Sinking the SHP That Drives Malignancy

Tyrosine kinases are enzymes that selectively phosphorylate specific tyrosine residues on diverse protein substrates and they play a pivotal role in signaling pathways that govern cell proliferation and survival. Receptor tyrosine kinases (RTKs) are transmembrane proteins that respond to extracellular stimuli and relay the signal to the internal milieu by phosphorylating targets that initiate a cascade of events. The activation of RTK kinases is tightly controlled and phosphorylation is thus counterbalanced by dephosphorylation, whereby the phosphate groups are removed by protein tyrosine phosphatases. Dysregulation of either of these functions can result in oncogenic transformation of cells, and this has led to research and development of inhibitors of these key enzymes. Tyrosine kinase inhibitors, such as those used to treat chronic myeloid leukemia, have been successful, but targeted therapy of phosphatases has lagged behind.

Src homology region 2 (SH2)–containing protein tyrosine phosphatase 2 (SHP2) is a nonreceptor phosphatohosphorylase encoded by the PTPN11 gene. SHP2 is a ubiquitous enzyme that promotes activation of the RAS-ERK signaling pathway within cells. It is highly expressed in hematopoietic cells where it transduces signals from hematopoietic growth factors, and thus, it plays an essential role in hematopoietic cell development. Mutations in PTPN11 that activate SHP2 are implicated in numerous cancers, including leukemias, and it was the first identified proto-oncogene that encodes a tyrosine phosphatase.

To discover new therapeutic targets for cancer, Dr. Ying-Nan P. Chen and colleagues screened a panel of 250 cancer cell lines with a deep coverage, pooled, short-hairpin RNA library that targeted 7,500 genes. Cell lines that were dependent on RTKs showed marked sensitivity to SHP2 depletion. Additional growth and complementation experiments verified that SHP2 is essential for the survival of these cells and that p-ERK activation was the mechanism of action.

Structural features of SHP2 are known, and the researchers used this information to guide subsequent screening strategies focused on identifying SHP2 inhibitors. SHP2 contains two tandem SH2 domains (N-SH2 and C-SH2) at the N-terminal end of the protein; a catalytic phosphatase domain toward the C terminal; and a C terminal tail with a proline-rich motif and two tyrosine residues, which can be phosphorylated. The active site of the enzyme is tightly regulated by the N-SH2 domain. Autoinhibition occurs when N-SH2 binds the catalytic domain and changes the conformation to prevent access to the substrate. The enzyme is activated when docking proteins with appropriately spaced phosphorytosine residues interact with N-SH2 and C-SH2 to expose the active site.

Catalytic inhibitors of SHP2 have been discovered but have not progressed to clinical use since they display low selectivity and potency, partly due to the highly solvated and polar nature of the catalytic pocket. The researchers therefore focused their attention on allosteric inhibitors to capitalize on the natural autoregulation of the enzyme. They partially activated recombinant SHP2 (residues 1-525) with a bis-phosphorylated peptide, 2P-IRS-1, and screened a library of 100,000 diverse compounds. Hits were prioritized by further screening against a truncated recombinant protein containing only the catalytic domain, as well as against a fully activated SHP2 molecule. Compounds that inhibited only the phosphatase domain were excluded, and further refinement led to the identification of SHP099, which was highly active with an IC50 = 71 nM. It also displayed high selectivity since it had no detectable activity against a panel of 21 phosphatases and 66 kinases and was also inactive against SHP1, the closest human homologue. To determine the mechanism of inhibition, the researchers conducted further biochemical studies and solved the crystal structure of the SHP2-SHP099 complex. This revealed that SHP099 bound to the central tunnel formed at the interface of the N-SH2, C-SH2, and phosphatase domains, which stabilized the protein in the inactive conformation.

The next step was to test whether the compound could inhibit the enzyme within cells by screening several cell lines, including a panel of 71 hematopoietic cancer lines, against a range of SHP099 concentrations, and by measuring the effect on p-ERK. Cancer cells with known mutations in oncogenic RTKs or cytoplasmic tyrosine kinases were sensitive to SHP099 inhibition, whereas cells with RAS or BRAF mutations were not affected. These findings confirmed the link between RTK dependence and SHP sensitivity that was demonstrated in their short-hairpin RNA screen. Off-target effects may occur, and thus, inhibitor-resistant alleles were developed that had mutations in key residues, which would disrupt binding of SHP099 but maintain the integrity of the three-domain regulatory interface. Cell growth and inhibition of p-ERK were used as endpoints, and the data demonstrated that SHP099 inhibits MAPK signaling and proliferation in RTK-dependent cells in a direct, on-target manner.

In vivo testing of SHP099 using a subcutaneous xenograft model in mice showed marked dose-dependent inhibition of tumor growth, and the treatment was well tolerated. This was extended to an evaluation of an in vivo protocol in a subcutaneous xenograft model in mice. The treatment was well tolerated and significantly reduced tumor size. This was extended to an evaluation of an in vivo protocol in a subcutaneous xenograft model in mice. The treatment was well tolerated and significantly reduced tumor size.

The discovery of SHP099 represents an exciting breakthrough and a promising strategy to treat RTK-driven cancers. SHP099 is the first example of a potent, highly selective, allosteric inhibitor of the protein tyrosine phosphatase SHP2. It is orally available and is well tolerated in xenograft mouse models. Additionally, it provides a valuable tool to investigate the biological functions of SHP2 and to explore its role in normal hematopoiesis and tumorigenesis.
Recruitment Across the Spectrum

One of the most important aspects of ASH’s mission is the commitment to recruit and retain the best talent in the profession. This goal is even more profound as there is a need to attract a diverse group of individuals to embark on careers in hematology. Thanks to the dedicated efforts of the Committee on Promoting Diversity, the Society has been doing an excellent job of diversifying the pool of new hematologists and drawing in medical school students leaning toward hematology careers. The work of the committee emerges in part from the understanding that a representative workforce benefits everybody, in areas ranging from patient satisfaction, to improved participation in clinical trials, to innovation in care and treatment. However, the Society recognizes that there is much more work to be done to build and sustain a truly diverse biomedical research workforce.

There have been countless studies showing that there is a disproportionately low percentage of minorities going into training, medical practice, and on to faculty positions. For example, in 2013, according to the Association of American Medical Colleges, Black or African American and Hispanic or Latino individuals made up only 11.5 percent of all biomedical doctoral degrees, while these groups comprise more than 30 percent of the overall population. As recently as 2010, less than 10 percent of all medical school faculty in the United States were African American or Hispanic. And in 2015, a U.C. San Francisco study found that “since the 1993 NIH Revitalization Act … less than 2 percent of the 10,000-plus cancer studies have included enough minorities to be relevant,” according to the National Institutes of Health’s very own inclusion standards. This stems from the lack of minorities with careers in research, who are more likely to conduct studies in minority populations and more likely to gain these communities’ trust.

We all might recognize that these patterns are unacceptable, but as a Society, we are called to do something about it! Closing these gaps means creating the right opportunities for diverse communities of students and trainees. It means opening up new pathways for under-represented groups to witness and experience the richness of our field and to bring their passion for science and patient care into the world of hematology. ASH’s Minority Recruitment Initiative (MRI) represents a vehicle for progress, a way for us to get involved and bring about change, and a seamless pipeline for talent that extends from medical school, to residency, to faculty.

This year, ASH announced three new programs to turn these ideas into reality. The greatly popular Minority Medical Student Award Program (MMSAP) will expand beyond its traditional summer timeframe, into a more flexible 12-month period. The new Minority Resident Hematology Award Program will provide research opportunities in hematology to residents in training, with funding to complete an eight- to 12-week project, also taking place over a flexible 12-month period. Projects can be either laboratory-based or clinical.

Lastly, participants in the ASH Institutional Representative (AIR) program will represent ASH locally within their own institution and/or at institutions within close proximity. Participants will advise trainees and hematology faculty on ASH award programs, facilitate the application process, and meet with trainees at all levels to discuss careers in hematology and provide career advice. These new programs will be rolled out in 2017.

All throughout this year, I and others have extolled what an exciting, fascinating time it is to be in hematology. If you want to help spread the word to those who are new to the field while promoting diversity, get involved with the MRI as a mentor, encourage students and colleagues to apply for MRI programs, and stay up to date on the AIR program in the coming months. Visit www.hematology.org/MRI to learn more.

Charles S. Abrams, MD
**The Hematologist Is Seeking Its Next Editor**

ASH is in the initial stage of the selection process for the next Editor in Chief of *The Hematologist* (term: 2018-2020).

Candidates with an MD or equivalent medical degree should have a broad and comprehensive knowledge of basic research and clinical investigation in hematology as well as an appreciation of its subspecialty areas; a distinguished research and publications record; high standing among peers; and demonstrated writing, reviewing, and editing skills.

Members of ASH are invited to submit the names of potential candidates, accompanied by a brief, informal endorsement and a description of the candidate’s editorial experience, to:

*The Hematologist: ASH News and Reports*

c/o Juana Llorens, Managing Editor

American Society of Hematology

2021 L Street, NW, Suite 900

Washington, DC 20036

jllorens@hematology.org

The application deadline is February 1, 2017.

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**Updated Grants Clearinghouse Database Available**

The ASH Trainee Council offers the Grants Clearinghouse as a service to aid trainees in their search for grant information. Browse this newly updated online resource and filter dozens of hematology grant opportunities at [www.hematology.org/Fellows/Grants/](http://www.hematology.org/Fellows/Grants/).

If you would like to notify us of updated information or suggest grants to add to the list, e-mail the ASH Training Manager at training@hematology.org.

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**Hit the Road to Support Hematology’s Future!**

The ASH Foundation Run/Walk is your chance to join fellow ASH annual meeting attendees and lace up for a great cause. This year, the Run/Walk will take place on Sunday, December 4, and follow along San Diego’s scenic Embarcadero. Choose a 3K or 5K route, and donate or fundraise as an individual, as a team, or as a sponsor for other participants. Friends and family can support your efforts by donating in honor of you or your team!

All proceeds (100%) from your registration fee and any donations will directly fund career development, research, and education programs supported by the ASH Foundation. To register yourself or your team, visit [www.hematology.org/runwalk](http://www.hematology.org/runwalk).

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**Complimentary MDS Summits: Five More Chances to Earn CME Credit**

There are many complexities associated with myelodysplastic syndromes (MDS) that a multispecialty team must address as a clinical-care unit. To address educational gaps associated with these complexities, the American Society for Clinical Pathology, ASH, and the France Foundation have designed comprehensive MDS-directed educational summits that feature live events designed by world-class subject matter experts.

These complimentary CME summits on the diagnosis, classification, and care of MDS are available in five U.S. cities, from now through December. Visit [www.hematology.org/MDS](http://www.hematology.org/MDS) for more information and to register.

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**ASH Announces Names of Recipients of Three ASH Awards**

ASH recently announced the names of awardees for three of its awards. Fifteen medical students and 15 residents were selected to receive the 2016 ASH HONORS Award, which supports talented medical students and residents in the U.S., Canada, and Mexico. Additionally, seven outstanding fellows were selected to receive the 2016 ASH Research Training Award for Fellows (RTAF), a year-long program aimed at encouraging careers in academic hematology by providing protected research time during training. Finally, 14 students were selected to take part in the 2016 Minority Medical Student Award Program, which offers an introductory mentored biomedical research experience to minority medical students.

Read more about these awards and this year’s winners at [www.hematology.org/newsroom/press-releases/](http://www.hematology.org/newsroom/press-releases/).
ASH Standing Committee Update

One of the most central underpinnings of ASH’s structure and governance is its group of 14 standing committees. These groups meet in person at least once throughout each calendar year and serve year-round to recommend policies, programs, and actions to ASH’s Executive Committee. In this serial feature, we will hear from the committee chairs who steer these groups regarding their mission, goals, and new initiatives in development.

Trainee Council: Who Are We? What Do We Do?

The ASH Trainee Council is a subcommittee of the ASH Committee on Training that was established back in 2001. The council’s mission is to advocate for hematology trainees everywhere regarding issues related to education, funding, and research, and to help them achieve career success. The council consists of 12 trainees, 10 MDs and two PhDs, from the United States, Canada, and Mexico.

The goals of the council are to: 1) provide a forum for trainees to discuss issues related to their career development; 2) represent trainees and their needs to ASH leadership and committees; 3) plan and organize Trainee Day and other trainee events at the ASH annual meeting; and 4) suggest outreach initiatives that would increase recruitment and retention of trainees in hematology. The council has led numerous projects, including, but not limited to, Grants Clearinghouse, “TraineeNews,” and Career Development Timelines.

Career Development Timelines are roadmaps created and transformed into an online searchable platform that is easier for trainees to navigate using relevant filters and keywords.

“The Grants Clearinghouse, available at www.hematology.org/Grants, is a database of more than 110 grant opportunities that fund trainees at all levels and/or junior faculty conducting hematology research. The database includes information of grant opening and closing dates, funding, and eligibility or citizenship requirements. Thanks to the work of the council, the database was transformed into an online searchable platform that is easier for trainees to navigate using relevant filters and keywords.

“TraineeNews” is another active project led by the council. The newsletter is usually written by council members, distributed quarterly to all trainees, and includes articles related to career development, funding, mentorship, job negotiation, and other important topics; board-style case study questions; and other important announcements relevant to trainees. All “TraineeNews” articles are archived in an online searchable database, which you can access via www.hematology.org/TraineeNews.aspx.

ASH Committee on Educational Affairs: Hitting the Road with a New Initiative

The Committee on Educational Affairs (CEA) is a vibrant and interactive group that is charged with oversight and recommendations for the planning, development, implementation and evaluation of continuing medical education (CME) and non-CME educational activities of ASH. These activities include live meetings, webinars, podcasts, videos, enduring print material, digital offerings, and collaborative educational projects with other societies and commercial vendors. The 14 appointed and eight liaison members of the CEA represent the diverse interests and mission of ASH and cover basic and clinical research in both nonmalignant and malignant hematology, health-care systems and outcomes, community practice and advocacy, medical education, graduate training, and global hematology.

Starting in 2016, the CEA embarked on a new initiative called the “Educational Roadmap.” The goal of this initiative is to create a substructure and mechanism within the CEA to allow us to preemptively recognize learner gaps and needs, identify opportunities to address these gaps in existing and future educational activities, and proactively recommend specific content and instructional formats that are inclusive, innovative, and aspirational. For this endeavor, we created six working groups within the CEA, each focusing on a core competency as defined by the Accreditation Council for Graduate Medical Education (ACGME); these include medical knowledge, patient care and procedural skills, systems-based practice, practice-based learning and improvement, professionalism, and interpersonal and communication skills. Working group members are tasked to identify learner gaps and generate recommendations using these competencies and their associated ACGME subcompetencies as a foundation, along with the core competencies of the Institute of Medicine (IOM), e.g., to provide patient-centered care, work in interdisciplinary teams, employ evidence-based practice, apply quality improvement, and utilize informatics.

Working groups also take into consideration the essential elements and commendation criteria from the Accreditation Council for CME when formulating their ideas and proposals.

During the summer CEA meeting on July 18 at ASH headquarters, the working groups put this effort into action. After a series of breakout brainstorming sessions, feedback discussions and final report outs, the groups generated aspirational goals and educational format recommendations for key topic areas including sickle cell disease, acute myeloid leukemia, systems-based hematology, and others.

This initial work will be presented to the Executive Committee for their approval and advice prior to moving forward to the next stage. These first exciting steps on the “Educational Roadmap” trail will eventually provide a “toolbox” containing broad content topics and versatile teaching methodologies that can be applied to the growing portfolio of ASH educational offerings.

Michael Linenberger, MD Chair, ASH Committee on Educational Affairs Fred Hutchinson Cancer Research Center, Seattle, WA

This Just In – ASH Is in the News!

The Committee on Communications is responsible for enhancing communication with ASH members and promoting the science and practice of hematology. The Committee is charged with developing and executing communications strategies that demonstrate the value and quality of hematology research and medical care to the press and to the public. To this end, the committee oversees the Society’s media relations efforts that seek to promote research presented at the ASH annual meeting and published in Blood, increase the visibility of ASH initiatives that promote clinical research in hematology, and increase the profile of ASH as a leading advocate on issues of importance to hematologists.

We have many exciting initiatives, including growing ASH’s social media presence at the annual meeting (hashtag #ASH16 for this year’s meeting, and #ASHsocial for the social media session), but I wanted to spotlight some of the great work we are doing in the media.

In January 2014, ASH launched a three-year pilot of the Media Experts Subcommittee in support of the Society’s renewed emphasis on media coverage. The goals of the subcommittee included making ASH more nimble in responding to press inquiries and better positioning ASH as the primary source for credible scientific information about blood and blood diseases. Members of the subcommittee are appointed to three-year terms and, following required ASH orientation and media training, are authorized to speak with the media as official Society spokespeople. The training includes an overview of best practices, instruction in effective messaging techniques, and on-camera practice interviews and critiques.

During the summer 2016 Committee on Communications meeting at ASH headquarters, the Committee evaluated the pilot program of the Media Experts Subcommittee. From early 2014 through July 2016, ASH experts have been quoted in more than 360 articles. Some of these articles include:

Sickle cell gene doesn’t elevate death risk, Stanford study finds
San Francisco Chronicle

Dear Science: If I drink coffee before giving blood, will recipients get a buzz?
Washington Post

Should you be worried about bruising easily?
US News and World Report

How to understand a lymphoma diagnosis
The Wall Street Journal

Breastfed children have slightly lower risk of childhood leukemia
USA Today

We are thankful for the opportunity to promote the great science and work of ASH and its members and look forward to much more.

~ Joseph Mikhael, MD Chair, ASH Committee on Communications Mayo Clinic Arizona, Scottsdale, AZ

Dr. Badawy indicated no relevant conflicts of interest.

Dr. Linenberger indicated no relevant conflicts of interest.
Fiscal Year 2017 Budget Process Continues Following Congressional Summer Recess

Returning from a more than seven-week break for the political conventions and the August district work period or “recess,” Congress faces a limited number of legislative work days in September before the expiration of the 2016 fiscal year.

Just prior to adjourning for the remainder of the summer, the House Appropriations Committee approved a draft fiscal year (FY) 2017 spending bill containing $33.3 billion for the National Institutes of Health (NIH) — $1.25 billion more than the FY 2016 enacted level. On June 9 of this year, the Senate Appropriations Committee voted overwhelmingly to advance its version of the FY 2017 NIH spending bill, which included a proposed $2 billion boost to NIH. However, there are still many impending legislative hurdles as the full membership of both chambers must still consider and pass these measures, as well as compromise on the differing funding levels for NIH and other federal programs.

Congressional spending bills are to be completed by October 1, which marks the beginning of FY 2017. However, despite House and Senate appropriators making significant progress on all 12 of the annual spending bills for the first time in years, it is unlikely that the House and Senate will vote and reach a final agreement on all of these bills prior to the start of the new fiscal year.

This leaves congressional leaders with two options: 1) Pass a temporary continuing resolution (CR) that extends federal agency and program funding unchanged from the current year or 2) shut down the government until an agreement on spending can be reached. With the November elections just weeks away, a government shutdown would be politically unfeasible. More likely, Congress will pass a CR that lasts through mid-November (and after the elections), when leaders will attempt to finalize FY 2017 funding.

Grassroots support is critical as the FY 2017 budget process continues. The Society needs the help of all of its members in continuing to focus attention on this and other issues of importance to hematology. Visit the ASH Advocacy Center (www.hematology.org/NIH) for the most up-to-date information on the status on the 2017 budget process and to see how you can contact your senators and representative to protect NIH funding in FY 2017.

ASH Observes National Sickle Cell Awareness Month

September is National Sickle Cell Awareness Month and ASH will be marking this occasion by announcing updates about the Society’s multifaceted initiative to address the burden of sickle cell disease (SCD), both in the United States and globally. As part of this initiative, the Society has engaged a broad array of stakeholders to identify the highest priorities needed to improve outcomes for individuals with SCD. Through this outreach, ASH learned about the exciting work that is happening in the field and decided there was a critical need for a coordinated effort to ensure further advancements in SCD care and research. To this end, ASH recently created the Sickle Cell Disease Coalition (SCDC) to help amplify the voice of the SCD stakeholder community, promote awareness, and improve outcomes for individuals with SCD. The SCDC focuses on promoting research, clinical care, education, training, and advocacy, and the growing

SCDC membership includes public health, research, and provider organizations; patient groups; faith-based organizations; federal agencies; and industry and funding organizations. The SCDC will serve as a platform to encourage stakeholders to work together to develop and implement important projects that will ultimately help improve outcomes for individuals with SCD.

To continue ASH’s efforts to raise awareness for SCD, the Society plans to launch a call to action on SCD during a press briefing on September 6 in Washington, DC. The purpose of the call to action is to engage and unite people over the need to improve SCD care, early diagnosis, treatment, and research. During the briefing, ASH and other partner groups will issue a report on the state of SCD care, along with what needs to happen to improve the lives of people with this disease. Stakeholder groups will be asked to sign on to the report and to commit to other activities aimed at making a difference in the lives of individuals with SCD. To continue the momentum after the launch, ASH and other stakeholder groups plan to continuously release updates about new SCD-related activities and resources, including reports about the progress that has been made and where efforts are still needed.

For the latest on ASH’s SCD initiative, visit www.hematology.org/advocacy/scd.

ASH Holds Foundational Workshop on Genome Editing

The ASH Workshop on Genome Editing took place July 14-15 in Washington, DC, and brought together more than 150 clinical and laboratory-based scientists, funders, and regulators to share cutting-edge research and tackle key scientific and clinical hurdles in the field of genome editing. Recognizing the potential of this groundbreaking science to correct genetic flaws, ASH has made genome editing a research priority. This workshop covered a broad swath of the genome editing landscape, including contemporary laboratory applications, burgeoning therapeutic potential, and regulatory considerations for such therapies. The workshop provided networking opportunities among academicians, industry scientists, government officials, and others in the field, to foster partnerships that can strengthen the discovery-approval-application pipeline for current and emerging genome editing processes. Special thanks to Representative Bill Foster (D-IL; pictured below) who stopped by the workshop.

New Digital Resources from The Hematologist

“Ask the Hematologist” Compendium

A new collection of The Hematologist’s most widely read department is now available. We have compiled articles spanning the years 2010 to 2015, and with the exception of some more recent works, we have asked authors to provide state-of-the-art updates to the content where applicable. Download the PDF or browse the web version at www.hematology.org/Thehematologist/Ask/5960.aspx.

New Monthly Podcasts

Did you know that The Hematologist podcast is now monthly? Check out the August podcast featuring Drs. Ken Anderson and Martin Tallman discussing what’s new on the horizon for this year’s Meeting on Hematologic Malignancies, and the September podcast on the events surrounding Thrombosis, as covered by Drs. Tracy George and Rama Gulapalli in this issue. Be sure to follow us on SoundCloud (www.soundcloud.com/ash_hematology) to stay up to date!
The WHO is New: 2016 Updates to the Classification of Myeloid Neoplasms

DAVID R. CZUCHELWSKI, MD; AND TRACY I. GEORGE, MD

1. Associate Professor, Department of Pathology, University of New Mexico, Albuquerque, NM
2. Professor, Division Chief, Hematopathology, Director, Hematopathology Fellowship Program, Department of Pathology, University of New Mexico, Albuquerque, NM

The forthcoming 2016 revision of the WHO Classification of Tumours of the Hematopoietic and Lymphoid Tissues was recently previewed in two detailed summaries prepared by key members of the clinical advisory committee. The classification, last updated in 2008, now shows even more abundant evidence of the impact of genetic markers on diagnosis and disease management. This Mini Review is intended as a brief and high-level summary of some of the most critical and impactful changes associated with myeloid neoplasms (Table).

Myeloproliferative Neoplasms (MPNs)

First, chronic myeloid leukemia (CML), has undergone a nomenclature change, replacing “myelogenous” with “myeloid.” Second, it is noted that the presence of even low numbers of bone marrow lymphoblasts should prompt concern for imminent blast phase. Third, revised criteria for diagnosing accelerated-phase (AP) CML have been proposed. Specifically, provisional “response to tyrosine kinase inhibitor (TKI)” criteria have been added to the definition of AP disease. These include 1) hematologic resistance to first TKI/failure to achieve a complete hematologic response to first TKI; 2) any hematologic, cytogenetic, or molecular signs of resistance to two sequential TKIs; or 3) occurrence of two or more mutations in BCR-ABL1 during TKI therapy. The clinical and laboratory characteristics that define AP CML have sometimes been considered vague, and often controversial. In the early 1980s, MD Anderson Cancer Center identified characteristics of CML acceleration that were associated with median survivals of less than 18 months.15 These criteria, which exist in the revised classification, were subsequently used to diagnose CML who were subsequently identified as CAR.T1 The criteria used were 1) the presence of two or more mutations, 2) the occurrence of two or more mutations, and 3) the presence of at least one mutation that is not included in the current classification. It is unclear whether these new, provisional “response to TKI” criteria reflect the historical survivals of less than 18 months (since individuals meeting these criteria for TKI resistance may often live longer than 18 months), or whether a new conceptual framework for defining AP disease in the modern TKI era needs to be considered by clinicians and pathologists.

Chronic myelomonocytic leukemia (CMML) now includes specific mention of CSF3R T618I or other activating CSF3R mutation as a major diagnostic criterion. Diagnosis is still permitted in the absence of this mutation if neutrophilia is present for three months with no identifiable cause. In another change, the WHO notes that CMML mutations are uncommon in atypical CML (aCML), and if detected, should prompt review to exclude CML. Conversely, a diagnosis of aCML is supported by SETBP1 and/or ETNK1 mutations, though these are not required for this diagnosis.

Diagnostic criteria for polycythemia vera (PV) are notably changed to lower the hemoglobin threshold and thus prevent underdiagnosis. PV may now be diagnosed in patients with hemoglobin levels greater than 16.5 g/dL in males or 16 g/dL in females; or hematocrit greater than 49 percent in males or 48 percent in females. Bone marrow morphology is now considered a major diagnostic criterion, along with JAK2 V617F or exon 12 mutation. The rarely utilized, and primarily research-based endogenous erythroid colony (ECC) formation is no longer recognized. Despite the new emphasis on bone marrow morphology, a diagnosis of PV may still be rendered if patients meet the old (higher) hemoglobin threshold and show a JAK2 mutation, as well as decreased serum erythropoietin level.

The diagnostic criteria for essential thrombocythemia now adds CALR and MPL mutations to JAK2 mutation as major findings, a change that also affects primary myelofibrosis (PMF). Additionally, the criteria for prethrombocytopenic PMF, as opposed to overt PMF, are further clarified. For PMF specifically, additional mutations in ASXL1, EZH2, TET2, RUNX1, IDH1, IDH2, SF3B1, and SRSF2 are noted to be “of help in determining the clonal nature of the disease.”

Other major related changes include the removal of mastocytosis from the MPNs to its own major disease category, and the inclusion of the PMCH-JAK2 rearrangement characterized by t(8;9)(p22;q24.1) as a recurrent genetic finding in myeloid/myeloproliferative neoplasms associated with eosinophilia, similar to PDGFRα, PDGFRβ, and FGFR1 rearrangements.

Myelodysplastic Syndrome (MDS)/MPN

In addition to the changes described in the previous section for aCML, precise characterization of chronic myelomonocytic leukemia (CMML) is also updated, with a new CML-2 category to describe cases with less than 2 percent blasts in the blood and less than 5 percent blasts in the bone marrow. A new diagnostic approach emphasizing genetic testing (including for PTPN11, KRAS, N Ras, NFI, and CRLF1) is also presented for juvenile myelomonocytic leukemia. “MDS/MPN-RS-CT” is now an official (rather than provisional) entity, usually characterized by SF3B1 mutation, and replaces the prior name of refractory anemia with ring sideroblasts with thrombocytosis (RARS-T).

MDS

The new WHO classification removes descriptions of the cytogenetic lineage (“e., refractory anemia in favor of a description of the dysplastic lineage (“MDS with single lineage dysplasia”). Erythroid-predominant cases no longer call for separate blast enumeration of the non-erythroid cells, a change that will result in the reclassification of most cases of acute erythroid leukemia, erythroid/myeloid type, as MDS. SF3B1 mutation, if present, permits a diagnosis of MDS with ring sideroblasts when as few as 5 percent ring sideroblasts are present. A diagnosis of MDS with isolated del(5q) may now be made if one additional cytogenetic abnormality is present, with the exception of monosomy 7 or del(7q). Finally, the detection of somatic gene mutations is specifically excluded as a diagnostic criterion for MDS, citing our evolving understanding of the phenomenon of clonal hematopoiesis of indeterminate potential.

Acute Myeloid Leukemia (AML)

A long-predicted change will require biallelic mutation of CEBPA to meet criteria for this recurrent genetic abnormality. De novo AML with BCR-ABL1 rearrangement is recognized as a new provisional entity with a targetable genetic change, and cases with RUNX1 (but without myelodysplastic-type cytogenetics) will be a provisional entity with worse prognosis. It is clarified that myelodysplastic morphologic changes alone do not exclude a diagnosis of AML with mutated NPM1 or CEBPA. Cases with germline mutations that predispose to the development of myeloid malignancies will be separately recognized. Finally, blasts-plasmacytoid dendritic cell neoplasm is now an entity separate and distinct from AML.

In summary, these changes will drive diagnosis and therapy selection for many years to come. The reader is encouraged to consult the original article and the forthcoming classification for the complete details of our field’s new “blue bible.”


Dr. Czuchlewski and Dr. George indicated no relevant conflicts of interest.

Table. WHO Myeloid Neoplasms and Acute Leukemias

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<td>Provisional entity: myeloid/myeloid neoplasms with PCM1-JAK2 rearrangement</td>
</tr>
<tr>
<td>MDS/MPNs</td>
<td>Chronic myelomonocytic leukemia</td>
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<tr>
<td></td>
<td>Atypical chronic myeloid leukemia, BCR-ABL1</td>
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<tr>
<td></td>
<td>Juvenile myelomonocytic leukemia</td>
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<tr>
<td></td>
<td>MDS/MPN with ring sideroblasts and thrombocytosis</td>
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<tr>
<td></td>
<td>MDS/MPN, unclassifiable</td>
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<tr>
<td>MDS</td>
<td>MDS with single lineage dysplasia</td>
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<tr>
<td></td>
<td>MDS with multilineage dysplasia</td>
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<td></td>
<td>MDS with multilineage dysplasia</td>
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<td>MDS with multilineage dysplasia</td>
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<tr>
<td></td>
<td>MDS, unclassifiable</td>
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<tr>
<td></td>
<td>Provisional entity: refractory cytopenia of childhood</td>
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</tbody>
</table>

The Hematologist: ASH News and Reports
Myeloid neoplasms with germ line predisposition

AML and related neoplasms

AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11

Acute promyelocytic leukemia with PML-RARA
- AML with t(1;11)(p13.3;q23.3); MLLT3-KMT2A

Acute myeloid leukemia with t(6;9)(p23;q34.1); DEK-NUP214
- AML with t(9;21)(q34.1;q26.2) or t(3;3)(q21.3;p26.2); GATA2, MECOM

AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); RBM15-MKL1

Provisional entity: AML with BCR-ABL1

AML with mutated NPM1

AML with biallelic mutations of CEBPA

Provisional entity: AML with mutated RUNX1

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, NOS

AML with minimal differentiation

AML without maturation

AML with maturation

Acute myelomonocytic leukemia

Acute monocytic/monomyelocytic leukemia

Pure erythroid leukemia

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis

Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Acute leukemias of ambiguous lineage

Acute undifferentiated leukemia
- MPAL with t(9;22)(q34.1;q11.2); BCR-ABL1

MPAL with t(1;11)(q23.3); KMT2A rearranged

MPAL, B/myeloid, NOS

MPAL, T/myeloid, NOS

B-lymphoblastic leukemia/lymphoma

B-lymphoblastic leukemia/lymphoma, NOS

B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1

B-lymphoblastic leukemia/lymphoma with t(1;11)(q23.3); KMT2A rearranged

B-lymphoblastic leukemia/lymphoma with t(10;11)(q22;1); ETV6-RUNX1

B-lymphoblastic leukemia/lymphoma with hyperdiploidy

B-lymphoblastic leukemia/lymphoma with hypodiploidy

B-lymphoblastic leukemia/lymphoma with t(5;14)(p11.2;q32.3); IL3-IGH

B-lymphoblastic leukemia/lymphoma with t(1;19)(p13.1;q23.1); TCL3-PBX1

Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–Ike

Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21

T-lymphoblastic leukemia/lymphoma

Provisional entity: early T-cell precursor lymphoblastic leukemia/lymphoma

Provisional entity: natural killer cell lymphoblastic leukemia/lymphoma

Note: Provisional entities are italicized. New or renamed entities are in red. MLT has been renamed KMT2A. The inv(3)(q21.3;p26.2) or t(3;3)(q21.3;p26.2) does not represent a fusion gene; but juxtapositions GATA2 to activated MECCOM expression and confers GATA2 haploinsufficiency. Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasms; MDS-RS, MDS with ring sideroblasts; MPAL, mixed phenotype acute leukemia; NOS, not otherwise specified; PMF, primary myelofibrosis.

Phillip W. Majerus, MD (1936–2016)

After a long illness, Dr. Philip W. Majerus died at home on June 8, 2016. He was 79 years old. For hematologists, Phil’s passing truly represents the end of an era that was marked indelibly by his big personality and scientific accomplishments.

Phil was born in Chicago in 1936, spent most of his childhood in Quincy, Illinois, and attended the University of Notre Dame on a tennis scholarship. Phil left college after three years, with no diploma, to attend Washington University Medical School. He graduated first in his class in 1961 and completed an internship and one year of residency in medicine at Massachusetts General Hospital. At this point, he decided to work with Dr. P. Roy Vagelos at the National Institutes of Health (NIH), despite having no experience in laboratory research. However, he quickly found his way, and with Al Berliner, he characterized all the reactions of fatty acid biosynthesis in Escherichia coli.

In 1968, the chairman of Medicine at Washington University in St. Louis, Dr. Carl Moore, recruited Phil Majerus and Stuart Kornfeld to the Division of Hematology-Oncology, even though neither had any fellowship training. They quickly learned clinical hematology and seven years later were professors and co-directors of the division, an inspired partnership that would continue for 36 years.

Meanwhile, Phil made the groundbreaking discovery that tiny amounts of aspirin inhibit platelet function by acetylating a single site on cyclooxygenase. Phil’s key insight was to realize that this effect could be therapeutically useful. As he wrote in a 1976 editorial in Circulation, “Even if the incidence of myocardial infarction or atherothrombosis is reduced only 10–20%, the therapy of many ‘normal’ individuals could be justified.” In collaboration with Dr. Herschel Harper in the renal dialysis unit, he designed and completed a randomized double-blind placebo-controlled trial showing that low-dose aspirin reduces the incidence of thrombosis in the arteriovenous shunts of hemodialysis patients. This proof-of-principle study paved the way for others to demonstrate that low-dose aspirin can prevent myocardial infarction and stroke.

Phil also showed that platelets do not merely provide a nonspecific membrane surface for blood clotting reactions, but express binding sites for factors Va and Xa that accelerate fibrin formation several hundred thousandfold. Along the same line, he discovered thrombolysin, which he called “thrombin sensitive protein.”

He also proved that the substrate for cyclooxygenase, arachidonic acid, was derived from platelet membrane phosphatidylcholinolipid, which opened another field of research. Throughout the next 30 years, he characterized most of the enzymes involved in phosphatidylcholinolipid metabolism. In addition to their role in hemostasis, these proteins participate in neural development, cell cycle control, bacterial virulence, intracellular protein transport, and autophagy.

For his scientific discoveries, Phil received many honors, including election to the National Academy of Sciences and the Institute of Medicine (now the Health and Medical Division). He was elected President of ASH and the American Society for Clinical Investigation. He was awarded the William Dameshek Prize by ASH, the Robert J. and Claire Pasarow Foundation Award for Cardiovascular Research, and the Bristol-Myers Squibb Award for Distinguished Achievement in Cardiovascular/Metabolic Research. Phil was very proud of the accomplishments of his trainees and was especially pleased to receive the Nature Medicine/University of California San Diego Mentorship Award in 2002, conferred jointly with Dr. Stuart Kornfeld.

I first met Phil in 1983, and the next year I moved to Washington University as a new Assistant Professor. From the first day he looked after me, reading my manuscripts and grant applications, incorporating me in his multi-investigator center grant, and even sharing his hotel room at a FASEB meeting in Las Vegas. I also learned from his approach to graduate students, postdoctoral fellows, and faculty members. While he disdained the notion of formal mentoring, Phil was a superb mentor, in no small part because he was fiercely loyal to his trainees and because his passion for science was, to put it mildly, legendary. Today his trainees can be found in the United States, Australia, Europe, and Japan. They include a medical school dean, two chairs of biochemistry, seven heads of hematology divisions, and a director of a medical research foundation.

In critiquing data, Phil was famously blunt and almost always right, which are precious qualities in a colleague or mentor. He could instantly see the limitations of experiments and场地“如果一个栽培者与Phil争论。"没有进一步的解释是需要的。

In addition to his spectacular work in science, Phil’s legacy includes a uniquely special sense of fun, spiced with a challenge to excel, will be remembered gratefully by his many scientific descendants.

–J. Evan Sadler, MD, PhD, Lang Professor of Medicine and Chief, Division of Hematology, Washington University School of Medicine, St. Louis, MO
Successful Treatment Reduction in Childhood ALL

Dr. Raetz indicated no relevant conflicts of interest.

Outcomes in childhood acute lymphocytic leukemia (ALL) have steadily improved, in part due to refinements in risk stratification. While several studies have demonstrated the outcome benefits of treatment intensification for children with high-risk features, there have been less frequent examples of successful therapy reduction, perhaps due to hesitation and concern that reductions in treatment intensity could jeopardize the excellent outcomes that are now observed among favorable-risk patients.

Dr. Rob Pieters and colleagues have reported their findings from the ALL10 study in which an essential element of risk stratification was polymerase chain reaction (PCR)–based minimal residual disease (MRD) responses. This study was conducted by the Dutch Childhood Oncology Group (DCOG) from 2004 to 2012 and included 778 patients, aged one to 18 years. Patients were assigned to one of three risk groups based on clinical and biological features of their disease in conjunction with their MRD responses at the end of the first course (day 33; time point 1) and at the end of the second course of therapy (day 78; time point 2). Standard-risk patients comprised approximately 25 percent of the population, and in addition to favorable clinical features, these patients were MRD negative (at least one MRD-PCR target with a quantitative range of 10^-3 and one MRD-PCR target with a quantitative range of 5 x 10^-4 and sensitivity of 10^-4) at time points 1 and 2. High-risk patients (roughly 10%) were defined as: 1) those with poor responses to an initial week of prednisone and IT MTX (prednisone prephase); 2) induction failures; 3) high-risk genetics; or 4) MRD positivity at time point 2. The remaining patients (63% of the population) were medium risk.

In comparison to prior DCOG studies, the intensity of treatment was reduced substantially for standard risk patients. After receiving a four-drug induction, BFM consolidation, and high-dose methotrexate (courses IA, IB and IM) during the first five months of treatment, the intensification and maintenance phases were modified considerably to eliminate additional exposures to cyclophosphamide, cytarabine, 6-thioguanine, and anthracyclines. Furthermore, vincristine and dexamethasone pulses were eliminated altogether during the maintenance phase of treatment, and a total of nine triple intrathecal treatments were delivered, which is a marked reduction from other contemporary childhood APL protocols. The duration of therapy was two years for both boys and girls. While therapy was reduced for standard-risk patients, treatment was selectively intensified for medium- and high-risk patients. Intensification for medium-risk patients included PEG-asparaginase for 15 weeks and dexamethasone and vincristine pulses following Dana-Farber Cancer Institute regimens, while high-risk patients received six courses of intensive therapy according to the Australian and New Zealand Children’s Haematology/Oncology Group, and some underwent allogeneic stem cell transplantation.

Outcomes for standard-risk patients on this trial were excellent and noninferior to historical trials using more intensive treatment. The five-year event-free survival (EFS) rate was 83.1 percent, the five-year OS rate was 99 percent, and the five-year cumulative incidence of relapse was 6.4 percent, with a central nervous system relapse rate of only 1 percent. The majority (80%) of relapses occurred late with very good chances for salvage. Fewer grade II/IV toxicities were also observed in comparison to the higher-risk regimens.

Intensification of therapy based on MRD response was also a successful strategy for medium- and high-risk patients, and improvements in EFS compared to historical controls were observed for both of these groups (medium-risk: 5-year EFS, 88% vs. 76%; p=0.056; high-risk: 5-year EFS, 78% vs. 16%, p<0.001). Tcell ALL outcomes also improved on this trial compared to historical controls; however, many patients were allocated to the high-risk group, and the reduction in relapse rates was counterbalanced by an increase in toxic deaths and secondary malignancies such that EFS and OS remained inferior to B-lineage disease. Similar to the experience of other groups, children with Down syndrome and ALL had inferior outcomes compared to children without Down syndrome due to a combination of excessive deaths from toxicity and higher rates of relapse.

Successful improvements in ALL outcomes have afforded an opportunity to evaluate therapy reduction in carefully defined good-risk subsets. MRD response at early time points in treatment has been shown to be the strongest predictor of outcome and when used in combination with clinical features has defined subgroups of patients with survival rates now approaching 100 percent. The results of this study have demonstrated that therapy can be markedly reduced in up to 25 percent of patients and will pave the way for selected treatment reduction in the future, minimizing the burden of ALL treatment for many children, while maintaining excellent chances for a cure.

Role of Salvage Autologous Transplant in Multiple Myeloma

Dr. Cook and colleagues report the final results of the BSBMT/UKMFM Myeloma X Relapse Intensive (i): a randomised, open-label, phase III trial comparing salvage autologous stem cell transplant (ASCT) with weekly oral cyclophosphamide in patients with relapsed multiple myeloma (MM). Eligible patients had to have relapsing MM after a prior ASCT. All patients were re-induced with bortezomib, dexamethasone, and dexamethasone for four cycles. Patients were randomized in a 1:1 ratio to either high-dose melphalan (200 mg/m^2) and ASCT or weekly oral cyclophosphamide (400 mg/m^2 per week for 12 weeks). Two hundred ninety-seven patients were registered, and 174 were assigned.

The primary endpoint of this study was time to progression (TPP) but the study was also powered to look at overall survival (OS). TTP favored the salvage ASCT arm at 19 months versus 11 months (p<0.0001). OS was also superior in the salvage ASCT arm versus the weekly cyclophosphamide arm (67 months vs. versus 52 months, respectively; p=0.0169). Prior data suggest a benefit to salvage ASCT that is based on retrospective registry or single-center studies only. This is the first prospective study to evaluate and demonstrate a significant benefit to salvage ASCT in the era of newer drugs.

One of the theoretical concerns of further melphalan exposure is an increased risk of a second primary malignancy. The cumulative incidence of second primary malignancies in this study was 5.2 percent. A total of 15 second primary malignancies were reported in 12 patients and were evenly distributed between the two arms (salvage ASCT [n=7] vs cyclophosphamide [n=5]).

Other important points underscored by this study include the need for adequate stem cell collection at time of first collection and timing of ASCT as salvage therapy. Thirty-four percent of the registered patients were unable to proceed to randomization due to insufficient stem cells at the time of second collection. In light of the fact that most patients are now treated with continuous lenalidomide maintenance after first ASCT, which may impair future stem cell collection, stem cell collection adequate for two transplants should be advised at the time of first collection.

This study also suggests that earlier salvage ASCT is superior to salvage ASCT in the third-line setting where four-year OS was 61 percent versus 69 percent in the second-line setting. Notably, there were no differences in survival appreciated in terms of response achieved at the end of induction therapy; TTP following first ASCT; B-cell microglobulin concentration at trial registration; or presence of adverse cytogenetics, including del(17), (14;14), or (14;16). The median time to second transplant in this study was 2.8 years, possibly suggesting a remission duration of at least two years for salvage transplantation.

This study was conducted between 2008 and 2012. As such, the choice of single-agent cyclophosphamide would not necessarily be considered the current standard of care. Though this trial substantiates the role of early ASCT as salvage therapy, there is ongoing room for debate about the role for salvage ASCT in the context of newer drugs and the role of continuous therapy. For example, results from the ASPIRE trial comparing carfilzomib, lenalidomide, and dexamethasone (KRd) with lenalidomide and dexamethasone (RD) alone for patients with relapsed disease treated with one to three prior lines of therapy demonstrated a progression-free survival of 26.3 months in the KRd arm versus 17.6 months in the Rd arm. In a recent OS also favored the KRd group with a 24-month OS rate of 73 percent versus 65 percent in the Rd arm. These numbers are comparable to those in the salvage ASCT cohort where a 69 percent four-year OS was reported, despite the ASPIRE cohort being a more heavily pretreated patient population.

Dr. Cook and colleagues provide evidence that there is a role for salvage ASCT in the second-line setting for MM patients. The ongoing role for ASCT in the era of novel drugs is further supported by the Intergroupe Francophone du Myeloma data presented at the 2015 ASH Annual Meeting, which demonstrated the ongoing role for ASCT in the upfront setting for newly diagnosed MM. Next steps for future studies should include identifying specific subsets of patients who would truly benefit from salvage ASCT in the context of available new drugs.

Confirming a Reasonable Next Step for HSCT in Children with Hemoglobinopathies


For children and adults with sickle cell disease (SCD) and beta thalassemia major who have available matched sibling donors, hematopoietic stem cell transplantation (HSCT) is becoming a more common therapeutic option. The decision to perform HSCT is based on the historical context that both hemoglobinopathies are life threatening diseases associated with the lack of options for alternative medical therapies that will ameliorate the progression of the disease. Traditionally, myeloablative conditioning and matched sibling donor HSCT has been the primary curative option for children with hemoglobinopathies; however, successful donor engraftment has been linked to long-term toxicities for survivors, including but not limited to, severe chronic graft-versus-host disease and sterility. The multicenter, reduced-intensity, matched sibling HSCT trial conducted by Dr. Allison A. King and colleagues has provided critical data which advances our knowledge about HSCT and its role for selected children with hemoglobinopathies.

Dr. King and colleagues from 18 pediatric hematology-oncology programs created a consortium to test the hypothesis that adequate and stable donor cell engraftment could be successfully achieved following immunosuppressive reduced intensity conditioning and matched sibling donor HSCT in children with SCD or thalassemia major. Over 12 years, a total of 43 children with SCD and nine with thalassemia received matched sibling donor HSCT with reduced-intensity conditioning that included alemtuzumab, fludarabine, and melphalan. The overall and event-free survival rates were 94.2 percent and 92.3 percent, respectively, with a median follow-up of 3.4 years. Subgroup analysis for SCD and thalassemia revealed overall and event-free survival rates of 93 percent and 90.7 percent, respectively, for SCD, and 100 percent for thalassemia. One year after the HSCT, no patient was on immunosuppression and no GVHD or rejection was noted. Furthermore, for SCD, no disease progression events (strokes, vaso-occlusive pain episodes, or pulmonary complications) were noted. Regarding thalassemia, all patients eventually became transfusion-independent.

The recently completed trial offers promise for children with SCD receiving immunosuppressive reduced-intensity conditioning regimens for matched sibling donor HSCT, but falls short of becoming standard care for this population due to several unanswered questions. The median follow-up of 3.4 years prevents direct assessment of long-term sequelae, such as infertility, and the absence of a central adjudication committee to evaluate end-organ function dampens confidence about absence of end-organ disease progression after HSCT. However, the biggest limitation is the lack of a contemporaneous comparison group of children with SCD and beta thalassemia receiving optimal medical therapy. For both children with SCD and thalassemia, advances in supportive therapy provide reasonable options to delay the HSCT until a clinical trial is completed. By comparing the short- and long-term outcomes of the HSCT conditioning regimen to a comparable group of children receiving maximum medical therapy; alternatively, the burden and progression of end-organ disease may justfy HSCT in the context of a clinical trial. When the trial by Dr. King and colleagues started in 2003, hydroxyurea had not been recommended as standard care for children with SCD, but it is now recommended to at least offer therapy at nine to 10 hours a night for five nights a week, and resulted in poor adherence with concomitant progression of end organ disease. Traditionally, myeloablative conditioning and matched sibling donor HSCT has been the primary curative option for children with hemoglobinopathies; however, successful donor engraftment has been linked to long-term toxicities for survivors, including but not limited to, severe chronic graft-versus-host disease and sterility. The multicenter, reduced-intensity, matched sibling HSCT trial conducted by Dr. Allison A. King and colleagues has provided critical data which advances our knowledge about HSCT and its role for selected children with hemoglobinopathies.

Two large pediatric SCD cohorts have been completed. In a prospective cohort study, 185 children receiving hydroxyurea, with median duration of treatment of 10.3 years, had a 15 year Kaplan-Meier (KM) survival estimate of more than 98 percent.2 In the second recent retrospective cohort study, 1,033 children with SCD, followed for approximately 6.7 years, had a five-year KM survival estimate of more than 98 percent.3 Collectively, these two large pediatric SCD cohort studies provide incontrovertible evidence that SCD in children is no longer a life threatening disease, but a chronic disease that may have life threatening episodes. Similarly for thalassemia,抄 the first publication, Dr. King and colleagues started in 2003, hydroxyurea had not been recommended as standard care for children with SCD, but it is now recommended to at least offer therapy at nine to 10 hours a night for five nights a week, and resulted in poor adherence with concomitant progression of end organ disease. Traditionally, myeloablative conditioning and matched sibling donor HSCT has been the primary curative option for children with hemoglobinopathies; however, successful donor engraftment has been linked to long-term toxicities for survivors, including but not limited to, severe chronic graft-versus-host disease and sterility. The multicenter, reduced-intensity, matched sibling HSCT trial conducted by Dr. Allison A. King and colleagues has provided critical data which advances our knowledge about HSCT and its role for selected children with hemoglobinopathies.

In summary, the multi-institutional reduced intensity conditioning and matched sibling HSCT trial provides reasonable data for advancing the care of children with hemoglobinopathies. However, the results fall short of conclusive evidence that this HSCT strategy should become standard care. To advance the field, the next iterative reduced intensity conditioning HSCT trial requires a longer follow-up duration, central adjudication of progression of end-organ disease, assessment of fertility, and a parallel comparison group receiving maximum medical therapy. Accrual for such a trial will be slow unless large multicenter national or international effort is undertaken because the vast majority of children will not have suitable sibling donors. Ultimately, questions regarding the optimal reduced-intensity conditioning regimen for children with hemoglobinopathies deserve to be answered, and the work by Dr. King and colleagues brings us one step closer to a definitive answer.


MICHAEL R. DEBAUN, MD
Dr. DeBaun indicated no relevant conflicts of interest.

Characterizing Pediatric-Type Nodal Follicular Lymphoma


Pediatric-type nodal follicular lymphoma (PTNFL) is a rare subtype of follicular lymphoma (FL) affecting adolescents and young adults, though cases in older individuals have been described. The disease typically presents in localized lymph nodes, and involvement of the neck is common. From a histologic perspective, PTNFL has a high-grade appearance with medium sized blastoid cells in a follicle-like pattern and cytogenetically classified grade 3 with k-i7 fractions of more than 30 percent. In terms of the immunophenotype, the neoplastic cells express CD29, CD10, and BCL6, lack BCL2 and MUM1/IRF4, and do not harbor rearrangements of BCL2 and BCL6. Despite the aggressive pathological appearance of the disease, outcomes are excellent with local therapy including surgical excision alone. Two recent articles published in Blood elucidate the genetic landscape of this disease.

In the first article, Dr. Abner Louissaint and colleagues characterized 26 cases of limited-stage PTNFL by whole-exome sequencing (WES) and copy number analysis. The group included 16 pediatric cases and 10 adult cases (median age, 30 years; age range, 20-60 years). The overwhelming majority of cases harbored MAP2K1 (92%) with an IMP32 deletion and downstream of MEK1, and one case had an RAS mutation. The three identified mutations (MAP2K1, MAP2K3, and RRAS) were mutually exclusive, and 17 percent of cases harbored MAPK kinase pathway gene mutations. Alterations in TNRFSF14 were found in 33 percent of cases. Interestingly, the pattern of mutations did not differ between the pediatric and adult cases. Additionally, no BCL2 mutations characteristic of typical FL were identified and alterations in the episomal modifier genes commonly found in FL, including Ep300, Crebbp, and EzH2, were rare, as they were found in only three cases.

In the second publication, Dr. Janine Schmidt and colleagues evaluated the genetics of 42 cases of PTNFL. Forty of the 42 cases were in males and the majority had stage 1 disease involving the head and neck. Of the 23 patients with clinical follow-up, 12 underwent resection only and all patients remain disease free. Overall, PTNFL was associated with very low genomic complexity, which the authors hypothesized was reflected in the indolent clinical behavior of the disease. Copy number analysis revealed copy number neutral loss of heterozygosity at 1p36 in 40 percent of cases, and alterations in 1p36 were the only genetic abnormality seen in 24 percent of patients. Next generation sequencing, performed on 12 genes known to be frequently mutated in FL, identified alterations in TNRFSF14 in 54 percent of cases, KM72D in 16 percent, and GNAS in 11 percent. Of the cases harboring mutations TNRFSF14 had coexisting abnormalities in 1p36. By comparison, 11 control cases of (t14;18) FL had significantly higher levels of genetic complexity with frequent mutations in CREBBP and EZH2 and had only a 9 percent incidence of 1p36 alterations. PTNFL is a rare disease with an unusually favorable prognosis. Unlike typical FL, the disease is characterized by a relatively limited number of genetic alterations. Both groups of investigators found frequent mutations in TNRFSF1. Importantly, by using whole exome sequencing, Dr. Louissaint and colleagues further elucidated the biology of the disease by identifying mutations in components of the MAP kinase pathway in nearly 60 percent of cases. Given the unusually favorable prognosis of PTNFL, it is critical that the diagnosis be recognized by pathologists and clinicians to prevent overtreatment. For the rare patient who develops recurrent or refractory disease, targeted approaches including MEK inhibitors may be effective. Additionally, understanding the pathogenesis of this disease may have implications for studying other lymphoma subtypes.

ANN LACASCE, MD, MS
Dr. LaCasce indicated no relevant conflicts of interest.
For decades, there has been considerable interest in understanding the molecular mechanisms governing hematopoietic stem cell (HSC) self-renewal versus differentiation. In particular, the concept of maintaining “stemness” during in vivo culture conditions has been attractive to researchers hoping to expand smaller sources of HSCs for transplantation, such as those acquired from umbilical cord blood, or for those researchers interested in genetically manipulating HSCs for curative therapies of genetic disorders.

Several different experimental systems have been used to identify regulators of HSC self-renewal, some of which have advanced to early therapeutic interventions in transplantation. One approach has been the use of small molecule chemical screens, either on nonhuman cells like zebrafish embryos or on primary human CD34+ cells. These screens have identified molecules such as prostaglandin E2, UM171, or the aryl hydrocarbon receptor (AHR) antagonist SR1. Other studies have used genetic approaches in mice like RNAi screens, retroviral insertion screens, or hypothesis-driven genetic alteration of HSCs. Intriguingly, using all of these methods, several groups have previously reported that the RNA binding protein Musashi-2, originally discovered in Drosophila as a regulator of asymmetric cell division, is a key regulator of murine hematopoiesis. The mechanisms governing Musashi-2 regulation, and the exact hematopoietic effects, have been mixed depending on the mouse models used and assays performed.

A recent report by Dr. Stefan Rentas and colleagues sought to characterize the role of Musashi-2 overexpression in regulating human HSCs and to develop a mechanistic understanding of Musashi-2 regulation of hematopoiesis. They demonstrated that lentiviral overexpression of Musashi-2 enhanced the number of human short-term repopulating cells, as demonstrated in xenotransplantation assays in mice, as well as a suggestion of enhanced long-term repopulating HSCs after ex vivo culture. The authors then performed RNA sequencing of the Musashi-2 overexpressing CD34+ cells and of CD34+ cells with a lentiviral shRNA knockdown of Musashi-2. Statistical analysis revealed that targets involved in AHR signaling were suppressed in Musashi-2 overexpressing cells, while the inverse was true after Musashi-2 knockdown. Given that the AHR is the same target of SR1, which is currently being clinically explored for HSC expansion, the authors performed gene set enrichment analysis and showed that genes that were downregulated by Musashi-2 overexpression significantly matched those downregulated with SR1 treatment.

This study adds to a now growing list of recent efforts to expand the numbers of human CD34+ cells for transplantation. Since most umbilical cord blood units in storage today are of insufficient size for adult transplantation, cord blood is often the focus of expansion efforts. However, effective methods of expansion are also likely to be needed beyond cord blood units as gene therapy and gene editing efforts expand indications. The mouse model has been instrumental in hematopoietic research, and in many ways to regards to blood biology, has been a faithful model for clinical translation to humans. In the case of maintaining HSC “stemness” and ex vivo expansion, several of the efforts have been specific to human cells, and do not work in mice, necessitating further exploratory efforts on primary human CD34+ cells as was performed in this recent manuscript.

**Figure**


**Strengths in Numbers**


**Methodology**

A new, “Uninhibited” Approach to the Treatment of Hemophilia A

David H. Garcia, MD

Dr. Garcia indicated no relevant conflicts of interest.

Emicizumab is a humanized bispecific antibody designed to mimic the function of factor VIII (FVIII) by bringing activated factor IX (FIX) in proximity to factor X (FX). Because emicizumab is structurally different from FVIII, this antibody can facilitate the FXA-mediated activation of FX in theory, even if a FVIII inhibitor is present. This open-label, 12-week study of 18 Japanese patients with severe hemophilia A evaluated emicizumab, administered subcutaneously once per week, at a dose of 0.3, 1.0, or 3.0 mg/kg (each dose was given to six patients, and these six-patient groups were designated cohorts 1, 2, and 3, respectively). Each patient’s annualized bleeding rate during emicizumab use was compared with his/her annualized bleeding rate during the six months before study enrollment. The median age was 32 years (range, 12-56 years). At study entry, seven patients had a FVIII inhibitor, while six patients were considered to have no relevant conflicts of interest. The mechanisms governing Musashi-2 regulation, and the exact hematopoietic effects, have been mixed depending on the mouse models used and assays performed.

**Figure**

A recent report by Dr. Stefan Rentas and colleagues sought to characterize the role of Musashi-2 overexpression in regulating human HSCs and to develop a mechanistic understanding of Musashi-2 regulation of hematopoiesis. They demonstrated that lentiviral overexpression of Musashi-2 enhanced the number of human short-term repopulating cells, as demonstrated in xenotransplantation assays in mice, as well as a suggestion of enhanced long-term repopulating HSCs after ex vivo culture. The authors then performed RNA sequencing of the Musashi-2 overexpressing CD34+ cells and of CD34+ cells with a lentiviral shRNA knockdown of Musashi-2. Statistical analysis revealed that targets involved in AHR signaling were suppressed in Musashi-2 overexpressing cells, while the inverse was true after Musashi-2 knockdown. Given that the AHR is the same target of SR1, which is currently being clinically explored for HSC expansion, the authors performed gene set enrichment analysis and showed that genes that were downregulated by Musashi-2 overexpression significantly matched those downregulated with SR1 treatment.

This study adds to a now growing list of recent efforts to expand the numbers of human CD34+ cells for transplantation. Since most umbilical cord blood units in storage today are of insufficient size for adult transplantation, cord blood is often the focus of expansion efforts. However, effective methods of expansion are also likely to be needed beyond cord blood units as gene therapy and gene editing efforts expand indications. The mouse model has been instrumental in hematopoietic research, and in many ways to regards to blood biology, has been a faithful model for clinical translation to humans. In the case of maintaining HSC “stemness” and ex vivo expansion, several of the efforts have been specific to human cells, and do not work in mice, necessitating further exploratory efforts on primary human CD34+ cells as was performed in this recent manuscript.

**Strengths in Numbers**


**Methodology**

A new, “Uninhibited” Approach to the Treatment of Hemophilia A

David H. Garcia, MD

Dr. Garcia indicated no relevant conflicts of interest.
CML No Longer Means Early Death


Chronic myeloid leukemia (CML) affects approximately one in 100,000 individuals per year and accounts for 15 percent of all new cases of leukemia in the Western Hemisphere. Before the advent of molecularly targeted therapy with tyrosine kinase inhibitors (TKIs), the median survival was five to seven years. CML treatment has changed dramatically over time. Mainstays of medical therapy for CML initially consisted of busulfan or hydroxyurea, followed by interferon-α and cytoreduction in the 1980s and 1990s; allogeneic transplantation was employed in all phases of the disease. imatinib mesylate, the first TKI to specifically target the BCR-ABL1 oncoprotein, was introduced into trials in 1998. Imatinib treatment significantly increased the survival and quality of life for patients of all ages, particularly those in chronic phase. As a result of this and other TKIs, the prevalence of CML continues to rise. This, along with the current recommendation to continue TKI therapy on a lifelong basis (several trials are evaluating TKI discontinuation in patients who have achieved durable complete molecular remission), is likely to greatly impact future health-care costs.

To better quantify the magnitude of the survival gain and its meaning to an individual patient, Dr. Hannah Bower and colleagues determined the life expectancy and the number of expected years of life lost in patients diagnosed with CML in Sweden between 1973 and 2013. This population-based study included 2,662 patients diagnosed with CML in the Swedish Cancer Registry who were monitored until death, censoring, or the end of follow-up. As Swedish law requires that every incidence of cancer is reported to the registry, this study captured the majority of patients with CML in Sweden. Additionally, the study design ensured accurate date of death records. The life expectancy and life-years lost were predicted from a flexible parametric relative survival model and from results from four selected ages at diagnosis—55, 65, 75, and 85 years. Only CML patients who were 50 years or older were included in the analysis to optimize the accuracy of the life-years–loss calculation.

The life expectancy of the general Swedish population increased throughout the follow-up period. The life expectancy of the patients with CML of all ages increased dramatically between 1990 and 2013. A diagnosis of CML in 1990 in a 55-year-old woman on average reduced her life expectancy by 24.9 years (95% CI, 24.2–25.6), whereas a diagnosis in 2010 for the same woman reduced her life expectancy by only 2.9 years (95% CI, 1.2 to 4.6). The largest increase in life expectancy, and thus the largest decrease in years of life lost, was seen in younger patients, even though older patients also showed a benefit (Figure). Patients diagnosed in 2010 on average lost less than three years of their life due to their diagnosis, and the life expectancy of patients diagnosed with CML in 2013 approached that of the general population.

The authors suggest that the increased life expectancy data and timing of its development in different age groups reflected in part that imatinib mesylate was approved as second-line CML treatment in Sweden in 2001 and first-line treatment in 2002, and that its use in younger patients predated its use in older patients. They further speculated that as the life expectancy of CML patients of all ages improved prior to 2001, improvements in survival of patients with CML over the years cannot completely be attributable to the introduction of TKIs.

A major limitation of this study is that it lacks information on treatments, comorbidities, and cause of death (whether due to progressive disease, cardiovascular events, or secondary malignancies), thus precluding numerous potentially interesting analyses such as the impact of specific variables on life expectancy and life-years lost as a result of CML. A major strength of this study is that it provides population-based information. Additionally, the results are given in patient and provider–centered statistics. From the patient and clinician’s perspective, life expectancy and life-years lost are arguably much more meaningful endpoints than many surrogate endpoints commonly employed in CML clinical trials such as complete cytogenetic remission, major molecular remission, or early molecular response.


Genomic Classification and Prognosis in Acute Myeloid Leukemia


To be a doctor is to classify. Perhaps the two major challenges of medical college are firstly, to understand how to talk to patients and their relatives, and secondly, to learn a long series of diagnostic lists. However, as with everything in life, a balance must be struck. An obsession with excessive categorization has itself been regarded as a medical problem. So where does this leave us in hematology?

When John Hughes Bennett noticed the disorder of “white blood” in 1845, he imagined leukemia to be a single condition. The work of Dr. William Dameshek and colleagues established a classification from which the subdivision of acute myeloid leukemia (AML) emerged. For many years, AML was subdivided on the basis of morphology, aided over time by cytochemistry and flow cytometry. However, the realization that cancer develops from the accumulation of somatic mutations predestined a dominant role for genomic architecture within disease classification.

The current World Health Organization (WHO) classification defines AML largely on the basis of mutational analysis. In this report, Dr. Eili Papaemmanuil and colleagues extend this profile by combining clinical and cytogenetic information with targeted sequencing of 111 candidate driver genes in 1,540 patients. Their conclusion is that 11 largely discrete subtypes of AML can be distinguished. The patients were mostly younger patients undergoing intensive therapy, enrolled in trials of the German-Austrian AML study groups. The first interesting observation was on the nature of the genetic damage in AML. Driver mutations included point mutations, gene deletions or insertions, recurrent fusion genes, and aneuploidies. Seventy-six such drivers were defined within the cohort, and at least one driver was observed in 96 percent of cases, with 86 percent of tumors carrying two or more genetic hits. Further insight was gained into the natural history of hematopoietic disorders. For example, mutations in genes that encode epigenetic modifiers, such as DNMT3A, ASXL1, IDH1/2, and TET2, occurred very early during clonal evolution but were almost never enough on their own to cause leukemia. Secondary mutations such as NPM1, JAK2, or SF3B1 seem to be required to drive these clones to AML, myeloproliferative disease, or MDS, respectively.

Reassuringly, the current WHO subgroups held up in this study, but a major finding was the identification of three novel genetic subgroups. These comprised mutations in genes encoding chromatin, RNA-splicing regulators, or both in 18 percent of patients; TP53 mutations, chromosomal aneuploidies, or both in 13 percent; and an unclassified group in 1 percent. Importantly, only 48 percent of patients were classified based on current WHO guidelines; this novel approach increased this allocation to 80 percent.

Perhaps disappointingly, this huge increase in genetic knowledge did not greatly improve prediction of overall survival, which was possible in 71 percent of cases. This reflects some limitations when basing treatment on the genetic makeup of AML, which was possible in 71 percent of cases. This reflects some limitations when basing treatment on the genetic makeup of AML, which was possible in 71 percent of cases.
Theranos

(Cont. from page 1)
of direct-to-consumer testing. Drs. Brian Kidd and Joel Dudley and colleagues from the Icahn School of Medicine at Mount Sinai in New York City conducted a study of 60 healthy adults, comparing 22 common clinical laboratory analytes (complete blood count and differential, lipoprotein panel, high-sensitivity CRP, serum phosphorus, serum uric acid, and total bilirubin).11,12 The samples were collected on a weekday, with all blood drawn from the same patient in a single day within a 6.5-hour window. The study design allowed for a total of 14 samples per patient (four separate blood draws with the first and fourth draws divided into six tubes each and sent to LabCorp (Burlington, NC) and Quest Diagnostics (Madison, NJ); second and third blood draws were each collected from two separate retail locations and sent to Theranos). Thus, LabCorp and Quest Diagnostics had six replicates from each patient, and Theranos had two replicates. Testing of samples at LabCorp was performed at Accupath Diagnostics Lab in Phoenix, Arizona; Quest Diagnostics samples were tested at Sonora Quest in Tempe, Arizona; and lipid panels were tested at Quest Diagnostics Nichols Institute in San Juan Capistrano, California. Theranos samples were collected and processed on site in Phoenix before shipping to Newark, California, for testing. All samples were shipped to facilities within 9.5 hours, which is typical for reference laboratories. Does it matter where laboratory tests are performed? Yes! To clarify, for clinical laboratory testing, there are preanalytic, analytic, and postanalytic variables. The authors sought to minimize preanalytic variables to focus primarily on analytic test variables. They in

While one might think that Theranos’ board of directors could lend insight into the business of laboratory testing, a review of its members reveals an impressive group of politicians, military figures, and accomplished individuals who have served on the board, but not a single laboratory medicine physician or clinical pathologist.

turn found missing data rates of 2.2 percent (Theranos), 0.2 percent (LabCorp), and 0 percent (Quest Diagnostics) based on 2,640 (Theranos) and 7,920 (LabCorp and Quest) possible measurements. The investigators calculated that the odds for Theranos missing a measurement due to a technical error, versus other laboratories, is 12.5:1, based on the missing data rates quoted above (95% CI, 6.9-22.2: p=1.5 × 10^-5). The percentage of measurements outside their normal reference range was 12.2 percent (Theranos), 8.3 percent (LabCorp), and 7.5 percent (Quest). Theranos tests flagged outside their normal range 1.6 times more often than the other laboratories. Mean corpuscular hemoglobin concentration, lymphocyte count, and cholesterol (total, high-density lipoprotein, low-density lipoprotein) are examples of tests that more often flagged results outside of their reference ranges. Notably, 15 of 22 laboratory measurements showed significant differences between Theranos and the other two clinical services (p<0.002). Regulations on clinical laboratory testing are among the most strenuous of any industry and are meant to control variability in testing in order to ensure the highest level of patient care. CMS regulates clinical laboratory testing based on guidelines outlined in the Clinical Laboratory Improvement Amendments (CLIA).11,12 These guidelines include mandatory participation in proficiency testing from a CMS-approved program using homogenous samples distributed to laboratories.11 CLIA, or an accrediting agency, determine acceptable criteria, including the total analytic error (TAE, method bias + total imprecision) for each analyte.11 In the article by Dr. Brian A. Kidd and colleagues, the authors state that a TAE of ≥ 10 percent would be considered insufficient for clinical decisions, and measurements once instrument imprecision is considered. Increased abnormal tests have consequences, including extra testing (and costs), extra visits to hospitals and clinics, and increased health-care service, which can potentially harm patients.11 Numerous questions remain about why testing by Theranos showed such unexpected variability in healthy adults. A detailed examination of the CMS inspection of the Newark laboratory, performed on November 20, 2015, reveals some potential answers.4 This 121-page document outlines numerous deficiencies that highlight a basic lack of understanding of clinical laboratory testing. Failed freezer temperatures; expired reagents; no training documentation; failure of the lab director to sign, date, or approve procedures prior to use; and proficiency testing failures with no investigation for multiple analytes are just a few of the findings.4 The fact that the original laboratory director was a dermatologist with no background in laboratory medicine should serve as a cautionary tale.11 While one might think that Theranos’ board of directors could lend insight into the business of laboratory testing, a review of its members reveals an impressive group of politicians, military figures, and accomplished individuals who have served on the board, but not a single laboratory medicine physician or clinical pathologist. This was addressed in April 2016 when a scientific and medical advisory board was added to Theranos, with a well-qualified group of laboratory professionals.15 The (Unknown) Science Behind Theranos

The science behind the technology driving the laboratory testing at Theranos remains elusive. Naturally, this has raised concern among physicians, given the potential effects of such untested technologies on patient safety.14 In the absence of any form of peer review of the science behind Theranos, it has become very difficult for pathologists to provide an informed opinion to clinicians and patients about the validity of the test results generated by the company. Publicly available sources such as newspaper articles and patent applications are no substitute for peer-reviewed scientific literature.

The publicly available facts regarding the testing pipeline at Theranos describe the use of a finger-stick method to obtain the patient’s blood sample into a patented collection device known as a “nanotainer.”13 The sample is then loaded onto a proprietary analysis machine code-named “Edison,”14 of which no specific information is publicly available. It is also believed that the sample is analyzed internally on the machine based on assay principles that also currently remain unknown.15 The data obtained are then wirelessly transmitted to a secure database with a portal interface from which the physician and the patient can then retrieve results.11

The main advertising claim of Theranos is the stated fear of the founder regarding venipuncture needles used in routine laboratory testing.11 The company patented the “nanotainer,” a 0.5-inch container device capable of storing one to two droplets of blood.13 This was advertised as a revolutionary method to obtain blood samples in a painless fashion to overcome the popular fear of venipuncture.11 Additionally, the low volumes of blood required to test samples were also advertised as a major selling point of the technology (“A tiny drop is all we need”). One popular news report claimed that Theranos needed 1/100 to 1/1,000 of the volume of regular laboratory testing.11 In the same article from 2014, it was reported that Theranos performed as many as 70 different laboratory tests from a single draw of 25 to 50 µL of blood obtained from the nanotainer.15 Subsequent news articles claimed a different number (approximately 30 tests) could be run on this blood volume. In October 2015, the U.S. Food and Drug Administration (FDA) called the nanotainer an “uncleared medical device” (Table).14 Subsequently, Theranos claimed to have stopped using the device for all of its tests except for the single FDA-cleared herpes virus test.11 The current status of the nanotainer in routine Theranos testing methodology remains unknown.

A cursory evaluation of the testing menu on the Theranos website shows availability of approximately 240 tests. However, there are no references to the exact collection method used in testing on the website. A review of the offered tests reveals that the testing menu at Theranos falls mostly into four different categories—clinical chemistry, immunology, DNA testing, and cell-based assays. Current point-of-care testing (POCT) methods use finger-stick-based techniques to obtain the necessary blood samples to enable POCT testing in clinics and homes. However, POCT tests represent a limited subset among the menu of tests available through routine laboratory testing needed in day-to-day clinical practice. A majority of the current science behind uses venipuncture-based access to obtain the required blood samples. The test validation performed by laboratory scientists also relies on the use of venipuncture-based sampling methods to ensure repeatable results.

Pertinent to this issue of sample collection (venipuncture vs. finger stick), a recent article by Dr. Meaghan M. Bond and colleagues16 examined the issue of drop-to-drop variation in the cellular components of blood. The authors analyzed the drop-to-drop variability of standard laboratory tests including hemoglobin, WBC count, WBC differential, and platelet count in six successive drops of blood collected from a single finger stick from 11 different donors.16 For example, the authors observed a significant difference in the coefficient of variation of the platelet counts obtained from finger stick compared with venipuncture (13% vs. 4.8%).

Table. Theranos Timeline

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>2003</td>
<td>Company founded by Elizabeth Holmes</td>
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<tr>
<td>2004</td>
<td>Theranos raised $6.9M</td>
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<tr>
<td>2010</td>
<td>Theranos raised $40M from single investor</td>
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<tr>
<td>2012</td>
<td>FDA launches investigation after complaint from Department of Defense official evaluating Theranos technology</td>
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<tr>
<td>2013</td>
<td>Walgreens and Theranos announce partnership. Theranos technology hailed as one of the top 10 medical and technological innovations in 2013.1</td>
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<tr>
<td>2014</td>
<td>Theranos raised $400M, valued at $9B with Holmes owning 50 percent of company</td>
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<tr>
<td>2015</td>
<td>Theranos lobbies for and wins approval in Arizona for a law allowing patients to order their own blood testing.</td>
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<tr>
<td>October 2015</td>
<td>Herpes simplex virus type 1 test approved by U.S. Food and Drug Administration (FDA); 261 other tests remain approved.</td>
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<tr>
<td>October 2015</td>
<td>FDA calls the nanotainer an “uncleared medical device.”14</td>
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<tr>
<td>November 2015</td>
<td>Centers for Medicare and Medicaid Services (CMS) inspects Newark, California, laboratory and finds numerous deficiencies in laboratory testing. These deficiencies could have posed “immediate jeopardy to patient health and safety.”15</td>
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<tr>
<td>February 29, 2016</td>
<td>Walgreens looks to cut ties with Theranos.</td>
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<tr>
<td>March 28, 2016</td>
<td>Kid Matt BA et al. article in the Journal of Clinical Investigation available online</td>
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<tr>
<td>April 7, 2016</td>
<td>Theranos adds medical board.14</td>
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<tr>
<td>April 13, 2016</td>
<td>News surfaces of CMS proposing a two-year ban for Holmes from owning and/or operating a laboratory, suspending Medicare and Medicaid payments and revoking Clinical Laboratory Improvement Amendments license from Newark, California, laboratory in a March 18 letter. Theranos had 10 days to respond; the response was not made public. No comment from CMS.</td>
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<tr>
<td>April 18, 2016</td>
<td>Theranos investigated by federal prosecutors, by CMS, and by the Securities and Exchange Commission</td>
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<tr>
<td>July 8, 2016</td>
<td>Federal regulators bar Elizabeth Holmes from owning or operating a medical laboratory for at least two years; certification of Newark, California, laboratory revoked (effective September 5, 2016); laboratory also prohibited from taking Medicare and Medicaid payments.</td>
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<tr>
<td>August 1, 2016</td>
<td>Elizabeth Holmes presents at the American Association of Clinical Chemistry, highlighting the “miniLab” as the new iteration of Theranos’ miniaturization technology.</td>
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respectively). This study illustrates the drop-to-drop variability of the finger-stick method for easy-to-perform laboratory tests. To our knowledge, no such analysis of the impact of finger-stick sampling on the other classes of tests offered on the Theranos menu (clinical chemistry, immunology, and DNA-based testing) has been performed. We believe a thorough peer-reviewed scientific analysis of finger stick sample reliability for analyte detection is critically important before it can be offered to patients in routine practice. In the current discussion, we have focused on the preanalytic components of the testing process, such as sample collection and reliability. Equally pertinent are the analytic and postanalytic components of any laboratory testing process. In the absence of reliable peer-reviewed data, it is difficult to determine the efficacy of the microfluidic-based testing approach that Theranos claims to use.

The idea of a “lab-on-a-chip” is not a new one. Groups around the world have been working on the development of microfluidic devices for many years. However, there are several challenges associated with the development of these devices for routine clinical implementation. Two of the most significant challenges are the lack of a scalable standardization of the microfluidic manufacturing processes and the lack of reusability of the microfluidic chips. These two factors increase the costs of microfluidic technology for routine clinical implementation. Laboratory medicine surely could benefit from innovative approaches such as microfluidic-based testing. However, these technologies should be implemented in clinical testing only after extensive peer review of the testing methodology. A previous College of American Pathologists study estimated that 70 percent of a clinical decision is directly correlated with the laboratory results provided, illustrating the seriousness with which laboratory testing needs to be taken.

As this edition of The Hematologist goes to press, Elizabeth Holmes gave a highly anticipated invited presentation at the annual meeting of the American Association of Clinical Chemistry on August 1, 2016. At this first scientific conference for the Theranos CEO, healthy skepticism was displayed as to whether substantive data would be presented. AACC President Patricia Jones echoed this in her introduction: Elizabeth Holmes: “We’re all aware that there have been some questions about whether we will see any science here today and the viability of Theranos’ technology.” In fact, the presentation lacked any details regarding the original “Edison” testing platform. Instead, a new device, the “miniLab,” was introduced to the public. Preliminary information provided about the inner components of the machine and an overview of data on precision and comparison studies. In the authors’ opinion, there is nothing unique about the spectrophotometer, luminometer, and basic hematology technology at the heart of the new miniLab device, which represents a miniaturization of various detection modules used in a standard clinical laboratory. The use of a new idea, amplification using the molecular biology assays and the slide-based cytometer for assessing lymphocyte subsets are newer technologies that have not been robustly tested in the clinical laboratory. While data presented appear to be robust and follow appropriate guidelines, the performance characteristics of this device at microfluidic volumes in real-world clinical applications are still uncertain and require validation. Thus, the aforementioned criticisms still stand and must be addressed in a thorough, transparent, and comprehensive manner. Theranos seems to be taking steps to address multiple shortcomings of their original testing model, including the need for mandatory scientific peer review. The introduction of a new scientific and medical advisory board, should also provide much-needed guidance. The ongoing trials of Theranos serve as an important case study in the need for physicians to have a firm understanding of the scientific principles of laboratory testing in order to provide the best level of health care for patients.

17. Palladino R. This CEO is out for blood. Forbes. 2014. Available at http://fortune.com/2014/06/12/theranos-blood-holmes/.

Dr. Gallapalli and Dr. George declare no conflicts of interest. Editor-in-Chief Dr. Jason Gotlib collaborated with Theranos on a pilot study whose results were published in 2014 (see reference 2). He received no funding.
FIxing Hemophilia B: Sustained Factor IX Expression of 30 Percent After Gene Therapy


CLINICALTRIALS.GOV IDENTIFIER: NCT02484092

FUNDING SOURCE: Spark Therapeutics and Pfizer

PARTICIPATING CENTERS: The Children’s Hospital of Philadelphia, University of Pennsylvania, University of Mississippi Medical Center, University of Pittsburgh, University of California Davis, Weill Cornell Medical College

ACCURAL GOAL: To be determined

STUDY DESIGN: This is a phase I/II, open-label, dose-escalation clinical trial evaluating the safety, tolerability, and pharmacokinetics of SPK-9001 in subjects with hemophilia B. The target population is adult males (a18 years) with hemophilia B (factor IX activity x 2% of normal). Exclusion criteria include hepatitis C virus and HIV viremia, adeno-associated viral (AAV)-Spark 100 neutralizing antibody titers 1:5 or greater, or history of a factor IX (FIX) inhibitor. The primary objective of the study is to evaluate the safety and tolerability of SPK-9001. The secondary objective is to characterize the kinetics of SPK-9001 vector-derived FIX transgene expression. Exploratory objectives include evaluating changes from baseline in clinical outcomes, including number of bleeding events and factor consumption. The vector is administered intravenously over one hour. Subjects are observed for 52 weeks.

RATIONALE: The SPK-9001 adeno-associated viral (AAV) vector was developed to achieve hemostatic levels of FIX activity at a dose low enough to avoid an immune response to the vector capsid, which has limited efficacy in prior clinical trials (Mingozzi F, et al. Nat Med. 2007;13:419-422). SPK-9001 consists of a bioengineered capsid, AAV-Spark100, with liver specific tropism analogous to AAV. The seroprevalence of preexisting neutralizing antibodies to AAV-Spark100 is approximately 40 per cent among sampled patients with hemophilia B (Anguera X, et al. J Thromb Haemost. 2015;13:S1243-325). The vector expression cassette encodes a single-stranded FIX-Padua transgene. FIX Padua is a naturally occurring missense mutation in the protease domain that confers approximately eightfold greater specific activity than wild type FIX (Simionetti P, et al. N Engl J Med. 2009;361:1671-1675).

Preclinical data on four subjects demonstrate that a single infusion of SPK-9001 at a dose of 5 x 10^11 vg/kg confers sustained FIX expression without need for immunosuppression. As of June 2016, four subjects have been followed for seven to 26 weeks following vector administration and achieved sustained mean (+ SD) FIX activities of 31.3 percent ± 5.7 percent (High KA et al. European Hematology Association 2016). There were no vector-related adverse events. Three of the four subjects were maintained on prophylaxis prior to study enrollment and safely stopped prophylaxis after vector infusion. Beyond a one-time factor infusion by a single subject two days following vector infusion, no subjects have experienced bleeding or required factor after vector administration. No subjects have experienced hepatic transaminase elevation more than 1.5-fold the normal level, a decrease in FIX activity, an immune response requiring immunosuppression, or development of an inhibitor to FIX.

COMMENT: Seminal data from Dr. Amit C. Nathwani and colleagues demonstrated long-term expression of FIX (1% to 7% of normal) in men with hemophilia B following AAV-mediated gene therapy (Nathwani AC, et al. N Engl J Med. 2004;351:1994-2004). While the clinical impact of sustained FIX activity around 5 percent is indiscernible, such levels of expression fall short of trough values achieved by prophylactic administration of extended half-life FIX products (Santagostino E, et al. Blood. 2016;127:1761-1769) and of natural history data suggesting that approximately 15 percent factor activity eliminates spontaneous hemorrhages (den Uijl IE, et al. Haemophilia. 2011;17:849-853). Experience so far suggests that SPK-9001 at a dose of 5 x 10^11 vg/kg may consistently meet or exceed these thresholds. This early success requires confirmation in a large number of patients and long-term monitoring of safety and efficacy. Nevertheless, the preliminary results suggest the possibility of a virtual “cure” for hemophilia B. Study recruitment and follow-up are ongoing. Challenges ahead for AAV gene therapy include developing strategies to overcome pre-existing AAV neutralizing antibodies and adapting gene therapy for the treatment of hemophilia A.

– Lindsey A. George, MD, and Adam Cuker, MD, MS

Dr. George and Dr. Cuker are investigators in the SPK-9001 trial. Dr. Cuker receives salary support from Spark Therapeutics.

BCL-2 Inhibition in Acute Myelogenous Leukemia

STUDY TITLE: A Phase 1b Study of ABT-199 (GDC-0119) In Combination With Azacitidine or Decitabine in Treatment-Naïve Subjects With Acute Myelogenous Leukemia Who Are ≥ 65 Years of Age and Who Are Not Eligible for Standard Induction Therapy

CLINICALTRIALS.GOV IDENTIFIER: NCT02203773

SPONSOR: AbbVie

ACCURAL GOAL: 160 participants

PARTICIPATING CENTERS: 22 sites in the United States, Australia, France, and Germany

STUDY DESIGN: This study is a phase Ib, nonrandomized, multicenter, open-label, prospective trial that will measure the safety of combination ABT-199 and hypomethylating agents in older adults with acute myelogenous leukemia (AML), who are treatment-naïve and not eligible for standard induction therapy. Secondary outcomes will measure depth and duration of responses (including minimal residual disease [MRD]) and survival. Eligible patients must be 65 years or older with a good performance status (Eastern Cooperative Oncology Group score, 0-2), having not received any prior AML treatment except hydroxyurea. Patients may not have received prior chemotherapy or hypomethylating agent for an antecedent hematologic disorder. Patients with favorable risk cytogenetics, including acute promyelocytic leukemia, are excluded from participation. Dose-escalated ABT-199 is administered orally daily with either standard doses of decitabine or azacitidine for the first five or seven days of each cycle respectively, for a minimum of four cycles.

RATIONALE: Reliance on the anti-apoptotic protein BCL-2 has been shown in preclinical studies to be important in the maintenance and survival of AML cells. Unlike disorders such as chronic lymphocytic leukemia (CLL), where there are discrete genetic causes for BCL-2 upregulation, specific genetic alterations that cause overexpression of BCL-2 are not known in AML. However, prior work has identified that AML cells are functionally more reliant on BCL-2 than normal hematopoietic stem cells, thus providing a therapeutic index for BCL-2 inhibition in AML. Therefore, there is hope that the efficacy of BCL-2 inhibition in AML might approach that seen with CLL, where ABT-199 treatment has resulted in sizable complete remissions, with some patients achieving MRD-negative status. Previously, a phase II study of ABT-199 as monotherapy in relapsed/refractory AML showed that five (16 percent) of 32 heavily pretreated patients achieved a complete response or complete response with incomplete blood count recovery (CR/Cri). The most common adverse events were nausea, vomiting, diarrhoea, and neutropenia.

COMMENT: Thus far, data presented at the 2015 ASH Annual Meeting showed promising results with the combination of ABT-199 and hypomethylating agents. Two dose levels of ABT-199 (400 mg and 800 mg) were evaluated in a total of 22 patients. The rate of CR/Cri was 60 percent and 67 percent in the azacitidine and decitabine arms, respectively. Compared with monotherapy, combination therapy resulted in thrombocytopenia and leukopenia, necessitating treatment disruptions in 55 percent of patients. No episodes of tumor lysis syndrome occurred. The thrombocytopenia was unexpected since ABT-199 does not inhibit BCL-XX, whose inhibition was found to be the cause of thrombocytopenia with prior generation BCL-2 inhibitors. Potential effects of the combination treatment on BCL-XX or other antiapoptotic proteins in platelets therefore, should be investigated. However, based on the positive results thus far, the U.S. Food and Drug Administration has granted ABT-199 a breakthrough therapy designation for use in combination with hypomethylating agents in this patient population. Updated results for a total of 34 patients were presented in June 2016 with similar results: CR/Cri of 71 percent, with no dose-limiting toxicity reached to date. Furthermore, a similar study using ABT-199 in combination with low-dose cytarabine in treatment-naïve AML patients aged 65 years or older (NCT02287233) reported a 44 percent response rate in 18 patients. However, this study did identify a dose-limiting toxicity of grade 4 thrombocytopenia at the 800 mg dose-level, and the 600 mg dose was recommended for phase II development with that particular combination.

The superior results with ABT-199 combined with other agents in the upfront setting as compared to monotherapy in the relapsed/refractory setting (44% to 70% vs. 15% to 13%, respectively) invites the question as to whether disease context or the combination of agents is responsible for improved efficacy. A phase III, randomized trial of ABT-199 in combination with hypomethylating agent versus hypomethylating agent alone in a treatment-naïve AML population would certainly answer this question, and these trials are currently being planned. In the meantime, the phase Ib combination of ABT-199 with hypomethylating therapy is currently recruiting participants and offers an option for those patients age 65 or older who are not eligible for standard therapy or other targeted agents.

– Justin Taylor, MD, and Omar Abdel-Wahab, MD

Dr. Taylor and Dr. Abdel-Wahab indicated no relevant conflicts of interest.
BRAFV600E gene mutation. The treatment of refractory hairy-cell leukemia with a dose BRAF inhibitor vemurafenib is highly effective in 2016;127:2847-2855.


In their Perspective article, Dr. Pieter Sonneveeld and colleagues present a consensus update of a cytogenetics-based definition for high-risk multiple myeloma. They use cytogenetic abnormalities to revise the definition of high-risk myeloma in the International Staging System and assess the impact of available treatable modalities on outcomes.


These two articles describe elegant genomic approaches driven by next-generation sequencing in unraveling the genetic abnormalities in patients with bleeding and thrombotic disorders. In their plenary paper, Dr. Ilenia Simeoni and colleagues describe exciting results using a high-throughput sequencing platform with 63 targeted genes in patients with heritable bleeding and thrombotic disorders. In their paper, Dr. Simon Stritt and colleagues describe a DIAPIH variant as a cause of inherited macrothrombocytopenia and hearing loss.


Dr. Sascha Dietrich and colleagues report that the low-dose BRAF inhibitor vemurafenib is highly effective in the treatment of refractory hairy-cell leukemia with a BRAFV600E gene mutation.


Dr. Gérard Michel and colleagues report that a single umbilical cord (UBC) transplant, if available in adequate cell numbers, is superior to a double UBC transplant in children and young adults with acute myeloid leukemia or myelodysplastic syndrome. Single-unit transplant is associated with equivalent engraftment and remission-free survival, lower rates of chronic graft-versus-host disease, and less transplant-related mortality.


Autoimmune lymphoproliferative syndrome (ALPS) is characterized by defective Fas signaling and lymphocyte accumulation that has been attributed to failure of apoptosis. Dr. Simon Völki and colleagues demonstrate that lymphoproliferation in ALPS is a more active process, with active proliferation and aberrant T-cell maturation induced by malignant target of rapamycin (mTOR) signaling. This suggests that rapamycin might be uniquely suited for treatment of the disorder.


Hepcidin is the master regulator of iron metabolism, acting by decreasing iron absorption from the gastrointestinal tract and release of iron from macrophages. Abnormally low hepcidin in the face of iron overload contributes to the pathophysiology of β-thalassemia intermedia, and iron availability controls the erythroid hyperproliferation in polycythemia vera (PV). Dr. Carla Casu and colleagues report that administration of minihedpin improves iron overload in murine β-thalassemia and decreases splenomegaly and polycythemia in a mouse model of PV. These exciting results promise to have a major impact on therapy for iron-overload syndromes and polycythemia.


Dr. Jakob Krejcić and colleagues provide the first clinical data demonstrating unexpected immune-stimulatory activity of the monoclonal antibody daratumumab. The prospect of triggering long-term memory antemyeloma immunity in patients at early stages of the disease offers encouraging potential for prolonged survival and possibly cure.


Recent studies have challenged the traditional dogma that the half-life of neutrophils in the peripheral blood is less than 24 hours. Dr. Julio Lahoz-Beneytez and colleagues re-examine this question using stable deuterium labeling and confirm that neutrophil half-life is 15-19 hours, as predicted several decades ago.
ASH Impact Series Podcasts and Videos Available on ASH On Demand

To help keep hematologists aware of the many clinical and research updates in the field, ASH offers a steady stream of educational content, featuring presentations by experts in hematology that provide current information on how to best diagnose and care for patients with hematologic conditions. The ASH Impact Series, available on ASH On Demand, includes educational content in the form of videos and podcasts covering a variety of hematology conditions and treatment approaches. This educational content is available free of charge. Topics discussed in the videos and podcasts include the latest chronic lymphocytic leukemia research in relation to new drug therapies, the most recent developments in the treatment of children with sickle cell disease, the newest targeted therapies for acute myeloid leukemia, novel drug combination treatments for multiple myeloma, and many other important developments.

To watch videos from the ASH Impact Series, visit www.ashondemand.org/FreeContent/Videos, or browse dozens of audio-only recordings from the Impact Series at www.ashondemand.org/FreeContent/Podcasts. For more information on ASH’s growing library of educational multimedia content, go to www.ashondemand.org.

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**WHAT’S ON THE WEB**

**MARK YOUR CALENDAR**

**September**
7 Translational Research Training in Hematology full application deadline
Washington, DC www.hematology.org/awards
14 CME Summit on Myelodysplastic Syndrome
Las Vegas, NV www.hematology.org/meetings
15 ASH Consultative Hematology Course
Chicago, IL www.hematology.org/meetings
15 CME Summit on Myelodysplastic Syndrome
Chicago, IL www.hematology.org/meetings
16-17 ASH Meeting on Hematologic Malignancies
Chicago, IL www.hematology.org/meetings
27-30 ASH Medical Educators Institute Workshop
Washington, DC www.hematology.org/educators

**October**
21 ASH annual meeting late-breaking abstract site opens
San Diego, CA www.hematology.org/annual-meeting
31 ASH annual meeting late-breaking abstract submission deadline
San Diego, CA www.hematology.org/annual-meeting

**November**
2 ASH annual meeting housing reservation deadline
San Diego, CA www.hematology.org/annual-meeting
3 Abstracts from the ASH annual meeting available online
San Diego, CA www.hematology.org/annual-meeting
4 ASH Consultative Hematology Course
Austin, TX www.hematology.org/meetings

**December**
3-6 CME Summit on Myelodysplastic Syndrome
San Diego, CA www.hematology.org/meetings
3-6 2016 ASH Annual Meeting & Exposition
San Diego, CA www.hematology.org/annual-meeting
5 ASH Consultative Hematology Course
San Diego, CA www.hematology.org/meetings