Perioperative Bridging Is Falling Down


The need for an elective surgery or invasive procedure is common among patients receiving warfarin for atrial fibrillation. In approximately one quarter of such procedures, bridging anticoagulation during perioperative interruption of warfarin is prescribed with the goal of reducing arterial embolism.1 Mounting evidence from nonrandomized comparisons suggests that this strategy may not achieve its stated goal and may increase major bleeding compared with interruption of warfarin alone.1,2 However, until recently, high-quality randomized controlled trial data were lacking.

Enter the BRIDGE (Bridging Anticoagulation in Patients who Require Temporary Interruption of Warfarin Therapy for an Elective Invasive Procedure or Surgery) trial. BRIDGE was a randomized, double-blind, placebo-controlled study of 1,884 adults on warfarin for chronic atrial fibrillation or atrial flutter who were scheduled for an elective procedure requiring interruption of oral anticoagulation. The study excluded subjects with a mechanical heart valve, recent embolic event, or major bleeding; renal dysfunction (creatinine clearance < 30 mL/min); or planned cardiac, intracranial, or intraspinal surgery. All subjects discontinued warfarin five days before surgery and resumed warfarin within a day afterward. Subjects were randomized in 1:1 fashion to receive subcutaneous dalteparin 100 IU/kg twice daily or matching placebo from three days to 24 hours before the procedure and for five to 10 days after the procedure. The study drug was resumed 12 to 24 hours after a minor procedure and 48 to 72 hours after a major procedure. The primary efficacy outcome was arterial thromboembolism (stroke, transient ischemic attack, or systemic embolism) at 30 days after the procedure. The principal safety outcome was major bleeding. All outcomes were confirmed by independent adjudicators who were blinded to treatment assignment.

Study subjects were predominantly elderly (mean age, 71.7 years) and male (73.4%). Mean CHADS2 score was 2.3. The most common procedures were gastrointestinal, cardiothoracic, and orthopedic. There was no difference in the incidence of arterial thromboembolism between the bridging group and the placebo group (0.9% vs. 0.4%; p=0.73). Major bleeding was significantly greater in the bridging group (3.2% vs. 1.3%; p=0.005), as was minor bleeding (20.9% vs. 12.0%; p<0.001). There were no fatal hemorrhages.

The saga of “The Tube” began for me in 1957. At that time, I was the House Physician to Sir James Paterson-Ross and Sir Eric Scowen on the Professorial Unit of St. Bartholomew’s Hospital in London, England. I was paid 400 pounds per year with room and board, for 24-hour, 365-day duty, while living in the hospital. One hundred pounds was withdrawn for food, but our shoes were cleaned! The job was considered an honor and allowed you to learn of the natural history of disease in the patients under your care. It certainly did this, but in the process you became a sleep-deprived autonomous robot – neither desirable nor safe.

One day in the long ward I cared for, I looked at a laboratory hematology report and said to myself, “How do I know that this slip bears any relationship to the blood sample I sent?” I also noted the equipment for collecting a blood sample and the makeshift system by which it got to the laboratory. Similarly at that time, as we gowned and masked to perform a lumbar puncture, the cerebrospinal fluid samples went to the laboratory in six 10-mL test tubes in a wooden test tube rack, with each tube having a cotton wool plug. This cavalier attitude toward a sample that had come from a patient’s spinal canal was deplorable!

I started to think of a new tube for collecting blood samples. A friend of my wife-to-be was the daughter of a Knight of the realm who ran a huge industrial enterprise. He kindly took my idea and produced a plastic tube with an attached stopper that would plug the tube. This was created by the relatively new wonder of injection molding, with plastic and a cotton wool plug. This cavalier attitude toward a sample that had come from a patient’s spinal canal was deplorable!

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**Making Sense of New Drug Discovery**

Dr. Douketis and colleagues have shown that, in patients with atrial fibrillation who require perioperative interruption of warfarin for an elective procedure, use of bridging anticoagulation increases bleeding without reducing thromboembolism. A recently published retrospective cohort study in patients with atrial fibrillation who require perioperative interruption of warfarin for an elective procedure: substudy of the RE-LY trial. Thromb Haemost. 2015;113:625-632.


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Be part of the excitement of the ASH Foundation Run/Walk! Last year the event surpassed the success of the inaugural race, and this year, ASH is setting the bar even higher. We encourage you not only to register, but also to build a team or grow the team you were part of last year. Together, we can move hematology forward through support of research, quality care, and education.

Can’t be there on Sunday? Join the ASH Foundation movement by donating, fundraising, or sponsoring other participants, such as trainees. Please be assured that 100 percent of the proceeds from your registration fee and any donations will directly fund programs supported by the ASH Foundation.

To register yourself or your team, visit https://webapps.hematology.org/runwalk/default.aspx.

To donate, visit https://webapps.hematology.org/runwalk/donate/donate.aspx.

Pack Your Sneakers for the ASH Foundation Run/Walk!

Complimentary MDS Summit: Three More Chances to Earn CME Credit

ASH, in collaboration with the American Society of Clinical Pathology (ASCP) and The France Foundation, is offering complimentary summits to improve the diagnosis and care of patients with myelodysplastic syndrome (MDS). Earn up to six AMA PRA Category 1 Credits™ at any of the following offerings:

- Chicago, IL, September 17, 2015 (just prior to the ASH Meeting on Hematologic Malignancies)
- New York, NY, October 9, 2015
- Tampa, FL, October 16, 2015

More information on the MDS Summits can be found at www.pathologylearning.org/mds/summits.

ASH Announces Six Oral Presentations at the Meeting on Hematologic Malignancies (MHM)

The ASH Meeting on Hematologic Malignancies in Chicago will feature discussions with top international experts in core hematologic malignancies who will share their own evidence-based treatment approaches through specialized “How I Treat” sessions. In addition to these, there will be several oral presentations selected from submitted abstracts. These oral presentations, being held Friday, September 18, and Saturday, September 19, will cover exciting breakthroughs across the five primary topic areas of the meeting. The schedule is as follows:

Friday, September 18, 3:00 p.m.-3:30 p.m.
- Adam J. Olszewski, MD, Alpert Medical School of Brown University, Providence, RI: “Management of HIV-Associated Hodgkin Lymphoma in the Antiretroviral Therapy Era: Analysis of the National Cancer Data Base (NCDB)”
- David J Chung, MD, Memorial Sloan Kettering Cancer Center, New York, NY: “T Cell Exhaustion/Senescence and Relapse in Multiple Myeloma after Autologous Stem Cell Transplantation”
- Jaleh Falah, MD, Memorial Hospital of Rhode Island, Pawtucket, RI: “Abbreviated Chemotherapy and Radiation for Early-Stage Diffuse Large B-Cell Lymphoma (DLBCL) in the R-CHOP Era: Analysis of the National Cancer Data Base (NCDB)”

Saturday, September 19, 9:10 a.m.-9:40 a.m.
- Ksenia Romanova, MD, Federal Research Center of Pediatric Hematology, Oncology, Moscow, Russia: “Low Risk of Avascular Necrosis in Children and Adolescents with Acute Lymphoblastic Leukemia Treated with Reduced Intensity Moscow-Berlin Regimens”
- Huisheng Ai, MD, Affiliated Hospital of Academy of Military Medical Sciences, BEIJING, China: “HLA-Mismatched Microtransplantation Vs HLA-Matched Nonmyeloablative Transplantation for Acute Myeloid Leukemia in Intermediate-Risk: Comparable Survival but Avoids of GVHD”
- Asher Alban Chanani-Khan, MD, Mayo Clinic, Jacksonville, FL: “Insights into the Management of Adverse Events in Patients with Previously Treated Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma: Experience from the Phase 3 HELIOS Study of ibrutinib Combined with Bendamustine/Rituximab”

MHM Videos with the Speakers

As the ASH Meeting on Hematologic Malignancies draws near, check out the MHM video clip series to hear directly from speakers about what to expect from their presentations on their treatment strategies for a variety of hematologic malignancies. Visit www.hematology.org/Meetings/Malignancies/4007.aspx to see clips from 15 of the “How I Treat” session presenters. The meeting will be held September 17-19, 2015, at the Fairmont Chicago, Millennium Park. Visit www.hematology.org/Malignancies for more information and to register.

ASH Meeting on Hematologic Malignancies: Irene Ghobrial, MD

ASH Meeting on Hematologic Malignancies: Richard E. Larson, MD

All Subsets
Ask the Hematologist

SHIRU NAKAO, MD, PHD
Professor of Cellular Transplantation Biology (Hematology/Reaproperty), Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

The Question
What is your approach to the diagnosis and treatment of patients with low-risk myelodysplastic syndrome (MDS) related to immune pathophysiology?

Case
A 55-year-old man was found to have pancytopenia during follow-up for hyperuricemia. The laboratory findings were as follows: WBC, 2.44×10^9/L with 26 percent neutrophils, 1 percent eosinophils, 8 percent monocytes, and 65 percent lymphocytes. RBC, 1.49×10^12/L; hemoglobin, 5.7 g/dL; mean corpuscular volume (MCV), 112.8 fL; platelets, 22×10^9/L; reticulocytes, 45×10^9/L; LDH=195 IU/L. The patient’s bone marrow (BM) was normocellular with 2 percent blasts and showed signs of dysplasia in erythroid precursors and granulocytes without increased ring sideroblasts. The mean corpuscular volume was decreased. The karyotype of the BM cells was 46,XY in 20 dividing cells. The patient was therefore diagnosed as having refractory cytopenia with multilineage dysplasia (RCMD) with International Prognostic Scoring System (IPSS) risk group of intermediate-1 (IPSS-revised risk category: intermediate). His physician suggested that he receive red blood cell transfusions and possibly hematopoietic stem cell transplantation or azacitidine therapy if the pancytopenia progressed further.

My Response
Distinguishing Cases of Immune-Mediated BM Failure
Diagnosing MDS is often challenging in patients with pancytopenia or bicytopenia who do not show definitive poor prognostic markers, such as karyotypic abnormalities or increased blasts or ring sideroblasts in the BM, as in the present case. The differential diagnosis of such cases of gray zone BM failure includes refractory cytopenia with multilineage dysplasia (RCMD) with indeterminate significance, and moderate aplastic anemia (AA). It is difficult to make an exact diagnosis based on the established diagnostic criteria because these disease entities overlap, and each diagnosis relies on a subjective judgement of the cell morphology by physicians and pathologists. 

Difficulties in Evaluating BM Cellularity in Patients with BM Failure
BM aspiration and trephine biopsies are essential for obtaining the differential diagnosis of BM failure syndromes, and the detection of a hypocellular BM is a prerequisite for diagnosing AA. However, assessing BM cellularity in patients with BM failure is often difficult, particularly when the cytopenias are not severe. Even when the BM of one bone site is grossly replaced with fat tissue as a result of the immune-mediated destruction of hematopoietic stem cells (HSCs), some hematopoietic rests remain in other bone sites and may show hypercellularity due to increased BM activity that compensates for the decreased hematopoiesis.

Laboratory Markers Representing Immune Pathophysiology of BM Failure
The presence of predominant thrombocytopenia is the most fundamental feature of immune-mediated BM failure that should prompt further examinations of other laboratory markers. An increased percentage of paroxysmal nocturnal hemoglobinuria (PNH)-type cells, which can be detected using high-sensitivity flow cytometry (FCM), represents a reliable marker of immune pathophysiology.

Another useful examination that does not require advanced techniques is measurement of the plasma thrombopoietin (TPO) level. Physicians may not need to examine the peripheral blood in patients with BM failure to determine the presence of other markers. The recent National Comprehensive Cancer Network guidelines recommend using hypomethylating agents for the treatment of MDS associated with thrombocytopenia; however, this option may be hazardous to patients, as BM failure in these cases is not based on the presence of abnormal stem cells with preleukemic features.

Figure 1 illustrates the mutual relationship of the markers that are potentially useful for diagnosing immune pathophysiology in patients who do not show any karyotypic abnormalities, increased blasts or ring sideroblasts in the BM. The precise role of these markers in the management of gray zone BM failure must be evaluated in a large prospective study because the predictive values of HLA-LLs and plasma TPO levels have only been assessed in Japanese patients with BM failure. When deciding on treatment, physicians also need to take patient age and disease duration into account, both of which are known to affect the response to IST.

Patient Follow-up
Based on the positive results for PNH-type granulocytes, we treated the patient with cyclosporine at a dose of 4 mg/kg/day. The pancytopenia gradually improved in response to this therapy, and the blood cell counts were normal within 6 months after the initiation of treatment as was follows: WBC, 3.0×10^9/L; RBC, 2.79×10^12/L; hemoglobin, 9.4 g/dL; MCV, 101.4 fl.; platelets, 57×10^9/L; reticulocytes, 7.5×10^9/L. He continues to receive cyclosporine without any signs of significant toxicities.

Dr. Nakao receives research funds and honoraria from Alexion.
Mutual relationship of the markers that are potentially useful for diagnosing immune pathophysiology in myelodysplastic syndromes: consensus statements and report from a working group. Haematologica. 2013;98:901-907.


Advances in understanding the pathophysiology of osteolytic bone lesions in recent years have contributed to the development of therapeutic approaches that target both the tumor cells and the microenvironment. The bone provides a complex microenvironment where tumor and bone cells, such as osteoclasts (OC), osteoblasts (OB), and osteocytes, interact and induce bone destruction. This “bone niche” provides a permissive niche for the tumor cells and helps in the growth and propagation of MM cells. Therefore, targeting this niche has the potential of not only treating bone disease but also impacting long-term disease control.

Currently, bisphosphonates are the mainstay of therapy for MM bone disease. Nitrogen-containing bisphosphonates such as pamidronate (PAM) or zoledronic acid (ZA) reduce OC activity and affect OC survival in vitro and reduce skeletal-related events (SREs) in vivo.2 Recent data have also demonstrated a survival advantage with the use of ZA.3 Accordingly, guidelines for the treatment of MM-related bone disease recommend bisphosphonate use in all patients (with and without bone lesions).3 Either ZA or PAM is administered every three to four weeks during initial therapy in patients with active disease.4 Although these agents have proven to be very useful in mitigating SREs, they are accompanied by a significant risk of long-term toxicities such as osteonecrosis of the jaw.5 This has fueled the investigation of biomarker studies,6 prompting the use of bisphosphonates at a less frequent dosing schedule, as in the Z-MArk study.7

The interplay between myeloma cells and bone marrow stromal cells induces OC activity through the secretion of cytokines such as CCL3 (also known as macrophage inflammatory protein [MIP]-1α), MIP-1β, tumor necrosis factor-α, interleukin (IL)-1β, IL-11, stromal-derived factor-1α, B-cell-activating factor, activin A, and vascular endothelial growth factor.8 Many of these factors work through the receptor activator of nuclear factor kappa B (RANK), its ligand (RANKL), and its inhibitor osteoprotegerin (OPG).9 Therefore, the RANKL:OPG axis represents an important target in the treatment of MM bone disease. In preclinical studies, CC-292 has been shown to block the OCs.5 Therefore, treatment of the underlying MM is critical for continued improvement of MM-related bone disease.

Recent efforts have focused on understanding the role of the OB in myeloma bone diseases. Several cytokines and proteins regulating the OB axis have been discovered. In particular, wingless (Wnt) signaling pathway plays an important role in OB differentiation and in the interplay between MM cells and OB. The secreted glycoprotein Dickkopf4 (Dkk4), a soluble inhibitor of the Wnt signaling pathway, is expressed by OB and MM cells. Additionally, high levels of Dkk1 have been found in patients with extensive bone disease.10 Promising preclinical data have demonstrated the potential use of anti-DKIk1 therapy in clinical settings in MM. BHD880, a human neutralizing IgG1 anti-DKIk1 monoclonal antibody, was evaluated in a phase 1b clinical trial in combination with ZA in relapsed patients. The inhibitor treatment was well tolerated, and beneficial effects were observed in some patients; however, no conclusion could be reached about the bone effect of BHD880 because of the concomitant use of ZA and anti-MM therapy.11 High levels of sclerostin, another Wnt inhibitor, have been found...
The Ubiquitous Tube

(Cont. from page 1)

then had the help of a colleague, Dr. Howard Davies, who edited the write-up of this research, and we shipped it off to the British Medical Journal.

Back in Edinburgh, I was reading The Times when I noticed an advertisement for a paid fellowship post at the Australian National University (ANU) in Canberra to work in virology. On route to Sydney, Australia, during a five-week trip on a P&O liner, I received a telegram from Moscow inviting me to join the International Committee on Standardization in Hematology (ICSH) as part of a committee to standardize the ESR. Swedish internist All Westergren was sitting on this committee; he had used the ESR as a pregnancy test. The committee was a part of the International Society of Hematology, which was planning to standardize hemoglobin and the ESR, two commonly used blood indices considered somewhat messy in the practical world.

While in virology at the ANU, I developed a reproducible mouse fibroblast system that cell biologists found useful. During this work, I noticed that a solution containing a pH indicator would change color when pipetted through soda glass, but not through Pyrex. It became obvious that the type of glass to be used for hematology was crucial. I was in Australia for five years in Canberra and later in Melbourne at the Baker Institute of Medical Research. Here I was working on ionspecific electrodes. One of my colleagues acted as a way station for blood samples arriving in modified evacuated tubes from Dr. Carleton Gajdusek in New Guinea on their way to the National Institutes of Health (NIH). He was working with Kuru in the Fore tribe, and developed his concept of “Prions,” for nature and the tubes was fixed. (See online-only Table 1 for details on the tubes.)

The transport standard brought the space age had produced a gadget that would give the connecting cable a resistance of 1,000 Ω instead of 1,000,000 Ω, which permitted the meter to meter to be greatly improved. I was just in time to join the ICSH in their standardization efforts for hemoglobin and ESR. Dr. Russell Eiters who was, at that time, a member of the ICSH was also a leader in the U.S. National Committee for Clinical Laboratory Standards (NCCLS), and he was involved in the standardization efforts for hemoglobin and ESR.

Dr. Dawson indicated no relevant conflicts of interest.

We were now all confounded as to what to do with specimens after laboratory acceptance until Dr. Roger Calam developed his subcommittee to solve the multitude of problems within the modern laboratory.

Thus, we now had five basic standardized procedures for the USA which I then took to my subcommittee of the ICSH, which included a dozen of the best international hematologists at that time from the United States, United Kingdom, Israel, Russia, Thailand, China, Australia, Canada, Germany, Sweden, and other nations. We met in Montreal and approved the five NCCLS hematology standards for international acceptance, only to find that Dr. S.M. Lewis (London), the Secretary of the ICSH, was not about to let a bunch of American standards become international. He canceled our international subcommittee and thereby its conclusions. His Dutch secretary supported his decision. Such is international politics.

I believe our NCCLS committee for hematology succeeded in creating five badly needed standards, which expanded in time to further standardize requirements for toxicology, heparin, and many others. I now feel that the results on laboratory reports really do represent the blood specimens themselves, using our standards would not be accredited.

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Those interested in standardization within medicine in general, and in the NCCLS in particular, can contact CLSI to become a member, to order individual documents, or for their catalog of hundreds of standards. Similarly, membership in bodies such as the SH and ICSH, which meet every two years, is ideal for CME. Their meetings are exciting, interesting, and provide opportunities for long-lasting overseas friends.

Dr. Dawson indicated no relevant conflicts of interest.

2015 ASH Annual Meeting Reminder

We hope you will join us for the 57th Annual Meeting in Orlando, Florida. If you haven’t already registered, keep in mind that the advance registration deadline is November 4, at 11:59 p.m. EST. If you are interested in submitting a late-breaking abstract, the submission website opens October 22 and closes October 29. The selection process for late-breaking abstracts is competitive; a maximum of six abstracts will be selected, regardless of the number of submissions. Reviewers will focus on identifying high-impact abstracts, and an effort will be made to balance the program such that both basic/ translational and clinical research are represented in the late-breaking abstracts, which will be available online November 5, at 9:00 a.m., EST.
The report accompanying the Senate bill includes language that ASH advocated for, including encouraging the National Heart, Lung, and Blood Institute (NHLBI) to further research efforts in gene editing and gene therapy to correct inherited blood disorders. It also urges the Department of Health and Human Services (DHHS) to continue coordinating all of its sickle cell disease (SCD) -related activities through its Intergency Working Group on Sickle Cell Disease. "Report language," as it is called, generally includes detailed research efforts in gene editing and gene therapy for inherited blood disorders. It oversees research that informs the delivery of medical advances to patients. The bills also seek to undo many of the programs created as part of the Affordable Care Act.

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As this issue of The Hematologist went to press, action on spending bills had stalled in both the House and Senate. At this time it is unclear when, or if, either of the bills would be considered in the full House or Senate. With Senate Democrats threatening to filibuster all spending bills that adhere to the sequestration budget caps and President Barack Obama promising to veto any spending bills adhering to the restrictive spending caps, any proposed increase for NIH is likely going to depend on a broader budget agreement to ease these budget caps. If Congress cannot reach a decision about spending bills by October 1, it has two options: 1) Pass a temporary continuing resolution that extends federal agency and program funding unchanged from the current year or 2) shut down the government until it can come to an agreement on spending.

ASH remains committed to working with Congress to replace sequestration, and ASH supports a balanced approach to deficit reduction that provides needed funding to NIH without further cuts to other important public health programs. ASH members, along with ASH staff, have met with numerous congressional offices to discuss the importance of biomedical research and the need to protect NIH from further funding cuts. The Society is also a sponsor and supporter of the third annual Rally for Medical Research Hill Day taking place September 17, 2015, in Washington, DC, during which hundreds of participants from more than 300 partnering organizations will be meeting with House and Senate offices to call on our nation’s policymakers to make funding NIH a priority and raise awareness about the importance of continued investment in scientific research that ultimately leads to more progress, more hope, and more lives saved.

How You Can Help
You can also have your voice heard in the halls of Congress and back home in your congressional district by participating in the Society’s advocacy efforts. Senators and representatives need to hear from their constituents about the negative impact that cuts in funding have had (and may continue to have) on hematology research. As Congress continues to formulate the FY 2016 budget, the Society encourages you to visit the ASH Advocacy Center (www.hematology.org/takeaction) and take action to support funding for NIH in FY 2016.

House Passes 21st Century Cures Act, Senate Legislation Expected This Fall
On July 10, 2015, the House of Representatives approved the 21st Century Cures Act (H.R. 6) by a bipartisan vote of 344-77. The bill reauthorizes the NIH for three years at funding levels that could represent an increase of up to $1.5 billion per year and creates an NIH Innovation Fund supported by $1.75 billion a year for five years in mandatory funding. A provision in the bill also clarifies the exemption of certain medical education events and materials from reporting under the Physician Payments Sunshine Act/Open Payments program.

Prior to the vote on final passage, the House defeated an amendment offered by Representative David Brat (R-VA) that sought to convert the NIH Innovation Fund from mandatory to discretionary funding. Passage of the amendment would have created uncertainty for the necessary funding for NIH and the U.S. Food and Drug Administration (FDA) in the 21st Century Cures bill. ASH would like to once again thank all of those who responded to the Society’s call to action and contacted their representatives to urge them to vote against this amendment.

The Senate is currently working to draft companion legislation to the 21st Century Cures bill, with the intent of releasing a draft bill this fall. ASH is monitoring this issue and will continue to provide updates as they become available.

NHLBI Supports New Sickle Cell Initiatives
It is an exciting time for those involved in the treatment and research of SCD as researchers are making great strides to address the multifaceted burdens of this condition. As a first step to a broader initiative, the Society hosted the ASH SCD Summit: A Call to Action in April 2015. The broad objectives of the Summit were to identify the highest priority actions needed to improve outcomes for individuals with SCD both in the United States and globally, and to map a plan to advance these actions in the short-term (less than five years) and long-term (five to 10 years). As the Society continues to refine and prioritize the issues pinpointed during the Summit and many other stakeholders who should be involved to help advance future efforts, a number of other SCD-related initiatives are happening.

On June 25-26, NHLBI hosted an SCD Forum – Engaging the Community: Developing Solutions. The overall goal of the meeting was to “help chart the future of SCD research.” More than 400 representatives from the SCD community participated in the forum in person and remotely, including patients and their families, advocates, health-care professionals, researchers, representatives from federal agencies, industry, and the media.

ASH cosponsored the forum, and Drs. David Williams (ASH President and member of ASH’s SCD Task Force) and John Strouse (member of the SCD Task Force and Committee on Quality) served as the Society’s representatives at the meeting. Dr. Williams presented an update on ASH’s SCD-related activities.

On July 20, the NHLBI released the following two grants focused on the care of individuals with SCD. The application deadline for both grants is November 12, 2015.

• SCD Implementation Consortium (SCDIC): Using Implementation Science to Optimize Care of Adolescents and Adults with SCD (R01) – This grant solicits applications to support clinical sites to improve the health and well-being of adolescents and adults with SCD in the United States through the development of multimodal, multisector interventions aimed at improving the rate at which patients with SCD receive routine primary care. Applications that consist of multidisciplinary teams of personnel from community and academic health-care institutions are highly sought. (http://grants.nih.gov/grants/guide/rfa-files/RFA-HL-16-010.html)

• Data Coordinating Center for Sickle Cell Disease Implementation Consortium (SCDIC): Using Implementation Science to Optimize Care of Adolescents and Adults with Sickle Cell Disease (U24) – This grant solicits applications for a Data Coordinating Center as part of the SCD Implementation Consortium, to support Clinical Sites that propose to improve the health and well-being of adolescents and adults with SCD in the United States through the development of multimodal, multisector interventions aimed at improving the rate at which patients with SCD receive routine primary care. (http://grants.nih.gov/grants/guide/rfa-files/RFA-HL-16-011.html)

In 2014, ASH inaugurated the ASH U.S. Food and Drug Administration (FDA) Speaker Series. This program consists of seminars in which ASH brings specialists to the FDA to educate FDA staff on important topics in hematology. On June 26, Dr. David Williams of Boston Children’s Cancer & Blood Disorders Center, presented information on novel cell therapies for untreatable diseases: gene therapy, induced immunodeficiencies and coagulopathies.
Computed Tomography Provides No Benefit over Routine Screening for Occult Cancer


Unprovoked deep-vein thrombosis and pulmonary embolism may be an early sign of undiagnosed cancer, with up to 10 percent of patients receiving a diagnosis of cancer within three years. In the natural history of venous thromboembolism (VTE), the majority with venous thromboembolism are two to four times more likely to be diagnosed with cancer when compared with the general population. A recent study, which examined patients with first-time splanchic venous thrombosis in 1,191 Danish patients, found that such patients were 33 times more likely to be diagnosed with cancer in the three months following this diagnosis. Litter cancer, pancreatic cancer, and myeloproliferative neoplasms were the most common tumors diagnosed.5 Given such statistics, screening for occult tumors in patients with unprovoked venous thromboembolism is thought to allow for early detection and intervention.

The question is, how aggressive should such screening be? Dr. Marc Carrier and investigators for the Screening for Occult Malignancy in Patients with Idiopathic Venous Thromboembolism (SOME) study conducted a multicenter, open-label, randomized controlled trial in Canada. 854 patients were randomized to undergo either limited screening or limited screening in combination with comprehensive computed tomography (CT) of the abdomen and pelvis, which included a virtual colonoscopy and gastrointestinal, biphasic enhanced CT of the liver, parenchymal panendoscopy, and unenhanced enhanced CT of the distal abdomen and bladder. Routine screening for occult cancer included basic blood testing, chest radiography, and screening for breast, cervical, and prostate cancer. The primary outcome was biopsy-proven cancer missed by the screening strategy and detected by the end of a one-year follow-up period. Results showed that 33 (3.9%) of total patients had a new diagnosis of occult cancer, with 14 (3.2%) of 431 patients diagnosed in the limited screening group and 19 of 423 (4.5%) in the CT plus limited screening group. There was no statistical difference between these two groups (p=0.28).

The primary outcome analysis showed four occult cancers missed in the limited screening strategy and five missed by the CT plus limited screening group (Figure). No significant difference between the two groups was detected in cancer-related mortality or in mean time to a cancer diagnosis. These results are consistent with a previous prospective, nonrandomized cohort study that compared a limited screening strategy for occult cancer with a strategy that included mammography for women and CT of the chest, abdomen, and pelvis in all patients. This study did not show any significant difference in the diagnosis of occult cancers or in overall mortality, with a follow-up of 2.5 years. An earlier randomized controlled trial involved patients with a negative limited screening result for occult cancer, in which subjects were randomized either to additional screening or no further testing. This trial was discontinued early due to recruitment difficulties but showed an increased detection of early-stage cancer in the extensive-screening group (nine of 14 [64%]) versus the limited-screening group (two of 10 [20%]; p=0.047). However, there was no significant difference in cancer-related deaths between these two groups during a two-year period. In conclusion, in this current study, SOME Investigators found a low prevalence of occult cancer in patients with a first unprovoked deep-vein thrombosis and/or pulmonary embolism. Furthermore, routine screening for occult cancer with CT plus limited screening for the abdomen and pelvis did not provide a clinically significant benefit over routine screening alone.


**Complement Component C3b and RBCs Provide a Potential Clearance Mechanism for Damaged Plasma Proteins**


Secreted proteins may become damaged, misfolded, and self-aggregated because they are subject to increased chemical stress such as oxidation, shear forces in the vasculature, and their own abnormality. Monoclonal immunoglobulin light chains in amyloid light-chain (AL) amyloidosis or various proteins in hereditary amyloidoses, may have an increased tendency to misfold and aggregate.2 Damaged plasma proteins may be deposited pathologically in extracellular spaces in various tissues. Clinical manifestations of these depositions include the systemic amyloidosis, glomerulonephropathies, neurodegenerative disorders including Alzheimer’s disease, and complications of diabetes mellitus.1 Extracellular chaperone proteins can bind denatured misfolded proteins and, thereby, prevent their aggregation while promoting their clearance from the circulation via interactions with specific cellular receptors.1 Extracellular chaperones such as clusterin and α2-microglobulin, which bind amyloidogenic proteins, often have other functions. An extracellular chaperone familiar to hematologists is haptoglobin, the acute-phase reactant that binds hemoglobin, but which uses a different site to bind misfolded proteins. In their report, Dr. Mahalakshmi Ramadas and colleagues at Stony Brook University demonstrate a new function for C3b as an extracellular chaperone for denatured plasma proteins and a potential new function for erythrocytes in the clearance of damaged/denatured plasma proteins.

Dr. Ramadas and colleagues, who previously showed that C3b bound covariantly to various native plasma proteins,1 used chemically denatured purified human vitamin D-binding protein, serum albumin, and α1-antitrypsin inhibitor to demonstrate that C3b reacted more avidly with the denatured as opposed to native forms of these plasma proteins. They subsequently showed that incubation of human erythrocytes with human serum, in which C3b was generated by cobra venom factor, resulted in C3b/erythrocyte complexes bound to the erythrocyte membrane. These complexes led to clearance of denatured plasma proteins by a series of events: 1) human erythrocytes, which express high affinity receptors for C3b in the form of clustered CD35 (complement receptor 1), bind the C3b/protein complex; 2) CD35 acts as a cofactor for C3b to its inactivation in the mononuclear phagocytes; and 3) C3b/erythrocyte complexes delivered to splenic and hepatic macrophages where receptors for C3b (CD11b/CD18 and CD11c/CD18) mediate phagocytosis of C3b/erythrocyte complexes with release of the erythrocytes back into the circulation.4 Lastly, the authors showed that denatured proteins in heat-treated human serum activate complement via the alternative pathway in a limited manner, such that C3b is formed without generating the pro-inflammatory C5a product.

The results of the study by Dr. Ramadas and colleagues show that denatured plasma proteins induce production of C3b, and C3b covariantly binds the denatured proteins. Complexes of C3b and denatured plasma proteins bind to CD35 on erythrocytes where factor I converts C3b to C3bi, which facilitates delivery of the C3bi/denatured protein complexes, and that the C3b/denatured protein clearance mechanism occurs in vivo. However, the findings are consistent with interactions of C3 with f-amyloid peptide, a major component of fibrillary deposits in Alzheimer’s disease brain lesions, and prior observations that 1) f-amyloid peptide activates alternative and classic complement pathways with formation of complexes of C3b and f-amyloid peptide, and 2) C3b-dependent binding of f-amyloid peptide to RBCs occurs via CD35.5 Therapeutic modalities that can harness C3b-mediated erythrocyte clearance of damaged and misfolded plasma proteins may thus have the potential to improve treatment of diseases characterized by tissue accumulation of misfolded or denatured proteins such as AL amyloidosis, diabetes mellitus, glomerulonephrosis, and neurodegenerative disease.


**MARK ROURY, MD**

Dr. Roory indicated no relevant conflicts of interest.
Hematopoietic Stem Cells Should Hold Their Breath


Much of what we know about hematopoietic stem cells (HSCs) revolves around our ability to take them out of a donor and transplant them into a conditioned recipient, as was discussed in greater detail in a Mini Review in The Hematologist. However, as is increasingly becoming clear, in many cases our experimental systems for HSCs may create observer effects, where the act of experimentation fundamentally changes the cells themselves. A recent report from Dr. Charlie Mantel and colleagues highlights just how sensitive HSCs can be to our experimental and clinical manipulations.

Several early reports have demonstrated that HSCs are present in regions of presumed hypoxia within the bone marrow. A recent direct measurement of local oxygen tension in the bone marrow of live mice showed that the bone marrow niche had considerably low oxygen (<32 mmHg) despite very high vascular density, with the lowest (~9.9 mmHg or 1.3%) found in deeper perisinusoidal regions. However, when HSCs are harvested, either for subsequent clinical transplantation or for experimentation, they are harvested and processed in ambient air, which is approximately 21 percent oxygen. The authors hypothesized that this exposure to ambient air could cause a phenomenon they term “stuporphysiologic oxygen shock/stress” or EPHOSS, and lead to reductions in HSC function.

They began by comparing mouse bone marrow that was harvested in ambient air, with marrow samples that were harvested in hypoxic chambers (3% oxygen content). Intriguingly, even exposures as low as 30 minutes in ambient air lead to almost 5-fold fewer phenotypically-defined HSCs than those that were maintained in 3 percent oxygen. When the bone marrow was harvested and competitively transplanted in hypoxic settings using wild-type mice, bone marrow harvested in ambient air from CypD knockout mice had significantly greater HSCs, with lower levels of ROS and progenitor activity, further supporting a role for EPHOSS and the MPTP in HSC regulation during harvesting in ambient air. While the authors could create a laboratory setup that allowed the harvesting of bone marrow from small mice and subsequent transplantation, all within a hypoxic chamber, the ability to replicate that method clinically would be expensive and cumbersome. Instead, the authors used the immunosuppressant cyclosporin A, which binds to CypD and prevents opening of the MPTP. When bone marrow was harvested in the continuous presence of cyclosporin A, the authors demonstrated a reversal of the deleterious effects of ambient air exposure, with significant increases in HSC recovery and competitive transplantation. These results translated to human cord blood samples harvested in cyclosporin A as well, with increases in immunophenotypically defined HSCs and repopulation into immunocompromised (SCID) mice. The authors therefore demonstrate a new pathway for regulation of HSCs and a new method of harvesting HSCs to prevent EPHOSS, either with collection in hypoxia or in the presence of cyclosporine A (Figure).

The report by Dr. Mantel and colleagues has many important findings for the field and raises further questions. Much has been done to enumerate the total number of HSCs in organisms, including seminal work by Dr. Janis Abkowitz that utilized both experimental data and simulations to estimate the number of HSCs as being between 11,000 to 22,000 in both mice and humans. Given that such results were based on air-exposed cells, it will be interesting to see if the total number of HSCs within an organism is actually significantly higher. The authors used a hypoxic chamber set at 3 percent oxygen and showed that 5 percent oxygen was not able to prevent EPHOSS and increase harvested HSCs. Most of the published work in hematopoiesis, particularly with the colony-forming assay, has used “hypoxic” chambers set at 5 percent oxygen. These results indicate that studies in hematopoiesis, or for that matter broad stem cell systems in other organs as well, may need to explore lower levels, or even gradients of oxygen exposure to accurately replicate in vivo conditions. Differentiation through the “hematopoietic tree” is typically thought of as a series of divisional events, where an HSC divides to form more mature progenitors, which then divide to form lineage-committed progenitors. However, in these studies, the authors saw very significant changes in the numbers of both phenotypic and functional HSCs in as little as 30 minutes of exposure to air. This surprising finding perhaps suggests that programming of an HSC can occur rapidly and independently of the hypoxic environment and warrants further exploration. Finally, the cyclosporine A protection of HSCs was also seen in human cord blood in both in vitro assays and in xenotransplantation studies, and merits further clinical exploration. Several clinical trials are underway exploring methods to ex vivo manipulate cord blood units, expand cord blood units, or modulate the host to enhance engraftment. This manuscript adds another approach and suggests that cord units should be harvested in the presence of an MPTP inhibitor.

Deadly Malaria Parasites Hijack CD55 to Invade Human Erythrocytes


Malaria remains a major global health threat and is particularly devastating in Africa, where it mainly kills children younger than five years who have not yet developed partial immunity to the disease. Plasmodium falciparum is the most lethal of the human malaria parasites and is able to rapidly develop resistance to antimalarial drugs, including the current artemisinin combination therapy. This therefore fuels the ongoing, urgent need to develop novel treatments, especially since there is currently no vaccine available. The red blood cell (RBC) stage of the parasite lifecycle is responsible for the pathogenesis of malaria, but the complex process of invasion and the concomitant interactions between host erythrocyte receptors and parasite ligands are not fully understood. One layer of complexity is added in the case of P. falciparum since it possesses several ligands that can target different receptors on the RBC surface, which facilitates entry into genetically variable human hosts and has contributed to the success of this deadly pathogen.

Mature erythrocytes are terminally differentiated cells with a limited proteome and no nucleus; therefore, genetic manipulation of the genome to identify factors influencing host susceptibility to infection is not possible. To overcome this challenge, Dr. Elizabeth Egan and colleagues from Harvard University exploited two recent technological advances of RNA interference and ex vivo production of RBCs to design a forward hematopoietic stem cell–based genetic screen. Since all the known host receptors for parasite invasion are erythrocyte membrane proteins encoding blood group antigens, the researchers selectively targeted this group of genes. They transduced hematopoietic progenitor cells with a pooled lentivirus short hairpin RNA (shRNA) library to knock down selected genes. These genetically manipulated cells were induced to proliferate and differentiate, and at the orthochromatic erythroblast stage, when they were mature enough for RBC development, they were infected with P. falciparum parasites expressing green fluorescent protein. Infected RBCs were isolated, and the relative amounts of each shRNA in the population were quantified by deep sequencing and compared with the profile of control knockdown cells not exposed to parasites.

Several hits were identified, including genes encoding proteins that directly interact with the invasion complex or its components, such as basigin and complement receptor 1 (CR1). The top ranked gene was CD55 or decay-accelerating factor (DAF). CD55 is a 70kDa glycoprotein, which is attached to the erythrocyte membrane by a glycosylphosphatidylinositol (GPI) anchor and carries the Complement Blood group antigen. It regulates the complement system and prevents complement-mediated destruction of erythrocytes. It is interesting to note that CD55 on epithelial cells serves as a receptor for some viral and bacterial pathogens.

To verify that malaria parasites utilize CD55 to invade RBCs, the authors used shRNA to knock down CD55 and demonstrated reduced invasion. Further evidence was provided by infecting CD55−/− cells from a Japanese patient with laboratory and clinical strains of P. falciparum indicating that CD55 is essential for the successful entry and development of parasites. The invasion process is complex and may be divided into several steps, including recognition of the host cell, re-orientation irreversible binding, and tight junction formation just prior to entry. Experiments with cytochalasin suggested that CD55 plays a role in the latter step of an irreversible step in the invasion process, particularly during the later stages of committed attachment, but the details still need to be determined.

These findings illustrate the feasibility of using a hematopoietic stem cell–based forward genetic screening approach to investigate host factors implicated in malaria pathogenesis. Additionally, these results highlight our lack of in-depth knowledge of host-parasite interactions and suggest that the role of complement in parasite invasion and clinical malaria should be re-examined. For example, how does the parasite–CD55 interaction affect the regulation of complement and the destruction of erythrocytes? The data also raise other intriguing questions. Can CD55 be exploited as a therapeutic target? What is the parasite ligand that interacts with CD55? The authors hope answering these questions will give important insights into the biology of a deadly pathogen and the host response to infection.
Targeted Fetal Hemoglobin Induction Therapy May Need a “Blood-Brain Barrier”


Funnell AP, Prontera P, Ottaviani V, et al. sp5p16.1 microdeletions encompassing and proximal to BCL11A are associated with elevated HbF in addition to neurologic impairment. Blood. 2018;126:89-93.

The normal developmental transition from the synthesis of fetal hemoglobin (HbF; α2γ2) to adult hemoglobin (HbA; α2β2), called hemoglobin switching, begins several weeks before birth and is largely complete by six months of age. In normal adults, HbF accounts for less than 1 percent of total hemoglobin. HbF is not uniformly distributed among all erythrocytes; it is restricted to a small population called F-cells. Some healthy adults have persistently elevated levels of HbF that are of no consequence. This variation in HbF levels is not inherited.1,2

In summary, these two reports indicate that haploinsufficiency of BCL11A in humans, whether by deletion of the gene itself or its promoter, results in elevated HbF. It is possible that this increased fetal hemoglobin will have therapeutic benefit in a subset of patients with severe β-thalassemia, the kinase DUSP4 (protein phosphatase activating kinase 4), and the histone deacetylase inhibitor 4-phenylbutyric acid. By comparing the results of these screens, 35 genes of interest were identified, and several concepts were made for 30 or 10,000 high cases, ectopic expression of the gene resulted in reduced viability in DLBCL lines, of which five were transcription factor genes and three had enzymatic activity (DUSP4, the kinase PRKCH, and the phosphatase C [f1, PLCB1]).

The group chose to study DUSP4 in more detail because it was strongly expressed in activated B-cells, was reactivated on demethylation in DLBCL cells, and has potentially important downstream targets, including JNK. Targeted binding of DUSP4 to the Cpg island upstream of the DUSP4 start site confirmed that it was extensively methylated in all 11 B-cell lymphoma lines studied. And in 10 of 13 primary DLBCL samples, irrespective of subtype, by contrast, normal B-cells from blood and tonsils had no myeloblastic lymphocytic leukemia (CGL) cells showed no methylation. Using an antibody specific to DUSP4, the group was able to examine a tissue array of several hundred different lymphomas, it appeared that the nuclear expression of the protein is much less common in DLBCL than in indolent types such as marginal zone follicular lymphoma or CGL, with an inverse correlation between promoter methylation and protein expression in a subset of 16 cases of DLBCL and CGL. A search of comparative genomic hybridization databases showed that DUSP4 deletion is relatively uncommon, with 6.4 percent of interest in DLBCL, and it appeared that it was extensively methylated in a subset of DLBCL and CGL.

To assess the effects of DUSP4 loss and re-expression in DLBCL, expression constructs were transfected into five DLBCL lines; this produced reduced viability and increased apoptosis but no disturbance of progression through the cell cycle. A point mutant construct lacking enzymatic activity was used as a control to show that a functioning DUSP4 protein was required to exert these effects. All showed similar patterns of promoter methylation compared to normal B-cells. To identify the genes that might be re-expressed following demethylation, several expression profiles were carried out on BTX and activated peripheral blood B-cells before and after treatment with 5-aza-2'-deoxycytidine and the histone deacetylase inhibitor 4-phenylbutyric acid. By comparing the results of these screens, 35 genes of interest were identified, and several concepts were made for 30 or 10,000 high cases, ectopic expression of the gene resulted in reduced viability in DLBCL lines, of which five were transcription factor genes and three had enzymatic activity (DUSP4, the kinase PRKCH, and the phosphatase C [f1, PLCB1]).

Epigenetic alterations are increasingly recognized as playing a key role in the pathogenesis of B-cell lymphomas, and genome-wide studies continue to yield important insights into the process, highlighting new opportunities for treatment based on the molecular phenotypes. This study gives further evidence that epigenetic dysregulation is important in the development of B-cell lymphomas. This is interesting thing is that it is effective across the molecular DLBCL subtypes, and the finding of synergy between JNK inhibition and Bruton tyrosine kinase in addition to a JNK inhibitor resulted in synergistic activity on ABC-type DLBCL lines in vitro.

This finding gives further evidence that epigenetic dysregulation is important in the development of B-cell lymphomas. DUSP4, which is silenced by methylation in many cases of DLBCL and by allelic loss in a few, seems to function normally as a tumor suppressor gene, providing negative feedback following activation via the B-cell receptor, CD40, or other pathways. Inhibition of JNK family members is a strategy that may restore one of the phycologic functions of abundant DUSP4. This is interesting because this approach is effective across the molecular DLBCL subtypes, and the finding of synergy between JNK inhibition and Bruton tyrosine kinase in addition to a JNK inhibitor resulted in synergistic activity on ABC-type DLBCL lines in vitro.

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DUSP4: A New Tumor Suppressor in B-cell Lymphoma With Some Very “Druggable” Targets


B-cell activation (eg, via B-cell receptor [BCR] and CD40 expression) induces the expression of dual-specificity phosphatase (DUSP4), which negatively regulates c-JUN N-terminal kinase (JNK) 1/2 by dephosphorylation, resulting in apoptosis. Inhibition of BTK and JNK1/2 acts synergistically to promote apoptosis of lymphoma cells.


Figure

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Development of cancer is usually thought to arise after gradual accumulation of mutations over time, but there is a more profound event termed “chromothripsis” that represents a catastrophic change of genome structure and widespread damage and acquisition of multiple mutations. This latter process involves a cluster of chromosomal rearrangements. The mechanism for the occurrence of the widespread damage was not clear for a long time, but current models have shed new insight.

The phenomenon of chromothripsis was observed after genome sequencing of a sample from a case of chronic lymphocytic leukemia that exhibited 42 genomic rearrangements involving the long arm of chromosome 14, with concomitant rearrangements of portions of chromosomes 1, 12, and 15. Chromothripsis was observed in about 3% of other cancers as well.

Dr. Cheng-Zhong Zhang and colleagues at the Dana-Farber Cancer Institute identified that the damage occurred after partitioning of intact chromosomes into micronuclei by combining live cell imaging with single-cell genomic analysis they termed “Look-Seq.” This group had previously identified the central role of the micronucleus in this process. These ‘tagging’ chromosomes are thought to properly attach to the mitotic spindle during cell division and are physically separated in micronuclei that have a separate nucleus. The mechanism for the occurrence of widespread damage was not clear for a long time, but current models have shed new insight.

The investigators created an experimental system by treating synchronized cells with nocodazole, a chemical that destabilizes the mitotic spindle. They observed DNA damage in 8 and G2, but not G0, after rupture of the micronuclear envelope by fluorescent marking for DNA strand breaks with agents such as γH2AX. Labeling cells with EdU (5-ethyl-2'-deoxyuridine) demonstrated that incorporation was restricted to the micronuclei than in the primary cell nucleus, indicating that the chromosome in the micronucleus are under-replicated. Look-Seq involved separation of single cells into 384 well plates after treatment with nocodazole. Live cell imaging showed cells with micronuclear envelope rupture during the beginning of S phase. They also used sRNA to knock down expression of the p53 tumor suppressor gene, preventing cell cycle arrest in G1 that would otherwise proceed. The micronuclear chromosome was then reincorporated into the primary nucleus of the daughter cell, allowing DNA damage repair. The under-replicated chromosome was unevenly distributed to the daughter cells, leading to copy number alteration in either 2:1 or 3:2 ratio, with the first number of the ratio representing the daughter cell that assimilated the mis-segregated chromosome. Whole genome sequencing was performed on 10 parent cells and nine daughters derived from the micronucleated cells. Each of the daughter cells had at least one chromosome with copy number asymmetry representing the mis-segregated chromosome that was formerly localized in the micronucleus. Rearrangements were enhanced (a mean ratio of 12.5-fold increase) in the mis-segregated chromosome (p<0.0001) and long-range rearrangements greater than 150 kb were more frequently observed. The researchers concluded that the long-range rearrangements arose from breakage of the chromatin in the ruptured micronucleus. Fragments of DNA were re-assembled in random order and orientation. For one daughter cell, 14 of 16 breakpoints formed an uninterrupted chain. For another daughter cell, both chromosomes 4 and 11 appeared to have been in the micronucleus, as both intra- and inter-chromosomal rearrangements were observed. Lastly, in one daughter cell, four 1 to 3 Mb circular chromosomes were observed that might correspond to the double minute chromosomes that can be present at high copy number and carry oncogenes.

This experimental model and technique of “Look-seq” provide insight into the mechanism of the “pulverization” of chromosomes that can be observed in cancer cells, with widespread chromosome fragmentation and reassembly. Loss of p53 permits survival in this setting by bypassing cell-cycle arrest.

The role of micronuclei that have been observed in many cancer types and normal cells in localizing individual chromosomes and mediating this damage is also highlighted. Perhaps even more direct evidence can be prevented in the future by methods that interfere with formation of the micronucleus.


Shattered Chromosomes: A Cataclysmic Origin of Cancer

MMSAP Returning Participant

Odia Eigbire-molen
Georgetown University School of Medicine
Research Project: Brain volumetric changes in children with sickle cell disease (SCD)
Research Mentor: Naomi Luban, MD, Children's National Medical Center and George Washington University School of Medicine
In His Own Words: How does this year’s project build upon last year’s? Last year, I learned the methodology used to determine brain volume changes and compared brain volumes of children with SCD with healthy children using magnetic resonance imaging to detect differences. I learned analysis techniques that I use in the current project, which is to determine changes in brain volume with age in children with SCD. We used brain images to assess the trajectory of brain volume changes in children with SCD, and we are looking for hematologic and other clinical risk factors that could contribute to changes in brain volume.

First-Time MMSAP Participants

L. Graham Rucker
The Ohio State University College of Medicine
Research Project: Targeting the PRMT5 enzyme for upregulation of fetal hemoglobin in SCD
Research Mentor: Robert Baiocchi, MD, PhD, Ohio State Medical Research Mentor
In His Own Words: How have your ASH mentors influenced you and contributed to your experience? My research mentor, Dr. Robert Baiocchi, has been very influential and supportive throughout my MMSAP experience. He introduced me to the fascinating and exciting field of hematology. I was able to interact with patients, see some procedures, and visit the NIH. Through my mentors, I was able to connect and collaborate with Dr. Michael DeBaun, the lead investigator of the Silent Cerebral Infarct Transfusion (SIT) Trial, which led to this year’s project. I don’t think these opportunities would have been possible without the support of my mentors.

Samantha Glass
University of Illinois at Chicago College of Medicine
Research Project: Health disparities in diffuse large B-cell lymphoma
Research Mentor: Christopher Flowers, MD, MS, Winship Cancer Institute, Emory University
In Her Own Words: I am amazed by the complexity of sickle cell disease and how a single amino acid substitution can have such a profound impact on the lives of those born with it.

Taisha Doo
University of Iowa Carver College of Medicine
Research Project: Effect of Type IIb Von Willebrand disease (vWD) on ADAMTS13 cleavage products on platelet binding
Research Mentor: Jose Lopez, MD, Bloodworks Northwest
In Her Own Words: We tested the hypothesis that proteolytic fragments of von Willebrand factor generated by ADAMTS13 can modulate VWF adhesive functions and impact hemostasis and thrombosis.

Kelli L. Currie
Mahway Medical College
Research Project: Children with SCD and history of parvovirus B19 infections and low baseline hemoglobin levels (<7.6 g/dL) have an increased odds of overt cerebral infarcts
Research Mentor: Michael Rutledge DeBaun, MD, MS, MPH, Vanderbilt University Medical Center
In Her Own Words: During my enriching research experience I have been able to hone my data analysis skills, shadow brilliant physicians, and work with a great team under Dr. Abel.

Tolulope Rosanwo
Case Western Reserve University School of Medicine
Research Project: SCD biochip: toward a simple and reliable way to monitor SCD: microfluidic/microfabrication/ bioengineering/SCD
Research Mentor: Jana A. Little, MD, University Hospitals, Case Medical Center
In Her Own Words: Our work with the SCD Biochip investigates how the cellular adhesion properties of a patient’s sickled red blood cells at baseline differ from when they are in crisis.

Jolene Afla Kokoko
University of California San Francisco School of Medicine
Research Project: SCD
Research Mentor: Carolyn C. Hoppie, MD, MPH, UCSF, Benioff Children’s Hospital Oakland, Children’s Hospital Oakland Research Institute
In Her Own Words: We explore the association of previously identified polymorphisms with hemoglobin F and the relationship of these variants with disease severity, including stroke.

Nicole M. Yordan Lopez
University of Puerto Rico, Medical Sciences Campus
Research Project: Antimutator effects of phenothiazine antipsychotics on T-ALL cells: defining if phenotype is associated with polymorphisms in GCH1
Research Mentor: Alejandro Gutierrez, MD, Boston Children’s Hospital
In Her Own Words: I did not have to follow a book of recipes, but was given the trust to design (and troubleshoot) a project that quickly felt my own. This was what made it truly enriching.

Felicia M. Austin
Howard University College of Medicine
Research Project: Phenotyping SCD patients for enhanced pain hypersensitivity/central pain: define if phenotype is associated with polymorphisms in GCH1
Research Mentor: James G. G. Taylor, MD, Medical College of Wisconsin & National Institutes of Health
In Her Own Words: My research experience has increased my awareness of the variability and severity of pain in the SCD population; I have acquired more knowledge and desire to involve clinical research as a physician.

Rigoberto E. de Jesus
University of Puerto Rico, School of Medicine
Research Mentor: Matthew J. Walter, MD, Washington University in St. Louis, School of Medicine
Research Project: Characterization of the DNA damage response induced by vosaroxin and azacitidine in patients with myelodysplastic syndromes
In His Own Words: I characterized the DNA damage response induced by vosaroxin and azacitidine in clinical samples taken from patients before initiating therapy on a clinical trial with these agents, to correlate the DNA damage response with their treatment response.

Tyler A. Hyman
The Ohio State University College of Medicine
Research Project: Evaluating the effect of arginine hydroxylase modulation on AML sensitivity to NK-mediated cytotoxicity
Research Mentor: Michael A. Caligiuri, MD, James Cancer Hospital and Solove Research Institute, OSU Comprehensive Cancer Center
In Her Own Words: I gained experience culturing AML cells and learned flow cytometry, real-time PCR, and immunoblotting to determine how AHR modulation impacts AML.

Katerina Konstantinoff
Washington University School of Medicine
Research Project: Phenotyping of megakaryocytes in granulocytic colony-stimulating factor (G-CSF): induction of hematopoietic stem cells (HSCs)
Research Mentor: Daniel Link, MD, Washington University School of Medicine
In Her Own Words: Tell us a bit about your research project. What led you to select it? My project consists of working to determine if megalakocytes play a role in the mobilization of HSCs by G-CSF. The translational research is to understand how such a role might be used clinically to move HSCTs from the bone marrow to the blood for transplant.

How do you see your project as contributing to the broader field of hematology or health care in general? This project aims to contribute to a more complete understanding of the mechanism of G-CSF–induced HSPC mobilization. I hope that this project and my future work will further strengthen the link between the lab and the clinic. On a broader scale, I think that the physician-patient relationship is built on trust and informed decision making, and improving treatment options through research will provide hope to many people now and in the future.

How has your research mentor influenced you or added to your experience in a way that was perhaps unexpected? Dr. Link is a leader in the field and an excellent mentor. He has been especially helpful in discussing the many opportunities available for research in my future career.

MGSSAA Recipient, 2011-2014

Colles Price, MS, PhD
Dana Farber Cancer Institute, Broad Institute of Harvard and M.I.T.
Research Mentor: William C. Hahn, MD, PhD, Dana Farber Cancer Institute, Harvard Medical School, Broad Institute of Harvard, M.I.T.
Research Project: Using genomics to understand novel identified cancer controlling susceptibilities
In His Own Words: How has the MGSSAA influenced the trajectory of your career and your work? It has been a great opportunity – one of the most amazing research experiences I have had in graduate school. I have presented several posters, which has enhanced my research and training. I have connected with new colleagues and obtained resources for my research from interested scientists and clinicians. The ASH community is collaborative and helpful so it is great for trainees. Giving a talk at ASH boosted my career substantially.

What sparked your interest in the role of microRNAs in leukemia? In my graduate work, I had the privilege of working with Dr. Janet Rowley, who provided me with insight into chromosomal rearrangements and their important role in cancer biology and hematology. My other mentor, Dr. Jianjun Chen, is an important leader in understanding noncoding RNAs in cancer. The combination of these two led to my interest in microRNAs in leukemia. While the field has made progress in understanding microRNAs, it is clear there is still much work left to do.

What advice do you have for those just starting medical school, who are considering a career in hematology? Find a network of colleagues. Not only will your classmates/friends be future scientific leaders, they also understand the challenges you are currently facing. Medical or graduate school does not need to be a solo endeavor; having a support system can be helpful. Also, it is of utmost importance to find good mentors throughout your career. As each mentor has his or her own strengths and weaknesses, it is important to have more than one. With the support of your colleagues and good mentors, a great career in hematology is guaranteed.
Myeloma-Related Bone Disease

In newly diagnosed MM patients, which correlates with MM disease stage and fractures. Encouraging preclinical data have highlighted the relevance of targeting sclerostin in MM.10 Data from clinical studies with the soluble activin A receptor RAP-011, its human version ACE-011 was evaluated in a phase IIa study in patients with MM and has demonstrated reduced bone pain and functional characterization of a specific antidote for ticagrelor. Blood. 2015;125:3484-3490.

Ticagrelor (Brilique or Brilinta) is a widely used reversible ADP P2Y12 antagonist for the treatment of patients with acute coronary syndromes. It reduces cardiovascular death, myocardial infarction, and stroke, but it also increases non-coronary artery bypass grafting-associated bleeding events. There is no specific treatment available for bleeding caused by antplatelet agents. In Blood this week, Dr. Andrew Buchanan and co-authors present the development of a specific and highly potent antidote for an antplatelet agent, fulfilling a long-sought need. The study marks an important advance with potential to improve patient safety.


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Clinical Trials Corner

Edoxaban in Venous Thromboembolism Associated with Cancer

STUDY TITLE: Cancer Venous Thromboembolism (VTE)

CLINICALTRIALS.GOV IDENTIFIER: NCT02073682

SPONSOR: Daichi Sankyo, Inc.

STUDY DESIGN: Randomized, open-label study

TARGET ENROLLMENT: 1,000 patients

PARTICIPATING CENTERS: Approximately 130 study sites in North America, Europe, and Australia/New Zealand

ACCRUAL GOAL: 1,000 patients

STUDY SYNOPSIS: This is a multinational, prospective, randomized, open-label, blind-evaluator (PROBE) noninferiority study comparing edoxaban to dalteparin for prevention of the combined outcome of recurrent venous thromboembolism (VTE) or major bleeding in patients with cancer-associated VTE. Eligible patients will have both confirmed deep vein thrombosis (DVT) or pulmonary embolism (PE: symptomatic or unsuspected) as well as active cancer. Key exclusion criteria include: poor performance status, creatinine clearance less than 30 mL/min, life expectancy less than three months, platelet count no higher than 50 x 10⁹/L, or more than 72 hours of therapeutic-dose LMWH prior to randomization. Participants will be treated with either edoxaban 60 mg by mouth daily versus dalteparin 200 U/kg subcutaneously daily for 30 days (followed by 150 U/kg daily thereafter).

RATIONALE: Patients with cancer are at increased risk of VTE, and evidence-based guidelines recommend that cancer-associated VTE be treated with at least six months of LMWH. Long-term LMWH is burdensome not only because it is expensive, but also because it must be administered subcutaneously.

COMMENT: Edoxaban, an oral direct factor Xa inhibitor, has recently been approved in many jurisdictions for the treatment of VTE. Although high-quality evidence indicates that edoxaban (along with other direct oral anticoagulants[DOACs]) is at least as safe and effective as standard therapy for DVT and PE, very few patients participated in the registration trials for these newer anticoagulants had cancer-associated VTE. Furthermore, the principal comparator in the registration trials of edoxaban and other DOACs was warfarin, not LMWH. Therefore, a trial comparing a DOAC to LMWH in patients with cancer-associated thrombosis is much needed.

CAR T Cells in Radiotherapy Diffuse Large B-cell Lymphoma

STUDY TITLE: A Phase 1-2 Multi-Center Study Evaluating KTE-C19 in Subjects With Radiotherapy Aggressive Non-Hodgkin Lymphoma (NHL)

CLINICALTRIALS.GOV IDENTIFIER: NCT02348216

SPONSOR: Kite Pharma, Inc.

PARTICIPATING CENTERS: Four centers in the United States

ACCRUAL: 124 participants

STUDY DESIGN: Kite Pharma is conducting a multicenter phase I/II study of KTE-C19 in patients with refractory diffuse large B-cell lymphoma (DLBCL), primary mediastinal large B-cell lymphoma, and transformed follicular lymphoma. The primary endpoints of the phase I and phase II portions of the study are safety and objective response rate, respectively. Collected via leukapheresis, peripheral blood mononuclear cells are cultivated with an anti-CD19 antibody and IL-2 and then transduced with a third-generation chimeric antigen receptor (CAR) T-cell construct containing an anti-CD19 scFv conjugated to a CD28 costimulatory molecule and CD3 zeta chain. Patients then receive fludarabine and cyclophosphamide followed by CAR T-cells at a target dose of 2 x 10⁹ cells/kg.

RATIONALE: Although the majority of patients with DLBCL are cured with upfront chemomimotherapy, approximately 35 percent of patients will experience relapse or have primary refractory disease. Fewer than half of these patients will be candidates for high-dose chemotherapy with autologous stem cell rescue as a result of failure to respond to second line therapy or due to age and/or medical co-morbidities. Additionally, disease recurrence after transplant is common. Despite advances in understanding the biology of DLBCL, including the identification of high-risk molecular features such as rearrangements and protein overexpression of MCL and BCL2,–2, effective therapies for relapsed DLBCL are desperately needed. Harnessing the immune system using CAR T-cells has emerged as a promising, novel approach in this setting.

CAR-D19 is expressed on nearly all normal and malignant B-cells with the exception of plasma cells, and is not found on hematopoietic stem cells or other cell types in the body. Anti-CAR-D19 CAR T cells are autologous T cells, collected by peripheral blood pheresis, engineered to express a single chain variable fragment (scFv), derived from a monoclonal anti-CD19 antibody, and linked to costimulatory molecules.

A recently published study of anti-CAR-D19 CAR T-cells from the NCI demonstrated impressive activity in a cohort of 15 patients with heavily pretreated B-cell lymphoma, nine of whom had DLBCL, including four with primary mediastinal large B-cell lymphoma (Figure; Kochenderfer JD, et al. J Clin Oncol. 2015;33:540-549. Mass MV, et al. Blood 2014;123:2625-2637). Their third generation CAR T-cell construct contained an anti-CD19 scFv conjugated to a CD28 costimulatory molecule and CD3 zeta chain designed to enhance T-cell activation. After undergoing pheresis, patients were pre-treated with cyclophosphamide and fludarabine to prevent destruction of inhaled anti-CAR-D19 CAR T cells. Of the seven evaluable patients with DLBCL (one was lost to follow-up and a second died approximately two weeks after CAR T-cell infusion of a presumed cardiac arrhythmia), four achieved a complete response, two had a partial response, and one experienced stable disease. Adverse events consisted primarily of acute reactions following cell infusion, including fever and hypotension, as well as reversible neurologic toxicity ranging from delirium to local neurologic deficits and myoclonus in the setting of elevated serum levels of interleukin-2 and interleukin-6.

COMMENT: CAR T-cell therapy represents a promising novel approach in patients with refractory aggressive B-cell lymphoma. Cytotoxic chemotherapy, consisting of combinations of drugs with diverse mechanisms of actions, even at high doses, has yielded disappointing results in relapsed DLBCL, particularly since the incorporation of rituximab in up-front therapeutic regimens. This appears to be particularly true in double hit and double protein-expressing lymphomas, which are especially resistant to chemotherapy and for which no effective salvage therapies have been developed. Employing an immunologic approach, which may be active across multiple underlying molecular drivers of disease, is highly appealing. If this approach proves effective in the refractory setting, it could then be studied as consolidation in previously untreated high-risk patients.

~Ann S. LaCasce, MD, MSE

Dr. Garcia has received consulting honoraria from Daichi Sankyo, the sponsor of the trial described here. Dr. Garcia is the U.S. National Coordinator for this trial.
As technology and the Web have evolved, so too have ASH’s online offerings. Now, beyond the ASH website, you can download ASH apps for your smartphone or tablet, follow ASH on Twitter (www.twitter.com/ASH_hematology), and find ASH videos on YouTube (www.youtube.com/user/ASHWebmaster).

You may have noticed that beginning with the May/June issue of *The Hematologist*, ASH has produced podcasts featuring interviews with selected authors, moderated by Editor-in-Chief Dr. Jason Gotlib and members of *The Hematologist’s* editorial board. These short, audio-only segments present new observations, commentary, and context around articles published in *The Hematologist*. Below are a few of the topics covered thus far, with new podcasts to be published bimonthly, with every issue. Keep an eye out for the red “speaker” logo (such as the one on the cover of this issue) indicating that an accompanying podcast is available:

- For the September-October 2015 issue, Contributing Editor Dr. Adam Cuker has a conversation with Dr. Tom Ortel about perioperative bridging through the lens of the BRIDGE trial data, which were published by Dr. Ortel and colleagues in the *New England Journal of Medicine*. In this podcast, Drs. Cuker and Ortel dive more deeply into the design, outcomes, and limitations of the BRIDGE study and discuss how its results may inform clinical practice and treatment of atrial fibrillation and other disorders.

- In his Diffusion article from the July/August 2015 issue of *The Hematologist*, Contributing Editor Dr. David Garcia discusses the PREPIC 2 study. The PREPIC 2 results, published in *JAMA*, highlight the benefits of well-managed anticoagulation in patients with high-risk pulmonary embolism. In this podcast, Dr. Garcia sheds additional light on the study as well as its ramifications throughout the field, with Dr. Gotlib leading the discussion.

- The strategic roadmap developed at the original ASH Meeting on Lymphoma Biology held in August 2014 was recently published in *Blood*. To continue this important conversation, Contributing Editor Dr. Ann LaCasce and guest author Dr. David Weinstock wrote a companion article, published in the May/June 2015 issue of *The Hematologist*. This podcast features additional insight from the authors on what could be in store for the roadmap and its steering committee, with Dr. Gotlib moderating.

Don’t miss out on this new collection of bonus content from *The Hematologist*. To stay up to date, follow ASH on SoundCloud at [https://soundcloud.com/ash_hematology](https://soundcloud.com/ash_hematology), or search “ASH_hematology” in iTunes or in the Podcasts app, and click “Subscribe.”

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**Mark Your Calendar**

### September

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<tr>
<td><strong>7</strong></td>
<td>Translational Research Training in Hematology full application deadline</td>
<td>Washington, DC</td>
<td><a href="http://www.hematology.org/awards">www.hematology.org/awards</a></td>
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<td><strong>17-19</strong></td>
<td>2015 ASH Meeting on Hematologic Malignancies</td>
<td>Chicago, IL</td>
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<td><strong>17</strong></td>
<td>ASH Consultative Hematology Course (CHC)</td>
<td>Chicago, IL</td>
<td><a href="http://www.hematology.org/meetings">www.hematology.org/meetings</a></td>
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<td><strong>17</strong></td>
<td>Workshop on the Diagnosis, Classification, and Clinical Care of MDS (DC3-MDS)</td>
<td>Chicago, IL</td>
<td><a href="http://www.pathologylearning.org/mds">www.pathologylearning.org/mds</a></td>
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<td><strong>23-26</strong></td>
<td>International Myeloma Workshop</td>
<td>Rome, Italy</td>
<td><a href="http://www.msv2015.it">www.msv2015.it</a></td>
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<td><strong>30</strong></td>
<td>Applications for Physician-Scientist Career Development Award due</td>
<td>Washington, DC</td>
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### October

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<td><strong>9</strong></td>
<td>Workshop on the Diagnosis, Classification, and Clinical Care of MDS (DC3-MDS)</td>
<td>New York, NY</td>
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<td><strong>16</strong></td>
<td>Workshop on the Diagnosis, Classification, and Clinical Care of MDS (DC3-MDS)</td>
<td>Tampa, FL</td>
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<td><strong>22</strong></td>
<td>ASH annual meeting late-breaking abstract site opens</td>
<td>Orlando, FL</td>
<td><a href="http://www.hematology.org/annual-meeting">www.hematology.org/annual-meeting</a></td>
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<tr>
<td><strong>29</strong></td>
<td>Deadline to submit late-breaking abstracts for the ASH annual meeting</td>
<td>Orlando, FL</td>
<td><a href="http://www.hematology.org/annual-meeting">www.hematology.org/annual-meeting</a></td>
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### November

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<tr>
<td><strong>4</strong></td>
<td>ASH annual meeting advance registration closes</td>
<td>Orlando, FL</td>
<td><a href="http://www.hematology.org/annual-meeting">www.hematology.org/annual-meeting</a></td>
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<td><strong>5</strong></td>
<td>ASH annual meeting late/on-site registration opens</td>
<td>Orlando, FL</td>
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### December

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<tr>
<td><strong>4</strong></td>
<td>Friday Satellite Symposia (FSS)</td>
<td>Orlando, FL</td>
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<td><strong>4</strong></td>
<td>Friday Scientific Workshops</td>
<td>Orlando, FL</td>
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<td><strong>5-8</strong></td>
<td>57th ASH Annual Meeting &amp; Exposition</td>
<td>Orlando, FL</td>
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Read *The Hematologist* online at [www.hematology.org/thehematologist](http://www.hematology.org/thehematologist), and catch up on the latest news in the field of hematology right on your desktop, mobile phone, or tablet.