A New Crystal Ball: Day-30 Mutation Analysis after AML Induction


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, such as FLT3, which activates signaling pathways. Driver mutations provide a survival or proliferative advantage to the cell that promotes the development of AML. There is also acquisition of additional subclonal mutations over time (including after treatment), 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. 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. 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 Kilo 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 (at either 100 or 200 mg/m²/day, and three additional similarly treated patients were added to this group. Of these 71 patients, 34 were refractory, 12 relapsed between six and 12 months, and 25 survived at least 12 months without an allogeneic transplant. For these three groups, there were no differences in the number of detectable subclones, the number of total genomic variants, or coding mutations.

The study group comprised 25 patients with sufficient DNA from paraffin-embedded samples at pre-treatment and 30 days post treatment, and an additional 25 patients for whom they had cryopreserved samples at diagnosis, first remission, or at relapse (Figure). All 50 patients achieved CR with less than 5 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 below the level of 5 percent of the cells with the mutation (< 2.5% variant allele frequency for a heterozygous mutation). All 18 patients with mutant NPM1 were also able to clear the mutation by day 30. There were 24 patients who had at least one mutation detectable after AML induction and outcomes in acute myeloid leukemia. JAMA. 2015;314:811-822.

Authors’ Note: This article is dedicated to the achievements of Paul Ehrlich on the occasion of the 100th anniversary of his death (August 20, 2015).

Between 1850 and 1915, the “new” fields of chemistry, biology, and medicine made revolutionary progress thanks to the seminal contributions of a number of outstanding scientists, including (among others) Louis Pasteur, Robert Koch, Emil Fischer, Rudolf Virchow, and Emil Behring. Inspired by this atmosphere of great discoveries, Paul Ehrlich, born in Strehlen, German Kingdom of Prussia, in 1854, became one of the most influential scientists of his time and a pioneer in the fields of hematology, immunology, chemotherapy, and pharmacology. In his career, Ehrlich connected cellular and molecular theories, discovered and exploited related biological principles, and demonstrated their practical implications. Through this contemporary approach,

(Cont. on page 3)
Leading the Charge for Precision Medicine

Nearly 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.hematology.org/Newsroom/PressReleases/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 mechanisms and standards. It was the realization in 2015 that ASH defined a new set of research priorities, and the areas of genomic profiling and chemical 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 delineate 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 can be used in treatment decisions that are clinically meaningful. To spotlight just a few of these opportunities, Dr. Jill Johnsen 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

Day-30 Mutation Analysis after AML Induction

(Cont. from page 1)

at the 5 percent level or higher, and these patients exhibited a reduced median EFS of only six months compared with 17.9 months for the 26 patients who cleared the mutations. The respective median OS rates for these two groups were 10.5 months versus 42.2 months. Moreover, for patients with intermediate-risk karyotype, similar reductions of median EFS (8.8 vs. 25.6 months) and OS (19.3 vs. 46.8 months) were observed if mutations were still detected on day 30. There has been considerable difficulty in finding utility in the identification of what now amounts to hundreds of mutations in AML. Investigators continue to struggle to find a practical application of next generation sequencing in terms of modifying treatment, with few exceptions (e.g., FLT3). As there are already alternative methods to identify MRD with rapid turnaround times (~1 day) and higher sensitivity, such as multicolor flow cytometry (sensitivity at least 0.1%) and FISH (sensitivity in the range of 1% to 7%), it remains to be seen under which circumstances the mutation testing described by this study would be superior to current methods. There may be selected circumstances (for example, patients with normal karyotype who have well-defined mutations associated with prognosis) for which such monitoring may be helpful, especially if a rapid method could be developed that would enhance the reliability of detection of mutations present in less than 0.1% of the cell population. Only the future will tell.


CORRECTION: There were inaccuracies in Figure 2 of the “Ask the Hematologist” article by Dr. Shinji Nakao in the September/October 2015 issue. The figure has been corrected, and the online article reflects these edits.
ASH Elects New Leadership

VICE PRESIDENT: Alexis Thompson, MD, MPH

Dr. Thompson will serve a one-year term as vice president followed by successive terms as president-elect and president.

COUNCILLOR: Jane Winter, MD

Dr. Winter will serve a four-year term as councillor.

COUNCILLOR IN CLINICAL PRACTICE: Steven Allen, MD

Dr. Allen will serve a four-year term as councillor.

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.

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.
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 (fg/dL). The laboratory findings were as follows: WBC, 7.4 × 10⁹/L with 29 percent neutrophils, 66 percent lymphocytes, and 5 percent monocytes; RBC, 3.94 × 10¹²/L; hemoglobin, 11.0 g/dL; mean corpuscular volume, 83.5 fL; platelets, 91 × 10⁹/L. The patient’s bone marrow was hypercellular (90%) with a marked lymphohistiocytic 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 plasma celloid lymphocytes were also observed. The karyotype of the bone marrow was 46.XY add(9)(p24); der(11)t(11;13)(p13;13)(q25) in four cells with a sideline containing all of these abnormalities and +13 in two cells, and an unrelated clone showing 45,X;Y in six cells, with 46,XY in seven cells. Fluorescence in situ hybridization (FISH) found the translocation of the 13q14.3 region and was negative for deletions of TP53, ATM, and LAMP1, and aneuploidy for chromosome 12.

My Response

Examining 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 feathered 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 mononucleosis, 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 "hatchet" cells. However, examination of RBCs and platelets should still be performed on the original blood smear because the albumin can affect platelet and erythrocyte morphology.

Reactive Lymphocytosis

Separating a monomorphic lymphocytosis from a pleomorphic lymphocytosis can help distinguish a lymphoproliferative disorder from a reactive lymphocytosis, respectively.1 Most reactive lymphocytoses show a wide range of sizes and shapes in lymphocytes. The classic example of a pleomorphic 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 lymphocytoses are pleomorphic, a few important exceptions are worth mentioning. The first is Bordetella 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 polycyclic B-lymphocytosis, which typically shows lymphocytes with distinct nuclear nuclets but will demonstrate a spectrum of morphologic changes including nuclear lamination 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 neutropenia and variable anemia should raise suspicion for large granular lymphocytic leukemia.1 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 clonality or KIR analysis, if involving T cells or natural killer cells, respectively.

Neoplastic Lymphocytosis

Lymphoma 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 blood lymphocytes. While CLL is the most common leukemia in adults in the western world and is frequently seen in peripheral blood (or its monoclonal B-cell lymphocytosis counterpart), peripheral blood involvement by bone marrow lymphoma is found in up to 30 percent of subjects in some studies.2 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.4 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 CCND1/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 × 10⁹/L or greater that had been called suspicious for a lymphoproliferative disorder after smear review by a pathologist.1 Using receiver operating characteristic (ROC) analysis, they found that an absolute lymphocyte count greater than 6.7 × 10⁹/L for patients 50 to 67 years of age, and 4 × 10⁹/L or greater for 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 × 10⁹/L in a consecutive three-month period and found that 6.8 × 10⁹/L was the best cut-off value for predicting a lymphoproliferative disorder with ROC analysis (sensitivity 90%, specificity 59%).5 In 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 FMC7, in addition to the usual phenotype by FISH for expression of t(11q23). Morphology in the current blood and bone marrow showed lymphocytoid lymphocytes and plasmacytoid cells and not the usual small round lymphocytes with coarsely clumped chromatin ("soccer balls") of CLL; the plasmacytoid cells were not readily identifiable 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 CD23, 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 SOX-11 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 D1.1 LEF1 was negative, providing no support for a diagnosis of CLL. 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 (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. Thus, 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

ASH NEWS AND REPORTS

The Hematologist: ASH NEWS AND REPORTS

ASH NEWS AND REPORTS
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 subsets simultaneously in complex, heterogeneous tissues such as blood and bone marrow, and measuring these cells individually for multiple physiological parameters, is now possible. A major recent advance in cytometric methods has been the development of mass cytometry, which replaces traditional, established labeling of antibodies with fluorochrome dyes, with the use of metal-labeled “mass tags,”2 which can be identified by a mass spectrometric readout.2 The metal tags currently available commercially are commercially available lanthanides, from 141-Pr to 176-Yb, 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 mass tag-labeled antibodies 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 and downstream signal 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 prognosis3 and sensitivity to drug treatments.4 Antibodies 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.5 Subsequent studies have applied similar approaches to identify pathophysiologic signaling responses in other myeloid neoplasms as well as lymphoid malignancies.6

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The multidimensional data derived from mass cytometry experiments were originally described in a study illustrating the signaling behavior of healthy human bone marrow mononuclear cells.1 In this study, 33 immunophenotypic markers were utilized to identify 29 cell subsets. In these populations, 18 antibodies specific for intracellular signaling modifications (mainly phosphorylation of signaling effectors) were assayed 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 SPADE (Spanning tree Progression 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.7,8 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).9 Both SPADE 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.10,11

Another recent study has added two new analytic algorithms, PhenoGraph and SARA (Statistical Analysis of Response Amplitude), to the mass cytometry analyst’s toolkit.3 These tools allowed the generation of a ‘multidimensional immunophenotypic-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 phenotypes enabled a prognostic predictive value that could not be achieved from cell surface immunophenotypes of the AML blast cell 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 release of small numbers of patients with AML and ALL.12 Expanding these studies to larger patient cohorts may enable...
Paul Ehrlich (1854–1915) and the Birth of Molecular Medicine

**Initial Theory**

- **Leukocyte differentiation by their dye staining properties**
  - Modern hematology
  - Leukocyte typing concept
  - Classification of immune cells
  - Classification of blood cell disorders

- **Side chain theory**
  - Receptor-ligand concept
  - Antibody recognition and diversity
  - Immunoglobulin receptor theory
  - Immunostaining reactions and immunomaps

- **Magic bullet theory (Zauberkugel/Theorie)**
  - Experimental pharmacology
  - Preclinical drug design and drug testing
  - Target expression profiling
  - Development of target-specific therapies

- **Development and application of specific drugs**
  - Translational medicine
  - Clinical oncology and hematology
  - Clinical pharmacology and drug validation
  - Development of anti-infective drugs

**Major Resulting Concept(s) and Disciplines**

- **Development and application of specific drugs**
  - Chemothrapy

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

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Ehrlich established the principles of molecular medicine. His groundbreaking concepts were not only immediately useful after their discovery 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).1-3

As a student, Ehrlich had already established the principles of modern hematology by describing distinct dye-staining properties of various leukocyte populations.4,5 During his studies, he employed both alkaline and acid dyes but also invented new neutral dyes. Using his dye armamentarium as well as the ability to differentiate the most leukocyte subsets from each other and also from other cell types.4,6 He also proposed terminologies for these cells and almost all were 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 1880, Ehrlich studied the red cell in detail and soon detected nucleated red cells in the blood and marrow; a few years later, he described putative maturation stages of red cell precursors that he called “normoblasts,” “megablasts,” “microblasts,” and “polikoblasts.”

Although his research covered most leukocyte populations, the favorite cell of Ehrlich was the mast cell.1 This highlights the fact that he examined not only blood but also epidermal and connective tissue 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 this apparent similarity, Ehrlich remained skeptical between tissue mast cells and blood basophils. However, despite this apparent similarity, Ehrlich remained skeptical.

During his career, Ehrlich received several honors and awards. Initially, he worked at the Charité in Berlin in association with Robert Koch. He became the Director of the Institute for Experimental Therapy in Berlin in 1896 and Director of the Institute for Experimental Therapy in Frankfurt in 1899. However, despite the brilliance of his discoveries and the awards 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 to mankind. Only his later establishment of the founding of the Geiger Speyer Haus, where he later established the principles of chemotherapy and development Salvarsan. In 1908 he received the Nobel Prize in Physiology or Medicine with his co-worker Elie Metchnikoff for his work on the basic insights into immunologic defense mechanisms.

In 2015, Vienna Cancer Stem Cell organized an international memorial meeting, with the goal of honoring Ehrlich and his contributions to science, and commemorating the 100th anniversary of his death (August 26, 1915). The authors of this article were members of the meeting faculty. For interested readers who would like more information about Ehrlich’s life and his achievements and contributions to science, please refer to the literature referenced here.1-3

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

<table>
<thead>
<tr>
<th>Initial Theory</th>
<th>Major Resulting Concept(s) and Disciplines</th>
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<tbody>
<tr>
<td>Leukocyte differentiation by their dye staining properties</td>
<td>Modern hematology, Leukocyte typing concept, Classification of immune cells, Classification of blood cell disorders</td>
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<tr>
<td>Side chain theory</td>
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</tr>
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<td>Development and application of specific drugs</td>
<td>Translational medicine, Clinical oncology and hematology, Clinical pharmacology and drug validation, Development of anti-infective drugs, Chemothrapy</td>
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Congress Averts Shutdown, Passes Short-Term Funding to Keep Federal Government Open Through December 11

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. This includes funding for NIH programs as the National Institutes of Health (NIH) at current levels through the first two months of the 2016 FY that began October 1. For the NIH, this means a short-term continuation of funding at the 2015 level of approximately $30.3 billion, minus an across-the-board cut of 0.2 percent applied to all programs (defense and nondefense) in order to comply with sequestration, through December 11, 2015.

Although the long-term funding of federal agencies and programs remained uncertain as this issue of The Hematologist went to press, congressional leaders were expected to begin negotiations in an attempt to complete work on the FY 2016 budget prior to the expiration of the CR on December 11. As Congress continues efforts to pass a FY 2016 budget, the Society will carry on in its advocacy efforts, urging lawmakers to reach a bipartisan agreement that replaces sequestration and allows for sustainable increases in biomedical research funding. However, with the two parties remaining locked in a showdown over 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 Society encourages you to personalize your letter, providing examples of why NIH funding is important to you and your research.

Mark Your Calendar: Policy and Practice Events at the ASH Annual Meeting

Join ASH leaders and colleagues at the ASH Grassroots Network Lunch on Saturday, December 5, from 11:15 a.m. to 12:15 p.m. in the Hyatt Regency Orlando, Orlando Ballroom N. In addition to learning about how you can participate in ASH’s advocacy efforts, communicate with Congress and the White House, and become an effective advocate for hematology, you will hear about the impact of federal deficit reduction on health care and biomedical research, and the importance of continued advocacy by the hematology community. The program will also feature an overview of ASH advocacy highlights from 2015 and a preview of the 2016 ASH advocacy agenda.

ASH Advocacy Leadership Institute Convenes for 2015

ASH’s Advocacy Leadership Institute (ALI), which was created in 2011, is an intensive two-day program for ASH members to learn about advocacy, health policy, and the legislative process, and to become engaged with the Society’s activities. In late October, 25 hematologists from throughout the United States came to Washington, DC, to attend the 2015 ALI. The first day of the Institute focused on learning about the legislative process and health policy. On the second day, participants were divided into groups for a full day of meetings with their respective Congressional delegation on Capitol Hill, in order to turn their knowledge into action in support of hematology. Participants learned about the major issues facing the field of hematology today, including budget cuts to NIH. ASH members called on their representatives to reverse the damaging impact that cuts to NIH have had on their research and their patients. In addition to advocating for research funding, this year’s ALI participants also urged legislation to provide insurance coverage parity for all cancer drugs. For more information on ALI please visit the ASH website at www.hematology.org/ALI or contact ASH Legislative Advocacy Manager, Tracy Roades, at troades@hematology.org

New Leadership Announced at FDA and CDC

In recent years, ASH has expanded the Society’s work with the U.S. Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) on important public health, research, and scientific-related issues. These collaborative efforts are helping to ensure that hematology-related issues are at the forefront of the Agencies’ agenda and that the FDA and CDC seek ASH’s expertise on hematologic matters. The Society was pleased to hear about the following leadership changes that were recently announced for the FDA and CDC:

Dr. Robert Califf Nominated for Commissioner of FDA. On September 15, President Barack Obama nominated Robert M. Califf, MD, MACC, a cardiologist and clinical researcher, to be the commissioner of the FDA. Dr. Califf joined the FDA as Deputy Commissioner for Medical Products and Tobacco in March 2015. His appointment follows a career at Duke University of over three decades. Dr. Califf’s nomination is subject to confirmation by the U.S. Senate.

Dr. Craig Hooper Selected as CDC’s New Director of the Division of Blood Disorders. On September 3, the CDC announced the selection of Craig Hooper, PhD, as the new Director of the Division of Blood Disorders (DBD) in the CDC’s National Center on Birth Defects and Developmental Disabilities. Dr. Hooper is the former Chief of DBD’s Laboratory Research Branch. His activities have revolved around translational research in hemostasis with an emphasis on the molecular and inflammatory aspects of thrombosis and hemophilia.

ASH looks forward to working with Drs. Califf and Hooper as the Society continues to strengthen its relationship with the FDA and CDC.

Save the Date for a New ASH Workshop on Genome Editing, July 14-15, 2016

The advent of novel gene-editing technologies (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. This workshop, which can be accessed at www.hematology.org/Research/Recommendations/Agenda.aspx. ASH is pleased to announce its new Workshop on Genome Editing, which will be held July 14 to 15, 2016, in Washington, DC. The workshop’s goals are to discuss current genomic 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 hematology. 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.
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 nodes 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 percent of patients) with five-year failure-free survival (FFS) of 38 percent, and a low-risk group (72 percent 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 68 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.
Broughton, P. G.; Durrant, D. L.; Iacopetta, B.; et al. Interleukin-1α (IL-1α)–induced megakaryocyte rupture mechanism of rapid platelet production described by Dr. Nishimura and colleagues at the University of Tokyo report a new mechanism of platelet release into the bloodstream involving interleukin-1α (IL-1α)–mediated rupture of megakaryocytes that shower large numbers of platelets into the vascular sinus. The mechanism also explains increased platelet size and decreased lifetimes compared with platelets of TPO-treated mice. The IL-1α–induced megakaryocyte rupture mechanism of rapid platelet production described by Dr. Nishimura and colleagues helps explain elevated levels 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.

Targeting the Purine Biosynthesis Pathway in Relapsed ALL


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

Utilizing this strategy, Dr. Benshang Li and colleagues performed whole-exome sequencing on matched 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 in 24 individuals. Overall, 17 distinct mutations were identified, with a frequency of 13 percent in the Chinese cohort and 2.7 percent in the German cohort. Notably, mutations in NT5C2, another gene involved in thiopeurine metabolism and among the most commonly observed relapse-specific mutations in childhood ALL, were also found in the German cohort (6.1%) but were mutually exclusive of PRPS1 mutations. Although treatment regimens varied between the Chinese and German cohorts, both included prolonged daily administration of thioguanine (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 subclone 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. All patients received thioguanine for relapse treatment. One patient exhibited loss of PRPS1 by short tandem repeat analysis, which would be unlikely to benefit from thioguanine. Therefore, the authors hypothesized that with exposure to thioguanine, PRPS1 knockout would emerge over the course of treatment, mutations in PRPS1 emerge from the selective pressure of these agents and confer resistance.

The authors next confirmed a gain-of-function mechanism of drug resistance showing that cells transfected with mutant PRPS1 demonstrated marked resistance to thioguanine, far exceeding that in cells transfected with wild-type PRPS1, whereas the reduced-function mutants and PRPS1 knockdown had little effect on drug resistance. To determine how PRPS1 knockdown affected the metabolism, the authors examined the impact of the mutations on thiopeurine prodiging conversion 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, thereby producing an abundance of substrates (purines) that competitively inhibited the normal conversion of thiopeurine prodrugs to active metabolites.

Finally, to test therapeutic strategies targeting the de novo purine biosynthesis, the authors inhibited enzymes in the purine biosynthetic pathway using CRISPR-Cas9 technology and treatment 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 thiopeurines, similar to activating mutations in NT5C2. 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 thiopeurines are the cornerstone of the most all treatment protocols, offering a potential strategy for reversing thiopeurine 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.

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 (rituximab or HER2 in gastric cancer), cytokines such as VEGF (bevacizumab), and now immune检查点 such as PD1 (e.g., pembrolizumab) have transformed oncology care and are routinely used across nearly all tumor types. Although treatment options for multiple myeloma (MM) over the last decade have converted the disease into a chronic condition for many patients, it is only now that the potential of 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 paradigm-shifting additions to MM treatment.

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 MM 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. Expression of SLAMF7 is nearly universal in stages 3 and 4 disease, except inpreneurological side effects of disease progression. Daratumumab is proposed to have several modes of action: 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.

As a single agent, elotuzumab does not show significant clinical activity. However, when it is combined with lenalidomide and dexamethasone, a phase II/III trial in relapsed or refractory MM showed an overall response rate of 84 percent with a median progression-free survival of 29 months.

Monoclonal antibodies are exciting new therapeutic agents in MM and have the potential to change the way we treat this hematologic disease.
Monoclonal Antibodies

(Cite from page 11)

therapy. Overall, elotuzumab and daratumumab (and unique monoclonal antibodies), with their unique mechanism 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

Microbiome

Dr. Andrew J. Yee, MD, Noopur S. Rajee, MD

Dr. Hoggatt indicated no relevant conflicts of interest.

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microbiome is key regulator of both innate and adaptive immunity. Classically, microbiomes are thought of as scavengers, phagocytosing dead cells and debris, but they can also act as sentries, 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 microbiomes 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 mouse or cells during transplantation. Work from Dr. Irwin Weissman’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. 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 microbiomes are the key cellular 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. Xiaojuan 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 the 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 vitro assays, the authors showed that dendritic cells, rather than macrophages, were the primary activators of T cells. Using a mouse model, in which CD11c+ dendritic cells could be specifically depleted in vivo, they then showed that depletion of dendritic cells resulted in a lack of effectiveness of anti-CD47 therapy, while depletion of tumor-macrophage-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 monotherapy, such as coupling anti-CD47 treatment with other chemotherapeutic agents. These experiments demonstrated that chemotherapeutic agents should be given prior to anti-CD47 rather than after (Figure) to allow for synergistic effects and maintenance of the responsive CD8+ T cell populations. Long-term immune surveillance.
It Takes a Village: A Fellow’s Narrative on Building a Strong Network of Mentors

OYEBIMPE ADESINA, MD

I grew up in a culture steeped in proverbs, which we often used to express our thoughts in allegorical context. 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 African American 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 healthcare costs approximate $1 billion because of the high acuity of care experienced on complications. One such resource-intensive condition is osteonecrosis of the femoral head (ONFH), which affects approximately 10 percent of all SCD patients. SCD-related ONFH is particularly morbid because of its rapid progression to femoral head collapse and the need for total hip arthroplasty at a relatively young age. There are no standards for preventive care or effective pharmacologic management, and I felt this was an area of unmet clinical need and further investigation. My broad aim was to evaluate therapies that could alleviate ONFH symptoms and potentially reverse bone changes in affected SCD patients.

To optimize my exposure to SCD patients, Professor Stan Hankins, then my CRTI mentor, and I spent considerable time with colleague Dr. Elliott Vichinsky at UCSF Benioff Children’s Hospital Oakland, the largest SCD research institution in Northern California. Dr. Vichinsky introduced me to our CRTI mentor Dr. 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 Sickle Cell Disease P30 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 Hip Abnormality Evaluation Scale (CHOHES) – a validated clinical tool for SCD patients with ONFH. I then successfully applied for the NIH KL2 Mentored Career Development award from the Stanford Center for Clinical and Translational Research and its SPARK (CIRMMT). This grant covered 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 CIRMMT, 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 end points 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 academic careers. My revised goals were aligned with the SCD priorities set forth by ASH because I wanted to repurpose 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 weeklong summer workshop, we received constructive criticism of our individual research proposals, presentation skills, and career development plans. The CRTI co-hosts, Drs. Sarah O’Brien and Joseph Mikhail, worked 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 Hanks, Anita Rajasekhar, Sara Vesely, and Lisbeth Welaak, and my co-participants Drs. Massaia Janhan, Jacqueline 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. 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 CIRMMT 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. Hoppe and Fung has led to success in 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.

I was inspired by the camaraderie among the faculty and my co-participants at the summer workshop, and I am looking forward to our reunion at the 57th ASH Annual Meeting in December. ASH CRTI has been a highlight of my fellowship education; it embodies the “village” mentality of experienced educators guiding the young and providing a platform to forge their place in society. While every fellow’s mentoring narrative is unique, I have come to appreciate the added value in constructing a mentoring network that not only embraces one’s home institution, but extends beyond to include regional and national SCD expertise (Drs. Hoppe, Fung, and Hankins), different medical subspecialists (Dr. Mary Leonard), and experienced academic researchers and biotech leaders (SPARK). CRTI consolidates these types of assets with a “deep bench” that provides many benefits to the junior investigator, not just during the year of dedicated mentorship, but throughout his/her entire career.

Serving as a mentor to Bimpe has been one of the most enjoyable and fulfilling aspects of my career. As I have learned first-hand, mentorship is probably the single most important predictor of success for a junior investigator venturing into the increasingly challenging area of translational research. From Bimpe’s studies, we will obtain new and important insights into the risk factors and mechanisms involved in ONFH, as well as potential approaches to treat this understudied complication of SCD. It is a small investment to support Bimpe as she joins the future generation of physician-scientists and I am honored to be part of her village.

– Dr. Carolyn Hoppe, UCSF Benioff Children’s Hospital Oakland

Dr. Hoppe 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 perceptive eye for important understudied 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 am fortunate to have met her and hope I can help bring her talents out in the open.

– Dr. Jane S. Hankins, St. Jude Children’s Research Hospital


Dr. Gotlib, Editor-in-Chief of The Hematologist, serves as Director of the Stanford Hematology Fellowship Program and has also mentored Dr. Adesina. Drs. Adesina, Hankins, and Gotlib indicated no relevant conflicts of interest.
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.

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

**STUDY DESIGN:** REACH is a prospective, phase II open-label dose escalation study of hydroxyurea for the treatment of children with sickle cell anemia (SCA) in sub-Saharan Africa, specifically Angola, Democratic Republic of the Congo, Kenya, and Uganda (McGuinn PT, et al. Pediatr Blood Cancer. doi:10.1002/pbc.25705). 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 severe comorbid illnesses (e.g., acute or chronic infectious disease, history of malignancy or severe malnutrition); previous 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 laboratory monitoring) at each of the clinical sites. Additionally, there is no proposal evidence that demonstrates the feasibility, safety, and benefits of hydroxyurea in this setting.

**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 regions is understated. Most babies in Africa with SCA die of acute anemia or infection in the first few years of life, often without a known diagnosis of SCA, because there is no early identification by NBS and little access to simple preventive measures and disease-modifying therapies (Grosse SD, et al. Am J Prev Med. 2010;41:588-596). To begin to address this need and challenge, a partnership between investigators in North America and Africa (the REACH investigators) was formed to generate and carry out a consensus-based, prospective, therapeutic research protocol. Moreover, this trial was made possible by a donation of hydroxyurea from a partner in the pharmaceutical industry.

Hydroxyurea is an oral medicine with an established safety and efficacy profile for individuals with SCA who are treated in high-resource settings. It is also the most plausible 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. Geneva, Switzerland), hydroxyurea is also unavailable or too expensive in much of Africa, especially considering the cost of concomitant laboratory monitoring. Additionally, there is no proposal evidence that demonstrates the feasibility, safety, and benefits of hydroxyurea in this setting.

A placebo-controlled trial of hydroxyurea was deemed unethical by the African site investigators, so REACH will assess benefits by using comparison to baseline and the known disease course of 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 resource-poor 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 (NCT01976416), 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 cerebral malaria, because it up-regulates the endothelial cell surface expression of ICAM-1, 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 vitro (Passos G, et al. Lancet. 1976;1:1269-1272), and an animal model demonstrates a protective effect of hydroxyurea against cerebral malaria (Pino P, et al. Parvovirus Immunol. 2006;26:675-680). The SPIN trial (NCT01976416) 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 2010-2015 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 with the use of 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.
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).

**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**

4  Friday Satellite Symposia (FSS)  
Orlando, FL  
www.hematology.org/meetings

4  Friday Scientific Workshops  
Orlando, FL  
www.hematology.org/meetings

5-8  57th ASH Annual Meeting & Exposition  
Orlando, FL  
www.hematology.org/annual-meeting

7  ASH Consultative Hematology Course (CHC)  
Orlando, FL  
www.hematology.org/meetings

**January**

15-16  Highlights of ASH in North America  
Seattle, WA  
www.hematology.org/highlights

Highlights of ASH in North America  
Toronto, ON  
www.hematology.org/highlights

22-23  Highlights of ASH in North America  
Atlanta, GA  
www.hematology.org/highlights

Highlights of ASH in North America  
Dallas, TX  
www.hematology.org/highlights

29-30  Highlights of ASH in North America  
San Diego, CA  
www.hematology.org/highlights

Highlights of ASH in North America  
New York, NY  
www.hematology.org/highlights

**March**

5-6  Highlights of ASH in Asia – Pacific  
Brisbane, Australia  
www.hematology.org/highlights

**April**

29-30  Highlights of ASH® in Latin America  
Natal, Brazil  
www.hematology.org/highlights