ASH Helps to Build Capacity for Cytogenetics Laboratories in Mexico

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In April, medical professionals from the United States and Mexico met in Mexico City to kick off a pilot program designed to standardize cytogenetics laboratory procedures for the diagnosis of hematologic malignancies in Mexico. This meeting came about after AMEH (La Agrupación Mexicana para el Estudio la Hematología [the Mexican hematology association]) asked ASH to help investigate ways to build capacity for hematologic cytogenetics laboratories in Mexico. The U.S.-Mexico Cytogenetics Laboratory Standardization Program was then established as a science-based educational collaborative partnership that includes ASH, AMEH, and the U.S. NCI Office of Latin American Cancer Program Development (OLACPD). The ultimate goal of this program is to improve the diagnosis and care of patients with hematologic malignancies utilizing high-quality cytogenetic analysis.

One of the first steps was to establish a Scientific Steering Committee consisting of a group of experts representing AMEH, ASH, and the NCI OLACPD to oversee the program. The committee

[Cont. on page 6]

CML Stem Cells: No Longer Addicted


The development of imatinib mesylate, a prototypical targeted agent, has had a major impact on the treatment of patients with CML and other Bcr/Ab1-related malignancies. Its use provides a paradigm for approaches that target oncogenes to which transformed cells, such as CML, have become addicted. Nevertheless, despite initial success, most patients with CML develop resistance to imatinib, and those with advanced disease (e.g., accelerated phase or blast crisis) do not respond as well as those with chronic-phase disease. Resistance has been characterized as Bcr/Ab1-dependent or Bcr/Ab1-independent, depending upon whether addiction to the oncogene persists.

Another complicating feature is that evidence suggests that primitive CML progenitors were not addicted to Bcr/Ab1 and that therapies directed exclusively at Bcr/Ab1 are unlikely to eliminate CML stem cells.

The results of this study, while potentially of great significance, have some sobering implications. While circumventing Bcr/Ab1-dependent forms of resistance to imatinib is hardly trivial, several approaches (e.g., developing more effective inhibitors, overcoming pharmacokinetic and pharmacodynamic barriers, etc.) come to mind. However, overcoming resistance in cells that are no longer addicted to Bcr/Ab1 is an entirely different matter; new approaches would have to be developed. In this context, the observation that HDAC inhibitors effectively enhance tyrosine kinase inhibitor activity against CML stem cells provides a possible mechanism by which CML stem cells make a contribution to disease persistence, which remains to be determined whether this is the case and, if so, by what mechanism. Assuming that CML stem cells might be addicted to Bcr/Ab1 in a way that is resistant to cancer therapy, the results of this study by Corbin et al. suggest that entirely novel approaches to go beyond inhibiting Bcr/Ab1 will be required for future advances in CML therapy. To this end, it may be necessary to travel well beyond the canonical Bcr/Ab1 pathway.


FEATURES

2 OP-ED: HEMATOLOGY PRIVATE PRACTICE IS BLEEDING TO DEATH – Community practitioner Dr. Thomas Bensinger sheds light on the difficulties facing community-based private hematology practices.

4 MINI REVIEW: THE CANCER-THERMOBISIS CONNECTION – Dr. Anna Falanga brings to the forefront the fact that thrombosis has a significant impact on the morbidity and mortality of cancer and talks about the importance of identifying which patients may be at higher risk than others.

7 ASH CAPITOL HILL DAYS FOCUS ON FUNDING FOR RESEARCH AND PREVENTING DRUG SHORTAGES – This report highlights the efforts of the ASH Committee on Government Affairs and Committee on Practice, which participated in Capitol Hill Days this spring.

15 PROFILES IN HEMATOLOGY: HILLBILLY HEMATOLOGIST – Puget Sound Blood Center’s Executive Vice President for Research, Dr. José López, shares the unique experiences that led him to a career in hematology.
Hematology Private Practice is Bleeding to Death

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Community-based private hematology practices are a valuable resource for the care and treatment of many patients with hematologic problems. Unfortunately, hematologists have recently seen a rapid increase in what had been a slow erosion of the incentives to enter and remain in such practices. Costs are higher; reimbursements are lower. Medicare is the greatest, albeit not the only, culprit. Examples of the "wounds" being inflicted on the practice community include:

1. Medicare has eliminated "consult" CPT codes 99241-99245 and substituted "new patient encounter" codes 99201-99205; of course, the new codes are paid at a lesser rate. I feel like Medicare has changed the name of what is demanded of me in my job solely to pay less for the same service.

2. Hematologists are trained to review the peripheral blood film under a microscope. Among other things, the following are identified: sickle cells, target cells, schistocytes, stomatocytes, eliptocytes, microcytosis, megaloblastic changes, and platelet clumping. Patients being treated for thrombotic thrombocytopenic purpura (TTP) require multiple blood film reviews for schistocytes. Yet, there is a long-standing Medicare policy not to pay for any peripheral blood film review in an outpatient setting and to only pay for one review in an inpatient hospitalization.

3. Medicare recalculates the cost of office-administered medications every three months but only adjusts for increased costs every six months. The payments are not retroactive to the time of the increase, leaving the physician responsible for the increased cost. The Patient Protection and Affordable Care Act (PPACA) that was enacted last year directed Medicare to develop Accountable Care Organizations (ACOs). The ACOs would mete out payments. This could put the hematologist in direct competition for any payment with hospitals and other physicians or agencies involved with a patient’s care.

4. The Food and Drug Administration (FDA) has also contributed to increasing unreimbursed costs in private practice. The FDA requires pre-approval every 28 days for some medications frequently prescribed by hematologists (e.g., thalidomide and lenalidomide). Pre-approval is a time-consuming process for offices that requires working with the patient on paperwork and follow-up surveys. While this generates important information for the manufacturer, I also think the manufacturer should pay for the office time rather than the physician. Private insurers also require extensive, unreimbursed paperwork and telephone-tag time. For example, I have never understood the gall of an insurance company to require pre-approval for something they are going to approve but not require that they have someone available by phone or fax to actually grant approval in a timely fashion.

5. Pre-approval for procedures such as CT scans, MRls, and PET scans is required by many private insurers. Usually the ordering physician is required to get the authorization for the doctor who will perform the study and be reimbursed for it. Medicare does not require pre-approval for these studies, but if the patient’s secondary insurance requires pre-certification, that is the rule that has to be followed. This means referring doctors must spend unreimbursed time to thoroughly investigate each patient’s secondary coverage requirements by company and by individual policy or run the risk of the radiologist not getting paid anything more than 80 percent of Medicare’s allowable rate. In every other instance that I am aware of, Medicare rules take precedence over secondary insurer rules. For example, when Medicare allows $100 for a drug or a procedure, but the secondary insurer allows more, the Medicare allowable is primary. In short: Medicare rules take precedence when it saves the secondary insurer money, and secondary rules take precedence when it saves the secondary insurer money.

Reimbursements by Medicare for the care of patients have been eroding for the last five years. Since many other carriers use Medicare as a reference point for their reimbursements, the amount paid to physicians has increased very little, if at all. At the same time, part of a significant increase in overhead is followed. This means referring doctors must spend unreimbursed time to thoroughly investigate each patient’s secondary coverage requirements by company and by individual policy or run the risk of the radiologist not getting paid anything more than 80 percent of Medicare’s allowable rate. In every other instance that I am aware of, Medicare rules take precedence over secondary insurer rules. For example, when Medicare allows $100 for a drug or a procedure, but the secondary insurer allows more, the Medicare allowable is primary. In short: Medicare rules take precedence when it saves the secondary insurer money, and secondary rules take precedence when it saves the secondary insurer money.

Reimbursements by Medicare for the care of patients have been eroding for the last five years. Since many other carriers use Medicare as a reference point for their reimbursements, the amount paid to physicians has increased very little, if at all. At the same time, part of a significant increase in overhead is the increased demand by insurance carriers for non-reimbursable services. Where is the incentive for a young physician whose primary interest is the community-based care of patients to choose hematology?

Hematologists individually, and ASH as our representative, need to rigorously educate both Medicare and the private insurance sector that a valuable resource for care and treatment of many patients with hematologic problems is being severely compromised.
ASH Members Named Fellows of the American Academy of Arts and Sciences

Clara Derber Bloomfield, MD
Distinguished University Professor; William G. Pace III Professor of Cancer Research; Cancer Scholar and Senior Advisor, The Ohio State University Comprehensive Cancer Center; Professor of Medicine, The Ohio State University

George Q. Daley, MD
Investigator, Howard Hughes Medical Institute; Samuel E. Lux IV Professor of Hematology/Oncology; Director Stem Cell Transplantation Program, Children’s Hospital and Dana-Farber Cancer Institute; Professor of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Chi Van Dang, MD, PhD
Johns Hopkins Family Professor in Oncology, Research Professor of Medicine, Cell Biology, Oncology and Pathology, Vice Dean for Research, Johns Hopkins University School of Medicine

Raymond J. Deshaies, PhD
Investigator, Howard Hughes Medical Institute; Professor of Biology, California Institute of Technology

Katherine Ann High, MD
Investigator, Howard Hughes Medical Institute; William H. Bennett Professor of Pediatrics, University of Pennsylvania School of Medicine

Seeking Editor of ASH Image Bank

ASH is in the initial stage of the selection process for the next Editor-in-Chief of the ASH Image Bank (term: September 1, 2011 – August 31, 2012). Candidates with an MD or equivalent medical degree should have a broad and comprehensive knowledge of basic research and clinical investigation in hematology as well as an appreciation of its subspecialty areas, a distinguished research and publications record, and demonstrated writing, reviewing, and editing skills. Candidate will be asked to oversee the Image Bank submission process. This will include review of image submissions, evaluation of current content, and active solicitation of under-represented collections. Members of ASH are encouraged to submit a letter of intent or provide the name of a potential candidate. Nominations should be accompanied by a description of the candidate’s editorial experience and a short, informal endorsement. Nominations must be received on or before August 30, 2011. Please submit letters of intent and names to imagebankeditorsearch@hematology.org.
The close relationship between cancer and thrombosis has been known since the days of Armand Trousseau, who first described the clinical association between idiopathic venous thromboembolism (VTE) and occult malignancy in 1865. Today, we know that cancer is associated with a hypercoagulable state and a four-fold increase in thrombosis risk, with chemotherapy elevating this risk even more. Epidemiologic and population-based studies provide detailed information on the scale of the problem and the identification of VTE risk factors, including those related to the tumor (tumor type, clinical stage, chemotherapy, use of anti-angiogenic drugs or erythropoietic growth factors, and insertion of central venous catheters), and those related to individual patient characteristics (sex, race, age, previous VTE history, immobilization, and obesity). Thrombosis has a significant impact on the morbidity and mortality of cancer; therefore, it is important to identify which patients may be at higher risk than others, especially before starting chemoradiotherapy or surgery.

As our knowledge of thrombosis risk accrues, more sophisticated methods of risk assessment are being developed. Prediction models for chemotherapy-associated VTE risk have become available and include many of the risk factors listed above as well as biological markers, such as leukocyte and platelet counts and circulating levels of tissue factor (TF), P-selectin, and D-dimer. The hope is that targeted thromboprophylaxis will become possible and predictive models may improve risk-benefit ratio. In this context emerges the important role of circulating cell-derived microparticles (MP), submicrometric membrane-bound vesicles that circulate in blood and can carry TF and procoagulant phospholipids on their surface. Variations in MP quantity and/or phenotype are relevant pathogenic markers of thrombosis and vascular damage and are associated with a higher risk of VTE in cancer patients. MP levels are currently under study as a criterion to enroll high-risk cancer patients in thromboprophylaxis trials.

Recently, a number of guidelines for the prevention and management of VTE in cancer patients have been released from major American and European scientific societies. After an initial period of wide heterogeneity, a consensus is emerging concerning thromboprophylaxis in hospitalized non-surgical and surgical cancer patients and treatment of VTE in cancer patients, although controversies remain concerning duration of prophylaxis after surgery, use of thromboprophylaxis in ambulatory patients receiving anti-cancer therapies, strategies for treatment of VTE after the first six months of therapy with low-molecular-weight heparin, treatment of VTE recurrences, and the role of new oral anticoagulants.

Critical for the design of appropriate pharmacologic interventions for cancer-associated VTE is a better understanding of multi-factorial mechanisms underlying the hypercoagulability. Among other factors, a prominent role is played by tumor cell-specific clot-promoting properties, which may also contribute to the process of tumor growth and dissemination. These include expression of TF by tumor cells, production of MP and inflammatory cytokines by tumor and/or host cells, and direct adhesion of tumor cells to platelets, leukocytes, and endothelial cells (Figure). TF of tumor origin is a key molecule that initiates blood clotting and also supports tumor growth and metastasis by coagulation-independent mechanisms, such as up-regulation of VEGF and activation of PAR-2. Coagulation system activation, with generation of thrombin and fibrin and activation of platelets, leukocytes, and endothelial cells, plays a crucial role in the progression of cancer. Recent extensive experimental evidence shows that platelets support tumor metastasis. Within the circulatory system, platelets (and fibrin) guard tumor cells from immune elimination and promote their arrest at the endothelium, favoring the establishment of secondary tumors. Experimental studies in mice demonstrate the role of platelet glycoproteins GPIb, GPVI, and P-selectin in supporting this process. Moreover, platelets exert a protective role in the maintenance of tumor vascular integrity and may represent a target for the specific destabilization of tumor vessels. Finally, MP shed by tumor cells and platelets, which carry pro-angiogenic factors, are newly identified players in maintaining tumor growth.

In the last decade, the story was advanced by the discovery of a complex scenario in which oncogenic events drive the procoagulant conversion of tumor cells. Oncogene and tumor suppressor gene-mediated neoplastic transformation driven by activation of MET, loss of PTEN, induction of Kras, and/or loss of p53 in several experimental models of human cancers has been associated with activation of clotting pathways as an integral feature of the transformation. Signaling pathways triggered by one or more of these genes can result in activation of blood coagulation and platelet function and/or suppression of fibrinolysis, which in some cases produced thrombosis and/or DIC in these models. Furthermore, a mutation of EGFR gene renders cancer cells hypersensitive to the action of coagulation proteins, such as TF, as a result, a microenvironment promoting tumor growth is generated.

In human malignancies, PML-RARα hybrid gene expression in patients with acute promyelocytic leukemia and JAK2V617F expression in patients with myeloproliferative neoplasms (MPNs) are associated with expression of a pro-thrombotic phenotype. Among other properties, platelets...
from JAK2-positive patients with MPN express increased TF on their membranes. From a better understanding of the molecular events associated with cancer thrombophilia, new targets for development of bifunctional drugs (i.e., those capable of attacking both the malignant process and the coagulopathy) may be identified. Perhaps one example of a targeted, bifunctional therapy is ATRA for treatment of APL. Until more specific targeted therapies are available, however, we must rely on anticoagulant drugs for prophylaxis and treatment of thrombosis.

In this context, it is worth noting that anticoagulant treatments have been reported to improve survival in cancer patients. Given the limitations of the available studies, the routine use of anticoagulants as primary anti-cancer therapy cannot be recommended. Nevertheless, the data from meta-analyses may provide a stimulus to test the hypothesis in properly designed, large randomized clinical trials. Additional efforts to develop therapies that rapidly correct the hypercoagulable state of cancer are required. As the molecular basis becomes better elucidated, development of drugs that will target both the malignant process and the resultant hypercoagulability is a realistic goal.

References

Above: Hematology trainees gather for a photo during the Highlights of ASH Latin America meeting in Punta del Este, Uruguay. Below: Young scholars who attended the Highlights of ASH meeting in Beijing, China, gather for a group photo.

The trainees were generously sponsored by the Coulter Foundation.

Meeting Program Announced for 2011 ASH State-of-the-Art Symposium

ASH will host its annual State-of-the-Art Symposium (SAS) meeting in Chicago, IL, September 23-24. This meeting will present the most current developments in key areas of hematology and give attendees the opportunity to explore the impact of new data on current practice. The topics that will be featured during this year’s SAS meeting include: monoclonal gammopathy consults, amyloidosis, relapsed myeloma, hematopoietic growth factors, monoclonal B-cell lymphocytosis, and large granular lymphocyte disorders. New this year, ASH is offering a session titled “The Great Debate: Instituting Myeloma Therapy – Synergy vs. Sequence,” which will feature two speakers weighing in on myeloma treatment strategies. A session on new oral thrombin inhibitors – when and when not to use – will also be offered. Registration is now open. For more information and to register for this meeting, go to www.hematology.org/Meetings/State-of-the-Art-Symposium.

CORRECTION

In the May/June issue, we neglected to give proper credit for use of the photo in the Ernest McCulloch obituary on page 7. Reprinted from The Lancet, 377/9765, Stephen Pincock, Ernest Armstrong McCulloch, Page 550, February 2011, with permission from Elsevier.

In the May/June issue, the link to the new pocket guide on ITP (News and Reports, page 3) was incorrect. The correct link is www.hematology.org/Practice/Guidelines/2934.aspx.
Cytogenetics Laboratories in Mexico

[Cont. from page 1]

includes AMEH Past President David Gómez Almaguer, MD, PhD; Hector Mayani, PhD; Diane Arthur, MD; Michelle Le Beau, PhD; Susana Raimondi, PhD; Jorge Gomez, MD, PhD; and me. Subsequently, four experienced cytogenetics laboratories representing different geographic areas of Mexico were selected to work in partnership with the Scientific Steering Subcommittee. The participating laboratories include Laboratorios de Analisis de Oncobematología in Mexico City, Neuvo Hospital Civil in Guadalajara, Laboratorios Mendel in Morelia, and Genética Estudios Pre y Postnatales in Mexico City.

On April 7-8, 2011, an initial workshop to plan a two-year pilot project for the cytogenetics standardization program was held in Mexico. The focus of the pilot project, which will be sponsored in part by the Wallace H. Coulter Foundation, is cytogenetic analysis of acute myeloid leukemia (AML). Representatives of the Scientific Steering Committee visited the four participating cytogenetics laboratories, and members of the Committee gave state-of-the-science presentations on genetics of AML, including the biologic and clinical implications of genetic abnormalities. These were followed by collaborative discussions between committee members and laboratory representatives about the process for implementing the pilot to ensure that the project was a Mexican-led initiative. Enthusiasm for the pilot project among the workshop attendees was unanimous.

Participating laboratories are now prospectively performing cytogenetic analysis at diagnosis on adult and pediatric patients with AML. Representative karyotypes and their interpretation will be entered into the Pediatric Oncology Network Database (POND) based at St. Jude Children’s Research Hospital. The cytogenetic data will be centrally reviewed by experts on the Scientific Subcommittee, and feedback will be provided to the participating laboratories. Diagnostic pathology material, such as bone marrow aspirates and relevant clinical and laboratory data, will also be reviewed and correlated with the cytogenetic results. Performance will be measured and compared with established short-term and long-term goals.

The Steering Committee and participating laboratories have contributed an enormous amount of time and energy to ensure a successful launch. Over the course of the two-year project, the aim will be to improve cytogenetic analysis in Mexico by creating centers of excellence that can then train other laboratories in the country. Ultimately, the goal of this collaboration between ASH, AMEH, and NCI is to improve outcomes for patients with AML.
ASH Capitol Hill Days Focus on Funding for Research and Preventing Drug Shortages

At the end of March, as Washington was in the midst of a budgetary showdown and preparing for a possible shutdown of the federal government, members of the ASH Committee on Government Affairs made visits to Capitol Hill to explain to Members of Congress and their staff the impact of proposed cuts in funding to the National Institutes of Health (NIH) on research to find cures and treatments for patients with serious hematologic diseases. Ultimately, as a result of the advocacy efforts of ASH and others in the research community, NIH fared better than many other federal agencies in the budget agreement Congress reached in mid-April; they escaped with a 1 percent cut in funding for fiscal year 2011.

In early May, just as congressional committees were beginning to examine the problems associated with the scheduled annual Medicare payment cuts to physicians that the sustainable growth rate imposes, and as shortages of chemotherapy and other critical drugs were making national headlines, members of the ASH Committee on Practice met with congressional offices to explain the negative effect these problems have on hematologists and their patients. In particular, ASH members highlighted the critical shortages of drugs used to treat patients with hematologic malignancies. ASH urged Members of Congress to conduct a hearing on the problem of drug shortages and support legislation that has been introduced in the Senate (S. 296, The Preserving Access to Life-Saving Medications Act) that would take the first steps toward addressing this problem.

Committee members visited nearly 100 congressional offices during the Committee Hill Days this spring. These meetings are crucial to ASH’s advocacy efforts, providing an opportunity for Members of Congress and their staff to gain insight on issues of importance to hematologists. ASH strongly encourages members to let the Government Relations & Practice Department know when you are in Washington, DC, and available to meet with your congressional delegation. Committee members visited nearly 100 congressional offices during the Committee Hill Days this spring. These meetings are crucial to ASH’s advocacy efforts, providing an opportunity for Members of Congress and their staff to gain insight on issues of importance to hematologists. ASH strongly encourages members to let the Government Relations & Practice Department know when you are in Washington, DC, and available to meet with your congressional delegation.

ASH Advocacy Leadership Institute: A Two-Day Workshop in October in Washington, DC

Call for Nominations

ASH is proud to announce the initiation of its Advocacy Leadership Institute to be held October 12-13. This is a unique opportunity for ASH members to come to Washington, DC, to learn about legislation and health policy affecting hematology.

Participants will receive intense training in policy-making processes and advocacy; guest speakers from the federal government, including the NIH and the Administration, will be featured. Participants will conclude the program with visits to the Congressional delegation on Capitol Hill.

Nominations are currently being accepted through August 15, 2011. The ideal candidate is an ASH member residing in the United States who is interested in health policy and advocacy and would like to be more engaged in ASH activities.

Please send your nominations to ASH Senior Manager for Scientific Affairs, Ulyana Desiderio, PhD, at udesiderio@hematology.org and include the following: 1) Nominator’s name/phone number; 2) nominee’s name/institution; 3) reason for nomination (short paragraph describing the nominee’s interest in this opportunity). Self-nominations are welcome.
Sequencing the Myeloma Genome


In this study, a large multi-institutional group led by investigators from the Broad Institute in Boston report the initial results from genome sequencing of multiple myeloma (MM) cells. Using the newest technologies of massively parallel DNA sequencing, they performed whole-genome sequencing and/or whole-exome sequencing on MM samples from 38 patients and found significant levels of mutation in many genes, including KRAS and NRAS, TP53, CCND1, DIS3, FAM46C, LRRK2, IRF4, PRMD1, BRAF, NF-κB, histone modifying enzymes, and components of the blood coagulation cascade. These studies confirmed previously known mutations and revealed novel mutations, which may yield insights into pathways involved in MM pathogenesis and suggest potential novel therapeutic targets. Ultimately, this study may help to identify hallmark genomic abnormalities in MM and allow for more effective personalized therapies.

Marked genetic heterogeneity has been demonstrated in MM, with important implications for tumor pathogenesis, prognosis, and treatment. For conventional therapy, hypodiploidy and t(11;14) have defined standard-risk MM with superior outcome, whereas hyperdiploidy, t(4;14), del (17p), and del(13q14) have defined high-risk MM with inferior outcome. However, novel therapies such as bortezomib can overcome, at least in part, the adverse outcome conferred by some t(14;14) but not others [del (17p) abnormalities]; the latter continues to define high-risk disease. Currently, mRNA (microarray), DNA (array comparative genomic hybridization [aCGH]) and single nucleotide polymorphism (SNP)), and microRNA (miRNA) profiling studies of clinically annotated samples from uniformly treated patients are being used to characterize molecular pathogenesis of MM, identify novel targets, and develop refined patient stratification and personalized medicine approaches in MM. Microarray profiling has revealed transcriptional changes correlating with evolution from monoclonal gammopathy of undetermined significance (MGUS) to smoldering MM (SMM) to active MM; this has led to transcript-based prognostic MM classification systems and new definitions of high-risk MM. Already genetic and molecularly distinct subgroups of MM have distinct biology and treatment options; for example, FGFR3 inhibitor therapy may be useful in t(4;14) MM and rituximab therapy in CD20-positive MM. DNA-based aCGH and SNP array studies have identified copy number alterations (CNAs), which predict for clinical outcome, including increased 1q and 5q gains and decreased 12p as a site of putative MM oncogenes, as well as decreased 12p as a site of putative MM suppressor genes. miRNA profiling studies can distinguish normal plasma cells from MM cell lines; patients whose tumors resemble the former have improved outcome versus patients with tumors resembling cell lines.

Previous studies in MM have revealed activating mutations of oncogenes (MYC, RAS, FGFR3), inactivation of various tumor suppressors (p53, p16, RB1), CNAs/mutations leading to activation of NF-κB pathway, and inactivating mutations and deletions of demethylase genes such as UTX. Importantly, this new MM sequencing study confirmed mutations of KRAS, NRAS, TP53, and NF-κB. It also revealed novel mutated genes involved in protein homeostasis, consistent with MM as the prototype cancer for therapeutic targeting with proteasome inhibition. Identification of novel CCND1 mutations also supports the central role of cyclin D1 in MM. Mutations in IRF4 and PRMD1 are also consistent with prior studies implicating these genes in normal and malignant plasma cell differentiation. Importantly, mutations in histone modifying enzymes confirm the inactivating mutations of UTX and further support efforts to target histone methyltransferases, such as MMSET. Of note and unexpectedly, mutations in BRAF, a gene encoding a serine/threonine kinase known to be involved in pathogenesis of melanoma, were described in 4 percent of cases, which may have immediate clinical application. Current and future studies are characterizing larger numbers of samples to identify less common mutations and define interrelationships of mutations; sequencing RNA for allele-specific expression, differential expression, and more accurate sample clustering; utilizing high-throughput gain/loss-of-function assays to identify driver mutation effects, with focused validation studies of leads; and performing longitudinal studies to assess evolution of changes with acquisition of drug resistance and disease progression.

The Quest for the Myeloma Cancer Stem Cell


Despite recent advances in drug development, multiple myeloma (MM) remains incurable for the majority of patients due to relapse and disease progression. Moreover, progression-free survival rates decrease progressively as relapses occur. A question that remains unanswered is why it has been impossible to cure myeloma. One potential mechanism is based on the concept of tumor dormancy, defined as persistence of non-dividing residual tumor cells for long periods of time. Evidence has emerged from experimental myeloma models suggesting that a balance exists between dormant tumor cells, which seem to persist in very small numbers, and the host microenvironment.1 Cross-talk between tumor cells and their microenvironment, angiogenesis, and anti-tumor immune responses play a role in the control of dormant tumor cells. Various mechanisms help maintain this equilibrium,2 including expression of immunoregulatory molecules, epigenetic modifications, and activation of autocrine loops.

Experimental models indicate that the dormant tumor cell population may be constituted of so-called cancer stem cells (CSCs) that enter a quiescent state but are capable of clonogenic growth, self-renewal, and differentiation into myeloma plasma cells. This model suggests that the long-term proliferative potential responsible for disease initiation, maintenance, and relapse is contained within specific subpopulations of biologically distinct tumor cells. Matsuji et al had first described the myeloma CSC, a rare cell population phenotypically resembling normal memory B cells (CD20 and CD27 positive) but lacking CD138. They found that cells expressing CD138+, an antigen present at high levels on MM plasma cells in virtually all patients, could not form tumor colonies in semi-solid media, in contrast to CD138-negative cells. The myeloma CSCs also appeared to be relatively resistant to a wide variety of anti-myeloma therapeutic agents, suggesting that they may persist following treatment and mediate tumor re-growth and relapse. However, controversy surrounds the exact phenotype and biology of myeloma CSCs.

Jakubikova and colleagues from the Dana-Farber Cancer Center in Boston have attempted to characterize these tumor-initiating subpopulations, also called side population (SP) cells, which are characterized by their “stem-like” features. They observed evidence for the clonogenic potential of SP cells in myeloma, as well as the ability of SP cells to regenerate original population cells. Moreover, SP cells revealed higher tumorigenicity compared to myeloma plasma cells. SP cells exhibited substantial heterogeneity in MM cell lines and primary MM cells but expressed CD138 antigen and had a higher proliferation index compared to non-SP cells.

This study provides more insight into the behavior of myeloma SP/CSC cells, providing further evidence for the role of the bone marrow microenvironment and the bi-directional interactions between the SP/CSC and microenvironment to sustain equilibrium between quiescence and self-renewal. The stem cell niche provides protection and nourishment to SP/CSC cells, and myeloma cell adherence to the bone marrow stromal cells increases the percentage, viability, and proliferation potential of the SP/CSC cells. Interestingly, immunomodulatory drugs lenalidomide and thalidomide attenuated this stimulatory effect of stromal cells, thereby significantly decreasing SP/CSC cell percentages. Defining the biological features and molecular characteristics of the MM stem-like/tumor-initiating cells that are responsible for tumor re-growth will facilitate the development of new strategies to prevent relapse and halt myeloma progression.


XAVIER LELEU, MD, PhD
Dr. Leleu indicated no relevant conflicts of interest.

KENNETH ANDERSON, MD
Dr. Anderson indicated no relevant conflicts of interest.

The Hematologist: ASH News and Reports
The Gatekeeper T315I Versus the Switch-Control Inhibitor DCC-2036: The Last Title Fight in CML?


Despite imatinib’s impressive milestones in CML and the success of the second-generation tyrosine kinase (TK) inhibitors in documenting in their ability to circumvent the majority of BCR-ABL resistance mutations, CML is still standing, albeit wobbly-kneed. With a frequency of ~15 percent among resistance mutations, the common ABL1T315I gatekeeper mutation exhibits pan-resistance to all of the currently approved TK inhibitors and has remained one major obstacle to vanquishing CML. From a clinical and drug development perspective, this challenge has carried the tonal equivalent to what Apollo Creed must have thought about Rocky Balboa in their first epic 15-round match: What is it going to take to knock this guy out?

Secondary resistance to TK inhibitors mediated by mutations such as ABL1T315I either impede drug binding due to steric hindrance in the ATP binding pocket (e.g., dasatinib) or result in a switch from the type II inactive to type I active conformation of the kinase to which these drugs can no longer bind (e.g., imatinib and nilotinib). This latter form of resistance, referred to as “conformational escape” is mediated by specific “switch control” amino acid residues within ABL1 that adopt orientations between the type I phosphorylated and type II unphosphorylated states. In this elegant report, collaborators from Tufts Medical Center, Emerad Biostructures, and Deciphera Pharmaceuticals used structural-based design to optimize development of a “switch control inhibitor” designed to interact with these critical residues in order to achieve a stable, inactive conformation state even in the presence of resistance mutations. These aims were realized by a series of synthetic maneuvers that culminated in the development of compound DCC-2036 with docking sites to both the switch control pocket (amino acids E282/R386) and the ATP hinge region of ABL1.

DCC-2036 exhibits an IC50 of 0.8 nM for ABL1 and 4 nM for ABL1T315I and exhibits low nonomolar inhibitory activity against SRC family of kinases, FLT3, and TIE2. In comparison, the IC50 for ABL1T315I for imatinib, dasatinib, and nilotinib range from 3,800 to more than 10,000 nM. Pre-clinical data indicate that DCC-2036 inhibits the proliferation of Ba/F3 cells transformed by BCR-ABL1T315I and downstream signaling effectors such as STAT5 and CrkL. In mice engraffed with BCR-ABL1T315I-transformed Ba/F3 cells or in a retroviral transduction/transplantation model of BCR-ABL1T315I -induced CML and B-cell ALL, DCC-2036 significantly prolonged survival compared with imatinib or dasatinib. DCC-2036 inhibited marrow-derived myeloid colonies from patients with either chronic-phase or relapsed-accelerated-phase CML and the L298V mutation.

A phase I clinical trial with DCC-2036 is currently underway to assess its safety and efficacy in patients with Ph+ CML or ALL that 1) exhibit the BCR-ABL1T315I mutation or 2) exhibit resistance or intolerance to a 2 TKIs with known efficacy. DCC-2036 joins AP24534 (ponatinib) as a novel agent with different mechanisms of action focused on T315I-resistant disease. Although it is anticipated that these TKIs will have a major impact on this disease population, nature has repeatedly shown that it will not easily surrender to the 10-count and fall to the canvas; resistance in some form is almost assured.

Solving the Clopidogrel Paraoxonase


Clopidogrel is the most important antiplatelet agent identified since aspirin. Its activity, however, is characterized by highly variable efficacy. This variability results from differences in their ability to convert to its active metabolite and from genetic determinants. The latter encompasses both clinical consequences, as evidenced by the observation that resistance to clopidogrel is associated with stent thrombosis in the setting of percutaneous coronary intervention (PCI) and myocardial infarction in coronary artery disease. Several of the enzymes responsible for the conversion of clopidogrel to its active metabolite have been identified. Cytochrome P450 isoforms are most widely studied and function in the conversion of clopidogrel to 2-oxo-clopidoğrel (Figure). Loss-of-function alleles of the cytochrome P450 2C19 gene are associated with increased stent thrombosis. A genome-wide association study linked CYP2C19 variants with differences in platelet response to clopidogrel. However, CYP2C19 variants account for only 12 percent of the heterogeneity in clopidogrel response. Bouman and colleagues from The Netherlands now show that paraoxonase-1, a second enzyme mediating clopidogrel metabolism, may contribute more significantly to response variability.

They used a metabolic profiling approach to identify enzymes responsible for clopidogrel metabolism. They broke the process down into two stages (Figure): cytochrome P450-mediated oxidation and esterase-mediated thiophene ring hydrolysis. Paraoxonase-1 was identified as the critical esterase in the second stage. In addition, it was noted that the Arg192 allele of paraoxonase-1 had a much better cleavage efficiency than the Gin192 allele.

The authors then conducted two clinical trials to evaluate the contribution of paraoxonase-1 gene (PON1) variants to variability in response to clopidogrel. In a case-cohort study in individuals who underwent PCI and received clopidogrel for six to 12 months, the authors found that individuals with the QQ192 PON1 variant (encoding the Gin192 allele) had a 12-fold increase in stent thrombosis compared with individuals with the RR192 PON1 variant (encoding the Arg192 allele). In addition, those with stent thrombosis demonstrated a lower plasma concentration of active metabolite and a higher concentration of 2-oxo-clopidogrel, indicating deficient paraoxonase-1 activity. Individuals with the QQ192 variant showed lower inhibition of platelet aggregation following clopidogrel administration. The results of this trial were corroborated in a second clinical trial in which nearly 2,000 individuals with acute coronary syndromes receiving clopidogrel were followed prospectively. This trial demonstrated a 10-fold increased risk of stent thrombosis and a nearly five-fold increase in myocardial infarction in QQ192 versus RR192 homozygous individuals. In contrast, individuals with the QQ192 variant demonstrated a significantly lower risk of bleeding. The authors calculated that the PON1 Q192R polymorphism explained 72.5 percent of the variability in ADP-stimulated platelet aggregation after clopidogrel administration.

The discovery of the PON1 Q192R polymorphism as a determinant of responsiveness to clopidogrel is an important advance in our understanding of this widely used drug. Yet some questions about this finding remain. The reasons why the PON1 gene region was not associated with clopidogrel response variability in a genome-wide association study of an Amish population is not entirely clear. Differences in populations or in methodology of platelet testing between the two studies might explain the discrepancy. Conversely, Bouman et al. did not demonstrate an impact of CYP2C19 genes, which has been previously associated with response variability, in their studies. These discrepancies notwithstanding, the impressive hazard ratio of thrombotic risk associated with the PON1 QQ192 variant in the setting of clopidogrel therapy makes a strong case for further evaluation of this gene variant. If follow-up studies are confirmatory, PON1 genotyping or PON1 activity measurements, perhaps in combination with assessment of CYP2C19, will help guide therapeutic decision-making. Such advances will be particularly useful in identifying candidates for newer alternative P2Y12 receptor inhibitors, such as prasugrel or ticagrelor, that do not require similar metabolism.

Figure

Clopidogrel

2-oxo-clopidogrel

Active metabolite

Clopidoğrel is metabolized via oxidation by cytochrome P450 isozymes followed by hydrolytic cleavage by paraoxonases, particularly PON1.

ROBERT FLAUMENHAFT, MD, PhD
Dr. Flaumenhaft indicated no relevant conflicts of interest.
Acetylation Deficiency and the Germinal Center of Doom: Lymphoma and the Sorting HAT


Evidence for epigenetic dysregulation in lymphomas continues to accumulate. Histone deacetylase inhibitors (HDACi) may reverse the bias toward transcriptionally repressed heterochromatin found in some tumors, restoring apoptotic and cell cycle regulation pathways (Figure). However, clinical results with HDACi to date have been relatively modest, and there is no reliable means to predict benefit.

The group led by Riccardo Dalla-Favera at Columbia University has performed whole-exome sequencing to produce entirely new information on the genetic changes that lead to malignancy. The therapeutic implication is that attempts to treat using inhibitors of HDAC may be most effective in those cases in which HAT gene dose having major consequences for their phenotype. The group then looked at follicular lymphoma, with similar results. Among 46 cases, 15 had CREBBP mutations, all clustered in the HAT domain and not found in the patients’ germline DNA, and four in EP300. Analysis of other types of low-grade B-cell lymphoma did not yield any similar abnormalities, suggesting they are particular to malignancy arising in the germinal center.

Protein expression in cases with CREBBP alterations showed the wild-type allele present but reduced protein levels, while EP300 alterations led to complete loss of expression, despite detectable mRNA. Reduced protein levels were also noted in some lines despite intact CREBBP and EP300, suggesting alternative mechanisms of inactivation in some lymphomas. The mutations in the two genes showed evidence of functional overlap, with a high proportion clustered in a region of high identity encoding the contact surface for coenzyme A, a cofactor in acetylation reactions. Transient transfection experiments confirmed that CREBBP proteins with mutations in the HAT domain lose the ability to acetylate BCL6 and p53. Studies using reconstituted double-knockout mouse embryo fibroblasts showed that the mutant protein blunted cyclic-AMP-induced transcription and endogenous histone acetylation and also reduced cell growth.

These observations suggest a previously unrecognized but surprisingly common pathogenic pathway in germinal center lymphomas, with a defect in one allele sufficient to produce important changes in the transcriptional profile of a cell despite the presence of a wild-type second copy. This suggests that HAT activity in B lymphocytes can be limiting, with small changes in effective gene dose having major consequences for their phenotype. The therapeutic implication is that attempts to treat using inhibitors of HDAC may be most effective in those cases in which HAT mutations have disturbed the normal balance of acetylation, although clearly other mechanisms will have a role in this. Why such a pathogenic mechanism should be particular to the germinal center remains a matter of speculation, but this paper highlights the power of whole-genome sequencing to produce entirely new information on the genetic changes that lead to malignancy.

Personalized Antiplatelet Therapy for ACS Patients Treated With PCI – Are We There Yet?

Clodipogrel suppresses adenosine diphosphate (ADP)-mediated platelet activation by inhibiting the platelet ADP P2Y12 receptor. Clodipogrel and aspirin are recommended for patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI) and drug-eluting stent (DES) implantation to prevent subsequent ischemic events. The platelet response to clodipogrel may vary, however; this variability can relate to both nongenetic factors and polymorphisms in the cytochrome P450 gene CYP2C19. High platelet reactivity on clodipogrel and loss-of-function alleles of CYP2C19 have been linked to adverse cardiovascular outcomes after PCI, particularly stent thrombosis. By comparison, CYP2C19 polymorphisms may not reduce the benefit of clodipogrel for ACS and atrial fibrillation. Thus, the relevance of platelet reactivity while on clodipogrel, the contributions of acquired and genetic co-factors to platelet function, and the potential benefit of intensifying clodipogrel dosing to “personalize” treatment for poorly responding patients remain undefined.

The Gauging Responsiveness With A VerifyNow Assay-Impact on Thrombosis and Safety (GRAVITAS) study, reported by Dr. Matthew Price from Scripps Translational Science Institute in San Diego, was designed to address whether higher-dose clodipogrel could enhance outcomes for patients with high on-treatment platelet reactivity after PCI and DES placement. This multicenter, randomized, double-blind, active-control trial enrolled 6,429 patients, 2,214 of whom had high platelet reactivity after clodipogrel initiation, as determined by a commercially available P2Y12 assay known as VerifyNow, which measures ADP-induced platelet agglutination. Patients were defined as poor responders if the assay showed P2Y12 reaction unit (PRU) values of 230 or higher. These patients were then randomized to receive either high-dose clodipogrel (600 mg loading followed by 150 mg daily) or standard dose (no additional loading followed by 75 mg daily) for six months. Primary efficacy endpoints included death from cardiovascular causes, nonfatal myocardial infarction (MI), or stent thrombosis. Pharmacodynamic assessments involved P2Y12 Novos at 30 days and six months. CYP2C19 genotype data were not reported. The rates of death at 30 days and six months from cardiovascular causes and all-cause mortality were not different among the high-dose and standard-dose clodipogrel cohorts. Similarly, there were no differences in nonfatal MI or stent thrombosis. High-dose clodipogrel resulted in significantly lower platelet reactivity at 30 days and six months compared with standard dosing, without increased bleeding events. In a secondary comparison, patients with high on-treatment platelet reactivity receiving standard-dose therapy had greater composite primary event rates than 586 observational cohort patients who did not have high on-treatment platelet reactivity at study entry; but these differences were not statistically significant.

The results of the GRAVITAS trial highlight the challenges of using population-based studies to define key targets and optimal goals for personalized therapeutic interventions. Despite GRAVITAS being the largest randomized trial to date of individualized antiplatelet dosing, it did not discern a significant effect of clodipogrel dose intensification. This may relate to the use of fixed drug dosing, genetic heterogeneity, nongenetic factors, variable pharmacodynamic responses, and unexpectedly low event rates among relatively modest sample sizes. Ongoing trials are investigating more potent ADP inhibitors that are not significantly affected by CYP2C19 polymorphisms. Additional study data are also needed to better define at-risk populations, the most reliable biological and/or genetic markers, and optimal on-target endpoints. Until further high-grade evidence is available, platelet inhibition strategies and pharmacogenetic testing for patients with ACS and PCI must remain “impersonal.”

The Way Things Work: Thrombin


Thrombin is the principal enzyme of hemostasis. It catalyzes the conversion of fibrinogen to fibrin and activates procoagulant factors V, VIII, XI, and XIII. Additionally, when bound to thrombomodulin, it activates protein C, an anticoagulant zymogen. Thrombin also activates platelets, regulates endothelial cell function, and has a host of direct actions on other cells. In addition to its catalytic site, thrombin contains a Na+‐binding site and two anion‐binding exosites, ABE1, which binds fibrinogen and thrombomodulin, and ABE2. Thrombin also contains an insertion site for its newly formed N‐terminus that develops following proteolytic cleavage of prothrombin. The insertion produces an internal salt bridge that results in reorganization and activation of the catalytic site.

Thrombin begins life as an inactive zymogen, prothrombin. The conversion of prothrombin to thrombin is catalyzed by factor Xa and includes two essential proteolytic cleavages, producing three intermediates: prothrombin 2, meizothrombin, and Fragment 1.2 (F1.2), which binds ABE2. Since the identification of these intermediates nearly 40 years ago,1 the mechanism(s) underlying the development of the enzymatic activity of thrombin and the remarkable differences in specificity of thrombin and other prothrombin derivatives have been the subject of intense investigation. More than 100 way structures have been reported in hundreds of papers describing the functional properties of thrombin and other prothrombin derivatives. Now Kamath et al. from Krishnaaswamy’s lab at Children’s Hospital of Philadelphia propose a novel mechanism by which thrombin activation and specificity is explained by a continuum of states from the enzymatically inactive zymogen, prothrombin, to catalytically active thrombin (Figure).

Using isothermal titration calorimetry to provide very accurate estimates of binding constants, the authors measured binding of F1.2 to four prothrombin derivatives that represent putative structural intermediates along the continuum from the zymogen, prothrombin, to thrombin. Binding to the derivatives increased with increasing zymogenicity. S195A prothrombin > TAT thrombin > S195A thrombin > FPR thrombin. Additionally, binding of F1.2 to S195A thrombin increased with progressively decreasing concentrations of Na+, consistent with conversion of S195A thrombin to a more zymogen-like state. In contrast, the binding of the thrombin inhibitor DAPA into the catalytic site of zymogen-like TAT thrombin decreased the binding of F1.2, consistent with conversion to an enzyme-like state.

DNA Methyltransferase Mutations Make Their Imprint on MDS and AML


Very so often, an innovative new technology comes along that is powerful enough to reveal entire new worlds. Hans Lippershey’s 1608 refracting telescope came quickly improved by Galileo and Kepler and used to detect extraterrestrial stars, planets, and satellites invisible to the naked eye. Antoine van Leeuwenhoek’s handcrafted microscopes brought previously unknown life forms to the attention of biologists after he published his first observations on “animalcules” in 1673. The introduction of the liben carved in the mid-15th century dramatically improved ocean-going travel and led to the discovery of Oceania and the “New World” by Europeans. And while DNA sequencing is far from new, it is only now, at the beginning of the 21st century, that unbiased high-throughput nucleic acid resequencing at a practical cost is revealing the molecular engines of human diseases in a way not previously possible, generating novel insights that will take many years to functionally validate and fully understand.

In the field of cancer biology, acute myeloid leukemia (AML) was the first malignancy scrutinized with whole-exome and whole-genome sequencing. Since 2008, AML genome projects coordinated by Timothy Ley, Elaine Mardis, and Richard Wilson at Washington University’s Siteman Cancer Center and the Genome Institute of the National Cancer Institute (NCI) in St. Louis led to the discovery of recurrent disease-associated mutations in genes encoding isocitrate dehydrogenase 1 and 2 (IDH1/2) among other proteins.1,2 Most recently this team reported that DNMT3A, which encodes DNA (cytosine-5)-methyltransferase 3A, is recurrently mutated in AML. This is an enzyme responsible for de novo cytosine methylation essential for processes such as DNA imprinting, modulating gene expression, and X-chromosome inactivation.3

WUGCI investigators analyzed 281 samples from patients with AML finding that 22.1 percent had DNMT3A mutations, including 18 different missense mutations, a smaller number of frameshift, nonsense, and splice-site mutations, and a 1.5-Mbp deletion. DNMT3A mutations were absent in patients with favorable karyotypes but were frequent (33.7%) in patients with intermediate-risk cytogenetic profiles; their presence predicted a markedly shorter survival (12.3 months with mutations vs. 41.1 months without). Surprisingly, there was no clear difference in global DNA methylation levels between AML cells with DNMT3A mutations and those without.

Tim Graubard is leading WUGCI’s efforts in myelodysplastic syndromes (MDS), and his team now report heterozygous DNMT3A mutations with translational consequences in 8 percent of 150 patients with MDS. DNMT3A mutations in MDS, as with AML, confer a higher risk of progression to AML, with a 1.5-Mbp deletion, and a higher risk of progression to AML. Patients with DNMT3A mutations were a median of nine months older than patients with wild-type DNMT3A but otherwise clinically indistinguishable.

DNMT3A mutations appear to predict poorer outcomes in both MDS and AML. In MDS, DNMT3A joins a growing list of recurrent disease-associated somatic mutations implicated in altering gene expression by disturbing epigenetic patterning and chromatin conformation, including TET2, EZH2, IDH1/2, ASXL1, UTX, and ATRX. Abrupt splicing patterns of DNMT3A and DNMT3B transcripts have also been reported in related myelodysplasia. Although the finding of recurrent DNMT3A mutations suggests a potential link to the clinical responses observed with DNA methyltransferase inhibitors such as azacitidine and decitabine, it is not yet clear whether there is a direct connection between these mutations that alter DNA methylation, MDS pathogenesis, and treatment response. Ongoing high-throughput sequencing projects in MDS promise even more discoveries that may better illuminate MDS pathology.


PETER B. LOLLAR, MD

Dr. Lollar indicated no relevant conflicts of interest.

DAVID P. STEENSMA, MD

Dr. Steenstra indicated no relevant conflicts of interest.
Human embryonic stem (hES) cells derived from human embryos have the features of self-renewal and pluripotency and provide an invaluable resource for research and potentially for cell therapy. Recent success in reprogramming somatic cells to generate inducible pluripotent stem (iPS) cells opens new avenues to make autologous pluripotent stem cells derived from a patient’s own cells. Two outstanding questions in the reprogramming field are: Are iPS cells normal and safe to use; how close are iPS cells to hES cells?

Early chromosome analysis based on karyotyping (Figure) revealed that most hES and iPS cells were karyotypically normal with some occasionally having extra copies or loss of a chromosome. Chromosomal abnormalities are relatively easy to identify and such cells are not used to model normal cell differentiation. Later studies using comparative genomic hybridization (CGH) to assess for deletion and/or additions of chromosomal DNA found again, with some exceptions, that most hES and iPS cell lines were free from larger than 10 megabase additions or deletions, and consistent differences between hES and iPS cells were not found. But, these assays miss the changes that occur in smaller regions. In the past 16 months, multiple publications have used more sensitive approaches to reveal many types of genetic and epigenetic differences between hES and iPS cells, such as changes in expression of protein coding and noncoding (e.g., microRNA) RNA in ES versus iPS cells.

Two recent reports highlight findings using genome-wide approaches that are highly sensitive for detecting small, but consistent, differences between hES and iPS cells. The paper by Laurent et al. from a multi-laboratory group headed by Jeanne Loring at The Scripps Research Institute reports data on single base pair changes in DNA and gain/loss of relatively short (less than 1 megabase) regions in hES and iPS cells in addition to other cell lines. A separate multi-laboratory group headed by Dr. Joseph Ecker from The Salk Institute performed in-depth analysis of DNA methylation throughout the genomes of ES, iPS, and other cell types. Although the analyses performed were different, with one identifying changes in DNA sequences and the other analyzing a somatic modification (methylation) of existing DNA sequences, both groups found that hES and iPS cells have many consistent differences.

Laurent et al. analyzed 69 ES, 37 iPS, and 72 other cell lines and identified genetic differences (predominantly amplifications and deletions) that were often in regions encoding genes involved in cell-cycle regulation and cancer, including recurrent duplications of oncogenes such as N-RAS, DNMT3B, and BCL2L1, and deletions of tumor suppressor genes. While most of the differences were not clearly cancer-related, the frequent association of the affected genes with cancer is cause for concern. The changes were found to occur at multiple stages of iPS cells’ growth including during reprogramming, over time in culture, and even during subsequent differentiation of the iPS cells. The changes identified did not correlate with the method used to create the iPS cell lines.

Lister et al. demonstrated that iPS and hES cells mostly shared similar patterns of DNA methylation, but they identified consistent patterns of differential methylation at about 120 short regions that were near genes. In most of these cases, the iPS cells were hypomethylated, suggesting a deficiency in resetting DNA methylation patterns during reprogramming. There were 11 regions that were consistently hypermethylated in iPS but unmethylated in somatic cells and ES cells. That they were found in most iPS lines studied suggests they may be important for the actual reprogramming. iPS cells also showed differential methylation patterns in quite large regions of genomic DNA near the centromeres and telomeres of the chromosomes.

While the potential clinical ramifications of these findings are not yet known, these data suggest that there is a lot of additional work that needs to be done to better characterize iPS cells before they are ready for clinical applications. By their very nature, undifferentiated hES and iPS cells form tumors (teratomas) when injected in vivo. The concern raised by these recent findings is that the differentiated cells, even if they could be completely separated from any remaining undifferentiated cells, would also be tumorigenic due to the genetic changes that have occurred over time. Every iPS cell line will need to be evaluated over time for acquisition of mutations.

The Hematologist: ASH News and Reports


drs. park and krause indicated no relevant conflicts of interest.

MicroRNAs in the Plasma: Not miRly a New Biomarker


Perhaps the most important factors in the treatment of chronic lymphocytic leukemia (CLL) are deciding when to initiate treatment and which treatment is best for a particular patient. The typical watch-and-wait approach dictates that treatment is not initiated until symptoms develop. This is mainly due to the risk of adverse side effects of common therapies, particularly when patients may not become symptomatic for many years following the initial diagnosis. However, if predicted correctly, aggressive cases of CLL would most definitely benefit from immediate treatment.

Many prognostic factors that have been determined to predict time-to-treatment or disease severity, such as ZAP70 expression, are difficult to reproducibly measure. Immunoglobulin heavy chain mutational status and select cytogentic markers can also classify disease and predict severity and are therefore very important diagnostic parameters. However, the detection of these abnormalities can be cost- and labor-intensive and is therefore not performed on a routine basis at most institutions. More accessible circulating markers, such as CD23, thymidine kinase, and β2-microglobulin, have not been as extensively validated as reliable markers. However, the ability to use plasma markers to predict disease remains a desirable objective.

The recent study by Moustay and colleagues from Luxembourg explores the use of microRNAs (miRNAs), particularly miR-20a, circulating in the plasma of CLL patients, to predict CLL disease status. These investigators found that many miRNAs are overexpressed in CLL patient samples compared to normal volunteers, not only in the cells but in the plasma compartment as well. The authors found that plasma levels of miR-195 and miR-20a are able to reproducibly distinguish CLL samples from normal controls, and the level of miR-20a correlates with disease severity. In addition, combined analysis of several plasma miRNAs, with particular emphasis on miR-20a, is able to distinguish between patients that are positive or negative for ZAP70 expression.

Interestingly, several miRNAs (miR-135*, miR-451, and miR-486-5P) show an unusual pattern of regulation, in that they are expressed at lower levels in the actual CLL cells compared to the serum. This suggests that the approaches described above, which focus only on the CLL cells, may not paint an accurate picture of the miRNA profile in patient samples. This is because they neglect to take into account miRNAs that are rapidly exported from the tumor microenvironment.

The overall importance of this study is not only that miRNAs can be used as prognostic factors to accurately predict disease state, but also that the composition of miRNAs in the plasma of patients might actually be a more accurate diagnostic tool. In addition, the development of recent technologies that allows analysis of the entire miRNome in a single assay without the need for RNA extraction or cDNA synthesis makes the detection of plasma miRNAs simple and cost effective, which makes this much more attractive as a clinical diagnostic tool.

ERIN HERTLEIN, PHD, AND JOHN C. BYRD, MD

Drs. Hertlein and Byrd indicated no relevant conflicts of interest.
**SIPPET Slowly: This One’s HOT!**

**STUDY TITLE:** Inhibitor Development in Previously Untreated Patients or Minimally Blood Component-Treated Patients With Severe Hemophilia A When Exposed to von Willebrand Factor-Containing Factor VIII (vWF/FVIII) Concentrates and to Recombinant Factor VIII Concentrates: an International, Multicenter, Prospective, Controlled, Randomized, Open-Label, Clinical Trial (The SIPPET Trial)

**COORDINATOR:** This independent study is sponsored by Fondazione Angelo Bianchi Bonomi.

**CLINICALTRIALS.GOV IDENTIFIER:** NCT01064284

**PARTICIPATING CENTERS:** 77 centers: 32 in nine European countries, 14 in three Asian countries, six in two African countries, 15 in the United States, 10 in four Latin American countries

**ACCRUAL GOAL:** 300 patients (129 randomized to date)

**STUDY DESIGN:** This is a prospective trial designed to compare inhibitor incidence in previously untreated patients or minimally blood-component-treated patients with hemophilia exposed to plasma-derived vWF/FVIII or recombinant FVIII products. Eligibility criteria are as follows: male sex, age >6 years, severe hemophilia A (FVIII < 1%) without evidence of an inhibitor, previously untreated or minimally treated (<5 exposure days) with blood components only.

**Patients are randomized to receive exclusively a single FVIII product (plasma-derived or recombinant), and they are then followed until they reach one of the following three endpoints: inhibitor development, 50 days of exposure to the FVIII product, or three years from enrollment. The primary endpoint is the proportion of patients who develop anti-factor VIII antibodies based on analysis performed following every three to four exposure days or every three months, whichever occurs first. Patients on prophylactic therapy will be tested every two weeks. The following secondary objectives are aimed at identifying risk factors for inhibitor development: age at first treatment; bleeding severity; surgery; treatment intensity; treatment regimen; vaccination history; concurrent infections; complications of venous access; delivery type; breastfeeding; FVIII gene defect; FVIII antigen concentration; HLA type; IL-10 G (promoter region microsatellite); TNF-α concentration; and CTLA-4 polymorphisms.

**RATIONALE:** The most challenging complication of hemophilia A is inadequate control of bleeding due to development of antibodies against FVIII. An unresolved issue is whether plasma-derived and recombinant FVIII products are associated with different risks of inhibitor development. A systematic review of published studies was uninformative because the analyzed trials differed in designs, case definitions, treatment regimens, patients’ characteristics, FVIII inhibitors assays, sample size, and duration of follow-up (Iorio A et al. J Thromb Haemost. 2010; 8: 1256-1265). The SIPPET trial is the first prospective, randomized study aimed at establishing whether recombinant and plasma-derived products confer a different relative risk of developing factor VIII antibodies.

**COMMENT:** Development of anti-factor VIII antibodies greatly complicates management of patients with hemophilia A, as treatment outcome using bypass agents, such as recombinant factor VIIa or factor IX complexes, is unpredictable and expensive. Eradication of inhibitors by inducing immune tolerance using scheduled FVIII infusions is effective in ∼70 percent of patients, but the process is demanding and costly. The SIPPET study is aimed at determining if the incidence of antibody development is influenced by the source of replacement product. How the findings will influence practice ultimately will depend on the magnitude of differences (if observed) and perhaps the willingness of patients to put aside concerns about safety if the plasma-derived product proves superior. Any randomized trial that involves a rare disease is a monumental undertaking, but the SIPPET trial, which involves patients and investigators in 19 countries on four continents, is particularly noteworthy.

>–Pier Mannucci, MD; Alessandro Gringeri, MD; and Margaret Ragni, MD, MPH

**Sequelae of DVT: Post-Thrombotic Syndrome**

**STUDY TITLE:** Acute Venous Thrombosis: Thrombus Removal With Adjunctive Catheter-Directed Thrombolysis (ATTRACT)

**SPONSOR:** Washington University School of Medicine

**COLLABORATORS:** McMaster University, Ontario Clinical Oncology Group, Babcsz Vascular, BSN Medical, Inc., Genetech, MIDRAD Interventional/Possis, Mid America Heart Institute, Society of Interventional Radiology Foundation VasCore at Massachusetts General Hospital

**CLINICALTRIALS.GOV IDENTIFIER:** NCT00790335

**PARTICIPATING CENTERS:** 57 institutions in the United States

**ACCRUAL GOAL:** 692 patients

**STUDY DESIGN:** This is an open label, randomized study with efficacy and safety endpoints. Eligibility criteria include the following patients who are 16 to 75 years old with a new (<14 day duration) symptomatic proximal deep-vein thrombosis (DVT) involving the iliac, common femoral, femoral vein, or a combination of these sites.

**ARM A, INTERVENTION:** Pharmacomechanical catheter-directed thrombolysis (PCDT) with intrathrombus delivery of recombinant tissue plasminogen activator (rt-PA) into the DVT over a period of up to 24 hours using one of three rt-PA delivery methods. Before and after PCDT, patients will receive standard DVT therapy as in the control arm.

**ARM B, CONTROL:** Initial anticoagulant therapy with unfractionated heparin, enoxaparin, dalteparin, or tinzaparin for at least five days, overlapped with long-term oral warfarin (target INR 2.0–3.0). Elastic compression stockings will be prescribed.

**PRIMARY OUTCOME MEASURES:** Cumulative incidence of post-thrombotic syndrome assessed by the Villalta scale within 24 months following randomization.

**SECONDARY OUTCOME MEASURES:** Efficacy: Severity of post-thrombotic syndrome (PTS), resolution of presenting DVT symptoms, the prevalence of valvular reflux and residual thrombus, the degree of clot lysis, and cost-effectiveness within 24 months of randomization. Safety: Major bleeding, symptomatic pulmonary embolism, recurrent venous thromboembolism, and death within 10 days and 24 months after randomization. This is designated as a safety issue.

**RATIONALE:** The purpose of this study is to determine if the use of adjunctive PCDT, which includes the intratherombus administration of rt-PA, can prevent PTS in patients with symptomatic proximal DVT as compared with optimal standard DVT therapy alone. Rt-PA is a thromolytic drug that is indicated for use in acute myocardial infarction, acute ischemic stroke, and acute massive pulmonary embolism in adults. Previous studies have established the capability of rt-PA to lyse venous thrombus in patients with DVT and suggest that successful rt-PA-mediated thrombolysis can prevent PTS, a morbidity, late complication of DVT that occurs in nearly 50 percent of patients.

Under imaging guidance, rt-PA is delivered directly into the venous thrombus using a catheter/device that is embedded within the thrombus. This method of rt-PA delivery, PCDT, is thought to be safer, more effective, and more efficient than other approaches. The question of whether PCDT using rt-PA improves long-term outcomes with acceptable risk and cost in patients with DVT has not been addressed. The ATTRACT trial will investigate the major clinical controversy of whether catheter-directed thrombolysis should be used routinely as part of first-line DVT therapy.

**COMMENT:** Despite optimum anticoagulation therapy, venous stasis syndrome, venous ulcers, and chronic leg swelling are common, costly sequelae of DVT. Why does PTS occur? Venous hypertension either primarily or secondarily underlies the pathophysiology, but inflammation, cytokines, and other factors likely play roles in the process. For several years the “open venous” hypothesis suggested that immediate thrombus removal may preserve venous valve function and prevent PTS. This study addresses many important questions regarding catheter-directed thrombolysis including safety, cost, and effectiveness in preventing PTS.

>–Gregory M. Vercellotti, MD

Drs. Mannucci and Gringeri are the principal investigators for this trial.

Dr. Vercellotti indicated no relevant conflicts of interest.
Alvin Mauer, MD
(1928 – 2011)

Alvin Mauer, MD, former president of the American Society of Hematology, died May 26, 2010, at the age of 82. Work by this distinguished physician, researcher, and mentor advanced the fields of hematology and oncology. Dr. Mauer encouraged collaboration of basic and clinical scientists at a time when bench-to-bedside approaches were in their infancy. His leadership enabled strong progress at Cincinnati Children’s Hospital, St. Jude Children’s Research Hospital, and the University of Tennessee — organizations he served with great passion and commitment.

Dr. Mauer was born in Le Mars, IA, in 1928. After service with the U.S. Army, he graduated from the University of Iowa College of Medicine and finished his medical education at Cincinnati General Hospital and Cincinnati Children’s Hospital. He then completed a three-year pediatric hematology fellowship at the University of Utah and later returned to Ohio to serve as director of hematology/oncology at Cincinnati Children’s Hospital.

In 1973, Dr. Mauer moved to Memphis, TN, to become the second director and CEO of St. Jude, an institution renowned for its work in childhood cancer and other catastrophic diseases.

Dr. Mauer was established to honor individuals who through their genius, talent, and energy have made outstanding contributions to health care and/or humanity.

Joel M. Rappeport, MD
(1939 – 2011)

Joel Rappeport, MD, died on January 16, 2011, after a long illness. He was an internationally recognized expert in bone marrow failure and hematopoietic stem cell transplantation.

Born June 10, 1939, in Quincy, MA, Dr. Rappeport graduated from Yale College in 1961 and received his medical degree in 1965 from Tufts University School of Medicine. He was a resident on the Tufts Medical Service at Boston City Hospital from 1965 to 1967. Following service in the Air Force Medical Corps, he was a resident at the Beth Israel Hospital, Boston, from 1969 to 1970.

Dr. Rappeport completed a fellowship in hematology at the Peter Bent Brigham Hospital where he joined the faculty, rising to associate professor of medicine at Harvard Medical School in 1984. At Harvard, Dr. Rappeport and colleagues David Nathan, Fred Rosen, and Robertson Parkman were pioneers in the development of bone marrow transplantation for neoplastic and non-neoplastic hematologic disorders. This collaborative effort at the Peter Bent Brigham Hospital and the Children’s Hospital Medical Center led to the first bone marrow transplantation unit in New England. He and his team were the first to do marrow transplants on patients with paroxysmal nocturnal hemoglobinuria and on those with congenital hematopoietic and metabolic defects such as Wiskott-Aldrich syndrome, Kostmann syndrome, and severe Gaucher disease. These successes paved the way for the use of hematopoietic stem cell transplantation to treat the more prevalent hemoglobinopathies, sickle cell disease and β-thalassemia major.

In 1987, Dr. Rappeport moved to Yale School of Medicine where he served as professor of medicine and pediatrics. Dr. Rappeport founded the allogeneic bone marrow transplantation unit at Yale-New Haven Hospital, which he directed from 1987 to 1997. He served as the director of the Sickle Cell Disease Program at Yale-New Haven Hospital from 1998 to 2003.

Dr. Rappeport fulfilled numerous advisory roles, including member of the Board of Directors and president of the New England chapter of the Aplastic Anemia Foundation of America, trustee and vice-president of the central Connecticut chapter of the Leukemia Society of America, and member of the Committee on Educational Affairs and an Education Program chair of ASH. He published more than 120 original scientific articles, reviews, and book chapters.

Dr. Rappeport was widely recognized as an outstanding clinician, teacher, clinical investigator, and mentor; he was greatly admired for his tireless dedication to his patients. His teaching and dedication to his patients earned him the Yale medical house staff Teacher of the Year Award in 2000-2001. He directed the hematology course for second-year medical students at Yale. He taught by example, imbuing his students, residents, and fellows with respect, responsibility, and a love of medicine and hematology. He had a highly developed sense of humor and irony that he put to good use on behalf of patients, prodding everyone to go the extra mile on their behalf. Nurses and aides were devoted to him. His total commitment to his patients inspired and empowered all who followed his lead.

—Bernard G. Forget, MD, and H. Franklin Bunn, MD

Under Dr. Mauer’s leadership, St. Jude doubled in size. He is credited with ensuring that the relationships between clinicians and basic scientists thrived as the hospital grew. During his tenure, Dr. Mauer also established the hospital’s Domestic Affiliate Program, which created St. Jude clinics in other cities, enabling some children to receive their protocol treatments closer to home.

Beginning in 1983, he served as chief of the division of hematology/oncology and later as professor emeritus of the department of medicine at the University of Tennessee Health Science Center.

A member of numerous scientific societies, Dr. Mauer was elected president of the Association of American Cancer Institutes and the International Society of Hematology. He also served two terms as president of ASH; he was the only member to have this honor. Among his many honors, Dr. Mauer was recognized with the prestigious St. Boniface General Hospital Research Foundation Award, which was established to honor individuals who through their genius, talent, and energy have made outstanding contributions to health care and/or humanity.

He is survived by his wife, Theresa; two daughters, Elizabeth Mauer Sweeney and Daria; and two sons, Timothy and Stephen.
My journey to hematology and blood research has been the result of a series of chance occurrences. I grew up in Peñasco, a small village at an elevation of 8,000 feet in the Sangre de Cristo Mountains of northern New Mexico that has been the home of my ancestors for countless generations. Peñasco was, and is, a poor village. When I was young, I did not have exposure to scientists or medicine. In retrospect, however, it is clear to me that my experiences as a child shaped my love for science and, eventually, hematology. My first scientific inquiry derived from the necessity of growing almost all of our own food, which exposed me to the behavior and breeding of all manner of farm animals; it taught me mammalian anatomy and allowed me to marvel at the wondrous ways that plants can manufacture food from dirt and air. My inquisitiveness was encouraged by my parents and challenged by my siblings. But none of this curiosity would have led me to a fulfilling career in hematology research had it not been for several key people who altered my path: my high school science teacher who encouraged me to apply to a summer program in science at a small science and engineering university in the state, my chemistry professor who offered me work in his laboratory so that I could pay my tuition, and my medical school counselor who encouraged my scientific leanings in a school devoted almost entirely to training primary-care physicians. Those experiences paved my way to a career in medicine, but the path to research was still murky.

It was an experience I had during my internal medicine residency at the University of Washington that sparked my interest in hematology. During the second year of residency, most UW residents did externships in distant community hospitals loosely affiliated with the residency program. I was sent to Billings, MT, to work with a local internist who had not anticipated my arrival. Being very busy, he sent me off to the hospital ICU to examine a young woman who appeared to be on the verge of death. Coincidentally, I had just read about the rare disease thrombotic thrombocytopenic purpura (TTP). After a cursory examination of the patient, I concluded that she did indeed have TTP. The patient and I were both lucky; I was right, and she received the lifesaving therapy of plasmapheresis. This diagnostic coup led to a request from the local medical community that I deliver a medical grand rounds presentation on TTP. I read every article I could find on the topic, and on platelets, since they clearly played a role in the disease. I became fascinated with the disease and with platelets and resolved to study them. I applied and was accepted to the hematology fellowship program at UW, then headed by former ASH President Dr. John Adamson. While I was a fellow, I queried many of my attendings about the research in their labs; few were interested. But Gerald Roth was interested, and, as a bonus, he happened to work on platelets. It was Jerry's idea that I apply for a fellowship program aimed at increasing the number of underrepresented minorities in medicine through the Robert Wood Johnson Foundation (now called the Harold Amos Medical Faculty Development Program). After one failed application, we enlisted the help of Dr. Earl Davie, renowned for his work on the basic science of blood coagulation. The second time was a charm, and it was through this program that I was able to meet many students with stories like mine; I am still in contact with many of these colleagues, including Arturo Molina, Alexis Thompson, and Griffin Rodgers.

The work I began with Drs. Roth and Davie led me to explore the molecular basis of platelet adhesion, first working on a platelet receptor, the glycoprotein IbαIX-V complex, and then on its vascular wall ligand, von Willebrand factor (VWF). The work took me through complex, and then on its vascular wall ligand, von Willebrand factor (VWF). The work took me through several academic appointments, from the University of Washington to the University of California, San Francisco, and the Gladstone Institute for Cardiovascular Disease, to Baylor College of Medicine, and finally back to Seattle with the Puget Sound Blood Center and UW, where I have had the opportunity to oversee and guide the growth of the Blood Center’s Research Institute. These travels have afforded me many opportunities, including working with Joel Moake, whose seminal work on the pathogenesis of TTP pointed to the role of ultra-large and hyperadhesive multimers of VWF. My opportunity to work with him and with other colleagues on the pathophysiology of TTP brought me back to the patient that initially sparked my interest in hematology. This work has also highlighted another reason I find hematology so attractive: it provides a scientist an opportunity to work on almost anything, from physics to human illness. I take to heart the old adage about hematology being “the study of blood and the organs through which it flows.”

As important to my career as my mentors and scientific colleagues have been, equally important and rewarding have been the opportunities to mentor and influence the many individuals who have been through my lab or that I have met in the clinic or through teaching. And it was an honor for me to serve for seven years as co-chair of the American Society of Hematology’s Minority Recruitment Initiative (now the Committee on Promoting Diversity) with Cage Johnson. I have always wanted to serve in the role of “the happy accident” that spurred a student’s interest in science.
The ASH website offers a convenient way for ASH members to find information relating to upcoming Society events and provides easy access to the many valuable products and services offered by ASH.

Spotlight on: Quick Links
In June, ASH introduced new site navigation features called “Quick Links” to help visitors quickly and easily access popular website content. Quick Links highlight website content that visitors repeatedly search for as well as content users access most often. Visitors will be able to access these Quick Links from the homepage and the Research, Practice, and Training sections of the website. Quick Links will be rotated regularly to ensure that visitors are directed to the most popular content on the ASH website.

July
19
Early-bird annual meeting registration opens to ASH members
San Diego, CA
www.hematology.org

August
1
Application deadline for active and international membership
Washington, DC
www.hematology.org

10
Annual meeting advance registration and housing open to members and non-members
San Diego, CA
www.hematology.org

11
ASH annual meeting abstract submission deadline
San Diego, CA
www.hematology.org

25-28
40th Annual Scientific Meeting of the Society for Hematology and Stem Cells
Vancouver, Canada
www.iseh.org

September
7-8
NIH State-of-the-Science Symposium on Myelodysplastic Syndromes
Bethesda, MD
www.nhlbi.nih.gov

23-24
2011 State-of-the-Art Symposium
Chicago, IL
www.hematology.org

22-24
Annual Meeting of the Society for the Advancement of Blood Management
Philadelphia, PA
www.sabm.org

October
8-9
The Malacca Strait International Hematology-Oncology Symposia 2011
Medan, North Sumatera, Indonesia
www.phldisumut-aceh.com

12-13
ASH Advocacy Leadership Institute
Washington, DC
www.hematology.org

19-22
2011 American Society for Clinical Pathology Annual Meeting & World Association of Pathology and Laboratory Medicine XXVI World Congress
Las Vegas, NV
www.ascp.org

21
ASH annual meeting late-breaking abstracts submission site opens
San Diego, CA
www.hematology.org