More than 250 mutations in acute myeloid leukemia (AML) have been observed on a recurrent basis in more than one patient. Some mutations are classified as initiating mutations that occur early, such as NPM1, DNMT3A, IDH1, and TET2, and some mutations are cooperating mutations, such as FLT3, which activates signaling pathways. Driver mutations provide a survival or proliferative advantage to the cell that promotes the development of AML.1,2 There is also acquisition of additional subclonal mutations over time (including after treatment),3 a process known as “clonal evolution.” Whole genome or exome sequencing of 200 genomes from patients with AML for The Cancer Genome Atlas (TCGA) revealed on average 13 gene mutations per genome, and 23 genes were found to be significantly mutated, with a higher-than-background mutation rate, likely representing driver mutations. Several other studies have been published since these earlier reports, including one showing that mutations in secondary AML arising from myelodysplastic syndrome (MDS) are largely in the same genes as primary AML.3 However, it has been difficult to define how this genomic knowledge will enhance our practice.

Chromosomal analysis by standard karyotyping and/or fluorescence in situ hybridization (FISH) permits subdivision of patients into favorable, intermediate, or unfavorable risk groups. There have been multiple attempts to identify biomarkers that can accurately predict the durability of response in AML, and to better subdivide intermediate-risk patients. One such marker for patients in complete remission (CR), classically defined as less than 5 percent bone marrow (BM) blasts, is minimal residual disease (MRD) by multicolor flow cytometry.4 Patients who attain CR but still have MRD of any amount identifiable by flow cytometry have a worse progression-free and overall survival (OS).

Dr. Jeffery Klco and collaborators, report that detection of mutations that persist on analysis of the day-30 BM is also predictive of shorter event-free survival (EFS) and OS in patients who achieve CR after initial therapy. Sixty-eight of the TCGA patients received induction chemotherapy with anthracycline (daunorubicin or idarubicin) and continuous infusion cytarabine (5-azacytidine) and 160 of those patients were treated with less than 50 percent BM blasts at a median of 34 days after the start of induction. Certain mutations did not clear by day 30. For example, of the 16 cases with DNMT3A mutations, only three cleared by day 30. Similarly, mutations in TET2 often persisted in remission. Mutations in FLT3, NRAS, and KRAS were cleared by day 30. For those patients who attained CR and whose mutations were detectable at day 30, mutations in NPM1 were also able to clear the mutation by day 30. There were 24 patients who had at least one mutation detectable at day 30 and none of these patients had detectable mutations at day 30. (Cont. on page 6)
**Leading the Charge for Precision Medicine**

N early nine months ago, in February 2015, I made a statement on behalf of ASH, applauding President Obama's funding proposal for the National Institutes of Health (NIH) as well as his unveiling of the Precision Medicine Initiative (PMI; www.ash.org/Newsroom/Press-Releases/2014/3645.aspx). The administration proposed $215 million in investments among NIH, the National Cancer Institute (NCI), U.S. Food and Drug Administration, and the Office of the National Coordinator for Health Information Technology, supporting projects including research, development of new databases, innovations in data collection and sharing, and creation of new requirements and standards.

It was also in 2015 that ASH defined a new set of research priorities, and the areas of genomic profiling and clinical biology are, quite literally, at the top of the agenda (www.hematology.org/ResearchAgenda).

Customized approaches to treatment have been around for some time, and hematologists have made many contributions using patient- and tumor-specific information to prevent, diagnose, and treat diseases. However, ASH’s sense of urgency is reflected in the development of the new ASH Research Agenda, renewed commitment to research funding at the federal level; advances in genome sequencing and bioinformatic analysis in the nascent era of big data; and other areas that will have critical implications for the future of informed, personalized clinical care. This year, ASH took direct action to further define the scope of our role in this crucial area, establishing the Task Force on Precision Medicine, whose chief priorities include exploring ways in which ASH can work directly with NIH and other entities to initiate and address gaps within genomically defined, precision medicine trials. The ultimate goal of the Task Force is to make sure that the opportunities for both malignant and nonmalignant hematology are fully explored, working in an advisory capacity to help the Society best realize the promise of precision medicine in patient care.

Interest in precision medicine goes well beyond the new task force. The 57th ASH Annual Meeting in Orlando next month will offer many opportunities to tap into some of the most fascinating breakthroughs in genomics and to learn how genomic data translate into information that is clinically meaningful. To spotlight just a few of these opportunities, Dr. Jill Johnson will chair the Education Session “Guiding Hematologic Care with Genetic Testing,” and Dr. Charles Mullighan will lead the Special Scientific Symposium “Precision Medicine in Cancer Therapy.” Dr. Louis Staudt of NCI will conduct a highly interactive Meet the Scientist session, answering questions on many aspects of precision medicine as well as discussing where the field is headed. Abstracts and talks will describe how specific disease-causing molecular defects in both malignant and nonmalignant conditions are being targeted by genetic approaches and with drugs that have surgical precision rather than using the blunt instruments of the past. It is so exciting to witness what seems like the tipping point in bringing these treatments to patients.

Serving as ASH President during such an eventful and auspicious time for the field has been an honor. In 2015, ASH made major strides in developing new clinical guidelines, in continuing to evolve as a global society, and redefining our leadership in sickle cell disease. And as part of an ongoing effort to support access to care and treatment, and related legislative goals, ASH targeted its advocacy efforts by working with Congress to address Medicare reimbursement and supporting the Cancer Drug Coverage Parity Act, which was introduced in Congress in June. ASH will continue to push for these legislative priorities at both the state and federal levels. As always, we made many, many lobbying trips to Congress to advocate for additional NIH research funding.

Looking ahead to 2016, many research and patient care-related agenda items lie before us that require ASH’s advocacy. I look forward to passing the gavel to Dr. Charles Abrams in Orlando, and welcome his leadership in the months ahead.

David A. Williams, MD
ASH Elects New Leadership

VICE PRESIDENT:
Alexis Thompson, MD, MPH
Sarah and A. Watson Armour Chair in Childhood Cancer and Blood Disorders; Hematology Section Head and Professor of Pediatrics at Ann & Robert H. Lurie Children’s Hospital of Chicago and the Feinberg School of Medicine at Northwestern University in Chicago
Dr. Thompson will serve a one-year term as vice president followed by successive terms as president-elect and president.

COUNCILLOR:
Jane Winter, MD
Professor of Medicine in the Division of Hematology/Oncology at Northwestern University’s Feinberg School of Medicine; member of the Robert H. Lurie Comprehensive Cancer Center in Chicago
Dr. Winter will serve a four-year term as councillor.

COUNCILLOR IN CLINICAL PRACTICE:
Steven Allen, MD
Professor of Medicine at Hofstra North Shore-LIJ School of Medicine; Associate Chief in the Division of Hematology, Department of Medicine; Attending Physician, Division of Medical Oncology/Hematology at Monter Cancer Center, North Shore-LIJ Health System; Associate Investigator at North Shore-Long Island Jewish Research Institute in New York
Dr. Allen will serve a four-year term as councillor.

57th ASH Annual Meeting Abstracts Available November 5

On November 5 after 9:00 a.m. EST, the complete ASH annual meeting schedule and program will be available on the ASH website. Read abstracts from the education and scientific programs, as well as oral and poster sessions, general sessions, special-interest sessions, and more. Browse the entire schedule by day, program, speaker, or keyword. Visit www.hematology.org/Annual_Meeting to get started.

Make ASH 2016 Meetings Part of Your New Year’s Resolutions

If education and exploration of emerging topic areas are on your list of 2016 New Year’s resolutions, consider attending one of the many ASH meetings and workshops held throughout the year. Start charting your course for learning by visiting www.hematology.org/meetings and stay tuned to future issues for registration deadlines, abstract submission details, speaker lists, and more.

• 2016 Highlights of ASH (see page 16 of this issue for complete dates and locations)
Eight locations, three continents, one great program. Catch up on new clinical research presented during the preceding ASH Annual Meeting in Orlando. This is your chance to evaluate your diagnostic techniques and therapeutic approaches and join leading hematology experts and colleagues to discuss how new research and clinical updates can be translated into novel patient care strategies.

• ASH Meeting on Lymphoma Biology, June 18-21, 2016, Colorado Springs, CO
Further your understanding of lymphoma pathogenesis and new therapies during this forum for scientific exchange and networking, designed especially for laboratory-based scientists, translational investigators, pharmaceutical scientists, and others interested in lymphoma science. This year’s keynote speakers will be Dr. Michael Stratton of Wellcome Trust Sanger Institute (“Genetics of Cancer”) and Dr. Hao Wu from Boston Children’s Hospital (“Elucidation of Macromolecular Interactions Using Structural Biology”).

• “NEW ASH Workshop on Genome Editing, July 14-15, 2016, Washington, DC
Genome editing technology is currently at the forefront of genetic engineering and has led to several transformative advances thanks to its simplicity, versatility, flexibility, and ability to precisely manipulate cellular genomes and correct mutations. This workshop will focus specifically on the therapeutic applications of genome editing to hematologic diseases.

• ASH Meeting on Hematologic Malignancies, September 2016, Chicago, IL
Join us for another offering of the premier showcase of experts in hematologic malignancies discussing the latest developments in clinical care and answering your most challenging patient care questions. Evidence-based presentations cover core malignancies, including leukemia, lymphoma, myelodysplastic syndromes, myeloma, and myeloproliferative neoplasms. To learn more, see page 16 of this issue and find out how you can view recordings and bonus content from the 2015 meeting.
Ask the Hematopathologist

TRACY I. GEORGE, MD
Associate Professor of Pathology; Director of the Hematopathology Fellowship Program, University of New Mexico School of Medicine, Albuquerque, New Mexico

The Question

What is your approach to lymphocytosis?

Case

A 71-year-old man with a history of atypical chronic lymphocytic leukemia (CLL) last treated in 2007 with a rituximab and chlorambucil-based regimen presents with an increasing M protein of 4.1 g/dL (IgG). The laboratory findings were as follows: WBC, 7.4 × 10^9/L with 29 percent neutrophils, 66 percent lymphocytes, and 5 percent monocytes; RBC, 3.94 × 10^12/L; hemoglobin, 11.0 g/dL; mean corpuscular volume, 83 fL; platelets, 91 × 10^9/L. The patient’s bone marrow was hypercellular (90%) with a marked lymphoid infiltrate present in nodular (paratrabecular and interstitial) and focal diffuse patterns involving 75 percent of bone marrow cellularity. Lymphocytes were small and round with condensed chromatin and occasional plasmaclastoid lymphocytes were also observed. The karyotype of the bone marrow was 46 XY add(9)(p24), del(11)(q13), +14 in four cells with a sideline containing all of these abnormalities and -13 in two cells, and an unrelated clone showing 45 X Y, -6 in six cells, with 46, XY in seven cells. Fluorescence in situ hybridization (FISH) found a deletion of the 13q14.3 region and was negative for deletions unrelated clone showing 45,X,–Y in six cells, with 46,XY in seven cells. Donor red blood cells were found in 90 percent of the circulating neutrophils, as well as small red blood cells with target cell morphology. The patient was treated in 2007 with a rituximab and chlorambucil-based regimen. The patient’s albumin was 3.7 g/dL and his creatinine was 0.9 mg/dL. The patient reported no prior transfusions or blood donation.

Examinining the Blood Smear

A slide review is appropriate in all patients with an unexplained lymphocytosis in order to confirm the automated cell counts or to perform a manual differential for leukocyte classification. In manually prepared blood smears, larger white blood cells tend to collect at the edges of the smear and in the heaviest edge. Good practice for slide review requires assessments of all cell types (leukocytes, red blood cells [RBCs], and platelets) in both quantity and quality. It is not uncommon for fragile leukocytes such as in CLL, infectious monoclonocytosis, or acute leukemia to smudge on blood smears. In these situations, a few drops of albumin can be added to peripheral blood before preparing the blood smear. These “albumin smears” allow for proper identification of leukocytes and reduce the number of “smudge” or “basket” artifacts.

Reactive Lymphocytosis

Separating a monomorphic lymphocytosis from a polyclonal lymphocytosis can help distinguish a lymphoproliferative disorder from a reactive lymphocytosis, respectively. More reactive lymphocytoses show a wide range of sizes and shapes in lymphocytes. The classic example of a polyclonal lymphocytosis is infectious mononucleosis, where the lymphocytes range in size from small and round to intermediate with abundant cytoplasm (reactive lymphocytes), to frank immunoblasts. It is this spectrum of morphology that points to a greater likelihood that a patient has a reactive lymphocytosis; younger age is also a helpful clue. The causes of a reactive lymphocytosis are extensive and include infections (viral, bacterial, and parasitic), autoimmune disease, vaccination, drug hypersensitivity, endocrine disorders, stress (trauma, cardiac, extreme exercise), smoking, and malignancy.

While most of these reactive lymphocytes are polyclonal, a few important exceptions are worth mentioning. The first is Boudettella pertussis, the causative agent of whooping cough. The lymphocytes of B. pertussis are small and deeply clefted with mature chromatin as shown in Figure 1. As this is commonly seen in the pediatric and pregnant populations, clinical correlation will readily separate this from lymphomas, which can show similar morphologic features (e.g., follicular lymphoma or Sézary syndrome). The second exception is polyclonal B-lymphocytosis, which typically shows lymphocytes with distinct nuclear clefts but will demonstrate a spectrum of morphologic changes including nuclear lobation and binucleate forms. This uncommon disorder is found in young to middle-aged female smokers with a high association with human leukocyte antigen DR7, and several genetic abnormalities have also been documented. The final exception is a large granular lymphocytosis. Increased numbers of large granular lymphocytes (reactive lymphocytes with scattered azurophilic granules) are commonly seen with viral infections, malignancy, after bone marrow transplantation, and following chemotherapy. These populations of large granular lymphocytes will wax and wane. However, persistence of a large granular lymphocytosis with accompanying neuropenia and variable anemia should raise suspicion for large granular lymphocytic leukemia. This is typically T cell in origin, though a chronic lymphoproliferative disorder of natural killer cells is also well described. Flow cytometry is recommended in these cases, followed by either T-cell cloning or KIR analysis, if involving T cells or natural killer cells, respectively.

Neoplastic Lymphocytosis

Lymphomas cells tend to be monomorphic in appearance. While a blood smear may contain a subset of lymphoma cells, these cells will resemble one another and stand out against a background of normal bland lymphocytes. While CLL is the most common leukaemia in adults in the western world and is frequently seen in peripheral blood (or its monosclonal B-cell lymphocytosis counterpart), peripheral blood involvement by bone marrow lymphoma is found in up to 30 percent of subjects in some studies. Lymphoma cells will show a wide variety of morphologic appearance, and this appearance raises a differential diagnosis as shown in Figure 2. Further identification of the type of lymphoproliferative disorder typically proceeds with flow cytometry. Although each laboratory has its own cocktail of antibodies used for flow cytometry, consensus guidelines have been published. While the results from flow cytometry narrow down one’s differential diagnosis to a short list, additional genetic or other ancillary studies are typically needed for confirmation, such as FISH for CD20/IGH to evaluate for mantle cell lymphoma. Additionally, bone marrow biopsy or biopsy of another involved site is often necessary for a final diagnosis.

A common question is when should flow cytometry be performed. Some studies have looked at this question in adults. In one study, the authors retrospectively reviewed flow cytometry results of 71 patients 50 years of age and older with an absolute lymphocyte count of 4 × 10^9/L or greater that had been called suspicious for a lymphoproliferative disorder by smear review by a pathologist. Using receiver operating characteristic (ROC) analysis, they found that an absolute lymphocyte count greater than 6.7 × 10^9/L for patients 50 to 67 years of age, and 4 × 10^9/L for greater or patients older than 67 years had a 95 percent sensitivity and 76 percent specificity for predicting an abnormal flow cytometry phenotype. A more recent retrospective single-center study examined 71 adults with newly detected lymphocytosis greater than 5 × 10^9/L in a consecutive three-month period and found that 6.8 × 10^9/L was the best cut-off value for predicting a lymphoproliferative disorder with ROC analysis (sensitivity 90%, specificity 59%). By my own practice, other triggers for flow cytometry include a persistent unexplained lymphocytosis or a morphology that does not correlate with the diagnosis, as discussed below.

Patient Follow-up

Review of the patient’s original diagnostic material confirmed that the atypical CLL diagnosis was given based on immunophenotypic expression of CD20, in addition to the usual phenotypic for CLL (CD20+, CD5−, CD23−). Morphology in the current blood and bone marrow showed lymphocytes and plasmaclastoid reactive by the usual small round lymphocytes with clumped chromatin (“soccer balls”) of CLL; the plasmacytid cells were not readily identified on the earlier bone marrow smears. I performed flow cytometry on the patient’s bone marrow and identified a light chain–restricted B-cell population that expressed CD19, CD20, CD10, and CD22, and lacked expression of CD5 and CD200. Additionally, a light chain–restricted plasma cell population was identified. Immunohistochemistry was performed on the bone marrow clot section. Cyclin D1 and SOX11 were negative in the B-cells, excluding the diagnosis of mantle cell lymphoma; SOX11 is a newer marker for mantle cell lymphoma that has been found to be expressed even in mantle cell lymphomas that lack overexpression of cyclin D1. (LEF) was negative, providing no support for a diagnosis of mantle cell lymphoma. Markers of follicle center cell origin, BCL6 and LMO2, were also negative, providing no support for a lymphoma of follicle center cell origin. The cytogentic karyotype, while abnormal, was not specific for any particular B-cell lymphoma. The lack of t(11;14) and t(14;18) argued against both mantle cell lymphoma and follicular lymphoma. Molecular testing for MYD88 L265P mutation was performed and was negative. DNA polymerase chain reaction analysis for immunoglobulin heavy chain gene (IGH) was performed on the 2007 bone marrow and on the current 2015 bone marrow. A clone was detected in both samples that was identical in amplicon size.

This case was presented at a multidisciplinary tumor board conference. While the immunophenotype of the lymphocytes switched from CD5 to CD10 expression, the IGH data supported that the same neoplastic clone was present in both the 2007 and current bone marrow samples. A low-grade B-cell lymphoma with plasmacytic differentiation was found, raising a differential diagnosis of lymphoplasmacytic lymphoma, versus a marginal zone lymphoma with plasmacytic differentiation. While the lack of a MYD88 L265P mutation argues against lymphoplasmacytic lymphoma, my colleagues have found this to be 96

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percent sensitive for a diagnosis of lymphoplasmacytic lymphoma in a study of 317 cases of low-grade B-cell lymphomas.15 A diagnosis of atypical CLL seemed less likely, as the lymphocytes lacked L1 expression—a marker identified based on gene expression profiling data that has been described as nearly 100 percent sensitive and specific for CLL.16 The lymphocytes also lacked expression of CD200 by flow cytometry, another lymphocytes lacked LEF1 expression—a marker identified based on gene expression

The differential diagnosis for a neoplastic lymphocytosis based on morphology in the peripheral blood smear. The bolded diagnosis represents the most likely. Further refinement of ancillary testing is listed in the third column. CLL, chronic lymphocytic leukemia; MCL, monoclonal B-cell lymphocytosis; MCL, mantle cell lymphoma; T-PLL, T prolymphocytic leukemia; FL, follicular lymphoma; HCL, hairy-cell leukemia; SMZL, splenic marginal zone lymphoma; HCLV, hairy-cell leukemia variant; LPL, lymphoplasmacytic lymphoma; T-LGL, T large granular lymphocytic leukemia; B-PLL, B prolymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; ALCL, anaplastic large-cell lymphoma. Adapted with permission from Chabot-Richards D, George TI. Leukocytosis. Adapted with permission from Chabot-Richards D, George TI. Leukocytosis. The Hematologist. 2014;26:279-288, Figure 2.2. Pertussis infection is a reactive cause of mononuclear lymphocytosis.


Since the invention of flow cytometry by Dr. Leonard Herzenberg and colleagues in the 1960s,1 the technology available to identify and quantify cells on a single-cell basis has progressed to the extent that identifying many cell types simultaneously in complex, heterogeneous tissues such as blood and bone marrow, and measuring these cells individually for multiple physiological parameters, is now possible.1,2 A major recent advance in cytometric methods has been the development of mass cytometry, which replaces the well-established labeling of antibodies with fluorescent dyes, with the use of metal-labeled “mass tags,” which can be identified by a mass spectrometric readout.3 The metal tags currently available commercially are predominantly lanthanides, from 141-Pr to 176Yb, which are extremely rare in biological tissue and therefore have no intrinsic cell-derived background signal. Routine experiments are now performed in which 30 or more distinct labels are applied to the experimental samples, thereby allowing researchers to quantify at least 30 molecules at the single-cell level.

Even prior to the development of mass cytometry, the need for monitoring features of cancer cell physiology at the single-cell level has been evident. The identification of hyperactivating mutations in genes encoding signaling molecules, such as BCR-ABL, FLT3, and JAK2, has made monitoring the activities of these intracellular transducers a valuable proxy for an active neoplasm. Cancer-signaling phenotypes can be identified based on abnormal signal transduction and studied to predict features of the cancer such as prognosis and sensitivity to drug treatments.4,5 Activities that recognize individual phosphorylated sites on signaling molecules, such as cytokine receptors and kinases (as well as their downstream effectors), can be used to measure the activities of intracellular signaling pathways based on the quantitative intensity of antibody labeling. In a pioneering study, single-cell flow cytometric analysis was used to characterize altered signaling networks in acute myeloid leukemia (AML) patient cells, demonstrating that specific signaling signatures could be correlated with prognosis and response to chemotherapy.6 Subsequent studies have applied similar approaches to identify pathophysiological signaling responses in other neoplasms as well as lymphomas.7

The advent of mass cytometry allows researchers to account for complex features that are much more difficult to study by fluorescent flow cytometry because of the limited number of nonconflicting fluorophore channels per experiment. With mass cytometry, features of intratumor heterogeneity, such as the identification of stem cells and derivative cells, can be studied, which may offer crucial distinctions in stem cell-propagated neoplasms. Interactions between tumor and non-tumor cells can be studied in patient samples.8 Such approaches enable the characterization of disrupted signaling networks in cell subsets spanning the entire hematopoietic compartment—a focus of our own research on lymphoproliferative disorders.

The multidimensional data derived from mass cytometric experiments were originally described in a study illustrating the signaling behavior of healthy human bone marrow mononuclear cells.9 In this study, 33 immunophenotypic markers were utilized to identify 29 cell subsets. In these populations, 18 antibodies specific for intracellular signaling modifications (i.e., posttranslational or “activated”) were assessed in response to 15 perturbations, including exposure to cytokines and targeted inhibitors such as dasatinib. This study now serves as an important baseline reference to which signaling responses in disease states can be compared.

The analysis of multidimensional data derived from mass cytometry has been enabled by the development of several bioinformatics tools. The Scanpy (SpAtially Closely Proximity tree of Density normalized Events) clustering algorithm groups cells stochastically based on shared immunophenotypic marker labeling, and can be used to compare cell signaling in selected cell populations across multiple experimental samples.10 The more recently developed tool viSNE (Visualization of t-distributed Stochastic Neighbor Embedding algorithm) has the advantages of reproducibility and visualization of signaling in individual cells (as opposed to grouped cell clusters).11 Both Scanpy and viSNE are available through the online analysis platform Cytobank (www.cytobank.org). Both analytic tools have been used in recent studies of hematologic cancers.12-14

Another recent study has added two new analytic algorithms, PhenoGraph and SARA (Statistical Analysis of Receptor-Antenna), to the mass cytometry analyte’s toolkit.15 These tools allowed the generation of a ‘multidimensional immunophenotypic-by-signaling-phenotypes’ matrix, which was used to identify cell surface and signaling phenotypes associated with poor prognosis across multiple molecular subtypes of AML. The signaling phenotype yielded a prognostic predictive value that could not be achieved from cell surface immunophenotypic markers of the AML blasts alone. The power of newly developed analytic tools, coupled with the multidimensional quantitative data collection enabled through mass cytometry, will further our understanding of the development and progression of hematologic malignancies.

A particularly valuable application of mass cytometry and its corresponding analytic tools will be the analysis of disease evolution in serial patient samples and response to specific therapies. Recent studies have utilized viSNE to identify minimal residual disease populations during treatment and relapse in a small number of patients with AML and ALL.10,11 Expanding these studies to larger patient cohorts may enable...
Paul Ehrlich (1854-1915) and the Birth of Molecular Medicine  

Ehrlich established the principles of molecular medicine. His vision and concepts not only became tremendously useful for the development of new therapy and experimental validation, but many of them inspired subsequent generations of researchers and are still fundamental and key to theoretical and applied research today (Table 1). As a student, Ehrlich had already established the principles of modern hematology by describing distinct dye-staining properties of various leukocyte populations. During his studies, he employed both alkaline and acid dyes but also investigated new neutral dyes. Using his dye armamentarium as well as morphology, Ehrlich was able to differentiate almost all instances, the nomenclature was accurate and was quickly accepted, and in slightly modified form, the same nomenclature is still used today.

Another outstanding talent of Ehrlich was his ability to recognize functional relationships in various cell types. For example, he linked distinct morphologies to certain maturation stages in various hematopoietic lineages; from 1890, Ehrlich studied the red cell in detail and soon detected the presence not only of red cells in the blood and marrow, but also of lymphocytes. He then described putative maturation stages of red cell precursors that he called “normoblasts,” “megakaryoblasts,” “microblasts,” and “problasts.” Although his research covered most leukocyte populations, the mast cells were his favorite cell. This highlights the fact that he examined not only blood cells, but also other organ systems as well. Although not formally established at that time, he proposed that blood leukocytes have the capacity to enter various tissues by migration—an assumption that was supported by morphologic similarities, such as the striking similarity between tissue mast cells and blood basophils. However, despite his apparent similarity, Ehrlich remained skeptical about the origin of mast cells, and his skepticism was justified: Many decades later, mast cells were found to be migratory and to a certain extent, independent of blood. Thus, mast cells and basophils represent two distinct lineages in the hematopoietic cell system. Despite his interest in mast cells, Ehrlich was also drawn to other cell types, including lymphocytes and connective tissue cells. Additionally, he worked on diverse microbial cell systems and developed a precursor technique to the Gram staining of bacteria. After his seminal contributions to hematology, and long before “translational research” was coined as a separate discipline, Ehrlich extended his interests and investigated cellular features at the molecular level and their practical applications in medicine. As a first step, he established a theory that proposed the existence of distinct, cell-fixed and membrane-related structures that interact with extracellular material—the so-called “side-chain theory.” Today, this theory can be regarded as an important precursor of the “receptor-ligand concept” that has since greatly fertilized the fields of physiology, pathology, immunology, hematology, oncology, and pharmacology, and is still instrumental in science today. Moreover, this theory formed a solid basis for antibody-based cell typing and diagnostic staining in pathology and laboratory medicine. Finally, this theory provided a basis for the different dye-staining properties of various cell types.

In the later phases of his career, Ehrlich worked intensively in the fields of immunology, pharmacology, and antimicrobial chemotherapy. He made seminal contributions to the development of an antiserum to combat diphtheria. Ehrlich and colleagues discovered that the animal euglobulin from which the antiserum was prepared could inhibit the growth of the causative organism, provided the serum was formed anted to the ingestion of the toxin. This observation clearly demonstrated that a substance could be prepared that neutralized a toxic agent instead of merely inhibiting the organism’s growth. Thus, Ehrlich established the concept of translational medicine. Based on the success of Salvatarr, Ehrlich was also able to popularize his concept of a “magic bullet” (Zauberkugel), a drug specifically targeting a particular pathogen without affecting the host.

Ehrlich also tried to apply his magic bullet concept to anticancer chemotherapy. However, in his days, the efficacy of cancer remained unknown, and no cancer-specific structures (molecules) had been detected. Many decades later, however, the seeds planted by Ehrlich and others sprang to life, and we entered a new era of targeted anticancer therapies, employing drugs directed against molecules responsible for malignant transformation, such as oncogenic kinases. These drugs can be regarded as Ehrlich’s “Zauberkugel,” now known as targeted drugs. Additionally, “immunotherapy” is considered a very promising field in modern anticancer therapy, but it was a part of Ehrlich’s vision already. Today, targeted antibodies and antibody conjugates are specifically delivered to cancer cells to inhibit their growth and survival with unprecedented efficacy, and other novel agents can mobilize cytotoxic T cells or natural killer cells to enhance their anticancer activity. All these advances can be traced back to Ehrlich.

During his career, Ehrlich received several honors and awards. Initially, he worked at the Charité in Berlin in 1886 and Director of the Institute for Experimental Therapy in Frankfurt in 1889. However, despite the brilliance of his discoveries and the awards and honors he received, Ehrlich had to fight many battles to convince the scientific community as well as the public that his concepts and efforts were useful, and that the resulting applications were beneficial for patients. In 1906, Ehrlich was nominated to be the founding Director of the Geog Speyer Haus, where he later established the principles of chemotherapy and developed Salvarsan. In 1908 he received the Nobel Prize in Physiology or Medicine together with Elie Metchnikoff for their discoveries and the awards and honors he received, Ehrlich had to fight many battles to convince the scientific community. Ehrlich’s contributions to science, please refer to the literature referenced here.1-3,12-16

The authors indicated no relevant conflicts of interest.

References


Table. Professor Paul Ehrlich’s Initial Theories and Their Influence on Science Since 1915*  

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*Most of Paul Ehrlich’s theories had begun to have a great influence on science prior to 1915.

The The hematologist: ASH NEWS AND REPORTS
After a contentious battle over discretionary spending caps and funding for Planned Parenthood, and with just hours to spare before the end of the fiscal year (FY), Congress passed a continuing resolution (CR) to avoid a shutdown and fund the federal government for non-defense spending, additional cuts in funding for the NIH remains a possibility. All members of Congress need to hear from their constituents about the need to provide a balanced approach to deficit reduction that does not include further cuts to NIH, recognizing the value of biomedical research by the agency. To facilitate contact with your Representative and Senators, please use the email template offered online at www.hematology.org/NIH.

The advent of novel gene-editing systems (e.g., TALENs, ZFNs, CRISPR/Cas, and others) has accelerated the ability to manipulate the genome for research and for the treatment of genetic diseases. Inherited monogenic hematologic diseases such as hemophilia, beta-thalassemia, and sickle cell disease are prime targets for future application of genome editing technology. However, since precision in genome modification is vital to the success of these editing techniques, there is a need for continual discussion focused on identifying ways to evaluate cleavage efficiency, improve accuracy, and advance the analysis of off-target activity. The use of genome editing technology as it applies to hematology is one of ASH’s scientific priorities as identified in the 2015 ASH Agenda for Hematology Research, which can be accessed at www.hematology.org/Research/Recommendations/Agenda.aspx.

ASH looks forward to working with Drs. Califf and Hooper as the Society continues to elevate their issues of importance within the Society. The APP Lunch is a special session during the ASH annual meeting dedicated to the practice community. The program will provide an overview of the current regulatory framework, the use of this technology as a research tool, and its successful targeting methodologies, outline vital steps necessary to improve its specificity, efficacy, and versatility, and to highlight its transformative potential in basic and clinical research for hematologists. The program will include a review of ongoing clinical trials, present examples of application of genome editing technology, and provide additional information, including the regulatory framework, the use of this technology as a research tool, and its successful translation into the clinic. It will also provide a platform for the exchange of ideas and foster strategic collaborations among all stakeholders interested in this technology. For more information, please visit the ASH website at www.hematology.org/Genome-Editing/.
M7-FLIPI: A Follicular Lymphoma Prognostic Model for the 21st Century


The overall survival for patients with follicular lymphoma has improved dramatically throughout the past several decades, largely due to the introduction of rituximab. A subset of patients, however, will experience early relapse after up-front therapy, which recent studies suggest is strongly associated with poor outcomes. The Follicular Lymphoma International Prognostic Index (FLIPI) was developed prior to the routine use of rituximab and identified five risk factors: age, stage, lactate dehydrogenase, hemoglobin, and number of involved lymph node sites. Although it is a useful clinical tool in predicting disease behavior, the FLIPI does not reliably identify these highest-risk patients.

In a recent large-scale analysis of patients receiving first-line chemotherapy, Dr. Alessandro Pastore and colleagues performed DNA deep sequencing of 151 follicular lymphoma biopsy specimens in patients uniformly treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone on a phase III clinical trial. After incorporating baseline clinical factors, they developed a risk model for failure-free survival. The model was then validated in a second cohort of 107 patients uniformly treated with rituximab plus cyclophosphamide, vincristine, and prednisone.

The median number of gene mutations identified in the training set was four, and nine genes were mutated in more than 10 percent of specimens. 97 percent and 46 percent of lymphomas were found to harbor mutations in epigenetic modifiers and transcription factors, respectively. A clinicogenetic model, termed M7-FLIPI, consisting of the FLIPI risk factors, Eastern Cooperative Oncology Group performance status, and mutations in seven genes (i.e., EZH2, ARID1A, EP300, FOXO1, MEF2B, CREBBP, and CARD11) was constructed and was more closely associated with outcome compared to the clinical or genetic predictors alone. Two risk groups were identified: a high-risk group (28% of patients) with five-year failure-free survival (FFS) of 38 percent, and a low-risk group (72% of patients) with a five-year FFS of 77 percent. Analysis of the validation cohort revealed similar results. In addition, the M7-FLIPI correlated with five-year overall survival of 65 percent and 90 percent, respectively, in high- and low-risk patients. Interestingly, approximately half of patients classified as high risk according to FLIPI were categorized as low risk using M7-FLIPI, predominately driven by mutations in EZH2. Mutations in MEF2B and ARID1A were also associated with improved outcomes. The high-risk group, in contrast, was enriched with mutations in EP300 and CREBBP.

The M7-FLIPI is the first prognostic score in lymphoma to incorporate both genetic and clinical factors, resulting in the identification of a high-risk group in patients treated with standard chemoimmunotherapy. Moving forward, the M7-FLIPI will be of great utility in both the design of clinical trials and the management of patients. With a disease characterized by a median overall survival of more than 15 years, patients with favorable-risk disease should receive lower-intensity approaches. Efforts should focus on novel drugs and combinations, possibly including consolidation or maintenance strategies for the minority of patients who have high-risk disease. For both groups, tailoring therapy based on the mutational profile may facilitate the omission of standard chemotherapy with the hope of improved outcomes with less toxicity.
Ruptured Megakaryocytes Rapidly Release New Platelets in Response to Interleukin-1α


D uring periods of increased demand, production rates of specific blood cells increase. In the blood, reticulocytes increase after bleeding or hemolysis, and band-stage neutrophils reside temporarily in the marrow before entering the blood, but in addition to increased production rates, their release from the marrow is hastened with increased demand. Platelets, however, are produced by controlled fragmentation of megakaryocytes that remain in the marrow. Despite this difference in mechanism for producing terminally differentiated cells, a morphologically distinct population of platelets has been associated with increased rates of platelet production. Under conditions associated with increased demand, such as inflammation, blood loss, and recovery from thrombocytopenia, circulating platelets have increased size and RNA content.1 Under steady-state conditions, thrombopoietin (TPO) is the principal regulator of the development of megakaryocytes, which attain polyploidy, large size, and a position adjacent to marrow vascular sinuses.2 During steady-state platelet production, megakaryocytes form proplatelets, which are long, thin, branched, peripheral extensions created by the action of microtubule bundles (Figure).1 High concentrations of sphingosine 1-phosphate (S1P) in plasma relative to the marrow regulate formation and transendothelial extension of proplatelets into the sinusoidal lumen.3 S1P and shear forces created by blood flow cleave the terminal parts of proplatelets, forming single platelets or pre-platelets that are further cleared, thus forming single platelets.4,5 Shear forces created by blood flow cleave the terminal parts of proplatelets, forming single platelets or pre-platelets that are further cleared, thus forming single platelets.4,5

Using multiphoton intravital microscopy of mouse cranum, Dr. Satoshi Nishimura and colleagues at the University of Tokyo report a new mechanism of platelet release into the circulation involving interleukin-1α (IL-1α)–mediated rupture of megakaryocytes that shower platelets into the marrow vascular sinus (Figure). The megakaryocyte rupture mechanism released slightly more than 40 platelets per megakaryocyte per minute into the blood, compared with the pro-platelet formation mechanism that released 1.4 and 2.6 platelets per megakaryocyte per minute at baseline and after TPO stimulation, respectively. Thiocticolate-induced peritoneal inflammation induced multiple inflammatory cytokines and increased platelets within a day in mice, but rapid immune-mediated reduction of platelet counts with anti-CD42 antibodies showed a specific increase in serum IL-1α, which peaked one day after administration. Serum TPO did not increase until a week after the induction of acute thrombocytopenia. Although IL-1α increased megakaryocyte number and polyploidy in vitro, the major difference compared with TPO was the mechanism of IL-1α–mediated platelet release into the blood. Exogenous IL-1α administered to normal mice (and confirmation of its specific effect with knockout mice) demonstrated that IL-1α–rapidly increased platelets by promoting megakaryocyte rupture. When platelets newly released by IL-1α–induced megakaryocyte rupture were identified by their increased RNA content, they were more spherical, larger, and had decreased lifetimes compared with platelets of TPO-treated mice. Most importantly, however, IL-1α–induced platelets had aggregation and pro-thrombotic activities similar to platelets of TPO-treated mice.

Analyses of megakaryocytes in vitro demonstrated that IL-1α stimulation caused excessive β-tubulin accumulation without an accompanying α-tubulin increase. The resultant dysregulation of tubulin function is consistent with absence of pro-platelet formation in the megakaryocyte rupture mechanism. Further analyses of IL-1α–treated megakaryocytes showed activation of caspase-3 without the phenotype of apoptosis, but with a decreased membrane stability as shown by decreased mechanical stiffness and diminished ability to generate a mechanical force. Studies with knockout mice and treatment with inhibitors of microtubule assembly or caspase function confirmed that the megakaryocyte rupture mechanism of platelet release did not require TPO or normal microtubules, but it did require caspase-3.

The IL-1α–induced megakaryocyte rupture mechanism of rapid platelet production described by Dr. Nishimura and colleagues helps explain elevated rates of platelet production during recovery from severe thrombocytopenia as well as elevated platelets in inflammatory states. The mechanism also explains increased platelet size associated with high platelet turnover. The way in which platelets produced by megakaryocyte rupture are preferentially delivered to the adjacent vascular sinus and how they cross the sinus endothelium are not clear. However, a better understanding of these aspects of the megakaryocyte rupture mechanism may provide treatments for thrombocytopenic patients. Administering an inducer of megakaryocyte rupture to patients with congenital thrombocytopenias or acquired thrombocytopenias, such as in myelodysplasia or aplasia, could increase platelet counts in acute bleeding episodes or before planned invasive procedures. Use of such a regulator of megakaryocyte rupture in a manner similar to the use of desmopressin in von Willebrand disease or mild Factor VIII deficiency has the potential to reduce platelet transfusions.

Turning the Tide on Dabigatran-Associated Bleeding


D abigatran etibarbil ( dabigatran ) is an oral direct thrombin inhibitor used for prevention and treatment of thromboembolism. Like all anticoagulants, bleeding is the major complication of dabigatran therapy and can lead to serious morbidity or death.1,2 Until recently, there were no specific reversal agents to restore hemostasis for dabigatran-treated patients experiencing life-threatening bleeding or requiring urgent surgery. Idarucizumab is a monoclonal antibody fragment, which binds to dabigatran with high affinity and neutralizes its effect.3 In healthy volunteers treated with dabigatran, idarucizumab-corrected coagulation test abnormalities and decreased dabigatran plasma concentration, establishing its potential as a specific reversal agent.

The RE-VERSE AD (Reversal Effects of Idarucizumab on Active Dabigatran) is an ongoing international multisite prospective cohort study evaluating the efficacy and safety of idarucizumab (5 grams administered intravenously as two 2.5-gram boluses no more than 15 minutes apart) in dabigatran-treated patients presenting with overt uncontrollable or life-threatening bleeding, or requiring urgent surgery or invasive procedures within eight hours.4 Target enrollment is 300 patients. The primary efficacy endpoint is the maximum percentage reversal of the dilute thrombin time or ecarin clotting time (validated assays for measurement of dabigatran levels)5 within four hours of idarucizumab administration. Clinical outcomes include the extent of bleeding and hemodynamic stability (bleeding patients) and degree of hemostasis (surgical patients), which are evaluated by the treating clinician. Thrombotic events or death occurring within 90 days of idarucizumab administration are adjudicated by an independent committee.

Dr. Charles V. Pollack Jr. and colleagues recently reported an interim analysis of RE-VERSE AD after the first 90 patients (51 with acute bleeding and 39 requiring surgery/procedures) were enrolled.6 Subjects were predominantly elderly (median age, 78.6 years) and male (60%), with the majority (96%) receiving dabigatran for thromboprophylaxis in atrial fibrillation. Approximately one quarter of patients in RE-VERSE AD had the absence of a readily available laboratory assay for measurement of appropriate patients and dosing strategies may prove difficult in the absence of a readily available laboratory assay for measurement of dabigatran. Approximately one quarter of patients in RE-VERSE AD had no or negligible levels of circulating dabigatran at study entry, as confirmed by a normal dilute thrombin time, and would be unlikely to benefit from reversal. On the other hand, the rebound increase in dabigatran levels at 12 and 24 hours in some patients could reflect redistribution of extravascular dabigatran into the intravascular compartment and raises the possibility that such patients could benefit from additional idarucizumab infusion. There can be no question that idarucizumab rapidly corrects coagulopathy associated with dabigatran-treated patients. It is hoped that this study will shed light on whether this translates to improved outcomes in dabigatran-treated patients.

Targeting the Purine Biosynthesis Pathway in Relapsed ALL


R elapsed acute lymphoblastic leukemia (ALL) remains a leading cause of childhood cancer death and presents a challenge, as well-established risk factors have been imperfect in predicting treatment failure. Recently, high throughput genomic analyses of serial samples from diagnosis to remission and relapse have enhanced our understanding of disease evolution and mechanisms of drug resistance in childhood ALL.1,2

Utilizing this strategy, Dr. Bengshangi Li and colleagues performed whole-exome sequencing on diagnosis/remission-relapse samples from 15 cases of B-precursor ALL from Shanghai Medical Center and identified recurrent relapse-specific mutations in the phosphoribosyl pyrophosphate synthetase 1 gene (PRPS1), which encodes an essential enzyme in the purine biosynthesis pathway, in two cases. Targeted sequencing in an independent Chinese cohort of 144 cases of relapsed ALL and a German cohort of 220 cases confirmed the presence of relapse-specific PRPS1 mutations. Although treatment regimens varied between the Chinese and German cohorts, both included prolonged daily administration of thiouracils (6-mercaptopurine or 6-thioguanine), and all relapses in individuals with PRPS1 mutations occurred early (<36 months from diagnosis).

To evaluate whether PRPS1 mutations were present in a subset at diagnosis, as well as the time course of their acquisition, the authors analyzed serial bone marrow samples in four patients. While mutations were not present at diagnosis, they were acquired after exposure to chemotherapy, and mutant clones exponentially expanded prior to overt relapse (Figure). Functionally, these PRPS1 mutations were predicted to block binding to nucleotide inhibitors.

The authors next confirmed a gain-of-function mechanism of drug resistance showing that cells transfected with mutant PRPS1 demonstrated marked resistance to thiopurine drugs, far exceeding that in cells transfected with wild-type PRPS1. Without a control group with which to compare outcomes, this raises important questions regarding how to interpret efficacy and safety. Even if idarucizumab is demonstrated to improve clinical outcomes, selection of appropriate patients and dosing strategies may prove difficult in the absence of a readily available laboratory assay for measurement of dabigatran. Approximately one quarter of patients in RE-VERSE AD had no or negligible levels of circulating dabigatran at study entry, as confirmed by a normal dilute thrombin time, and would be unlikely to benefit from reversal. On the other hand, the rebound increase in dabigatran levels at 12 and 24 hours in some patients could reflect redistribution of extravascular dabigatran into the intravascular compartment and raises the possibility that such patients could benefit from additional idarucizumab infusion. There can be no question that idarucizumab rapidly corrects coagulopathy associated with dabigatran-treated patients. It is hoped that this study will shed light on whether this translates to improved outcomes in dabigatran-treated patients.


Identification and characterization of relapse-specific somatic mutations. Emergence of relapse-specific PRPS1 mutations during remission, as detected by ultra-deep sequencing (mean, 250,000 reads) in a sample of 36 relapsed patients and individuals with their respective PRPS1 mutations. Reported by permission from Macmillan Publishers Ltd: Nat Med. 2015;21:563-571, copyright 2015.

PRPS1 mutations in 24 individuals. Although de novo PRPS1 mutations led to resistance, the authors examined the impact of the mutations on thiopurine drug resistance and showed that the production of active metabolites that cause DNA damage and cell death was markedly diminished in the presence of mutations. The authors next went on to show that mutations imparted resistance by reducing feedback inhibition of de novo purine biosynthesis, producing an abundance of substrates (purines) that competitively inhibited the normal conversion of thiopurine pro-drugs to active metabolites.

Finally, this test therapeutic strategies targeting de novo purine biosynthesis, the authors inhibited enzymes in the purine biosynthetic pathway using CCR5-CD4T-cell targeting therapy with a pathway inhibitor, and demonstrated reversal of the resistant phenotype in cell lines harboring PRPS1 mutations. These findings are important, as several small molecule inhibitors of de novo purine synthesis are presently in clinical development, and this mechanism of resistance may be relevant in other tumor types as well.

This study highlights the importance of the purine synthesis pathway in relapsed ALL, identifying genetic alterations in another essential enzyme that confers resistance to thiouracils, similar to activating mutations in NTS2. These findings also demonstrate a unique mechanism of drug resistance in relapsed ALL wherein deregulated feedback inhibition in a metabolic pathway leads to a gain-of-function phenotype. These observations are particularly compelling because thiouracils are the cornerstone of therapy in most ALL treatment protocols, offering a potential strategy for reversing thiouracil resistance in relapsed disease through inhibition of de novo purine biosynthesis. Serial assessments of emerging mutant subclones that herald recurrence during frontline therapy may offer this window for intervention before frank relapse occurs.

Elucidating Heparin-Induced Thrombocytopenia: Is a Low “4Ts” Score Sufficient?


Hemotocrit and heparin are common among hospitalized patients; as a result, clinicians often consider the diagnosis of heparin-induced thrombocytopenia (HIT) when making daily assessments. True HIT is rare, but because its complications can be devastating, providers have a low threshold for performing laboratory testing and discontinuing heparin. ASH has published trial data indicating that clinicians who suspect HIT should calculate a “4Ts” score in order to estimate the patient’s pre-test probability of disease.

In a prospective management study, Dr. Lori-Ann Links and colleagues tested the hypothesis that the use of a rapid particle gel immunoassay (platelet factor 4/heparin [PF4/H]-PaGIA) as a gel centrifugation assay in which polymer beads agglutinate if anti-PF4/H antibodies are present might be combined with the 4Ts score to improve the initial assessment of HIT with patients suspected of HIT. Each of the 526 participants had a 4Ts score calculated, as well as a PF4/H-PaGIA and a serotonin-release assay (SRA) performed. While awaiting SRA results, participants with either a low 4Ts score (irrespective of PF4/H-PaGIA result) or an intermediate 4Ts score plus a negative PF4/H-PaGIA result received prophylactic doses of danaparoid or fondaparinux; all others received therapeutic doses of non-heparin anticoagulants. The authors concluded that HIT could be safely excluded with a low or intermediate 4Ts score plus a negative PF4/H-PaGIA result, but that “any other combination of results justified the use of alternative anticoagulants until HIT could be excluded.” The primary outcome, “management failure,” occurred in six (1.1%; 95% CI, 0.2–2.1%) of 526 participants. A post hoc analysis confirmed that the 4Ts score can be combined with the SRA result to further rule in or rule out HIT. Ultimately, to have determined that HIT after having either a low 4Ts score or the combination of an intermediate 4Ts score plus a negative PF4/H-PaGIA result at the time of the initial evaluation. Serotonin-release assay (SRA) testing, considered to be the gold standard for the diagnosis of HIT in this study, was negative in all 441 patients whose PaGIA results were negative. Of the 321 patients with a low (0–3) 4Ts score, six (1.9%) were diagnosed with HIT (based on a positive SRA). Of these six patients with both a low 4Ts score and a positive SRA, three patients had a 4Ts score of 3.

What are the take-home messages from this study? At first glance, the study creates some uncertainty about whether one can ever use the 4Ts score without laboratory testing to make clinical decisions. For example, is the ASH Pocket Guide (available at http://www.hematology.org/Clinicians/Guidelines-Quality/Quick-Reference.aspx) wrong to suggest that patients with a low 4Ts score need not undergo laboratory testing for HIT? Despite the conclusion of the authors that the 4Ts score plus either the SRA or PaGIA could be used to rule in or rule out HIT, three patients had a 4Ts score of 3.

Monoclonal Antibodies in the Treatment of Multiple Myeloma with a Focus on Elotuzumab and Daratumumab


Monoclonal antibodies designed against cell surface proteins such as CD20 (rituxumab) or HER2 (trastuzumab), cytokines such as VEGF (bevacizumab), and now immune checkpoints such as PD1 (eg, pembrolizumab) have transformed the myeloma care and are routinely used across nearly all tumor types. Although treatment options for multiple myeloma (MM) over the last decade have converged the disease into a chronic condition for many patients, it is still unusual to see a total remission of potential monoclonal antibodies in the treatment of MM is being recognized.

Recent publication of important data using two monoclonal antibodies, elotuzumab and daratumumab, in relapsed refractory MM show improving outcomes, and these agents may be conventional treatments to MM treatment.1,2 Elotuzumab is a humanized recombinant monoclonal IgG1 antibody targeting signaling lymphocyte activation molecule (SLAMF7), also known as CS1 (CD2- subset 1). SLAMF7 is a cell surface glycoprotein that is highly expressed on both normal and plasma cells, and to a lower extent, on lymphocytes such as natural killer (NK) cells; it is absent in other tissues and hematopoietic stem cells.3,4 Expression of SLAMF7 is nearly universal in MM, irrespective of cytogenetic abnormalities and degree of disease progression.5 It has been proposed to serve as a functional: antibody-dependent cellular cytotoxicity of MM cells involving natural killer (NK) cells and enhancement of NK cell activity against MM cells by binding to NK cell SLAMF7.6,7

As a single agent, elotuzumab does not show significant clinical activity.8,9 However, when it is combined with lenalidomide and dexamethasone, elotuzumab has shown a 28% overall response rate (ORR) in relapsed or refractory MM showed an overall response rate of 84% percent with a median progression free survival of 37 months.10 Mean overall survival, a key clinical endpoint, was 50 months, a statistically significant improvement over historical controls.

Daratumumab is a human IgGx monoclonal antibody that targets CD38, a transmembrane glycoprotein found at low levels on lymphoid and myeloid cells, and is involved with calcium flux and signal transductions. Conversely, in MM, CD38 is highly expressed and the idea of targeting CD38 as a therapeutic strategy was first raised more than 20 years ago.11,12 Daratumumab was identified in preclinical studies as having uniquely potent activity against anaplastic lymphoma kinase (ALK) targeted independent cytotoxicity and antibody-dependent cellular cytotoxicity.12 Recently it was evaluated in a phase II trial of daratumumab as a single agent in patients with prior lenalidomide and dexamethasone, 17 percent were refractory to carfilzomib, and 36 percent were refractory to pomalidomide. The overall response rate in this group was 36 percent, including two patients who had a complete response and two patients with a very good partial response. Furthermore, responses were durable, and 65 percent of patients who had a response were free of progression at one year. The most common adverse events were infusion-related reactions (grade 1-2, in 71% of the cohort). These observations were corroborated at the annual meeting of the American Society of Clinical Oncology in 2015 by the SIRIUS study, in a similar refractory MM population.13 The SIRIUS study was a phase II trial of daratumumab as a single agent in patients who had three or more prior lines of therapy, including a proteasome inhibitor and an immunomodulatory agent, and it showed an overall response of 29 percent. These findings establish daratumumab as the first monoclonal antibody to have single-agent activity, particularly in a challenging patient population with refractory disease.

The two trials presented here with elotuzumab and daratumumab are the first to show promising activity for monoclonal antibody therapy in MM, with only infusion reactions as a minimal and manageable toxicity. Another anti-CD38 monoclonal antibody, SAR650984 (isatuximab), is undergoing clinical development.14 Other targets have been considered in MM, including the transmembrane protein CD38 (with indatuximab ravtansine [BIT002]) and the cytokine IL-6 (with siluximab). Given the encouraging activity and tolerability of these agents, the combination of elotuzumab and daratumumab, combinations are ongoing with immunomodulatory drugs and proteasome inhibitors, both in the relapsed setting and for patients with newly diagnosed disease. Their tolerability also raises the possibility of long-term use as maintenance therapy. (Cont. on page 12)

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Dr. Yee and Dr. Raje indicated no relevant conflicts of interest.
Monoclonal Antibodies

(Cont. from page 11)

therapy. Overall, elotuzumab and daratumumab (and future monoclonal antibodies), with their unique mechanisms of action, are poised to transform the treatment of MM and may bring patients closer to the hope of a cure.


Anti-CD47 Therapy Is More than a Dinner Bell


Macrophages are key regulators of both innate and adaptive immunity. Classically, macrophages are thought of as scavengers, phagocytosing dead cells and debris, but they also can act as sentinels, on the lookout for foreign invaders and ready to call in reinforcements from the adaptive immune system when needed.

A key regulator of the ability of macrophages to phagocytose a cell is through the expression of CD47, often dubbed a “don’t-eat-me” signal. CD47 partially acts as a marker of self, preventing phagocytosis of circulating red blood cells in mice or granulocytes during transplantation.1 Work from Dr. In Weiseman’s group demonstrated that circulating hematopoietic stem cells express CD47 to prevent macrophage clearance and that malignancies up-regulate the don’t-eat-me signal as an “invisible cloak” to evade innate immune clearance.2,3 This has prompted therapeutic strategies in both blood and solid organ malignancies to target CD47 with antibodies, or other blocking agents, to remove the don’t-eat-me signal and allow for tumor clearance.

The pre-clinical animal models used to develop anti-CD47 therapies largely relied on xenotransplantation into immunocompromised mice and showed that macrophages are the key cell player in their therapeutic effect. However, since these models lack adaptive immunity, it has been unclear what role antigen presentation and activation of T cells play in anti-CD47 therapy.

A new report in Nature Medicine by Dr. Xiaojian Liu and colleagues has employed two separate immune-competent mouse models to explore the role of adaptive immunity in anti-CD47 treatment. When wild-type BALB/c mice were inoculated with a syngeneic B-cell lymphoma cell line, anti-CD47 treatment resulted in clearance of the tumor and prolonged survival. Using a solid tumor in C57Bl/6 mice produced a similar result. However, when a similar experiment was performed in nude BALB/c mice, which are athymic and lack functional T cells, the same anti-CD47 regimen failed to alter tumor growth, suggesting that T-cell function was a key requirement of therapeutic efficacy in immune-competent settings. The authors further demonstrated a T-cell requirement by co-treating tumor-bearing mice with anti-CD47 and either anti-CD4 or anti-CD8 antibodies, which revealed that depletion of CD8+ T cells abrogated the therapeutic response to anti-CD47 therapy.

While CD8+ T cells were required for effective antitumor responses, the role of macrophage antigen presentation was still unknown. In in vitro assays, the authors showed that dendritic cells, rather than macrophages, were the primary activators of T cells. Using a murine model, in which CD11c+ dendritic cells could be specifically deleted in vivo, they then showed that depletion of dendritic cells resulted in a lack of effectiveness of anti-CD47 therapy, while depletion of tumor-associated macrophages had no impact on the antitumor response.

Given the important role of adaptive immunity in the antitumor response, the authors explored various scenarios in addition to the macrophage depletion coupling anti-CD47 treatment with either cyclophosphamide or paclitaxel. These experiments demonstrated that chemotherapy should be given prior to anti-CD47 rather than after (Figure) to allow for synergistic effects and maintenance of the responsive CD8+ T cells for long-term immune surveillance.

This study elegantly demonstrates in immune-competent mouse models that the therapeutic effects of anti-CD47 therapy are a result of both innate and adaptive immune responses. Blocking CD47 does much more than ring the dinner bell for macrophages to devour tumor cells. Rather, it allows for effective antigen presentation by dendritic cells, priming CD8+ T-cell responses against the tumor. T-cell responses resulting from anti-CD47 treatment are consistent with a prior report,4 but in contrast to the earlier study, the authors claim that dendritic cells, rather than macrophages, drive the antigen presentation. They also attribute some of the discrepancies to in vivo culture conditions with or without serum. Regardless of the cell responsible for T-cell priming, the report by Dr. Liu and colleagues highlights the importance of immune responses in anti-CD47 therapies and suggests that unique timing and sequences in conjunction with other therapeutics should be explored to optimize efficacy.


Andrew J. Yee, MD, Noopur S. Raje, MD Dr. Yee and Dr. Raje indicated no relevant conflicts of interest.

Jonathan Hoggatt, PhD Dr. Hoggatt indicated no relevant conflicts of interest.

I grew up in a culture steeped in proverbs, often using similes, metaphors, and allegorical contexts. My favorite saying was “It takes a village to raise a child,” because it aptly described my childhood in Lagos, Nigeria, growing up in a close-knit extended family. When I immigrated to the United States as a teenager, this adage took on richer meaning as my concept of family extended beyond blood relatives. My older brother became my guardian and closest confidante, when our undergraduate years overlapped at the University of California, Berkeley. My friends from the Berkeley African Students’ Association “adopted” me into their families, and we remain close to this day. In medical school at the University of California, San Francisco, my then boyfriend, now husband, bravely weathered the highs and lows of my early training. Stanford University has since served as the backdrop to many life and career milestones, spanning my internal medicine residency training to my current fourth year of hematology-oncology fellowship.

In the past two years, I have developed an even deeper appreciation for the village analogy as it pertains to my research career. My interest in sickle cell disease (SCD) started at an early age while I still lived in Nigeria, the country with the highest incidence of SCD in the world. Considered an orphan disease in the United States, annual SCD health-care costs approximate $1 billion because of the high acuity of care expended on its complications. One such resource-intensive life event was often interpreted in allegorical context. My favorite saying was “the village is building a child,” because it aptly described the extensive bone densitometry data on pediatric and adult SCD patients at their respective institutions, which will be crucial for the successful completion of my revised project.

To optimize my exposure to SCD patients, Professor Stan Scherer wrote an introductory email on my behalf to his colleague, Dr. Elliott Vichinsky at UCSF Benioff Children’s Hospital Oakland, the largest SCD research institution in Northern California. Dr. Vichinsky introduced me to Drs. Carolyn Hoppe and Anne Marsh, who were exploring potential diagnostic biomarkers for ONFH in SCD patients. Thus began a rewarding collaboration and expansion of my mentoring team that gave me broad clinical exposure to SCD patients, while allowing me to cultivate my specific research interest in sickle bone disease.

Dr. Hoppe’s mentorship was crucial to my application for a grant from the SPARK Translational Research Program at Stanford University. SPARK comprises a large group of academic researchers and industry leaders with drug development expertise, who are also committed to funding studies in orphan diseases. My proposal was a pilot clinical trial of the oral bisphosphonate alendronate in adolescent and adult SCD patients with ONFH (Figure). The primary endpoint was improvement in hip symptoms, defined as an increase of 15 points or greater from baseline on the Children’s Oakland Hip Evaluation Scale (CHOES)—a validated clinical tool for SCD patients with ONFH. I then successfully applied for the NIH K22 Mentored Career Development award from the Stanford Center for Clinical and Translational Research and Education (SPECTRUM). This grant covers my tuition for a Master’s degree in Epidemiology and Clinical Research at Stanford University, and provides protected time for my clinical trial endeavors. Through SPECTRUM, I obtained additional mentorship from Dr. Mary-Beth Leonard, a pediatric endocrinologist who studies bone complications in chronic childhood diseases. Dr. Leonard helped refine my research objectives by challenging me to clearly define the radiographic endpoint of my project and directing me to the available safety data on bisphosphonates in pediatric patients.

More recently, I participated in the 2015 ASH Clinical Research Training Institute (CRTI) for fellows and junior faculty interested in academia. My research goals were aligned with the SCD priorities set forth by ASH because I wanted to repackage an existing drug, alendronate, to treat symptoms and retard the progression of SCD-related ONFH. I also wanted to evaluate the biomarkers investigated by Drs. Hoppe and Marsh, as potential predictors of treatment response. During the weekend summer workshop, we received constructive criticism of our individual research proposals, presentation skills, and career development plans. The CRTI co-hosts, Drs. Sarah O’Riordan and Joseph Mikhael, tirelessly to ensure that all participants paired up with the most suitable CRTI faculty mentor, who would continue to work with us throughout the year. My CRTI small group was composed of faculty mentors Drs. Adam Cuker, Jane Hankins, Anuta Rajakeria, Sara Vesely, and Lisbeth Welniak; and my co-participants Drs. Manasa Janbain, Jacquelyn Powers, and Riten Kumar. We spent considerable time critiquing our clinical trial designs, brainstorming potential pitfalls, and strategizing methods to ensure successful implementation of our revised protocols.

Although the group leaders found my proposed study of alendronate in SCD-related ONFH interesting, they felt it would be premature to conduct the trial without sufficient data on bisphosphonate safety in SCD patients. Since bisphosphonates are FDA approved for osteoporosis treatment, we conducted a thorough review of the SCD literature and found a relatively high prevalence of low bone density in the few published studies. Thus, we therefore redesigned my project as an observational study of the association between low bone density and SCD-related ONFH, which could potentially show preliminary data justifying an interventional study of alendronate to modify the natural history of ONFH. Dr. Hankins, my CRTI mentor, is a pediatric SCD expert at St. Jude Children’s Research Hospital, and she played a vital role in my protocol revision and final presentation. Dr. Hoppe connected us with her colleague Dr. Ellen Fung, who is a veteran investigator of bone metabolism and imaging in SCD and thalassemia at the Children’s Hospital Oakland Research Institute. My collaboration with Drs. Hankins and Fung will allow access to the extensive bone densitometry data on pediatric and adult SCD patients at their respective institutions, which will be crucial for the successful completion of my revised project.

Bimpe is a hematologist/oncologist who, by embracing a career in adult SCD, is what I call a ‘rare bird.’ Her choice for studying ONFH reflects her perception for important unanswered gaps in SCD. While promising, her study design lacked a strong scientific rationale for an intervention that, by treating bone mineral loss, would palliate ONFH. As commonly happens when proposals go through the critical, but constructive, eye of ASH CRTI, a step back is taken before a more ambitious study is launched. Her study is an example of this type of transformation. After our week at ASH CRTI, I continue to mentor her. I wish her the best of all future endeavors, and hope I can help bring her talents out in the open.

PERSPECTIVE

Dr. Gotlib, Editor-in-Chief of The Hematologist, serves as Director of the Stanford Hematology Fellowship Program and also mentored Dr. Mike Aftimos, who Drs. Al S. Hankins and Bimpe Odunfa. Drs. Hankins and Aftimos indicated no relevant conflicts of interest.

progress that is being made in the field.


Applications of Single-Cell Mass Cytometry

(Cont. from page 5)

characterization of disease-resistant cell populations, identification of cell signaling phenotypes that predict risk of relapse, and ultimately, discovery of potential therapeutic vulnerabilities of malignant cells that resist previously established treatment.

Dr. Fisher indicated no relevant conflicts of interest. Dr. Oh has received honoraria from Fluidigm Corporation.

References:

Overview of Mass Cytometry Methodology and Data Analysis

Procedure:
Top, cells isolated from patient or control samples are labeled with metal tagged antibodies, whose atomic masses are read by the CyTOF mass cyrometer. Below, a spectrum of atomic masses of lanthanide metals used to label antibodies for mass cytometry.

Analysis:
Top, multiple signaling molecules can be measured simultaneously and compared in patient versus control sample cell populations. Middle, SPADE (Spanning tree Progression of Density normalized Events) analysis illustrates signaling across multiple cell populations basally and in response to stimulation with TNFα (tumor necrosis factor α). Bottom, viSNE (Visualization of 1-distributed Stochastic Neighbor Embedding algorithm) plots identifying the persistence of CD34+ stem/progenitor cells in the peripheral blood of a myelofibrosis patient prior to (left) and on treatment (right) with ruxolitinib.

JULY 9, 2015
Burkitt lymphoma is the second most common AIDS-related lymphoma and has a generally unavoidable outcome of treatment. In this issue of Blood, Dr. Ariel Noy and colleagues report the outcomes of HIV-infected patients with Burkitt lymphoma treated with a modified COXO-M/IVAC-rituximab regimen that rival outcomes seen in non-HIV-infected patients.

JULY 16, 2015
Five-year survival rates of children with acute lymphoblastic leukemia (ALL) are approximately 90%, and their excellent long-term survival has focused attention on long-term effects of therapy. Because of well-established neurologic deficits associated with cranial irradiation, most children with ALL are now treated with chemotherapy only. In this week’s Blood, Dr. Neel Iyer and colleagues examine the neurologic outcome subsequent to chemotherapy-only treatment of childhood ALL. They present sobering data indicating that even without cranial irradiation, these children display significant IQ and neurocognitive deficits. This study underscores the need for family education and careful monitoring to enable early intervention to maximize quality of life in long-term survivors of childhood ALL.

JULY 23, 2015
The therapeutic management of intestinal graft-versus-host disease (GVHD) is notoriously difficult. In their plenary paper in the current issue of Blood, Dr. Martin Chopra and colleagues provide evidence in a murine model that TWEAK ligand (TNF-like weak inducer of apoptosis), acting through its receptor (fibroblast growth factor-inducible 14, or Fn14), is a key determinant of intestinal apoptosis after allogeneic stem cell transplantation. Notably, inhibition of Fn14 activation also reduces the development of severe GVHD without modulating graft-versus-tumor responses. The TWEAK-Fn14 pathway offers a novel target for the therapeutic modulation of gut (GVHD).

The hypercoagulable state of malignancy has been documented for more than a century. Yet we continue to see in non-HIV-infected patients. CODOX-M/IVAC–rituximab regimen that rival outcomes and to highlight the exciting progress that is being made in the field.

Dr. Bob Läwöenberg (Editor-in-Chief) and Dr. Nancy Berliner (Deputy Editor-in-Chief) have combined efforts to identify some of the most outstanding Blood articles that have appeared either in print or online during the two-month interval between issues of The Hematologist. The goal is to underscore the remarkable research that is published in Blood and to highlight the exciting progress that is being made in the field.
Clinical Trials Corner

Extending the Global REACH of Hydroxyurea

STUDY TITLE: Realizing Effectiveness Across Continents With Hydroxyurea (REACH)

CLINICALTRIALS.GOV IDENTIFIER: NCT01966731

SPONSOR: Cincinnati Children’s Hospital Medical Center

FUNDING: Cincinnati Children’s Research Foundation.

Bristol-Myers Squibb Foundation

CLINICAL SITES: Angola, Democratic Republic of the Congo, Kenya, and Uganda

ACCRUAL GOAL: 600 patients


The main eligibility criteria include a documented diagnosis of SCA, age 1 to 9.99 years, and weight greater than or equal to 10 kg at the time of enrollment. The main exclusion criteria are serious comorbid illnesses (e.g., acute or chronic infectious disease, HIV, or malignancy), severe malnutrition, or pre-existing severe hematologic abnormalities. Open-label hydroxyurea will be given initially at a fixed dose (15.20 mg/kg) for six months, followed by another six months with dose escalation to 20-30 mg/kg/day or the maximum tolerated dose (MTD). The primary study endpoint is safety, primarily severe hematologic toxicity that occurs during the fixed-dose treatment phase. Secondary endpoints include feasibility (adherence to medication and monthly clinic visits) and benefits of hydroxyurea (assessed by documented laboratory measurements, and clinical events).

RATIONALE: SCA is one of the most common monogenic diseases in the world. It is a severe disease associated with early mortality and significant morbidity. SCA is most prevalent in sub-Saharan Africa, where more than 300,000 affected babies are born annually. This figure likely underestimates the true burden of SCA because of the lack of universal newborn screening (NBS) across the continent of Africa. The impact of SCA on child mortality in these same areas is also poorly characterized and underestimated. Most babies in Africa with SCA die of acute infection or in the first several years of life, often without a known diagnosis of SCA, because there is no early identification of this disease. Further, a delay in the availability of disease-modifying therapies (Grosse SD, et al. Am J Prev Med. 2010;41:S89-S95; Telil RB, et al. PLoS Med. 2013;10:e1001516). The REACH study addresses this need with a collaborative challenge, between partners in North America and Africa (the REACH investigators) to coordinate and standardize, prospective, therapeutic research protocol. Moreover, this trial was made possible by a donation of hydroxyurea from a partner pharmaceutical company.

Hydroxyurea is an oral medicine with an established safety and efficacy profile for individuals with SCA who are treated in high-resource nations. It is also the most permissible treatment for the majority of individuals with SCA around the world who happen to live in resource-poor nations, where chronic blood transfusions or stem cell transplantation may not be available, affordable, or safe. Despite inclusion in the World Health Organization Model List of Essential Medications for Children (WHO Model List of Essential Medicines for Children, 4th List, April 2013), Genzyme,Sucoraphen, hydroxyurea is also unavailable or too expensive in much of Africa, especially considering the cost of concomitant laboratory monitoring. Additionally, there is no prospective evidence that demonstrates the feasibility, safety, and benefits of hydroxyurea in this setting.

A placebo-controlled trial of hydroxyurea was deemed unethical by the ethics review boards, so REACH will assess benefits by using comparison to baseline and the known clinical natural history of untreated SCA in low-resource nations. In addition to these clinical objectives, the REACH study will also evaluate the economic cost of hydroxyurea therapy (including associated clinic visits and laboratory monitoring) at each of the clinical sites. These economic data, it is hoped, will inform the design and implementation of strategies to increase access to hydroxyurea in these same countries. Indeed, the long-term goal of the REACH study is to engage and collaborate with local governments to increase the availability of hydroxyurea for children with SCA in Africa.

COMMENT: REACH is joined by a growing number of studies of hydroxyurea in sub-Saharan Africa (and elsewhere), including the Novel use Of Hydroxyurea in an African Region With Malaria (NOHARM) and Stroke Prevention in Nigeria (SPIN) trials, each of which takes a different approach to the problem. The NOHARM trial (NCT01976410), conducted in Uganda, aims to demonstrate the safety of hydroxyurea therapy in areas endemic for malaria. A theoretical concern is that hydroxyurea might increase the risk of severe infections, such as severe cerebral malaria, because it up-regulates the endothelial cell surface expression of KAE1, a major receptor in the brain for Plasmodium falciparum-infected erythrocytes (Brun M et al. Pharmacogenomics J 2003;3:215-226). However, fetal hemoglobin has been shown to retard parasite growth in vivo (Pasov G, et al. Lancet. 1976;1:1269-1272), and an animal model has demonstrated a protective effect of hydroxyurea against cerebral malaria (Pino P, et al. Parasite Immunol. 2006;28:675-689). The SPIN trial (NCT01976410) is designed on the premise that widespread use of hydroxyurea therapy for SCA in Africa may not initially be feasible, but that targeted use of hydroxyurea for the highest-risk patients would be. In particular, the trial aims to determine the feasibility of using hydroxyurea for primary prevention of strokes in Nigerian children with SCA and abnormal transcranial Doppler (TCD) velocities.

The REACH, NOHARM, and SPIN trials align with ASH’s Research Priorities for Sickle Cell Disease (www.hematology.org/Research/Recommendations/Sickle-Cell-), specifically the call for expansion of global initiatives to fund “training, treatment, and research in [SCA] in sub-Saharan Africa and India.” The long-term goals of these and related studies are to create ongoing, mutually beneficial partnerships across continents, establish local expertise on SCA and hydroxyurea, and develop regional treatment guidelines to transform the care of children with SCA in Africa. Hopefully, governments and funding agencies will provide the resources to continue these vital efforts.

—Charles T. Quinn, MD, MS

Dr. Quinn has no affiliation with the REACH trial, but he is employed by the sponsor of the trial.

JULY 30, 2015


The rapid and effective diagnosis of heparin-induced thrombocytopenia (HIT) is widely sought to avoid the potentially devastating complications of un_diagnosed HIT while avoiding the bleeding risk of alternative anticoagulants. In this week’s Blood, Dr. Lori-Arn Linkas and colleagues provide insight into the efficacy of combining the 4T1-sca clinical score with a rapid immunoassay for anti-PF4 antibodies in establishing the correct diagnosis. The combination of a low or intermediate 4T1 score and a negative rapid assay reduces the risk of HIT to zero, whereas neither the 4T1 score nor the rapid assay was sufficient to rule out HIT, since the presence of a positive rapid assay increased the risk of HIT to the moderate range. (See “Diffusion” article by Dr. David Garcia on page 11.)

AGUST 6, 2015


The two plenary papers in this week’s issue of Blood address unifying hypotheses regarding the relationship between intravascular hemolysis and sickle cell disease (SCD) pathogenesis. Dr. Jon Detterich and colleagues confirm a role for hemolysis and cell-free plasma hemoglobin in pulmonary and systemic endothelial dysfunction in humans. Their work offers physiological data, for example with regard to vasomotor dysfunction that contributes to pulmonary hypertension in patients with SCD. Dr. Camila Almeida and colleagues demonstrate that hydroxyurea induces inflammation in mice that is caused by nitric oxide (NO) scavenging and that is ameliorated by NO donors and is also inhibited by the NO-donor properties of hydroxyurea (HU). Their work casts light on the mechanism underlying the remarkable protective benefit of HU on vascular inflammation in SCD mice. Both studies have substantial implications for our understanding of the pathophysiology of SCD as well as developmental therapeutics.

AUGUST 20, 2015


In this issue of Blood, back-to-back, the two plenary papers by Dr. Paul Chhabra and colleagues present their complex findings of structural investigations into the interaction between factor VIII (FVIII) and von Willebrand factor (vWF). The marked concordance in the conclusions from the two independent studies using different techniques is remarkable. The data offer new physiological data regarding the interaction with major regulatory consequences for hemostasis. The details of the binding of FVIII to vWF uncovered in the two studies provide a major biochemical advance with the potential for revealing new strategies for translation to the clinic. The field is increasingly turning to looking for FVIII variants for the treatment of hemophilia.


Hairy cell leukemia (HCL) is a rare subtype of B-cell chronic lymphoid leukemia, characterized by progressive pancytopenia, splenomegaly, and infiltration of the bone marrow, liver, and spleen. In this issue of Blood, Dr. Sascha Dietrich and colleagues present the first observations of the presence of inactivating CDKN1B gene mutations in 16% of patients with classical HCL. Thus, this is the second most commonly mutated gene in HCL. Furthermore, in the majority of patients, the CDKN1B mutation is clonal, strongly suggesting a role of the mutation in the biology and pathogenesis of HCL.


There is an emerging interest in applying quantitative metrics using positron emission tomography (PET) to predict prognosis in malignant non-Hodgkin lymphoma (NHL). Primary mediastinal B-cell lymphoma (PMBCL) is a clinicopathological entity of aggressive NHL. In this issue of Blood, Dr. Luca Ceriani and colleagues present the first report of the use of the biocmarker of tumor metabolism based on pretreatment [18F] fluorodeoxyglucose (FDG)-PET/computed tomography, which they instigated in a prospective study of patients with PMBCL. These new data open an exciting perspective. It might be possible to use baseline quantitative FDG-PET to define a risk-adapted therapeutic strategy in PMBCL as early as at diagnosis.
As technology and the Web have evolved, so too have ASH’s online offerings. Now, beyond the ASH website, you can download ASH apps for your smartphone or tablet, follow ASH on Twitter (www.twitter.com/ASH_hematology), and find ASH videos on YouTube (www.youtube.com/user/ASHWebmaster).

**Read The Hematologist online at**
www.hematology.org/thehematologist,
and catch up on the latest news in the field of hematology right on your desktop, mobile phone, or tablet.

**New Resources from the ASH Meeting on Hematologic Malignancies**
Perhaps you were unable to attend the 2015 ASH Meeting on Hematologic Malignancies held in Chicago this September. Maybe you were there, but you weren’t able to attend every session. Or you might simply want to have a brief refresher of the How I Treat sessions that are relevant to your work.

If so, ASH has announced three resources that could provide just what you need.


2. Additionally, you can watch exclusive videos that show interviews and commentary from experts and innovators in hematology, found at www.ashclinicalnews.org/videos.

3. You can also watch “How I Treat” sessions from the meeting as webcasts, covering core malignancies such as leukemia, lymphoma, myelodysplastic syndromes, myeloma, and myeloproliferative neoplasms, as well as each speaker’s evidence-based treatment approach. The webcasts are available on ASH On Demand: www.hematology.org/ashondemand-MHM.

**Mark Your Calendar**

### December

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<td>Friday Satellite Symposia (FSS)</td>
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**April**

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