Piecing Together the Bone Marrow Niche


The bone marrow microenvironment is a complex, tightly regulated network that provides a supportive environment for hematopoietic stem cells (HSCs) to develop into mature blood cells. This microenvironment is critical for maintaining hematopoiesis, the process of blood cell production. In their recent publication, Dr. Anastasia N. Tikhonova and colleagues use single-cell RNA sequencing to analyze the intricate interactions within the bone marrow, providing insights into the cellular composition and functions of the bone marrow microenvironment.

The authors initially isolated putative mesenchymal HSC niche components such as blood vessels, mesenchymal stromal cells, and osteo-lineage cells to obtain single-cell sequencing data from bone marrow in steady-state conditions. T-distributed stochastic neighbor embedding analyses showed clear separation of single-cell sequencing data from bone marrow in steady-state conditions. The authors further identified two endothelial, four perivascular, and three osteo-lineage cells were identified.

The advent of next-generation sequencing has seen the exponential expansion of knowledge of the molecular and cellular complexities of hematopoietic cells in normal and malignant contexts. Until recently, these techniques have been applied to bulk populations of cells, with inherent limitations in sequencing depth and resolution of rare cell populations, especially in admixtures of normal and malignant cells. Single-cell sequencing facilitates interrogation of cells at single-cell resolution allowing dissection of clonal composition and heterogeneity of cell populations without need for computational inferences of subclonality.

In their recent publication, Dr. Anastasia N. Tikhonova and colleagues use single-cell and bulk mRNA sequencing to delineate spatiotemporal relationships, patterns of hematopoietic factor production and functional consequences of gene expression changes within the bone marrow microenvironment in mice during steady-state and regenerative hematopoiesis. They couple these techniques with robust identification of cell subpopulations and functional in vitro and in vivo correlative studies.

The authors initially isolated putative cellular HSC niche components such as blood vessels, mesenchymal stromal cells, and osteo-lineage cells to obtain single-cell sequencing data from bone marrow in steady-state conditions. T-distributed stochastic neighbor embedding analyses showed clear separation of the lineage populations. Interestingly, within each lineage, several subpopulations were identified: two endothelial, four perivascular, and three osteo-lineage subpopulations. Biomarkers were able to distinguish each of these subpopulations, even to the level of arterial vasculature versus sinusoidal capillaries or mesenchymal stromal cells destined for adipogenesis or osteogenic differentiation. The authors

(Cont. on page 16)
**President’s Column**

**Strength in Numbers: How ASH Plans for the Future**

The mission of ASH is broad: furthering the understanding, diagnosis, treatment, and prevention of blood-related disorders. Its membership is diverse: more than 17,000 hematologists from nearly 100 countries, all with heterogeneous professional needs. So how does ASH think and act creatively to keep moving forward? How do we prepare for the future while effectively managing a complex portfolio of publications, meetings, educational and training activities, advocacy efforts, and global outreach?

There is no right answer to this, and professional societies differ in their approaches. ASH relies on a large and diverse group of volunteers who represent the numerous clinical and research disciplines that touch our field. They serve on ASH committees, editorial boards, taskforces, and working groups, and provide their unique perspectives in a team-based approach to address specific aspects of our mission.

Helping to organize and harness the know-how and talents of our volunteers is the ASH staff. Abby led by our long-time executive director Martha Liggett and deputy executive director Matthew Gertzog, ASH staff members specialize in the wide variety of skills needed to keep an organization of this size moving forward, including fundraising, meeting planning, project management, accounting, and strategic development. Over the past two and a half years as an ASH officer, I have chosen to lead taskforces, coordinate the meetings and workshops and can bear witness to the deep and diverse talent pool serving ASH. The combination of dedicated volunteers, a robust committee structure, and a highly skilled staff is the “special sauce” that keeps ASH moving forward. Providing oversight is the ASH executive committee — five officers and eight councilors elected by membership, from members who serve as a volunteer board of trustees for the organization.

As 2019 president, I am asked to oversee the board’s meetings and shepherd the Society as we make decisions that affect our financial outcomes, our membership, and our patients. My experience has been that board members share common values and a commitment to the ASH mission. When we meet, we “put on our ASH hats” and place the best interests of the Society above self-interests or the interests of our home institutions.

Through strategic thinking, our board can rise above routine responsibilities and get to the meat of our work. We define strategic priorities, ensure that we maximize our efforts to be successful in these priorities, minimize or eliminate efforts in activities that are not strategic priorities, and identify new opportunities that align well with our mission. We listened to the voices of our members and their representatives on the board that focused the Society’s efforts on our multipronged sickle cell disease and precision medicine initiatives, the creation of Blood Advances, development of evidence-based ASH clinical practice guidelines, the ASH Foundation, and the ASH Research Collaborative. These programs are in various stages of development, from fledging to adolescence, and are positioning our Society for the long-term success as a leading professional organization for hematologists.

I learned recently that an activity that distinguishes boards that are truly exceptional is the willingness and ability to spend precious board time in “generative” discussions, designed to generate questions rather than deliver answers, and thus to identify problems before they become obvious and define future areas for strategic planning. At our annual retreat, board members, editors, standing committee chairs, and staff leaders devoted time for such discussion apart from our usual agenda, thus committing to be exceptional in our approach. Using the format of a formal debate, we discussed existential threats and opportunities that may lie ahead and how we might be better prepared to address them. We focused specifically on workforce development, asking whether hematology can continue to thrive as a “stand-alone” discipline recognized as a key specialty that brings value to clinical and research teams. We also discussed whether our strategy of positioning ASH as an umbrella society that broadly covers all areas of the discipline will remain attractive to members, as more narrowly focused societies emerge and grow. The discussion was spirited and productive and will undoubtedly lead to new ideas to test and implement.

I hope that this column serves as a reminder to the entire membership that the Society is always seeking out “new blood,” and nominations for leadership and committee positions are strongly encouraged. I hope that it also illuminates the everyday hard work that transforms the needs of members into actionable policies and programs. My experiences so far as ASH President make me confident that our Society is in good hands. We are inclusive, mission-focused, strategic, and forward thinking in our approach to governance.

But ASH has a great understanding of the challenges facing patients and trial development in SCD. “For me, being able to set up this network so that it will help with accrual is a key piece,” she continued, “but so is having this system where there is clear communication to industry about what the expectations should be to do these trials successfully.”

“ASH fits that great niche between industry and the community of providers who care for individuals with SCD.”

In June 2018, ASH hired Dr. Charles Chesson to serve as director of the CTN and recruited Dr. LaTasha Lee to serve as Senior Manager of Partnership Engagement. “Their first year of work has been to operationalize the endeavor, setting up the infrastructure and organizing both the patient engagement groups and nascent industry partnerships. Getting patients involved has been one of the important development goals of this milestone effort. So far, four of a planned eight patient engagement meetings have been undertaken. In these meetings, individuals diagnosed with SCD, and their caregivers and families discuss barriers to participation in trials and outline what they consider to be key research priorities. Following these meetings, Drs. Chesson and Lee aim to publish a comprehensive review of their findings, which will take the form of the first ever SCD Patient-Centered Research Agenda. The hope is that this agenda will influence research and be a driving factor in the development of additional treatment options for individuals living with SCD.

Simultaneous with patient engagement, the plan to approve the first wave of SCD is on track and will be announced in the early fall or winter of 2019. Each of the sites is required to form a community advisory board as part of their application, ensuring that the views of patients, caregivers, teens, adolescents, and older adults with this disease are represented.

Dr. Abrams thinks that there is a growing momentum. “We’ve partnered with SCD community groups and patient national groups. We’ve been engaging with the National Institutes of Health with the DOA on several occasions. We have met with patient groups, and more recently, we’ve been engaging local sites that potentially would be members of the network.”

“There has been a lot of interest both by sites and by biotech and pharmaceutical companies,” he continued. “These milestones are getting buy-in from everyone. It’s been a joy to see that people are all stepping forward and saying, ‘We want to help with this.’”

**The Sickle Cell Clinical Trials Network: One Year In**

*Cont’d from page 1*

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The two ASH members elected to the National Academy of Sciences are:

Timothy J. Ley, MD
Lewis T. and Rosalind B. Apple Chair in Oncology, Division of Oncology
Washington University School of Medicine, St. Louis

Nancy A. Speck, PhD
Investigator, Abramson Family Cancer Research Institute; Professor, Department of Cell and Developmental Biology
University of Pennsylvania School of Medicine, Philadelphia

ASH Members Elected to the National Academy of Sciences

The National Academy of Sciences has announced the election of 100 new members and 25 foreign associates, including two ASH members. Election to the National Academy of Sciences is considered one of the highest honors in the sciences and recognizes distinguished and continuing achievements in original research.

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Housing Fraud Alert

As the launch of the registration and housing website for this year’s annual meeting approaches, please keep in mind that SPARGO, Inc., is the only official housing provider for the 61st ASH Annual Meeting and Exposition. Do not be misled by “pirate” housing companies and travel agencies that aggressively pursue attendees with supposedly significant discounts. ASH has no affiliation with these organizations and does not endorse their services. Deceptive tactics by these companies include:

- Informing attendees and exhibitors that the ASH hotel room block is sold out and that if you do not book with them immediately, you may not get a room
- Distributing fliers or promotional materials that appear to be issued by ASH
- Using ASH’s name and/or logo to falsely represent themselves as being affiliated with ASH
- Offering to make registration/housing arrangements for you via email

Please note that all registration and housing arrangements must be made via our secure website. Be sure the domain of the site you are on ends in www.hematology.org or spargoinc.com. If you encounter any of the scenarios listed, contact the ASH Housing Center immediately at ashhousing@spargoinc.com. Visit www.hematology.org/Annual-Meeting/4256.aspx for more information.

2019 ASH Annual Meeting Upcoming Deadlines

The 61st ASH Annual Meeting will take place December 7-10, 2019, in Orlando. The meeting will provide an invaluable educational experience and the opportunity to review thousands of scientific abstracts highlighting updates in the hottest topics in hematology. Attendees can also network with top minds in the field as well as a global community of more than 25,000 hematology professionals from every subspecialty. Visit www.hematology.org/Annual-Meeting for more information and to see what’s in store this year in Orlando.

- • ASH Foundation Run/Walk registration opens – July 10, 2019, 11:00 a.m. Eastern Time
- • Members-only registration and housing opens – July 24, 2019, 11:00 a.m. Eastern Time
- • Abstract submission deadline – August 1, 2019, 11:59 p.m. Pacific Time
- • Advance registration and housing open for nonmembers – August 14, 2019, 11:00 a.m. Eastern Time
- • Call for late-breaking abstract submissions – October 21-31, 2019


Various recommendations for pain management in patients with sickle cell disease (SCD) proposed by ASH were included in the final report. The report also highlighted a clarification from the Centers for Disease Control and Prevention (CDC) stating that the CDC Guideline for Prescribing Opioids for Chronic Pain is not meant to deny clinically appropriate opioid therapy to any patient who suffers acute or chronic pain from conditions such as cancer or SCD, but was developed with the purpose of providing recommendations for primary care clinicians who prescribe opioids for patients with chronic pain outside of active cancer treatment, palliative care, and end-of-life care.

To access the final report, visit www.hhs.gov/ash/advisory-committees/pain/reports/index.html. You can also find an informative Q&A with Dr. Varuna Singh, the Chief Medical Officer of the HHS Office of the Assistant Secretary of Health and Chairperson of the Pain Management Inter-Agency Task Force, at www.hhs.gov/blog/2019/05/10/patient-centered-care-is-key-to-best-practices-in-pain-management.html.
The Case
A 46-year-old woman with a long history of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma since 2011 presented urgently to a local hospital after being found unresponsive in her bedroom by family. Cardiopulmonary resuscitation was started immediately, emergency medical services were quickly notified, and she was defibrillated, intubated, and stabilized throughout the next 48 hours. The presenting rhythm identified by EMS was ventricular fibrillation. A cardiac catheterization was done that revealed normal coronary arteries, and the only major finding was that her left ventricular end diastolic pressure was 25 mm Hg (normal, < 12 mm Hg). An electrocardiogram (ECG) done the next day indicated overall normal left ventricular function (left ventricular ejection fraction, 55%). A subcutaneous implantable cardioverter defibrillator (ICD) was implanted the next day without difficulty. One week later, she was healing well from any wounds, and the ICD was functioning appropriately.

Her medical history was notable for CLL/small lymphocytic lymphoma, with her initial presentation in November 2011 with peripheral lymphocytosis and lymphadenopathy; the florescence in situ hybridization was negative. Her treatment included fludarabine and six cycles of rituxan from December 2011 to May 2012, with a complete response. She then had recurrent disease with diffuse lymphadenopathy and bone marrow involvement noted in May 2015. Ibrutinib 420 mg was administered from May 2015 to present, and computerized tomography imaging on June 24, 2016, indicated the resolution of lymphadenopathy.

Of note, her other medications included dextroamphetamine-amphetamine, synthonoid, and desvenlaxamine ER. Additionally, she reported drinking alcohol heavily for three days prior to her cardiac arrest.

The Question
Does ibrutinib therapy increase the risk of serious rhythm disturbances?

The Response
The question of whether ibrutinib is causative of atrial fibrillation has been the subject of much discussion and investigation. Several articles have suggested that it might occur quite frequently in patients with CLL, in one study up to 13 percent, and the consequences of atrial fibrillation in patients who were on ibrutinib resulted in a high percentage having to discontinue therapy, most commonly owing to an increase in bleeding events. It is very clear that ibrutinib therapy is associated with a higher rate of atrial fibrillation than the “ambient” rate found in patients with CLL who are not on that therapy, and the risk of bleeding is a particularly vexing problem that may lead to discontinuation of this treatment. What is particularly challenging in this case is whether ibrutinib is associated with a more serious and life-threatening rhythm disturbance than just atrial fibrillation. As it turned out, in this patient, there was an episode of sudden death in which she was quickly and effectively resuscitated. This is very good news, however, from a detailed history and extensive evaluation, the cause of ventricular tachycardia or ventricular fibrillation (VT/VF) was not apparent. She had no structural reason for VT (normal left ventricular ejection fraction and normal valvular structures) but was on ibrutinib and dextroamphetamine-amphetamine (a powerful stimulant). She did have significant alcohol exposure, and that certainly may have contributed. Her baseline ECG in 2014 and the repeat after the event in May 2019 did not reveal any major abnormalities other than nonspecific anterior T wave changes (Figure 1). When a topical wireless monitor was placed, the QT interval appeared prolonged and varied significantly when accessed via the transmission (Figure 2, black arrows). It has been observed that ibrutinib may be associated with VT, but it is not clear that it is a prolonged QT-based mechanism. Furthermore, this patient had not been on ibrutinib for more than two weeks on the May 2019 ECG or when the wireless monitor was placed.

The current electrocardiogram (ECG) on top (May 2019) after ibrutinib is withheld for at least two weeks. The ECG on the bottom (April 2014) precedes the initiation of ibrutinib initially. No significant changes are noted between these two tracings.

Recommendations
It is difficult to associate VT or sudden death with a particular medication. As is frequently the story in cases of sudden cardiac death, there are many possible explanations. In this case, the patient was on ibrutinib, but also dextroamphetamine-amphetamine and had significant alcohol exposure. Exactly what was required to create the milieu for a “perfect storm” and resultant VT is conjecture. After the event, it appeared as though she did have intermittent prolongation of the QT interval that is only detected with prolonged monitoring. Her future therapy for CLL should probably be an alternative agent, mainly because this sudden death event is at least possibly associated with ibrutinib. Nevertheless, it is difficult to identify a safe drug if there are no telltale signs on the baseline standard 12-lead ECG. This case highlights that a topical patch monitoring system for an extended period may identify important findings to establish a connection between a medication and serious rhythm disturbances. It is unlikely that such an association could ever be established with an intermittent 12-lead ECG as is the current standard recommendation.


Dr. Lenihan indicated no relevant conflicts of interest.
Autoimmune Disorders and the Development of Myeloid Disease: Should We Always Blame the Therapy?

The Case

A 64-year-old woman was referred to our clinic with pancytopenia and fatigue. The patient reported a four-year history of systemic lupus erythematosus (SLE; synovitis, malar rash, oral ulcers, pleuritis, effusions, anti-Smith antibody) that had previously been treated with pulse cyclophosphamide and concomitant oral prednisolone followed by maintenance azathioprine for severe nephritis. The bone marrow (BM) aspirate at diagnosis showed 56 percent blasts consistent with acute myeloid leukemia (AML). BM cytogenetics revealed a chromosome 7 deletion. Azathioprine was promptly discontinued. Molecular testing revealed lack of NPM1, FLT3, CEBPA, or TP53 mutations and presence of pathologic mutations in DNMT3A and ASXL1. After detailed discussion with the patient about lower- and higher-intensity therapy options in the context of adverse cytogenetics with monosomy 7, she elected to proceed with intensive chemotherapy, to be followed by allogeneic hematopoietic stem cell transplantation.

This case illustrates one of the dreaded downstream events in individuals with severe autoimmune disease (AD) — the development of therapy-related myeloid neoplasm (t-MN). An elevated risk of AML/myelodysplastic syndromes (MDS) has been reported across several ADs, including SLE, rheumatoid arthritis (RA), inflammatory bowel disease, and multiple sclerosis. Large scale population-based studies have shown the risk to be between 1.3 and 2.1 higher in patients with a previous history of AD compared to population baseline.1,2 Historically, this risk has been tied to given cytotoxic therapies, but that is likely too simple. Rather, current thought is that genetic susceptibilities, immune stimulation of the BM, and immune surveillance defects cooperate in the development of t-MN. Supporting this is the early emergence of myeloid malignancies in the natural course of AD and development of MDS/AML among patients who have had no prior treatment exposure for their AD.

Etiologies

One of the first questions that this patient and others will ask is why t-MN occurs? For years, many hematologists blamed prior treatment; however, it is increasingly understood that there is a multifactorial pathogenesis to AD-associated MN development. The risk of t-MN varies by the type of AD and the duration and type of genotoxic agent exposure. Among the commonly used drug classes in the management of AD, secondary leukemogenesis has been described extensively with the use of alkylating agents (cyclophosphamide), antimetabolites (azathioprine), and topoisomerase inhibitors (mitoxantrone).3,4 For example, prior azathioprine exposure has been associated with as high as a sevenfold elevated risk compared to the azathioprine-hazard-pooled-toxicity database due to direct DNA mutagenic effects. However, it is not just the treatment. Leukemogenic susceptibility to genotoxic agent exposure is strongly influenced by individual genetic predisposing factors. These factors include metabolism phenotypes, DNA damage repair pathways, and possibly the concomitant presence of clonal hematopoietic of indeterminate potential (CHIP). Patients with preleukemic CHIP have a significantly higher risk of developing t-MNs than patients without clonal hematopoiesis, and screening for CHIP at the time of primary cancer diagnosis can help identify patients at risk for t-MN.5 The risk of t-MN in breast cancer and Hodgkin lymphoma survivors is correlated with the extent of radiotherapy and the duration and type of chemotherapy.6 Importantly, the elevated risk of MN with conventional cytotoxic therapies does not seem to be shared by biologic therapies such as tumor necrosis factor-alpha (TNF-α) inhibitors.7

What about treatment-naïve patients? One hypothesis is that HLA-associated susceptibility can partially explain the occurrence of AML in patients with AD who have never been treated or who avoid cytotoxic agents. For example, HLA-RB7 carrier status has been associated with an increased predisposition to both AD and AML.8 Another important molecule implicated in hematologic malignancies (HMs) involving the myeloid lineage is interferon-1 (IFN-1). Polymorphisms within the IFN-1 receptor antagonist gene have been associated with both AD and secondary AML.9

The BM microenvironment is known to play an important role in AML/MDS pathogenesis and progression. A proinflammatory tumor microenvironment can contribute to overall tumor progression by promoting various aspects of cancer cell proliferation and survival. A central proinflammatory mediator, implicated in both ADs and leukemias,10 is nuclear factor of B (NF-κB). Constitutive NF-κB signaling can be achieved either through intracellular autogenic activation or extrinsically by cytokine factors produced in the tumor microenvironment milieu. Finally, leukemic myeloid cells develop a variety of escape mechanisms to evade peripheral T-cell immune surveillance and destruction. Further impairment of a disrupted T-cell immunologic surveillance by exposing the BM to immunosuppressive agents could potentially lead to their clinical emergence.

SLE and t-MN

Only a few studies have specifically investigated the risk of myeloid leukemia in SLE, which is the pre-existing condition in this case. Analyses by Dr. Lena Björnärd and colleagues11 and Dr. Arvi Parikka-Patel and colleagues12 reported an elevated risk of myeloid leukemia in SLE, with a standard incidence ratio of 3.4 and 2.86, respectively. Similarly, Dr. Björn Lidström and colleagues reported an increased risk of AML in a Swedish national cohort of 6,438 patients with SLE.13 Notably, the leukemia risk in this study was confined to the patient subset with preceding prolonged cytopenias, especially leukemia. The median latency period between SLE and leukemia diagnoses was five years. The risk of myeloid leukemia was restricted to the subgroup characterized by more men and older age at onset of SLE. Further, the study did not identify a difference in the frequency of cytotoxic exposure between the case and control cohorts, suggesting that prior cytotoxic exposure is not a major cause for AML development in SLE.14 Another study investigated the effect of latency of AML development in SLE and showed a decremental risk for myeloid leukemias with longer SLE latency.14

Newer Treatments for AD?

Another question that arises, especially during consultation on cases of AD, is whether or not there are increased risks when the agents dubbed "disease-modifying antirheumatic drugs" (DMARDs) are used. These medications are commonly used in patients with RA and in conditions such as ankylosing spondylitis, psoriatic arthritis, and SLE. While data on the increased risk of development of certain cancer types (e.g., melanoma, nonmelanomatous skin cancers, and lymphomas in patients with RA treated with anti-TNF-α therapy) are conflicting, there is no substantial increase in the risk of leukemias in patients treated with anti-TNF-α therapy as compared with those treated with any nonbiological DMARDs.15 In a Swedish Cancer Registry study by Dr. Johan Askling and colleagues, a significant association between RA and AML risk was observed only in the inpatient advanced and early-arthritis cohorts, but not in the TNF-α-blocker group, which argues for a DMARD approach as a critical risk factor in AML development.16 Data from the same study also suggested no effect of RA latency on the risk for AMLs. While a significant, albeit weak, association between DMARD use and HM risk has been noted with azathioprine and cyclophosphamide therapies, the occurrence of leukemia cases even among patients who had no prior exposure to cyclophosphamide or azathioprine suggests a link to RA-related immune stimulation itself.17

Future Directions

The excess risk of MNs in AD varies by the particular type of AD, reflecting biologic differences between AD entities. Unfortunately, studies designed to investigate the role of...
AD-directed therapy in MN risk have yielded conflicting results, and data correlating patient and AD features with subsequent MN development are not forthcoming. Additionally, we still do not have a complete understanding of the molecular defects underpinning secondary leukemogenesis in AD. Important future research agendas include evaluation of molecular defects and identification of risk factors associated with MN development in AD. A more detailed characterization of biologic mechanisms through focused efforts directed toward delineating pathophysiologic pathways will not only further our understanding of the association between the two entities but also help identify patients who are at a higher risk for developing post-AD MNs and who may therefore benefit from preemptive strategies, especially now with the availability of potentially safer and more effective biologic treatment alternatives.

Editors’ Note: This feature is based on a more robust review article published in March of 2019 in Best Practice &Research Clinical Haematology.

8. Brusamolino E, Anselmo AP, Klersy C, et al. The risk of acute leukemia in patients treated for Hodgkin’s disease is significantly higher if [see listed modality programs than after chemotherapy alone and is correlated with the extent of radiotherapy and type and duration of chemotherapy: a case-control study. Haematologica. 1998;83:812-821.
Three Identical Strangers, One Pressing Question

KRISTEN O’DWYER, MD
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Interest in documentaries has been on the upswing, as anyone who follows podcasts or subscription television can attest. It’s not just technology, the increasing number of media platforms, or the incredibly expanding amount of contemporary footage that has fueled this turnaround. The format of the documentary provides something that neither fiction nor newscasts can accomplish. While broadcast news may be able to deliver the current truth or a vision of it, documentaries provide scope. They look backward across long periods, which allows viewers to see the entire historical picture compressed and illuminated. Unlike fiction, where the bargain with the reader is that something recognizable but unreal will happen, documentaries ask us to acknowledge what is true.

Reclaimed footage, a compelling story, and innovative direction can spotlight stories that might have been otherwise forgotten. At their best, they can also force us viewers to rethink the present. That’s what happened to me on viewing “Three Identical Strangers,” a 2018 documentary film that has remained among the most popular offerings on Netflix since its release.

(Spoiler alert: Those who would like to watch the film without spoilers should do so now before reading further.)

The story tells of triplet boys born to a teenage single mother in 1961, relinquished to an adoption agency in New York City, and placed in separate adoptive families. Neither the families nor the boys — Eddy Galland, David Kellman, and Robert “Bobby” Shafran — were told of the other identical siblings’ existence, but a chance encounter by two of the brothers at community college in 1980 is what sparked an explosion of events set in motion. And what started as a joyful reunion turned into a painful discovery of the circumstances leading to their separation and the knowledge that they were not alone: other identical siblings were born as a result of multiple births (5 sets of twins and 1 set of triplets; 13 families) in the 1960s by Dr. Peter Neubauer, a prominent child psychiatry. The study was done under the pretext of routine evaluation of adopted children. The study ended in 1996, and the design, purpose, and findings of the study have never been published, nor have they been revealed to the 13 families. Its contents were placed under seal at Yale University until 2965 by Dr. Neubauer, who died in 2008.

“Three Identical Strangers” raises many questions, and foremost among them is concern about the medical ethics associated with the Neubauer study. In a Newweek interview following the film’s release, the director, Tim Wardle, explained that he did not think those involved with the study were immoral. “It’s easy to judge the past by today’s standards,” he stated, “but you have to make some allowances.” Indeed, evidence exists that Neubauer and other prominent contemporary psychiatrists believed twins caused a burden to parents and the family structure, and the study was designed to examine the effects of nature versus nurture. The study, a decades-long experiment commenced in the 1960s by Dr. Peter Neubauer, a prominent child psychiatrist, involved the film subjects as part of a cohort of multiple births (5 sets of twins and 1 set of triplets; n=13). The 13 families involved in the study all had used the same adoption agency. Dr. Neubauer acquired data on the twin pairs, triplets, and their families in the form of filmed direct observation and interviews of the children, parents, and siblings; psychological tests; and school reports under the pretext of routine evaluation of adopted children. The study ended in 1996, and the design, purpose, and findings of the study have never been published, nor have they been revealed to the 13 families. Its contents were placed under seal at Yale University until 2965 by Dr. Neubauer, who died in 2008.

The issues raised by the film remain timely and challenge us as clinical researchers to ask what our research community is doing today that will not stand up to the ethical test of time. We are reminded of the vital need to be vigilant in our examination of our own actions and those of our collaborators to ensure they are appropriate when designing and conducting research studies, to listen closely to concerns raised by colleagues, to consider the perspectives of patient advocates and other stakeholders, and to periodically take a step back and look at what we’re doing with an eye as critical and focused as that of these filmmakers.

While I was moved to tears by the poignant stories of the triplets growing up together made clear the high human cost of the study. Footage of the triplets distressed reaction as a clinical researcher was eclipsed by my visceral response as a parent. Having been invited to write this essay and discuss how the movie affected my professional worldview, I can report my distressed reaction as a clinical researcher was eclipsed by my visceral response as a parent. Footage of the triplets sharing a crib in their infancy juxtaposed with interviews of them as adults reflecting on the lost opportunity to grow up together made clear the high human cost of the emotional pain inflicted on these children and their adoptive parents. I invite readers who have seen the film and those inclined to view it now to share your thoughts.
X-linked severe combined immunodeficiency (X-SCID) is a rare inherited disorder of the immune system caused by mutations in the common \( \gamma \) chain (IL2RG). This condition is characterized by near-absent T and natural killer (NK) cells with present but dysfunctional B cells. Without immune reconstitution, X-SCID is generally fatal in the first year of life. The standard of care for X-SCID is allogeneic hematopoietic cell transplantation (HCT), with best outcomes achieved using HLA-matched sibling donors (MSD). However, less than 20 percent of infants will have an MSD, and transplantation with unrelated and haploidentical donors is complicated by increased rates of graft-versus-host disease (GVHD) and delayed immune reconstitution. Furthermore, high-dose chemotherapy is required for B-cell reconstitution in these patients, raising concerns for potential late effects related to the use of alkylating agents in early infancy.

Gene therapy is an appealing alternative for infants without an MSD because it allows for use of milder conditioning regimens and eliminates the risk of GVHD, abrogating the need for immunosuppressive therapy. Early gene therapy trials in Europe using traditional \( \gamma \)-retroviral vectors without conditioning demonstrated successful reconstitution of the T-cell compartment in patients with X-SCID. However, five of 20 patients developed T-cell leukemia due to insertional oncogenesis; one patient died, and all patients remained dependent on immunoglobulin replacement. A subsequent international trial using a self-inactivating (SIN) \( \gamma \)-retroviral vector had an improved safety profile with no reports of myelodysplasia or leukemic transformation; however, humoral immunity was not restored in the absence of chemotherapy conditioning. More recently, Dr. Suk See De Ravin and colleagues at the National Institutes of Health (NIH) used a SIN-lentiviral vector with low-dose busulfan conditioning to treat five older male patients ranging from seven to 23 years of age with persistent immune dysfunction despite haploidentical HCT in infancy. Encouragingly, follow-up from the two older patients demonstrated both T- and B-cell reconstitution with clinical improvement and independence from immunoglobulin replacement.

Dr. Ewelina Mamcarz and colleagues presented results of a dual-center, phase II clinical trial out of St. Jude Children’s Research Hospital and the University of California, San Francisco observing SIN-lentiviral gene therapy in eight consecutive male infants newly diagnosed with X-SCID and without an MSD. The group used the same lentiviral vector as the previous trial to produce bone marrow–derived CD34+ cells and low-dose targeted busulfan with a goal area under the curve (AUC) of 22 mg h/L. Median age at gene therapy was 3.5 months (range, 2-1.4 months). Interestingly, three patients had maternal T-cell engraftment, and five had a history of previous infection, including one patient with active cytomegalovirus (CMV) and two patients with active disseminated bacille Calmette-Guérin (BCG) at time of gene therapy. Median CD34+ cell dose was 6.73 ± 106/kg (range, 4.46-15.10 ± 106/kg), and median vector copy number (VCN) was 0.40 per CD34+ cell (range, 0.16-1.13 per CD34+ cell). Conditioning was well tolerated with no significant busulfan-related toxicity, and all patients were engrafted without the need for blood product transfusions. There were no significant new immune complications, and the preexisting CMV and BCG infections were resolved with immune reconstitution. All patients were alive at a median follow-up of 16.4 months (range, 6.7-24.9 months). VCN has remained stable with time, and clonal TCR repertoire analyses demonstrated highly polyclonal integration patterns in all patients.

One patient had persistent maternal T cell engraftment and received a gene therapy boost 12 months after his first infusion with improvement in marker-marked T cells. Of note, this patient received the lowest CD34+ cell dose and had the lowest VCN of the graft. T-cell recovery was rapid in the remaining seven patients with all patients achieving normal T-cell subset counts (CD3+, CD4+, CD8+) within two to four months after infusion, including normal naïve T-cell counts (CD3+CCR7+CD45RO−; Figure). T-cell receptor (TCR) excision circles were present in all seven patients at three months, indicative of thymopoiesis, and T-cell proliferation to phytohemagglutinin was normal by four months. Spectrotyping demonstrated polyclonal TCR-Vβ repertoires in all patients. B-cell recovery was also rapid with normal B-cell counts by two months, and four patients were able to come off intravenous immunoglobulin replacement (IVIG) at 15 to 23 months after gene therapy. Three patients have also mounted protective antibody responses to vaccines, indicative of functional T- and B-cell reconstitution.

In summary, early results from this phase II trial are encouraging, with 100 percent survival and seven of eight patients achieving rapid and robust T- and B-cell reconstitution following SIN-lentiviral gene therapy with low-dose busulfan. Immune reconstitution was more rapid than typically seen following T-cell depleted unrelated and haploidentical donor HCT, and there were no cases of viral reactivation in this trial. Reassuringly, vector integration site analyses have demonstrated polyclonal integration patterns with no clonal expansions, though follow-up is admittedly short. Ultimately, long-term follow-up is needed to demonstrate durability of immune reconstitution and long-term safety of the lentiviral vector product. Nevertheless, results from this trial suggest that gene therapy is a viable treatment option for X-SCID patients without an MSD and may be superior to HCT with alternative donors. As more patients are treated with gene therapy and as safety and efficacy improve, one can foresee a time when gene therapy becomes the new standard of care for X-SCID.

Double-hit Diffuse Large B-cell Lymphoma: More Common Than We Think?


The disease formerly known as double-hit diffuse large B-cell lymphoma (DH DLBCL), now called high-grade B cell lymphoma with MYC and BCL2/BCL6 rearrangements, may be more common than previously thought. The entity is currently defined by the detection of rearrangements of the relevant genes using fluorescence in situ hybridization (FISH) probes, and most series estimate the incidence to be approximately 8 percent of all DLBCL cases.

Dr. Kahl indicated no relevant conflicts of interest.

Horn H, Ziepert M, Becher C, et al. MYC status in concert with BCL2 and BCL6 expression predicts outcome in the same attention for novel drug development and clinical trials (Figure).

Figure


Dr. Brierley and Dr. Mead indicated no relevant conflicts of interest.

Is Double-hit Diffuse Large B-cell Lymphoma More Common Than We Think?

Rumors of the future, then most of the high-risk patients with GCB fit under this definition. The other groups is needed before such a proposal can be adopted. If this broader category of the cases harboring traditional DH rearrangements by FISH. Presumably, validation by gene expression profiles and mutational analysts, observed low expression of genes associated with immune signatures and inflammatory signatures. Despite the MYC signatures by gene expression profiles and mutational analysis, MYC rearrangements were not present by FISH testing, suggesting other mechanisms signatures. Despite the MYC signatures by gene expression profiles and mutational analysis, MYC rearrangements were not present by FISH testing, suggesting other mechanisms for MYC dysregulation. Embedded in this biology are some suggestions of therapeutic strategies to exploit (histone modifying agents) and avoid (immunotherapy).

So, should the definition of this poor-prognosis DLBCL be revised from “DH DLBCL” to “DH signature DLBCL?” This change would create a new, larger category with about half of the cases harboring traditional DH rearrangements by FISH. Presumably, validation by other groups is needed before such a proposal can be adopted. If this broader category is the future, then most of the high-risk patients with GCB fit under this definition. The remaining GCB DLBCL cases (the ones without a DH signature) have an outstanding program of greater than the fraction cured with standard R-CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine, and prednisone) and perhaps do not require the same attention for novel drug development and clinical trials (Figure).


RNA Isoforms, Innate Immunity, and Leukemogenesis: IRAK4 As a Novel Therapeutic Target in MDS/AML


Occurrent somatic mutations in core components of the spliceosome, the central cellular machine that coordinates processing of pre-messenger RNA (pre-mRNA) to mature mRNA during gene transcription, are prevalent in myelodysplastic syndromes (MDSs) and other myeloid neoplasms. These mutations are heterozygous, mutually exclusive, and cause aberrant splicing due to altered splice-site recognition. As a result, the spliceosome fails to correctly exclude introns, resulting in a panoply of differentially expressed isoform variants. Functional assessment to identify the key isoform variants that contribute to the development of hematopoietic malignancy represents a major challenge.

In May’s issue of Nature Cell Biology, researchers based at the Cincinnati Children’s Hospital Medical Center, in collaboration with groups in New York, St. Louis, Bethesda, and Oxford, described a powerful experimental approach to identify such functional isoform variants. The researchers first evaluated exon usage in 160 acute myeloid leukemia (AML) samples from 53 children (GCB-ALL), specifically focusing on genes that were explicitly regulated at the level of mRNA isoform switching, rather than gene expression variance. This identified 877 genes demonstrating high variance of isoform expression that could be used to segregate patients into three groups with distinct clinical prognoses. The group with the most adverse prognosis demonstrated significant enrichment of common mRNA isoforms in innate immune pathway genes. The most significantly altered gene undergoing isoform switching was interleukin-1 receptor–associated kinase 4 (IRAK4), a serine/threonine kinase acting downstream of the toll-like receptor and interleukin-1 receptor superfamily, interacting with key cell-signalling regulators such as MYD88 to control proinflammatory gene expression. The inclusion or exclusion of exon 4 of IRAK4 determines whether a long (IRAK4-L) or short (IRAK4-S) mRNA isoform is generated. Dr. Molly A. Smith and colleagues demonstrated that certain AML human primary cells and AML cell lines preferentially express the IRAK4-L RNA and protein isoform, when compared with healthy cord blood and bone marrow CD34+ cells, which predominantly express IRAK4-S.

Next, they used a range of different experimental approaches to demonstrate that IRAK4-L is a key component of the mydosome, interacting with MYD88 to activate NF-κB and MAPK pathways, whereas IRAK4-S preferentially activates MAPK signalling. Immune-competent mouse xenotransplantation studies demonstrated that IRAK4 binds MyD88 directly near the N terminal domain, which is lost in IRAK4-S. Leukemic cell function in vitro and in vivo was shown to be dependent on IRAK4-L, including through treatment with CA-4948, an IRAK4 kinase inhibitor in clinical development. Importantly, neither genetic nor pharmacologic inhibition of IRAK4 had a major impact on normal hematopoiesis.

Finally, the authors evaluated the genetic alterations which result in aberrant IRAK4-L splicing and demonstrate correlation between IRAK4-L expression and the hotspot S34F mutation in U2AF1. Selective IRAK4 inhibition in U2AF1 mutant primary patient cells suppressed serial engraftment of MDS cells in a xenograft model, suggesting that inhibition of IRAK4-L impairs U2AF1-mutant MDS-propagating cells in vivo.

Overall, this comprehensive functional analysis of a targetable oncogenic RNA isoform elegantly demonstrates interactions between innate immunity, pathogen stimulation, and leukemic transformation. The authors also proposed that IRAK4-L expression might underlie aberrant IRAK-1 activation recently reported in MDS and AML. The mechanism of aberrant IRAK4-L expression in non-U2AF1 mutant AML cases remains unclear. These findings provide proof of principle that aberrant splicing associated with spliceosome mutations creates therapeutic vulnerabilities relating to specific isoforms, presenting an alternative strategy to target these poor prognosis malignancies in addition to the use of small-molecule modulators of the spliceosome. In conclusion, and as broadly, this study also highlights the importance of understanding the mechanisms of leukemogenesis where control of innate immunity is disturbed, and whether there is overlap between pathways activating innate immunity and those regulating hematopoietic stem/progenitor cells during MDS/AML development.

In summary, Dr. Smith and colleagues identified a poor-prognosis subtype of MDS/AML defined by the accumulation of certain mRNA isoform changes in innate immune pathway genes. The preferential expression of IRAK4-L occurs as an event downstream of the key mutation S34F in U2AF1. IRAK4-L is present in numerous MDS and AML cell lines and primary cells. IRAK4-L retains an N terminal domain that interacts directly with MyD88 to promote maximal activation of NFκB when compared to the short isoform, which promotes MAPK activation. A selective IRAK4 inhibitor halts leukemic growth both in vitro and in transplantant experiments of primary cells into immunosuppressed mice, providing a putative novel therapeutic strategy in MDS/AML.
Piecing Together the Bone Marrow Niche

Cont. from page 1)

were further able to trace differentiation of these latter cell populations along a continuum using relative expression of various differentiation markers. To ascertain interactions between the microenvironment and hematopoietic cells, they profiled expression levels of prohematopoietic factors within individual cells and populations, identifying the specific populations that produce factors with known functions in the cell extrinsic support of HSCs, such as stem cell factor and stromal cell-derived factor 1.

To determine changes during stress hematopoiesis, mice were given a marrow-ablating dose of 5-FU and the analysis was repeated. In this case, the authors observed an adipocyte-primed cluster not present at steady state and a global reduction in the osteolineage. They saw an increase in active cycling cells pre-empting steady-state and a global reduction in the osteolineage. These mechanistic insights are elegantly translated using genetically modified mouse models, demonstrating the integral functions of Dll4 in hematopoiesis, particularly after chemotherapy. The study helps to piece together the complex, three-dimensional, living jigsaw puzzle that is the bone marrow niche, highlighting the integral roles of these cells in supporting hematopoiesis in health and disease. The level of detail of the bone marrow microenvironment provides opportunities for discovery of cellular interactions that can be targeted therapeutically for use in the clinical setting and raises new questions. For example, are there aberrant interactions present that maintain leukemia and stem cell survival? Can these interactions be inhibited using small molecules or antibodies? Can altering prohematopoietic signaling from the microenvironment promote bone marrow regeneration following cytotoxic or immunogenic insults? Can age-related decline in HSC function be stalled or reversed?

Single-cell sequencing is currently limited in the numbers of cells that can be studied (thousands to tens of thousands), but can uniquely provide resolution to detect subtle changes within small subpopulations of cells and will build on bulk sequencing studies. The data and techniques showcased in Dr. Tikhonova and colleagues’ study represent seminal gains in knowledge in the hematopoietic microenvironment and have wide-ranging significance for future studies into normal blood formation, ageing, bone marrow regeneration, and hematopoietic malignancies.


On the Road to a Better CAR: Identifying the Variables Associated With a Durable Response to CAR-T Cell Therapy in Relapsed/Refractory B-ALL


D19-directed chimeric antigen receptor T-cell (CAR-T) therapy induces complete remission (CR) in approximately 80 percent of children and adults with relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL). In those responders, approximately 75 percent (range, 53-93%) have no detectable minimal residual disease (MRD) by multiparameter flow cytometry.1 Robust CD19 CAR T-cell expansion, followed by a targeted cytotoxic attack of the CD19 lymphoblasts, is the likely mechanism associated with high remission rates. However, some patients will maintain long-term remission, with relapse rates of approximately 25% (range, 21-50%) reported in phase I and II clinical trials.2-4 Although the clinical characteristics and laboratory biomarkers that determine the duration of the antimicrobial responses of CAR T therapy have not been specifically defined, successful long-term remission is linked to CAR-T cell persistence beyond the induction of CR5 and disease burden at the time of T-cell infusions.6,7 Additionally, whether additional consolidation therapy in the form of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is required and/or beneficial for all relapsed B-ALL patients following CAR-T therapy has not been determined and remains an unanswered clinical question. Thus, understanding of the clinical and laboratory variables that contribute to long-term remission will help define the optimal use of CAR-T immunotherapy for children and adults with B-ALL.

Dr. Kevin Hay and colleagues reported long-term follow-up data of 53 adult patients (age >18 years) with relapsed/refractory B-ALL who were treated at the Fred Hutchinson Cancer Research Center on a phase II clinical trial between August 2013 and April 2017 and provided an analysis of the factors associated with event-free survival (EFS). Patients on the trial received lymphodepleting chemotherapy (cytapheresis-based regimen with or without fludarabine) followed by infusion of autologous T cells engineered to express a CD19 CAR incorporating a 4-1BB costimulatory domain. Disease response was performed three to four weeks after CAR-T infusion followed by a bone marrow and/or positron emission tomography–computed tomography and cerebral spinal fluid flow studies from patients with extramedullary disease. A subset of patients with an identified leukemia clonogenic by high-throughput (HTS) of the immunoglobulin heavy chain locus had HTS performed on the remission marrow to assess the depth of response of the clone.

The MRD-negative CR rate was 85 percent (n=45). Eight patients (15%) showed no response. At a median follow-up of 30.9 months, the median EFS among the 45 patients who had an MRD-negative CR was 7.6 months, and the median survival (OS) was 20 months. Among the eight patients with no response, the median EFS was 0.8 months, and the median OS was five months. In the subset of patients with an identified leukemia clone by HTS prior to CAR-T therapy (n=28), who achieved an MRD-negative remission by flow cytometry and HTS (n=20), the EFS was superior compared to patients with an MRD-negative remission by flow cytometry, but with a persistent clone identified by HTS (n=8; 6.4 months vs. 3.6 months; p=0.038). Twenty-four (49%) of the 45 patients who achieved MRD-negative CR had a relapse (median time to relapse, 3.5 months). Fourteen (88%) of the 22 patients had a relapse with CD19+ blasts, and six patients (36%) with CD19- blasts. The two-year cumulative incidence of relapse for patients with CD19+ blasts was 34 percent compared with 14 percent for CD19- blasts.

Of the 45 patients who had an MRD-negative CR, 18 (40%) proceeded to allo-HSCT. The median time from CAR-T infusion to transplantation was 70 days (range, 44-138 days). At a median follow-up of 28.4 months, the two-year estimated EFS and OS for the transplant cohort was 62 percent and 72 percent, respectively. The two-year cumulative incidence of relapse following transplantation was 17 percent, and the median relapse mortality was 25 percent.

Univariable logistic regression analyses were used to evaluate baseline and treatment-related factors associated with achieving an induction response. These analyses showed that a robust CAR T-cell expansion was strongly associated with the high rates of MRD-negative CR, and central nervous system leukemia prior to lymphodepleting chemotherapy was associated with no response to treatment (odds ratio, 0.08; p<0.013). Next, the investigators developed a stepwise multivariable regression model that associated with superior EFS among the patients who achieved MRD-negative CR. Three factors associated with better EFS include normal pre-treatment LDH (HR, 1.39 per 100 U/L increment increased), higher platelet count (HR, 1.05 per 50,000 cells/mm3 increased), and CD19+CD13+CD10-CD14+CD33-CD34+ cells as part of lymphodepleting chemotherapy regimen (HR, 0.34). Patients with a normal serum LDH, with a platelet count higher than 100,000/mm3, and who received fludarabine-containing lymphodepleting chemotherapy, had a predicted two-year EFS of 86% (95% CI, 78-93%), respectively, compared to an EFS of 13 percent and OS of 29 percent among patients without these three favorable characteristics. Allo-HCT was incorporated into the multivariable model as a time-dependent covariate to evaluate its independent effect on EFS for patients in MRD-negative CR after CAR-T therapy.

Patients who proceeded to allo-HCT in MRD-negative CR had superior EFS compared to patients who did not proceed to HCT (HR, 0.39; 95% CI 0.13-1.15; p=0.088).

In conclusion, Dr. Hay and colleagues have provided important long-term follow-up data on their cohort of adult patients with relapsed/refractory B-ALL and examined the variables associated with a durable CAR-T cell therapy response. The authors identified lower serum LDH concentration, higher platelet count prior to lymphodepleting chemotherapy, and the use of fludarabine in the lymphodepleting chemotherapy regimen to be associated with durable remissions. The relationship between a lower serum LDH and a higher platelet count as well as a higher frequency of durable remissions suggest that aggressive disease kinetics and tumor burden and the cumulative effects of prior salvage regimens on the marrow may limit CAR-T therapeutic effects. The use of fludarabine in lymphodepleting chemotherapy has been associated with CAR-T cell expansion and persistence, and these follow-up data support that association. Additionally, a multivariable analysis associated allo-HCT with better EFS in patients in MRD-negative CR following CAR-T therapy. Confirmation of the results in larger cohorts of patients will require a larger cohort of patients. Detection of MRD-negative CR is required for consolidation treatment after CAR-D19 T-cell therapy in the relapsed patient will require randomized clinical trials.


CAROL FRIES, MD, AND KRISTEN O’DWERY, MD

Dr. Fries and Dr. O’Dwerly indicated no relevant conflicts of interest.
Superficial Thrombophlebitis: The Less Dangerous Cousin of Deep Vein Thrombosis That Can Still Cause Harm


S
uperficial thrombophlebitis (ST) is a painful thrombotic condition that presents as a tender, erythematous, palpable cord with localized edema. Unlike patients with acute deep vein thrombosis (DVT), patients with ST do not necessarily require anticoagulation. How then do we decide which patients require anticoagulant therapy? And if anticoagulants are recommended, which agent, dose, and duration should be prescribed?

The decision to prescribe anticoagulants for superficial thrombophlebitis is primarily based on the risk of progression into the deep venous system. Factors such as length of the ST (≥5 cm), proximity to junction with the deep veins (<3 cm from saphenofemoral junction or perforator veins within the popliteal fossa), pregnancy, hormones, active inflammatory disease, prior venous thromboembolism (VTE) or ST, and active cancer increase the potential for progression. Patients without any of these risk factors can generally be treated with pain control measures (topical nonsteroidal anti-inflammatory drugs [NSAIDs] and compresses) in addition to clinical surveillance for worsening of symptoms. For patients with one or more of these risk factors, guidelines suggest anticoagulants be considered.1–5

To answer the question of which agent to use for treatment of ST, Dr. Marcello Di Nisio and colleagues recently published a clinical synopsis of their Cochrane review.6 Randomized controlled trials evaluating low-molecular-weight heparin (LMWH), fondaparinux, rivaroxaban, heparin spray gel, tenoxicam, naproxen, graduated compression stockings, and saphenofemoral disconnection were included in the review. The primary outcome measure was symptomatic VTE. Secondary outcome measures included ST extension, progression to DVT or ST, and symptom control.

In brief, when compared with placebo, only fondaparinux reduced the risk of symptomatic VTE,7 whereas fondaparinux, LMWH, and NSAIIDs all reduced the risk of ST extension (Table). The largest trial comparing two active treatments showed no difference between fondaparinux 2.5 mg daily and rivaroxaban 10 mg daily for symptomatic VTE or ST extension.8 There was insufficient evidence for all other treatments. Key limitations for the two largest trials included restriction of enrollment to low-risk patients in the fondaparinux versus placebo trial,9 and the small number of events in the rivaroxaban trial.10

The clinical synopsis by Dr. Di Nisio and colleagues shows that the jury is still out on the right agent to use for treatment of ST. Fondaparinux is the most promising to date, but it requires 45 days of costly injections for a 1 percent absolute risk reduction for VTE.

My personal approach to treating ST starts with a risk assessment (site and length of the ST; risk factors as mentioned previously) followed by an ultrasound to exclude DVT (unless an isolated short segment confined to a varicose vein below the knee). If the ST is larger than or equal to 5 cm long and/or within 3 cm of the saphenofemoral junction, I perform a risk assessment for bleeding. If the risk of bleeding is low, I recommend either fondaparinux, LMWH, or NSAIIDs for 45 days (for pregnant patients LMWH is a safe alternative). If the bleeding risk is high, I arrange for a repeat ultrasound within five to seven days to rule out progression into the deep veins.

For all patients, I recommend topical diclofenac four times daily because it leads I offer better pain control than oral NSAIDs, with fewer adverse effects. Duration of treatment is continued beyond 45 days if pain continues, but it is not based on resolution of the palpable cord (which turns brown and is no longer tender) or of ultrasound abnormalities, which can persist indefinitely. Lastly, I review the symptoms of VTE. Upper extremity superficial thrombophlebitis was not covered in the referenced Cochrane review, but my approach is similar though the requirement for anticoagulants in these patients is less common.


Table. Summary of Placebo-Controlled Trials for Treatment of Superficial Thrombophlebitis

<table>
<thead>
<tr>
<th>Agent</th>
<th>Comparator</th>
<th>No. of Studies</th>
<th>Symptomatic VTE RR (95% CI)</th>
<th>Extension of ST RR (95% CI)</th>
<th>GRADE Quality of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fondaparinux 2.5 mg sc daily x 45 d</td>
<td>Placebo</td>
<td>1 (n = 3,002)</td>
<td>0.15 (0.04-0.50)</td>
<td>0.08 (0.03-0.22)</td>
<td>Moderate</td>
</tr>
<tr>
<td>LMWH prophylactic dose x 8-12 d</td>
<td>Placebo</td>
<td>1 (n = 222)</td>
<td>1.22 (0.38-3.8)</td>
<td>0.44 (0.26-0.74)*</td>
<td>Low</td>
</tr>
<tr>
<td>LMWH therapeutic dose x 8-12 d</td>
<td>Placebo</td>
<td>1 (n = 222)</td>
<td>0.85 (0.23-3.06)</td>
<td>0.46 (0.27-0.77)*</td>
<td>Low</td>
</tr>
<tr>
<td>NSAIIDs</td>
<td>Placebo</td>
<td>1 (n = 211)</td>
<td>0.91 (0.25-3.8)</td>
<td>0.46 (0.27-0.78)</td>
<td>Low</td>
</tr>
</tbody>
</table>

Abbreviations: LMWH, low-molecular-weight heparin; RR, relative risk; sc, subcutaneously; VTE, venous thromboembolism.

*Also included recurrent superficial thrombophlebitis.

LORI-AHN LINKINS, MD
Dr. Linkins has received payment for data adjudication of the MARINER trial (rivaroxaban for prophylaxis in medical patients).

Seeking Insight Into the AML Subpopulations and Microenvironment Through Single-cell RNA-Seq


The most recent World Health Organization and European LeukemiaNet classification system uses genetic and molecular findings to distribute cases of acute myeloid leukemia (AML) into favorable, intermediate, or adverse risk categories, which in turn influence treatment strategies for patients.1–5 Despite advances in treatment stratification, relapse after initial remission is common in all categories, and a subset of patients also remain refractory to induction chemotherapy. This has led to an interest in better understanding tumor biology to inform development of novel therapies, with a resultant resurgence of interest in transcriptomics. The recent massive literature has shown that heterogeneity within tumors plays an important role in recurrence and resistance.1 However, comprehensive molecular profiling in AML that includes transcriptomics has not yet been widely adopted despite the publication of several AML risk stratification models that incorporate combinations of somatic mutations, immunologic gene expression, and genetic expression profiling. The prior studies used various methods of assessing gene expression profiles of entire AML samples without single-cell resolution.

In their recent article, Dr. Peter van Galen and colleagues used a wide range of single-cell transcriptomics and genotyping to assess bone marrow (BM) monocytic cells from 16 patients with AML at diagnosis and during treatment, and BM aspirates from five healthy donors. Adapting Single-cell Gene Expression technology, the researchers performed high-throughput single-cell RNA sequencing (scRNA-seq) as well as single-cell genotyping by short-read and long-read sequencing to detect and phase AML mutations in individual cells. A machine learning classifier distinguished malignant cells from normal cells based on mutation status and classified cells on the basis of monocytic (monoblasts to monocytes) to myeloid (progenitor to mature neutrophils)1 according to their similarity to normal BM cell types.

The analysis revealed six distinct malignant AML cell phenotypes within the AML cases. The proportion of these cell types varied across the AML cases studied as well as over the course of treatment for each AML. The gene signature profiles of these six phenotypes were used to score bulk expression profiles of 179 diagnostic AML aspirates from the Cancer Genome Atlas (TCGA). The TCGA cohort could be clustered into seven different malignant tumor cell composition profile groups that, not surprisingly, demonstrated correlation with both characteristic genetic lesions (i.e., acute promyelocytic leukemia mapped to a high granulocyte-macrophage progenitor signature while CBFB-MYH11 cases mapped to high monocytic expression), progenitor-like cell (i.e., French-American-British morphologic subtypes). Additionally, mutational dysynchrony was found by transcript profiling that is reminiscent of the aberrant immunophenotypes of leukemic blasts and maturing cells seen typically by flow cytometry.

Interestingly, within the somatic mutational subtypes, the investigators found differential behavior between NPM1 subgroup—one with a strong HSC/progenitor phenotype in cases with co-occurring NPM1 and FLT3-ITD mutations and a second with a more differentiated monocyte- to dendritic-cell-like signature with LAMA5 mutations. Likewise, within the FLT3 mutated cases, different cellular hierarchies were identified. Genotyping of one case showed three subclones: “A” with a FLT3 p.A680V mutation, “B” with an additional FLT3-ITD mutation on the opposite allele, and “C” with a FLT3 p.N841K mutation only. Interestingly, most cells in subclones “A” and “B” expressed signature genes associated with progenitor-like cells, and the majority of subclone “C” cells expressed genes associated with differentiated monocyte-like or conventional dendritic-like cells. In these examples, the more progenitor-like phenotype typically was associated with an adverse prognosis.

Another area of recent investigative interest has been the study of antitumor immune responses within AML. Although therapeutic trials of immunomodulating treatments in AML have been limited to date, preliminary data have indicated a role of T cells in dysregulation in impairing antileukemic immune responses.1 Dr. van Galen and colleagues also looked at the T cell signatures in the AML and control samples. They found that AML BMs contained fewer T cells and cytotoxic T lymphocytes but relatively more Tregulatory cells (also confirmed by immunohistochemical analysis of CD25 and FOXP3). Additionally, the investigators performed in vitro assays of T cell activation that showed a strong inhibitory effect associated with the presence of leukemic cells from an acute

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Dr. Khatri and Dr. Kim indicated no relevant conflicts of interest.
myelomonocytic leukemia cell line, MUTZ-3. Cultures enriched with monocYTE-like (CD14+1) MUTZ-3 cells were also particularly suppressive of T cell activation, decreasing T cell activation by 10-fold (p<0.0001). Review of the transcriptome data from the AML cases in this series showed that monocYTE-like AML cells preferentially expressed pro-inflammatory cytokines, suggesting the notion that these cell types may be driving the immune-suppressive biology of AMLs.

In summary, this study provides comparative transcriptomic and genomic assessment of single cells, confirming the differential transcriptome between AML and control marrow, between different cytogenetic and morphologic subtypes of AML, and between different somatic mutational subtypes of AML. Taking advantage of the single-cell phenotyping capability of this method, the authors also examined the microenvironment of AML, demonstrating the role of monocYTE-like cell lines in inhibiting immune response to AML and different T cell subset distributions in AML, paving the way for future studies observing immunosuppression in AML. Despite these findings and several other gene expression profiling AML stratification models,1 transcripts have yet to find a place in routine clinical practice. Additional work is needed to parse out the clinical interplay between more traditional prognostic markers and the adverse progression associated with progenitor-like signatures in clonal and subclonal populations, the effect of immunosuppressive monocYTEs, and the T cell subset milieu. Additionally, by using the presence of mutations, including genes such as DNMT3A and TET2, to distinguish the malignant clonal cells from the background normal cells, the authors did not discriminate between clonal hematopoiesis and leukemic hematopoiesis. These complexities will require further elucidation prior to clinical adoption. However, it is clear that the use of single-cell profiling provides the added granularity needed to take our understanding of AML biology to the next level.

3. Welch JS, Ley TJ, Linco DC, et al. The origin and evolution of mutations in hematopoiesis. These complexities will require further elucidation prior to clinical adoption. However, it is clear that the use of single-cell profiling provides the added granularity needed to take our understanding of AML biology to the next level.

PET-adapted Strategies in Advanced Stage Hodgkin Lymphoma: Big to Small, or Small to Big, or Does It Even Matter?

The preferred treatment for advanced-stage Hodgkin lymphoma (HL) has historically differed with geography, with oncologists in the United States favoring chemotherapy with ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), Europeans have favoring escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone). ABVD is associated with an inferior progression-free survival (PFS), but its proponents argue that 1) this decrement in PFS is less important than the increased toxicity, including serious infection, infertility, and secondary malignancies, seen with escBEACOPP and 2) there is no overall survival (OS) benefit attached to the more toxic regimen. In an attempt to avoid excessive toxicity in patients with a high risk of relapse after allogeneic transplantation. N Engl J Med. 2016;375:143-153.

In the AML2011 study, reviewed here in the context of the existing literature, all patients with advanced-stage HL were randomized to standard treatment (n=413) consisting of six cycles of escBEACOPP, or to PET-driven treatment (n=410) where the results of PET2 were used to determine subsequent therapy. PET2-positive patients (Deauville 4 or 5) received additional escBEACOPP and PET2-negative patients (Deauville 1-3) received ABVD. PET2 was negative in 88 percent of patients in both standard treatment and PET-driven treatment groups, respectively. The primary endpoint of the study was five-year PFS, which was similar in both groups (80.2% vs. 85.7%, respectively) and compares favorably with the PFS results of studies of ABVD alone (2-5 year PFS 77-79%), strategies of PET-driven treatment intensification such as RATHL (3-year PFS 67.5%), and a new standard for advanced-stage HL, AVD+brentuximab, from the ECHERON-1 study (2-year PFS 82.1%).

1. Dr. Jacobson indicated no relevant conflicts of interest.
2. CARON A. JACOBSON, MD

This is a well-designed and important study for the treatment of advanced stage HL and, in recognizing that six cycles of escBEACOPP exposes a majority of patients to increased toxicity unnecessarily, seeks to limit this exposure using a PET-driven treatment de-escalation approach. In doing so, they preserve an excellent over five percent of five-year PFS, which is approximately five to 15 percent better than ABVD alone, dose-escalation strategies following two cycles of ABVD, or AVD+brentuximab. However, OS in this study is not markedly improved over that seen in studies of ABVD alone (2-5 year PFS 77-79%).
Poorer Pain Control With Normal Saline Bolus in Children With Sickle Cell Acute Vaso-Occlusive Pain


A new multicenter study challenges dogma of normal saline boluses (NSBs) to treat acute vaso-occlusive pain in sickle cell disease (SCD).

“When I come home from the hospital my legs are very swollen…and they hurt…I gain 10+ pounds and it takes over six weeks for my legs to get back to their normal size.”

This scenario has been retold to me repeatedly by my patients with SCD. So much so that I have adjusted my usual practice of liberal hydration during acute pain to one of limited hydration with hypotonic fluids for 24 to 48 hours at no more than maintenance fluids. In my review of the literature to support my practice, I noted that the use of intravenous hydration had become the community standard for supportive management of acute vaso-occlusive episodes (VOEs) in SCD in addition to parenteral analgesics, with little supportive evidence. Supplemental hydration is routinely used regardless of the hydration state of the individual patient, with little consistency in the amount, type, and rate of administration. The proposed rationale for supplemental hydration (hypotonic with saline) during sickle VOEs is that this will shift toward interventions that result in rehydrating the red cell rather than increasing intravascular plasma volume with NSB. Furthermore, rehydration using hypertonic fluids (normal saline) may be expected to only have an impact on increasing intravascular volume and vasodilation and do very little to rehydrate the sickle erythrocyte.

The Saline Against Lactated Ringers or Plasma-Lyte in the Emergency Department (SALT-ED) trial and the Isotonic Solutions and Major Adverse Renal Events Trial (SMART) both suggest that giving balanced crystalloids or NSB during acute respiratory failure with lower incidence of adverse kidney events within 30 days compared to saline (4.7% vs. 5.6%; adjusted odds ratio, 0.92; 95% CI, 0.70-0.96; p=0.01).1

Recently, investigators challenged the dogma of giving NSBs as part of acute VOG management, who tend to be treated in practice in most emergency departments (EDs). Using microfluidic in vitro models of the post-capillary venules, Dr. Mark A. Carden and colleagues demonstrated that exposing sickle red blood cells (RBCs) to hypertonic fluids reduced erythrocyte deformability and disrupted vascular occlusion compared with normotonic fluids.1 This provided clinical rationale to investigate the clinical impact of NSB on pain outcomes in patients with SCD. They then conducted a rigorously designed multi-institution retrospective cohort study of the use of supplemental fluids for acute respiratory failure with 20 outcomes of 400 children (median age, 13.8 ± 4.9 years) with SCD. Not surprisingly the majority (66%) of patients received a fluid bolus (despite no signs and symptoms of dehydration), with hyperosmolar normal saline almost exclusively (99.2%) representing the fluid of choice. The average volume infused during the ED stay was 18.2 ±9.5 ml/kg. Patients who received an NSB had similar pre-triage pain scores and similar overall opioid consumption compared with those who did not receive an NSB (18.4 ±11.0 vs. 18.5 ±11.2, p=0.82). They expectedly reported lower pain scores (0,100) spent more time in the ED (p=0.01), and had higher rates of admission (p=0.01). Due to the significant negative association of NSB with better pain outcomes, they supported a single-institution report1 and is supported by findings in non-SCD cohorts, investigators concluded that the routine use of NSBs in patients without clear indication of dehydration is not indicated.

So, why is it so easy to stick to the “tradition” of hydrating patients with SCD using NSB despite no evidence to support this practice, even in the absence of dehydration? There has hitherto been no evidence to support that NSBs in euveolic patients with VOEs, and yet it remains common practice in most pediatric EDs.

While the study by Dr. Carden and colleagues was limited by its retrospective cohort design, it does provide strong rationale for more research to determine the appropriate type of intravenous fluids for this population, if any. In my experience in the ED to manage VOEs. More physiologic, balanced salt solutions may be better for our patients with SCD given their unique red cell physiology as these are more likely to promote red cell rehydration by driving K+ and water into the RBC. My anecdotal practice of limited hypotonic fluids is indeed supported by the evidence after all.


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Rationale: FLT3 is a common mutation seen in a large number of participants with FLT3/ITD AML. The rationale for randomization on their trial.

The BMTCTN1502 study started enrollment and will generate helpful data for patients and physicians. It is accruing well and on target to complete enrollment within 24 months.

Three prospective clinical trials that compared the performance of three different DOACs with the LMWH dabeparin (Table) have been completed and published in the past year. One trial, the ADAM trial, which showed that edoxaban was noninferior to dabeparin with respect to the composite endpoint of recurrent VTE or major bleeding, while the rate of recurrent VTE was lower, the rate of major bleeding was higher with edoxaban than with dabeparin. Given the noninferiority of edoxaban, the clinician practicing evidence-based medicine can, with confidence, use edoxaban instead of LMWH in patients with cancer and acute DVT or PE. However, in the U.S. edoxaban is not a widely used DOAC. Therefore, the question is whether the two DOACs mostly used in the U.S. — apixaban and rivaroxaban — can be used instead of LMWH.

The SELECT-D (n=406) trial was set up as a pilot trial to compare rivaroxaban with dalteparin. It found, similar to the HOKUSAI trial, a lower risk of recurrent VTE with rivaroxaban, but at the cost of more clinically relevant nonmajor bleeding. The results provide evidence that rivaroxaban is also an effective alternative to LMWH for the treatment of VTE in patients with cancer. The ADAM trial (n=300, published so far only as an abstract), designed to investigate the safety of apixaban compared to dabeparin, found that apixaban was noninferior to dabeparin with respect to bleeding and significantly lower VTE recurrence than LMWH, suggesting the clinical utility of apixaban for the acute treatment of VTE in cancer.

Given the relatively small size of the trial, the confidence in the findings and generalizability to all cancer patients is limited. Therefore, a larger, more definitive study of a size similar to the HOKUSAI trial is needed to ensure clinicians that apixaban is noninferior to the current gold standard LMWH in the treatment of acute VTE in patients with cancer.

Acute DVT or PE in Cancer Patients: Apixaban Reasonable to Use? 

STUDY TITLE: Apixaban for the Treatment of Venous Thromboembolism in Patients With Cancer: A Prospective Randomized Open Blinded End-Point (Probe) Study (CARAVAGGIO)

ACCRUAL GOAL: 1,168. As of May 21, 2019, 1,115 patients have been randomized worldwide.

STUDY DESIGN: This is an open-label randomized treatment trial. Individuals on the experimental arm receive oral apixaban, 10 mg twice daily for seven days, after which they are transitioned to a once daily (total treatment period, 6 months). Subjects on the active comparator arm receive dalteparin, 200 IU/kg subcutaneously once daily for one month, and thereafter, 150 IU/kg once daily for five months. The primary outcome is recurrent venous thromboembolism (VTE) during six months on the trial. Patients eligible if they (1) newly diagnosed, objectively confirmed symptomatic or unsuspected proximal lower-extremity deep-vein thrombosis (VTE) during six months on the trial. Patients eligible if they (1) newly diagnosed, objectively confirmed symptomatic or unsuspected proximal lower-extremity deep-vein thrombosis (VTE) during six months (or symptomatic pulmonary embolism, PE, or unexpected PE in a segmental or more proximal pulmonary artery, and 2) any type of cancer (other than basal-cell or squamous-cell carcinoma of the skin, primary brain tumor or intracerebral metastasis, and acute leukemia). Exclusion criteria include poor Eastern Cooperative Oncology Group (ECOG) Performance Status (grade 3 or 4), life expectancy of less than six months, active bleeding or high-risk bleeding that contraindicates anticoagulation; and thrombocytopenia, venous or arterial insertion, or thrombolysis used to manage the acute episode. Sponsors include the Fondo Foundation (Federazione of the Associations of Italian Hospital Managers, www.fasiogor.org), an Italian internal medicine society, and the University of Perugia, Italy.

Rational: Based on somewhat dated society guidelines from 2015 to 2016, low-molecular-weight heparin (LMWH) is still the gold standard treatment for patients with cancer who have VTE. A new American Society of Clinical Oncology cancer guideline is expected to be published soon, and based on recent clinical trial data of the studies listed in the table, it may well list edoxaban and rivaroxaban together with LMWH as treatment options preferred over vitamin K antagonist therapy in patients with cancer and acute VTE. Given the inconvenience of subcutaneous injections and the cost of LMWH, the question that many oncologists have is whether direct oral anticoagulants (DOACs) — any of them or just selected ones — can be used instead of LMWH.

—— Amy DeZern, MD, MSIS

Dr. DeZern indicated no relevant conflicts of interest.
The Hematologist: ASH News and Reports

APRIL 11, 2019

This article presents novel insights into the pathogenesis of bone marrow fibrosis in JAKV/VI7-driven myeloproliferative neoplasia. Drawing on a series of elegant experiments in mouse models, the investigators provide compelling evidence for the critical role of the nuclear vitamin D receptor and bone marrow macrophages in the pathogenesis of idiopathic myelofibrosis.

APRIL 18, 2019

Dr. Lu Zhang and colleagues report impressive phase II results for an oral regimen of thalidomide, cyclophosphamide, and prednisone for the treatment of siltuximab-resistant idiopathic multicentric Castleman disease.

APRIL 25, 2019

and


Investigators using complementary approaches report critical insights into the structure and function of ADAMTS13. Their studies reveal interactions that allosterically regulate the proteolytic activity of ADAMTS13 on von Willebrand factor.


Voxelotor (GBT440) binds to the a-globin chain of hemoglobin and increases hemoglobin-oxygen affinity, thereby decreasing the polymerization tendency of deoxygenated sickle hemoglobin. The results of this phase I/II study of voxelotor in patients with sickle cell disease show a median 1 g/dl increase of hemoglobin concentration and a decrease of markers of hemolysis.

MAY 2, 2019

Dr. Miguel Ganuza and colleagues examine whether steady-state hematopoiesis in the aging mouse displays a decline in clinical complexity reflecting human clonal hematopoiesis of indeterminate potential (CHIP). As with human hematopoietic stem cells, they demonstrate loss of heterogeneity and myeloid skewing, but studies implicate gene mutations different from those in humans.


Dr. Andrew D. Zelenetz and colleagues report on phase Ib results of a trial combining venetoclax with R-CHOP or G-CHOP for the treatment of non-Hodgkin lymphoma, demonstrating very high response rates and manageable toxicity.

MAY 9, 2019

In a plenary paper, the investigators demonstrate that megakaryocytes, mainly recognized for their role in the generation of platelets, are also capable of preventing the spread of viral infection, indicating a role in immunity.


After nearly four years of follow-up, the authors present impressive sustained, progression-free, and overall survival benefits following the use of the Bruton tyrosine kinase inhibitor ibrutinib in patients with high-risk relapsed or refractory chronic lymphocytic leukemia.


In this study, the investigators shed light on the mechanism of pancytopenia in inflammation, a long-standing clinical observation lacking an explanation.

Interferon-γ (IFN-γ), a mediator of the inflammatory response, suppresses hematopoietic stem and progenitor cell function by binding to thrombopoietin, thereby blocking its signaling via its own c-MPL receptor.

MAY 16, 2019

Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is frequently treated aggressively. Dr. Sven Borchmann and colleagues demonstrate in a retrospective study of 167 patients that active surveillance yields excellent outcomes equivalent to those of early therapy.


Dr. Philipp Karschina and colleagues report on the diagnostic features and management of chimeric antigen receptor (CAR) T cell-related neurotoxicity, and they discuss biomarkers that predict outcomes.

MAY 23, 2019

In this plenary paper, the authors describe a promising novel therapeutic strategy using CRISPR-Cas to correct j-thalassemias resulting from aberrant donor or acceptor splice sites.

MAY 30, 2019

Most patients with hemophilia B have mutations in the factor IX (FIX) gene which lead to a protein that is functionally inactive. Using mouse models of hemophilia B, Dr. Brian Cooley and colleagues demonstrated that this circulating species impairs response to FIX prophylaxis, perhaps explaining the wide range of individual responses to infused FIX products in patients with hemophilia B.

JUNE 6, 2019

Sickle cell disease significantly alters the composition of the clot in a mouse model of venous thrombosis, a finding which adds to our understanding of its prothrombotic effect. Entrapped sickled red blood cells increase fibrin deposition in venous thrombi and enhance clot resistance to fibrinolysis.
A Woman With Thrombocytopenia Status
Post–Partial Gastrectomy

MARYAM ASIF, MD, 1 KYLE PARKER, MD, 1 AND FATIMA ALDARWEESH, MD 1
1. Pathology Resident, University of Chicago, Chicago, IL
2. Resident Pathologist, University of Chicago Medical Center, Chicago, IL
3. Assistant Professor, Associate Medical Director of Blood Bank and Transfusion Service, University of Chicago, Chicago, IL

A 62-year-old woman presented to the emergency department with abdominal pain and weakness; she has a medical history significant for end-stage renal disease secondary to focal segmental glomerulonephritis status after directed donor kidney transplantation two years ago. A computed tomography (CT) scan revealed pneumoperitoneum. She emergently underwent exploratory laparotomy with distal gastrectomy and gastrojejunostomy.

Upon admission, her platelet count was 96 × 10^3/µL (reference range [RR], 150-450 × 10^3/µL), approximately five days post-surgery her mental status waned, and platelet count decreased to 9 × 10^3/µL.

Other notable tests produced the following results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.0</td>
<td>11.5-15.5</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>3.6</td>
<td>0.5-1.4 (baseline, 3.4)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>1,280</td>
<td>116-245</td>
</tr>
<tr>
<td>International normalized ration</td>
<td>2.3</td>
<td>0.9-1.1</td>
</tr>
<tr>
<td>Mean corpuscular volume (fL)</td>
<td>87</td>
<td>81-99</td>
</tr>
<tr>
<td>Haptoglobin (mg/dL)</td>
<td>&lt; 20</td>
<td>51-192</td>
</tr>
<tr>
<td>D-dimer (µg/mL)</td>
<td>&gt; 20</td>
<td>&lt; 0.40</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>7.2</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>Unconjugated bilirubin (mg/dL)</td>
<td>2.7</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>Conjugated bilirubin (mg/dL)</td>
<td>4.5</td>
<td>0-0.3</td>
</tr>
<tr>
<td>Tacrolimus level (ng/mL)</td>
<td>20.8</td>
<td>5-20</td>
</tr>
<tr>
<td>ADAMTS13 functional level</td>
<td>24%</td>
<td>69-133%</td>
</tr>
<tr>
<td>ADAMTS13 inhibitor</td>
<td>&lt; 0.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>C3 complement level (mg/dL)</td>
<td>78</td>
<td>83-188</td>
</tr>
<tr>
<td>C4 complement level (mg/dL)</td>
<td>12</td>
<td>18-45</td>
</tr>
<tr>
<td>PF4 ELISA (OD)</td>
<td>0.152</td>
<td>&lt; 0.4</td>
</tr>
</tbody>
</table>

Abbreviations: ADAMTS13, A disintegrin and metalloproteinase with thrombospondin-1–like domains 13; ELISA, enzyme-linked immunosorbent assay.

What is the diagnosis?
A. Thrombotic microangiopathy, thrombotic thrombocytopenia purpura
B. Thrombotic microangiopathy, other
C. Heparin-induced thrombocytopenia
D. Antibody-mediated renal allograft rejection

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

Drs. Asif, Parker, and Aldarweesh indicated no relevant conflicts of interest.