Opportunities and Challenges for Use of Human Pluripotent Stem Cells in Hematopoietic Developmental Biology and Regenerative Medicine

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Mouse Pluripotent Stem Cells
The defining features of pluripotent stem cells (PSCs) are their unlimited proliferative capacity and their ability to differentiate into all somatic cell types as well as germ cells. Mouse embryonic stem cells (ESCs), the first known non-malignant PSC type, are derived from the early epiblast. They were originally isolated in 1981 and have since been used with great success as in vitro models of mammalian development. Highlights in hematopoiesis include their differentiation into hemangioblasts, erythroid cells, lympho-hematopoietic progenitors, and hematopoietic stem cells (HSCs). Mouse ESCs have also been key to numerous in vivo analyses of gene function and to proof-of-principle studies that demonstrate the feasibility of PSC-based therapies for degenerative disorders, such as immunodeficiency and sickle cell anemia. However, it is important to note that several aspects of human biology and pathology of particular relevance to hematopoiesis are only poorly replicated in mouse models. Examples include developmental programs of globin gene switching and pathways such as Fanconi anemia (FA), Trisomy-21-associated M7 acute myelogenous leukemia, and poorly defined genetic syndromes (e.g., thrombocytopenia with absent radii). Furthermore, mouse models often fail to mirror responses to pharmacologic agents such as TNFα.

Human PSCs
Pluripotent human ESCs, derived from the late epiblast, were first isolated in 1998. More recently, another breakthrough was achieved: the direct reprogramming of differentiated human somatic cells into induced PSCs (iPSCs) by forced expression of pluripotency genes. Generation of human iPSCs is significant since it provides a straightforward source for normal as well as patient/disease-specific PSCs that are remarkably similar to embryoid-derived PSCs while avoiding the ethically charged use of human embryos or eggs. Reprogramming is also a fascinating process per se, the study of which will undoubtedly continue to further our understanding of epigenetics, stem cell biology, cell fate regulation, cancer, and aging.

Human ESCs may be obtained from embryos produced by IVF, parthenogenesis, or, at least in theory, nuclear transfer, whereas iPSCs can be achieved by direct reprogramming of somatic cells, such as skin fibroblasts. Independent of their origin, these PSCs have the potential to give rise to all somatic cell types, including those of the hematopoietic compartment, thus allowing human tissue ontogeny to be studied in vitro. PSC-derived somatic cells may be used to model diseases such as leukemia, to screen drugs, or as therapeutic agents in cell-based therapies.

Using Human PSCs to Study Hematopoietic Ontogeny
Informed by prior mouse studies, several groups were able to develop protocols for directed differentiation of human PSCs into hemangioblasts, multi-lineage progenitors, lymphoid cells, and even HSC-like cells. However, several challenges remain. For example, in vitro differentiation tends to replicate normal ontogeny, thus favoring development of early embryonic or fetal cells over more mature lineages. In particular, generation of adult-type erythroid cells or bona fide HSCs, so far, has met with little success. Furthermore, many studies make liberal use of undefined reagents, such as animal sera or support cells, which may obscure a detailed mechanistic understanding of the patterns of cellular development and hinder clinical use of these protocols. Furthermore, we are only just beginning to understand why individual stem cell lines can differ dramatically in their intrinsic potential to form particular lineages, a fact that limits our ability to generalize findings obtained with any small set of lines.

Using Human PSCs to Study Hematopoietic Disorders and Malignancies
One group has already produced disease-corrected hematopoietic progenitors from FA patients using iPS technology. In addition, human ESCs or iPSCs from Trisomy-21-, SCID-, SBDS-, and thalassemia embryos or patients have been generated and await further analysis.

Human PSC technology is particularly powerful, as it allows for the modeling of diseases without specific knowledge of the underlying gene mutation(s), or of diseases that result from tissue-restricted somatic mutations (e.g., leukemia). It will be interesting to see if multi-step progression of proliferative disorders can be reconstructed in vitro and whether drug screening in such models will lead to improved treatment modalities. Further, PSC technology can provide genetically diverse sets of human cells for drug toxicity testing.
Letter to the Editor

NHLBI Clinical Trial for Treatment of TTP

To the Editor:

The National Heart, Lung, and Blood Institute (NHLBI) Transfusion Medicine/Hemostasis Clinical Trials Network has opened a clinical trial to study the role of rituximab in the treatment of thrombotic thrombocytopenic purpura (TTP). The Network was formed in 2002 as a consortium of 18 academic centers to provide opportunities for clinical research on uncommon hematologic disorders. The Study of TTP and RituXimab (STAR trial) randomizes patients to standard care (plasma exchange, corticosteroids) or standard care plus rituximab (the standard regimen of four weekly infusions), begun before the sixth plasma exchange. Eligibility is based on clinical criteria and does not require ADAMS13 deficiency. Rituximab is supplied free of charge to the patient by Genentech, Inc. The primary objective is to improve early (before day 21) treatment response. Our hypothesis is that rituximab will reduce the failure to achieve an early treatment response from 38 percent to 16 percent. Two hundred thirty-eight (238) patients, 119 in each treatment arm, will be enrolled. The complete protocol is accessible at www.clinicaltrials.gov/ct2/search.

We want hematologists to be aware of this study. If you are near one of the Network centers (listed on the Web site), you may consider whether a patient with TTP should be enrolled. We are enthusiastic about the potential of the STAR trial to define the appropriate role of rituximab in the treatment of TTP.

Joseph E. Kiss, MD, University of Pittsburgh
Lynne Uhl, MD, Beth Israel Deaconess Hospital, Harvard Medical School
James N. George, MD, University of Oklahoma

Letters to the Editor Solicitation

The Hematologist welcomes letters of up to 200 words. These letters may be in response to editorials or on any subject of interest to our readers. Please include a postal address, e-mail address, and phone number. Publication will be based on editorial decisions regarding interest to readers and space availability. We may edit letters for reasons of space or clarity. The Hematologist reserves the right to publish your letter, unless it is labeled “not for publication.”

Letters should be sent to:
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President’s Column

ASH’s Voice in the Health Reform Debate

As this issue of The Hematologist goes to press, the U.S. Senate and House of Representatives are preparing for votes on health reform legislation. The legislation to overhaul the nation’s health-care system is constantly evolving: key congressional committees are advancing proposals, but battle lines continue to sharpen between Democrats and Republicans. Meanwhile, the major stakeholder interest groups — insurers, pharmaceutical companies, hospitals, physicians — spar over provisions involving the role of the federal government, who will be covered and who will cover them, and how we will pay for reforms.

ASH members likely have been following the debate and are familiar with the discussion concerning the development of a public insurance option, elimination of coverage denials based on pre-existing conditions, creation of an employer mandate, and other important issues. However, it may not be apparent to our membership how ASH has been representing hematology in this debate.

Over a year ago, the Committee on Government Affairs proposed major principles for health-care reform, which were approved by the Society’s Executive Committee. These principles provide a broad context for Society involvement and include support for universal access to affordable health care, evidence-based medicine, and continued federal investment in biomedical research. The complete principles can be viewed at www.hematology.org/Advocacy/Policy-Statements.

As the new Congress began discussing reform earlier this year, members of ASH’s Committee on Government Affairs and Committee on Practice drilled down further and visited congressional offices to discuss the need to maintain access to specialized hematology care. They made the following recommendations: Congress should maintain policies that ensure patients have direct access to hematologists, legislation must recognize the value of cognitive services and improve Medicare payment for these services, legislation should not establish policies that increase payment to primary-care services by reducing payment for cognitive services, and Congress should eliminate the Medicare Sustainable Growth Rate formula and provide physicians with an adequate annual update in fees.

By spring, congressional debate concerning health reform began in earnest, and there was significant congressional interest in addressing the increasing shortage of primary-care physicians. While ASH supports the concept of a primary-care bonus, the Society became concerned that the “budget-neutral” proposals under consideration would require any bonus to be financed through reductions in other services, including cognitive services.

Recognizing that this was a concern other cognitive specialties may share, ASH took the initiative to reach out to all of the internal medicine subspecialty societies to organize a joint advocacy strategy. Consequently, an ad hoc coalition of internal medicine subspecialty societies developed a proposal that would provide a bonus payment for evaluation and management (E/M) services provided to patients suffering from the chronic conditions already identified by the 2009 Medicare Special Needs Plan Chronic Condition Panel (SNCP). By using a patient-centered approach to determine eligibility, all physicians treating patients with the chronic conditions identified by the SNCCP would be rewarded for the provision of care.

As of the beginning of July, nine physician groups and a growing list of patient advocacy organizations have shared this proposal with Congress. ASH has helped coordinate joint visits to key congressional offices. While the outlook for this specific proposal is not clear, this multispecialty-society effort has helped educate Congress about evaluation and management services and has helped increase the visibility of what our members and other physicians in heavily cognitive subspecialties do to treat patients. I encourage you to keep track of the health reform debate and ASH’s efforts through the Society’s Web site. Please do not hesitate to share your concerns and questions with the Government Affairs staff at grassroots@hematology.org.

Nancy Berliner, MD

President’s Column
Janet Rowley Receives Presidential Medal of Freedom and Wins Prestigious Gruber Foundation Prize

Long-time ASH member, Janet D. Rowley, MD, of the University of Chicago has had a very special summer. On August 12, she was honored at the White House by President Obama with the Presidential Medal of Freedom, the highest civilian award in the United States. She shares this honor with a group of 16 highly distinguished international figures, including Sen. Edward Kennedy, film star Sidney Poitier, civil rights leader Rev. Joseph Lowery, retired Supreme Court Justice Sandra Day O’Connor, and retired Archbishop Desmond Tutu of South Africa. Dr. Rowley was also named the 2009 winner of the $500,000 Peter and Patricia Gruber Foundation Genetics Prize for her groundbreaking research on recurring chromosome abnormalities in leukemias and lymphomas. These were among the first studies to show that cancer is a genetic disease.

Election Ballots Due September 30

Active members in good standing should have received election materials for this year’s ASH leadership election for Vice President and Councillors. The results of the election will be announced in the November/December issue of The Hematologist.

New Opportunity: Translational Research Training in Hematology

Applications are now being accepted for the Translational Research Training in Hematology (TRTH) award. This joint venture between ASH and the European Hematology Association (EHA) gives early-career researchers the tools, mentoring, and access to resources beneficial for a successful career in hematology. The application deadline is September 15. To learn more, visit www.hematology.org/trth.

John Leonard Appointed to ABIM Subspecialty Board

ASH member John P. Leonard, MD, was named to the hematology subspecialty board of the American Board of Internal Medicine (ABIM). ABIM’s Subspecialty Boards are composed of experts in both academic medicine and practice, all of whom must be ABIM-certified in their particular subspecialty. Members of these boards apply their individual and collective knowledge toward the development of the policies, standards, and requirements for Certification and Maintenance of Certification in their subspecialty, with special focus on developing the cognitive exam that physicians must take to certify or maintain their certification in that field. Dr. Leonard is the Richard T. Silver Distinguished Professor of Hematology and Medical Oncology at Weill Cornell Medical College, New York Presbyterian Hospital.

Remembering James McArthur, MD, Long-Time Editor of the ASH Education Program Book

ASH member James R. McArthur, MD, died Sunday, July 12, 2009. From 1973 until several years past his retirement, he served as director of the University of Washington’s morphologic slide bank, which eventually became the ASH Image Bank, and for 25 years he was the editor of the ASH Education Program Book. In 1998, Dr. McArthur received the Exemplary Service Award, which recognizes long-standing service to ASH. Dr. McArthur was a professor emeritus in the Division of Hematology at the University of Washington. Read Dr. McArthur’s obituary in the Seattle Times. Go to www.legacy.com/obituaries/seattletimes.

ASH Announces 2009 Recipients of the Research Training Award for Fellows

ASH awarded five individuals the ASH Research Training Award for Fellows, a grant that encourages junior researchers to pursue careers in academic hematology by supporting protected time to conduct research during their fellowship training. The program provides grants of $50,000 for a one-year period to third- and fourth-year trainees. The 2009 ASH Research Training Award for Fellows recipients are:

- Janice M. Staber, MD, University of Iowa Children’s Hospital, Iowa City, IA
- Omar I. Abdel-Wahab, MD, Memorial Sloan-Kettering Cancer Center, New York, NY
- Sascha A. Tuchman, MD, Duke University Medical Center, Durham, NC
- Daniel A. Pollyea, MD, Stanford University, Stanford, CA
- Laura E. Hogan, MD, New York University School of Medicine, New York, NY
A Girl Who Cries Blood – Or Does She?

George Buchanan, MD

Dr. Buchanan is Children’s Cancer Fund Distinguished Chair in Pediatric Oncology and Hematology, and Director of Pediatric Hematology-Oncology at the University of Texas Southwestern Medical Center.

One of the most common clinical problems that hematologists deal with is diagnosis and management of patients with prolonged, excessive, or unusual bleeding. As a pediatric hematologist interested in hemorrhagic disorders for three decades, I thought until recently that I had “seen it all.” But I was wrong. This past January, I was asked by the ASH Communications Department staff to consider working with the National Geographic Channel in filming a story about a 13-year-old girl in India who was said to “cry tears of blood.”

Somewhat hesitantly, I agreed to become involved. Little did I then imagine what was to follow. My odyssey during the subsequent months started with discussions involving the prospective film’s producer/director and his crew, based in London. They described an unusual patient named Twinkle, whose story had surfaced in press reports and on the Internet. They shared with me some of her medical records. Several years earlier she had begun having unexplained, spontaneous bleeding from her eyes, scalp, hands, and feet on almost a daily basis. Several evaluations at medical centers in Northern India resulted in no specific diagnosis. As a result of the unusual bleeding, the girl was expelled from school and shunned by her friends.

It was felt by the National Geographic Channel that telling Twinkle’s story and attempting to find the cause of her bleeding and identify a “cure” would be of wide-spread interest. The plan was to have a hematologist from America travel to India to observe her bleeding, assist with making a correct diagnosis, and deliver effective treatment. I wasn’t sure whether ASH was honoring me or punishing me by recommending that I be that hematologist! But in late March, I traveled to India with the film crew, met the patient and her family, and — working collaboratively with a hematologist and other staff at a hospital in Mumbai — reviewed the existing medical records and ordered tests. I also observed Twinkle’s bleeding … well, more specifically, observed Twinkle after she bled.

The bottom line of this adventure is that the National Geographic Channel documentary was successfully filmed and will be internationally televised beginning on Sunday, September 13. Does Twinkle really cry tears of blood? What is her diagnosis? To find out, you’ll have to watch the program! Although it may not fully clarify what is wrong with Twinkle, I am hopeful that it will inform the thousands of viewers about the diagnosis and management of bleeding disorders — and the important role of hematologists in that process — as well as provide a fascinating overview of a girl and her family in rural India whose life has been changed by a perplexing problem.

COver StOry

Therapeutic Use of Human PSCs

Much of the excitement around human PSCs comes from the credo that patient-specific cells can be generated, undergo gene repair (if necessary), be induced to differentiate into therapeutically valuable cell types, and subsequently be re-introduced into patients to replace or ameliorate the affected cells or tissue. PSC-derived HSCs could benefit those in need of a bone marrow transplant, since finding a suitable marrow donor remains a major obstacle. In contrast to conventional bone marrow transplants, HSCs from a single donor could be provided to several recipients and could be prepared devoid of lymphocytes to avoid graft-versus-host concerns. Additional clinical benefits of these HSCs include induction of tolerance to solid tissue transplants of the same donor type and graft versus autoimmunity.20

While human PSC-derived, clinical-grade blood cells are not yet a reality, Geron Corp. is forging ahead with an experimental therapy for spinal cord injury that uses iPSC-derived HSCs to ameliorate the affected cells or tissue. PSC-derived HSCs could benefit those in need of a bone marrow transplant, since finding a suitable marrow donor remains a major obstacle. In contrast to conventional bone marrow transplants, HSCs from a single donor could be provided to several recipients and could be prepared devoid of lymphocytes to avoid graft-versus-host concerns. Additional clinical benefits of these HSCs include induction of tolerance to solid tissue transplants of the same donor type and graft versus autoimmunity.

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The Road Ahead

The development of human PSC-based therapies faces several challenges, such as funding restrictions on ESC derivation and use, an uncertain intellectual property landscape, and a regulatory framework that was not developed with such therapies in mind. Scientists also struggle to find safe and efficient methods for patient-specific ESC or iPSC production as well as for subsequent large-scale differentiation into transplantable cells.

Other issues include the question of whether human iPSCs are indeed equivalent to conventional ESCs (Emerging evidence suggests that substantial differences exist and much more needs to be learned.), and the lack of long-term clinical data. Nevertheless, while the obstacles undoubtedly may be daunting in the aggregate, each individual challenge can likely be overcome. After all, it took several decades from the first development of monoclonal antibodies to their broad success in clinical use. Likewise, if given sufficient time and support, human PSC technology promises to revolutionize regenerative medicine.

6. Rideout WM, Hochedlinger K, Kyba M, et al. Stringent purification of the differentiated cells is therefore of paramount importance. For added safety, the cells can be encapsulated or irradiated prior to transplantation, but this will often significantly limit the effectiveness and durability of the therapy.

Human PSC-derived erythroid cells constitute a cell type that can be irradiated without detrimental effects on function. The therapeutic effects of irradiated erythroid cells will necessarily be transient, as is the case with conventional blood transfusion. However, the combination of a clinical need, the absence of HLA restriction, and the possibility of avoiding a critical safety concern through irradiation make it likely that PSC-derived erythrocytes will become one of the first clinical success stories. Indeed, large-scale production is feasible, and a consortium headed by the Scottish National Blood Transfusion Service will attempt to produce clinical-grade universal donor erythrocytes.

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Watch the premiere of “The Girl Who Cries Blood” Sunday, Sep- tember 13, 2009, at 9:00 p.m. ET/PT on the National Geographic Channel. If you happen to miss the show on September 13, go to http://natgeotv.com/blood to learn more.

Pluripotent Stem Cells

[Cont. from Page 1]
ASK THE HEMATOLOGIST

Mikkael A. Sekeres, MD, MS
Dr. Sekeres is Director, Myelodysplastic Syndromes Program, Department of Hematologic Oncology and Blood Disorders, Cleveland Clinic Taussig Cancer Institute.

(Editor’s Note: This question was submitted through the Consult-a-Colleague Program. Dr. Sekeres was asked to respond.)

CLINICAL PROBLEM

A 70-year-old gentleman presented with a two-month history of fatigue and bleeding gums. A recent CBC revealed a WBC of 38,600/µL, with a differential of 20,400 neutrophils/µL, 6,900 lymphocytes/µL, 400 monocytes/µL, 3,000 metamyelocytes/µL, 6,500 myelocytes/µL, 700 progranulocytes/µL, and 700 myeoloblasts/µL. His hemoglobin was 9.4 g/dL, and his platelet count was 23,000/µL. A bone marrow biopsy revealed 80 percent to 90 percent cellularity with markedly decreased megakaryocytes with mono-lobate forms and decreased erythroid maturation with evidence of dyserythropoiesis. The M:E ratio was 23:1 and the differential was left-shifted, with 2 percent myeoloblasts, 7 percent progranulocytes, 54 percent myelocytes, 15 percent metamyelocytes, 5 percent bands, and 8 percent segmented neutrophils. Moderate fibrosis was present. G-Banded chromosomal analysis was normal, 46 (XY). The BCR/ABL1 translocation was not detected by fluorescence in-situ hybridization (FISH) analysis; nor was the JAK2 (V617F) mutation identified. The final diagnosis by our pathologist was myelo-dysplastic syndrome/myeloproliferative syndrome categorized as unclassifiable (MDS/MPD-U) by WHO classification. Leuk May 2010;23(5):218-24. [Epub ahead of print]

The patient described here appears to have a more proliferative disease, given the leukocytosis. My approach, then, more proliferative or more dysplastic), and an estimate of prognosis. In one series, MDS/MPN-U represented only 2 percent of patients with MDS, with a median overall survival of 21 months (range 10-61).4 The international prognostic scoring system (IPSS) for MDS cannot be applied for many patients with this syndrome, as it excluded patients with a WBC >12,000/µL, but given the median survival of less than two years, I would consider this group of patients equivalent to the higher-risk IPSS groups and, thus, would initiate some sort of therapy now.

The details of this patient’s history illustrate nicely how our ability to elegantly diagnose a rare bone marrow neoplasm exceeds the sophistication of the drugs we have available in our arsenal to treat it effectively.

DR. SEKERES’ RESPONSE

The details of this patient’s history illustrate nicely how our ability to elegantly diagnose a rare bone marrow neoplasm (and exclude the diagnoses of other molecularly characterized abnormalities) exceeds the sophistication of the drugs we have available in our arsenal to treat it effectively. MDS/MPN overlap disorders come in many flavors: as a true overlap condition at initial presentation, with evidence of dysplasia of cellular elements and myeloproliferative components of these disorders are related to lymphomas, multiple myeloma, as well as leukemic and hemorrhage/thrombosis. Assigned colleagues will respond to inquiries within one to two business days either by email or phone. To get started, visit www.hematology.org/Practice/Consult-a-Colleague.

ASH does not recommend or endorse any specific tests, physicians, products, procedures, or opinions, and disclaims any representation, warranty, or guaranty as to the same. Reliance on any information provided in this article is solely at your own risk.

The Hematologist: ASH News and Reports
ASH Continues to Advocate for Larger Increase for NIH
The House of Representatives has approved its version of the fiscal year (FY) 2010 bill funding the National Institutes of Health (NIH). The House bill includes $30.967 billion for NIH, an increase of $500 million over the President’s request and $842 million over FY 2009. Additionally, as this issue of The Hematologist went to press, the Senate Appropriations Committee had approved its version of the bill, which included funding for NIH equal to the President’s request, but a time frame for a vote by the full Senate had not yet been announced. Spending bills need to be passed by October 1, which marks the beginning of the federal fiscal year.

ASH has supported an increase of at least 7 percent for NIH. For the latest information concerning NIH funding, including use of funding provided by the American Recovery and Reinvestment Act (ARRA), please visit the ASH Web site at www.hematology.org.

Obama Administration Releases Final Guidelines on Human Stem Cell Research
The Obama administration released final guidelines governing human embryonic stem cell (hESC) research to implement the President’s March 9 Executive Order that will allow research involving many of the approximately 700 existing cell lines to be eligible for federal funding. The guidelines establish policy and procedures under which the NIH will fund extramural stem cell research. The previous policy implemented by President George W. Bush had allowed federal dollars to pay for some stem cell research, but only if studies were performed on the 21 cell lines that had existed prior to August 2001.

The final guidelines reflect several changes in the rules the administration proposed in April. These changes were made in response to criticism by the scientific community, including ASH, that was directed at new restrictions that might have unintended consequences. For example, they modified the rule requiring that donors of fertilized eggs sign extensive consent forms, which would have made unintended consequences. For example, they modified the rule requiring that donors of fertilized eggs sign extensive consent forms, which would have made

Francis Collins Confirmed as NIH Director
The U.S. Senate has unanimously confirmed Francis Collins, MD, PhD, as director of the National Institutes of Health (NIH). Dr. Collins is the former director of the National Human Genome Research Institute (NHGRI) at NIH, where he led the effort to complete the Human Genome Project. Dr. Collins left his position as director of NHGRI in 2008 to explore other writing and professional opportunities but has continued at NHGRI’s Division of Intramural Research as a special volunteer.

Some of Dr. Collins’ early research focused on sickle cell disease, thalassemia, and the hereditary persistence of fetal hemoglobin. His research has also led to the identification of the genes responsible for the M4 type of adult acute leukemia.

CMS Proposes Payment and Policy Changes for Physicians for FY 2010
The Centers for Medicare & Medicaid Services (CMS) released its proposed Medicare Physician Fee Schedule for 2010. Once again, physicians are facing a significant reduction in Medicare payment unless Congress acts. CMS projects a negative update of -21.5 percent for the proposed 2010 Medicare Physician Fee Schedule based on the application of the Sustainable Growth Rate (SGR) formula. A complete summary of the proposed rule and analysis of its impact on hematologists, along with ASH’s comments to CMS, may be found on the ASH Web site. Specific areas of concern include the overall impact on hematology/oncology, changes to the SGR, elimination of consultation codes, and changes to physician quality programs. The final rule is expected to be published later this fall, and, unless otherwise specified, the new payment rates and policies will apply to services furnished to Medicare beneficiaries on or after January 1, 2010.

CMS Proposes 2010 Rule for HOPPS
CMS has proposed its rule for the hospital outpatient prospective payment system (HOPPS) for 2010. CMS projects that overall CY 2010 payments to the more than 4,000 hospitals subject to HOPPS will be $31.5 billion. This reflects an increase in the hospital market basket of 2.1 percent. Hospitals are required to participate in a quality reporting program in order to be eligible for the full increase. A complete summary of the proposed rule along with ASH’s comments to CMS may be found on the ASH Web site. Specific areas of interest to hematologists, along with ASH’s comments to CMS, may be found on the ASH Web site. Specific areas of interest to hematologists, along with ASH’s comments to CMS, may be found on the ASH Web site. Specific areas of interest to hematologists include payment for hematologic-related services, classification of allogeneic bone marrow transplant procedures, quality reporting, and payment for drugs and radiopharmaceuticals.

Patient Advocates Networking Reception
Brad Schwartz, MD, Chair of ASH’s Committee on Communications, presented information about the Society’s advocacy efforts and public education campaign at the annual hematology Patient Advocates Networking Reception on June 8, 2009. More than 30 representatives from hematology-related patient advocacy groups and the National Heart, Lung, and Blood Institute attended.

This event was one of several activities ASH has initiated to reach out to patient advocacy groups and share information about the Society’s public education campaign, Blood: The Vital Connection, and ASH’s advocacy efforts to increase federal funding for biomedical research at the National Institutes of Health. As a result, several of the patient groups now share ASH’s short film “Research Saves Lives” with their members and have engaged in ASH’s advocacy program.

NIH Proposes Between $150 and $300 Million for Stem Cell Research
NIH has proposed a range of $150 million to $300 million for extramural stem cell research under the American Recovery and Reinvestment Act (ARRA). The proposal is still subject to final legislation and will need to be approved by Congress. The Obama administration released final guidelines governing human embryonic stem cell (hESC) research to implement the President’s March 9 Executive Order that will allow research involving many of the approximately 700 existing cell lines to be eligible for federal funding. The guidelines establish policy and procedures under which the NIH will fund extramural stem cell research. The previous policy implemented by President George W. Bush had allowed federal dollars to pay for some stem cell research, but only if studies were performed on the 21 cell lines that had existed prior to August 2001.

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he 51st ASH Annual Meeting and Exposition, which will be held December 5-8, 2009, at the Ernest N. Morial Convention Center in New Orleans, promises to offer a whirlwind exhibition of the latest discoveries and clinical approaches in the hematologic field and abundant opportunities for professional development. The Society continues to plan a jam-packed meeting, so you will want to make sure you don’t overlook certain aspects of the meeting. A number of relatively recent additions to the annual meeting program have been added that warrant a careful inspection before booking flights and making hotel reservations with the flurry that usually accompanies registration for the ASH annual meeting.

To help with this planning, the Schedule At-a-Glance is already posted on the ASH Web site (www.hematology.org/schedule). Here, we see www.hematology.org/schedule for the ASH annual meeting.

Although not a formal part of the ASH meeting, these symposia, which are sponsored by nonprofit and for-profit organizations, provide interesting information for both clinicians and researchers. Also on Friday, December 4, is Trainee Day (www.hematology.org/trainee-events). This program, open to trainees only, is a half-day workshop designed to support and encourage trainees in the field of academic hematology and to enhance their career development. This program will be presented through didactic and interactive small-group breakout sessions that will provide attendees with abundant time for discussion, questions, and answers.

On Saturday, December 5, the ASH annual meeting officially begins with multiple 7:30 a.m. Attendee registration opens at 7:00 a.m. Throughout the meeting, ASH highlights of the previous day’s events and previews of upcoming presentations. This year, outstanding hotels, on shuttle buses, and at the convention center at the crack of dawn. A annual meeting is succinctly provided by our ASH President Nancy Berliner, MD, the Preliminary Program presentations, special Prize, and Stratton Medal, New this year will be a the current and future ef-

For the last several years, the final day of the annual meeting (This year it’s Tuesday, December 8.) panned format different from previous years and is worthy of careful review. Similar to last year, a abstracts session will also be offered on Tuesday morning. Back for its fourth year, the Special Basic Science of Hemostasis and Thrombosis with invited presentations by leaders in the field and sessions will take place Tuesday morning and afternoon. In a recent Hematologist article, Dr. Bruce Furie discussed the special symposium on Saturday presented by the Quality-of-Care Subcommittee that will focus on forts to improve the national blood supply.

With such a rich menu of opportunities and approximately 4,000 abstracts presented over four days, it will be impossible to attend every session one might hope to. The Best of ASH session,

So with the promise of Louisianan hospitality, cuisine, and the science and medical breakthroughs, we can count on a experience this coming December.
Optimal therapy for patients with genetic hematologic diseases would be to take the patient’s own cells, correct the genetic abnormality, and return the cells to the patient for long-term functional engraftment. Such therapy would require a combination of cell and gene therapy. In this paper, investigators from the laboratories of Juan Carlos Izpisua-Belmonte in Barcelona and Juan Antonio Bueren in Madrid published proof-of-principle studies showing that cells from patients with Fanconi anemia (FA) can be genetically repaired and reprogrammed into inducible pluripotent stem (iPS) cells, which can then be guided to differentiate down the hematopoietic lineage in vitro.

FA is characterized by genomic instability and hypersensitivity to DNA damage. Patients generally present in childhood with anemia and/or susceptibility to infection and subsequently progress to complete bone marrow failure. Also, the increased susceptibility to DNA damage throughout the body increases the risk for developing solid malignancies. The iPS studies presented here present an option for repairing the hematopoietic system of these patients but not their other cell types. Bone marrow transplantation has been used to treat FA but is hampered by the risks of performing allogeneic transplantation as well as the high toxicity of chemotherapy in patients with FA.

IPS cells can be derived from skin fibroblasts and other dividing somatic cell types in vitro by introducing specific genes or gene products that reprogram the mature somatic cell nucleus so that the gene-expression pattern is modified to mirror that of embryonic stem (ES) cells. The most widely used current protocols use viral vectors to transfer four specific genes: OCT4, Sox2, KLF4, and c-MYC. Similar to ES cells, IPS cells can grow indefinitely in the laboratory and have the capacity, under specific in vitro conditions, to differentiate down every cellular lineage, including the hematopoietic lineage. In this work, the investigators were unable to reprogram skin fibroblasts from patients with FA into IPS cells unless they first “repaired” the genetic mutation by introducing a normal copy of the mutant FA gene. Cells were successfully reprogrammed only after the normal FA complex was restored; this teaches us new information regarding the genes required for IPS formation. The new IPS cell lines no longer showed a hypersensitivity to DNA-damaging agents. More importantly, hematopoietic cells derived from these patient-specific IPS cells also showed normal DNA repair in response to DNA-damaging agents.

There are significant hurdles to overcome before such an approach could be used therapeutically for humans with hematologic diseases. First, IPS cells will need to be safe. The goal is to develop IPS cells without permanent incorporation of the reprogramming genes (several of which are oncogenes), and to be able to differentiate the cells into entirely normal hematopoietic stem and progenitor cells that can reconstitute the hematopoietic system in the long term without any risk of malignancy. Investigators have succeeded in reprogramming adult somatic cells into IPS cells using either adeno viral vectors, which do not incorporate into the genome and eventually are lost, or with purified proteins that have been engineered to cross the cell membrane, and are thus only transiently available to reprogram the nucleus. However, even without permanent incorporation of the exogenous transgenes, the resultant IPS cells are still immortalized pluripotent cells that can form teratomas in vivo and can mutate in culture over time. Second, even though IPS can be differentiated into all types of blood cells in vitro, we do not yet know how to differentiate ES or IPS cells into normal, long-term, reconstituting hematopoietic stem cells.

Thus, in genetic diseases, such as FA, it is possible to create patient-specific skin fibroblasts in which the mutation has been corrected and to then convert them into IPS cells, which later can be differentiated into blood cells. This new therapeutic strategy can be applied to many other genetic diseases by first correcting and then differentiating IPS cells into healthy tissues that these patients lack.

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Dr. Krause indicated no relevant conflicts of interest.

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Dr. Chao indicated no relevant conflicts of interest.

Proof of Principle for Combined Cell and Gene Therapy for Fanconi Anemia

Bad Fat
B18-Fluorodeoxyglucose PET Scanning in Multiple Myeloma


In this paper, Bartel et al., from Barlogie’s group at the University of Arkansas for Medical Sciences, compared the utility of F18-fluorodeoxyglucose positron emission tomographic (FDG-PET) imaging to skeletal x-ray survey and magnetic resonance imaging in multiple myeloma (MM). In the context of their Total Therapy (TT2) program, FDG-PET was the leading independent factor predictive of decreased event-free and overall survival. Importantly, suppression of FDG uptake after induction therapy predicted for a superior outcome after transplantation. This study demonstrates the potential utility of FDG imaging for staging and assessing response to therapy in MM.

Bone disease detected by skeletal survey occurs in 80 percent of patients with MM and is the major factor limiting performance status and quality of life. Magnetic resonance imaging is more sensitive, since it can detect marrow infiltration even before bone destruction. However, a very sensitive technique to assess sites of MM activity, localized or diffuse within the bone marrow, is needed both for staging at diagnosis and for assessing response to therapy. Those patients with nonscreatory MM or extramedullary MM are important MM subgroups, in whom such technologies are necessary for optimal treatment. FDG-PET scanning represents such a technology, with FDG uptake correlating with biology and disease activity, and concomitant PET detecting anatomic abnormalities. Importantly, suppression of FDG activity has shown prognostic value in studies in lymphomas and solid tumors, and FDG scanning has been approved for reimbursement by CMS. To date, however, there have not been studies examining the role of FDG-PET scanning in staging or prognosis of uniformly treated MM patient populations.

The authors of this study have been pioneers in evaluating TT treatment programs — double autologous transplant protocols — which have sequentially evolved to incorporate thalidomide (TT2) and novel therapies bortezomib and lenalidomide (TT3). In the TT3 studies, sustained complete remissions, even in patients with cytogenetic abnormalities, are now being observed. In other studies, the combination of bortezomib, lenalidomide, and dexamethasone used as initial therapy has been shown to achieve 100 percent response rates, with 71 percent very good partial response or better. These high response rates measured with standard International Myeloma Working Group criteria have identified the need for more sensitive techniques to evaluate both extent of disease at diagnosis and response to treatment. In this study, the authors showed that the number of focal lesions on PET, the presence of extramedullary disease, and the number of osteolytic lesions adversely impacted event-free and overall survival. More than three focal lesions on PET conferred adverse prognosis even in the low-risk subgroup defined by gene array, whereas patients with high-risk MM, defined by gene array, uniformly did poorly. Most importantly, the complete suppression of FDG activity prior to first transplantation conferred superior outcomes in both low- and high-risk gene-defined subgroups; conversely, the lack of suppression was associated with poor outcome and indicates the need for alternative strategies.

These studies have important clinical applications in MM since they suggest that FDG-PET scanning is useful both for staging and assessing response to therapy and may, therefore, allow for individualized treatment strategies. Further prospective trials of FDG-PET scanning in uniformly treated MM patients, particularly in the context of novel treatment paradigms achieving high extent and frequency of response, are needed to confirm its value and ultimately incorporate its use in guidelines for disease diagnosis, staging, and assessing response to therapy in MM.

Bypass Gene Therapy for Hemophilia


The formation of inhibitory antibodies to factor VIII or IX following therapy with either plasma-derived or recombinant factors remains a challenging problem in hemophilia management. Recombinant human activated factor VIII (rFVIIIa) could potentially provide secondary prophylaxis as a bypass agent in hemophilia complicated by inhibitor formation.1 The short half-life (2.7 hours) and expense, however, limit this approach. Continuous expression of FVIIa gene therapy would obviate the need for multiple, costly rFVIIIa infusions. To this end, Margaritis and colleagues, from Kathy High’s group at Children’s Hospital of Philadelphia, have developed a FVIIa transgene that is intracellularly processed and secreted as FVIIa.2 Building on previous success in mice,3,4 Margaritis et al. have now performed FVIIa gene transfer in dogs with hemophilia.

Outbred canine models of both hemophilia A and B have proven predictive value in the evaluation of gene therapy for hemophilia. To assess the feasibility of gene transfer of FVIIa, investigators infused a type B adeno-associated virus vector expressing canine FVIIa (cFVIIa) into three dogs with hemophilia A and one dog with hemophilia B. Efficacy of therapy was assessed by measuring circulating cFVIIa levels, whole-blood clotting time and PT, as well as by thromboelastography. The dog with hemophilia B received the smallest vector dose and demonstrated little detectable change in cFVIIa levels. Dogs with hemophilia A received progressively higher doses of vector and demonstrated sustained cFVIIa levels between 1.3 and 2.5 µg/ml. A stable reduction in whole-blood clotting times and PT, as well as a near normalization of thromboelastography reaction times, was observed. More importantly, no spontaneous bleeding was observed in more than 45 months of cumulative observation for the dogs with hemophilia A (21 spontaneous bleeds were expected) or more than the 34-month observation period for the dog with hemophilia B (15 spontaneous bleeds were expected). The observation that no spontaneous bleeds occurred in the dog with hemophilia B was particularly interesting, since this dog had cFVIIa expression below detectable levels. Since FVIIa expression levels of 2 µg/ml or more in mice were associated with early mortality and fibrin deposition in the heart and lungs,5 investigators screened dogs for indications of thrombosis. Thrombin-antithrombin (TAT) complex levels, D-dimer, and fibrinogen levels remained within normal limits after several months of treatment. Liver and kidney function tests were also essentially normal. These results suggest that the cFVIIa gene transfer strategy was both effective and safe.

Hemophilia A and B are suitable candidates for gene therapy because they result from single, known genetic defects and since expression of a fraction of normal levels has a marked clinical benefit. Yet, 20 percent to 30 percent of individuals with severe hemophilia A and ~5 percent of individuals with severe hemophilia B develop inhibitory antibodies to factors VIII and IX. Bypass agents, such as prothrombinase complex and rFVIIa, have been used to treat bleeding in hemophiliacs with inhibitors. FVIIa has also been used for secondary prophylaxis in hemophiliacs with inhibitors.6 Continuous expression of FVIIa via gene transfer could address the problems of short half-life and expense that limit prophylactic FVIIa use. Successful FVIIa gene transfer in dogs with hemophilia is a critical step in the development of this approach for human trials. Problems complicating human hemophilia gene therapy trials with other vectors — low transduction efficiency, hepatotoxicity, immunogenicity of expression, and immunogenicity — were not observed. Success in dog models has not consistently translated into success in humans, and the issue of thrombosis as a potential complication of FVIIa gene therapy remains a concern. Nonetheless, this study provides the hope of a compelling solution to a dreaded complication of hemophilia.

Somatic mutations in JAK2 in Philadelphia-chromosome-negative (Ph-) myeloproliferative disorders (MPD), FLT 3 and other genes in acute myelocytic leukemias (AML), and numerous others reported in myelodysplastic syndromes (MDS) are clearly not sufficient to explain the full genesis of these disorders. Thus, the recent finding of a multitude of mutations of the tumor suppressive gene TET2 in numerous malignant hematologic entities has created a lot of excitement.

TET2 is a homolog of the gene originally discovered at the chromosome Ten-Eleven Translocation (TET) site in a subset of patients with acute leukemia. TET2 was first found in AML patients with deletions of chromosome 4q24 and was suggested to be a tumor suppressor gene. Delhommeau and colleagues, from a group headed by Drs. Bernard and Vainchenker in France, reported at the 2008 ASH Annual Meeting and now in the New England Journal of Medicine that mutations and deletions in this gene were found in bone marrow cells from a significant proportion of patients with Ph− MPD (both JAK2V617F positive and negative), AML, and MDS. The TET2 gene spans 150 kb and has 11 exons, and since mutations are distributed throughout the entire gene, their delineation was a formidable task. They demonstrated that the TET2 loss-of-function mutations originate in pluripotent hematopoietic stem cells but seem to favor myeloid rather than lymphoid proliferation, and that in many patients both alleles were affected. In five patients with MPD who also had the JAK2V617F mutation, elegant in vitro studies coupled with transplantation of hematopoietic stem cells into immuno-deficient mice demonstrated that TET2 mutations preceded the JAK2V617F mutation.

An independent study of MDS patients by Langemeijer and colleagues, from Jensen’s group in the Netherlands, also submitted for publication in 2008, reached similar conclusions. Using Single Nucleotide Polymorphism (SNP) microarrays, they analyzed 102 MDS patients for copy number alterations and loss of heterozygosity. Approximately one-quarter of the patients had abnormalities at the TET2 locus. In most patients with large deletions at the 4q24 TET2 locus, mutations were also present in the non-deleted allele. Interestingly, the burden of the second mutant TET2 allele was more variable, suggesting that mutations are acquired sequentially with the progression of disease and that multiple clones may exist in the same individual. One of the studied subjects later progressed to AML, and in the leukemic blasts, the 4q24 deletion and the nonsense TET2 mutation were retained. This is quite different from leukemic transformation of JAK2V617F-positive MPDs, where transformed leukemic cells are generally negative for JAK2 mutations.

These papers were followed by others from additional groups who confirmed the findings and showed that TET2 mutations are also seen in systemic mastocytosis and chronic myelomonocytic leukemia, and that the frequency of TET2 mutations in MPD increases with age but does not alter the disease severity. These studies raise the important question of whether the TET2 mutation could be the pre-JAK2V617F somatic event responsible for MPDs.

An important study published online in Blood from Saint-Martin and colleagues from the French Group of Familial Myeloproliferative Disorders led by Bellanne-Chantelot demonstrated conclusively by studying families with multiple cases of MPD that TET2 mutations cannot be disease-initiating, as the mutations differ among affected relatives. Further, they found that in some instances the TET2 mutations followed, rather than preceded, the appearance of the JAK2V617F mutation. Their work also suggests that the TET2 mutations can increase the risk of transformation to myelofibrosis, contrary to what was reported in a study by a group from the Mayo Clinic.
Bugs and the Vasculature: 60 Trillion Endothelial Cells Can Save Your Life


T

ty years ago Charles Janeway proposed the concept of innate immunity that transformed immunology. 1,2 These ideas suggested that innate immune recognition of microbes depended upon receptors that detected conserved microbial products using pathogen-associated molecular patterns. This evolutionarily conserved mechanism led to the discovery in drosophila of Toll-like receptors (TLR) that could bind ligands of bacteria, viruses, and fungi that were non-self. Today, TLRs are targets for novel therapies to treat sepsis, skin diseases, and cancer. In vitro culture techniques of the vascular endothelium developed some 40 years ago revolutionized our concepts of its function from a bland inert barrier to an active cell-regulating inflammation, thrombosis, and immunity. The vasculature and innate immunity have become intimately intertwined. Andonegui et al., from the laboratory of Paul Kubes, demonstrated that endothelial cells play a critical sentinel role for binding Lipopolysaccharide (LPS) via a TLR-4 receptor and preventing E. coli lethality. TLR-4-/- mice were mated with C57BL/6 mice, and the fertilized eggs were micro-injected with an endothelial-cell-specific TLR-4 transgene to produce mice called EndotheliumTLR-4 mice that exclusively express TLR-4 on the endothelium. Mice without TLR-4, endothelial LPS, and peritoneal neutrophils accumulate in the lungs of wild-type animals but did not accumulate in the peritoneum. However, conversely, the EndotheliumTLR-4 mice showed reduced pulmonary neutrophils and increased peritoneal neutrophils. Furthermore, EndotheliumTLR-4 mice injected with E. coli cleared the bacteria more effectively and did not die, while 50 percent of wild-type mice died within 24 hours. When LPS was administered intra-peritoneally, EndotheliumTLR-4 mice cleared neutrophils in the lung but when these mice were transplanted with bone marrow from TLR-4 replete wild-type mice, similar neutrophil accumulation occurred as in wild-type mice. Thus, TLR-4 on neutrophils and macrophages was necessary to respond to an intraperitoneal LPS challenge.

This study confirms that the 4.000 square meters of 60 trillion endothelial cells play an essential role in host defense. Remarkably, TLR-4 has been linked to multiple diseases ranging from atherosclerosis to prostate cancer and even Alzheimer disease. Endothelial cell TLR-4 polymorphisms may explain an individual’s susceptibility to multiple processes. What is remarkable in this paper is how activation of macrophage/neutrophil TLR-4 may induce a cytokine storm ultimately to the detriment of the host, while exclusive expression of endothelial cell TLR-4 cleared an IV injection of bacteria and permitted survival of the host. Neutrophils/macroage cytokines were critical to the mortality, as TLR-4-/- mice cleared bacteria very slowly (<50 percent at 168 hours), but did not die.

This paper provokes an observation and some questions from this hemato-

logist. Most of our chemotherapy and hematopoietic stem transplant patients survive prolonged neutropenia with bacteremia. Are the 60 trillion endothelial cells all those bacteremia? Does chemotherapy or immunotherapy after endothelial cell TLR-4 function? Are some indiv-

iduals with certain polymorphisms of TLR-4 more susceptible to sepsis? The signal transduction cascade downstream from TLR-4 includes two discrete pathways, MyD88 and TRAM/TRIF, leading to activation of NF-κB, p38 MAP kinase, JNK kinase, and others. Will pharmacologic targeting of these signaling molecules modulate TLR-4 and alter sepsis morbidity? 3,4 Clearly, two decades later Janeway’s concepts of innate immunity suggest new ways to improve our patients’ outcomes with hematologic diseases.


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Dr. Vercelotti indicated no relevant conflicts of interest.

Molecular Profiling in Lymphoma Comes of Age


T

he emergence of gene-expression analysis has added a powerful new weapon to the oncologist’s armamentarium in the classification of neoplastic diseases and in the identification of prognostic indicators. The basic premise underlying this approach is that tumors that appear to be identical from a purely morphologic standpoint may in fact harbor highly disparate genetic profi-

ciles. A corollary of this notion is that individual genetic patterns may be associ- cated with specific biologic behaviors and may result in divergent responses to chemotherapy and/or in overall survival.

One of the first attempts to validate this concept occurred in the case of diffuse large B-cell lymphoma (DLBCL), an aggressive, heterogeneous, immunologic, thymic, and lymphoid malignancy. Molecular profiling of DLBCL helped to identify three distinct subtypes. These include germinal center (GC)-DLBCL, which arises from germinal center B cells, and activated B-cell (ABC)-DLBCL, which arises from post-germinal center B cells. A third subtype, which is relatively rare, is the primary mediastinal B-cell lymphoma (PMBL). Most notably, ABC and GC-DLBCL display numerous differences from each other, including distinct karyotypic profiles, aberrations in microRNA expression, and dys-regulation of various signaling path-

ways. One of the most striking differences between the ABC and GC subtypes is that the latter cells are characterized by constitutive activation of NF-κB and appear to be dependent upon its activation for survival. This is based on results from preclinical studies indicating that ABC-DLBCL cells are significantly more sensitive to agents that interrupt the NF-κB pathway than their GC counterparts. Significantly, ABC-DLBCL lymphomas are significantly less responsive to conventional chemotherapy than the GC and PMBL subtypes and are associated with a significantly shorter five-year survival. 2,3

Until recently, molecular profiling provided potentially useful prognostic information for patients with DLBCL but gave little specific guidance in the optimal selec-
tion of agents or regimens. However, this situation may have changed with this recent report by Donleavy et al., from NCI and the Roswell Park Cancer Center. In a large multi-institutional trial, they compared responses of patients with refractory ABC- or GC-DLBCL to a regimen consisting of dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) with bortezomib. The latter agent was selected, because in preclinical studies bortezomib has been shown to inhibit NF-κB by preventing degradation of IκBα, a protein that traps NF-κB in the cytoplasm, thus preventing its nuclear translocation and activation of NF-κB-responsive genes. While bortezomib has significant activity in multiple myeloma and mantle cell lymphoma, it has little single-agent activity in DLBCL. The authors hypothesized that an NF-κB antagonist such as bortezomib might be particularly active against the ABC-DLBCL subtype.

The results of this study, which involved a total of 49 patients, were quite striking. Although in controls, patients with ABC- and GC-DLBCL had equally poor outcomes, responses of ABC-DLBCL patients to bortezomib-containing chemotherapy were significantly better (P<0.001) than those of patients with GC-DLBCL (83% 5 CR and 5 PR) versus 13 percent (1 CR and 1 PR), as was survival (14 months vs. 3.4 months). The authors concluded that bortezomib-containing regimens are most appropriate for relapsed DLBCL with the ABC dis-

tease subtype.

The significance of this study is that it raises the possibility that targeted therapy may be effective in a broad range of malignancies in addition to such well-established models as Bcr/Abi+ CML and lung cancer associated with mutant EGF Receptor. Several pertinent questions remain. For example, the trial design included refractory patients who had failed prior adriamycin-containing regimens. It will be interesting to learn if the addition of bortezomib improves responses in patients with ABC-DLBCL in the upfront setting. In addition, while it is very tempting to speculate that bortezomib was effective in ABC-DLBCL patients as a result of the NF-κB dependence of this subtype, it is possible that alternative bortezomib actions might have been responsible for improved outcomes. Whatever the an-
swer, the results of this study provide a strong impetus to continue to explore the therapeutic implications of molecular profiling in DLBCL and other hematologic malignancies.


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Dr. Grant indicated no relevant conflicts of interest.
Activation of Prothrombin by a Potential Staphylococcus Aureus Virulence Factor: A Lesson in Classical Enzymology


Most of the trypsin-like serine proteases in the coagulation and fibrinolytic mechanisms are formed by proteolytic activation from an inactive precursor, called the zymogen. During this process, a peptide at the NH2-terminus of the zymogen is cleaved. In a process referred to as molecular sexuality, the newly formed NH2-terminus of the nascent enzyme forms an internal salt bridge that results in conformational activation of the catalytic site. Prothrombin (proT) normally is proteolytically activated to thrombin by factor Xa. However, staphylocoagulase, a secretory product of Staphylococcus aureus, activates prothrombin non-proteolytically. In 2000, Friedrich et al., from the laboratory of Paul Bock at Vanderbilt University, reported that staphylocoagulase accomplishes this by inserting a dipeptide from its NH2-terminus into proT to substitute for the normal internal salt bridge.1

Von Willebrand factor-binding protein (vWbp) is another secretory product of S. aureus and is homologous to staphylocoagulase. Kroh et al., from the Bock laboratory, now have found that vWbp also is a potent activator of proT. Like staphylocoagulase, vWbp non-proteolytically activates proT by a molecular sexuality mechanism. However, unlike staphylocoagulase, there is a distinct lag period between the addition of vWbp to proT and the development of enzymatic activity. Approximately 40 years ago, Carl Frieden used the term hysteresis, from the Greek meaning “lagging behind,” to describe a mechanism in which enzymes develop activity only after a slow conformational change that follows the formation of the initial enzyme-substrate complex.2 Bock trained under Frieden, and, thus, he and his co-workers were prepared to determine whether the vWbp-proT complex is a hysteretic enzyme. They found that the behavior of the vWbp-proT complex indeed fit a kinetic model consistent with the hysteretic mechanism. In the model, vWbp binds rapidly to proT. The resulting complex slowly equilibrates into an active vWbp-proT* complex that binds the substrate, S, with high affinity (Figure). There is a lag phase as the substrate (e.g., fibrinogen) waits for the active vWbp-proT* complex to form. Once formed, the fibrinogen rapidly latches on and is converted to product.

Kroh et al. propose that during staphyloccocal endocarditis vWbp binds to von Willebrand factor that has been deposited at sites of vascular injury. Because a substrate is required in vivo to pull the vWbp-proT complex toward the active state, they suggest that the hysteretic mechanism restricts activation of proT by vWbp to areas rich in the substrate, fibrinogen. Thus, the initial formation of fibrin on infected heart valves may be catalyzed by vWbp-proT, followed by additional staphylocoagulase-mediated fibrin deposition and vegetation growth.

This study is important because it describes a novel enzyme, vWbp-proT, and rigorously tests a kinetic mechanism that accounts for the lag behavior displayed by the enzyme. This behavior is then interpreted in the context of an important disease setting, staphylococcal endocarditis.


Figures

Hysteretic behavior of the vWbp-proT complex

Lenalidomide and Pomalidomide Meet RhoA


The immune-modulating therapeutic agent lenalidomide is a broadly active therapeutic agent for a variety of hematologic malignancies including multiple myeloma, del(5q) myelodysplastic syndrome, acute myeloid leukemia, non-Hodgkin lymphoma, Hodgkin disease, and chronic lymphocytic leukemia. This has prompted considerable interest in developing third-generation immune modulation agents, such as pomalidomide. Several of these are currently in clinical trials for multiple myeloma where promising responses have been observed. One of the perplexing things about this class of medications is the diverse potential mechanism(s) of action, including immune effector cell activity enhancement, tumor microenvironment interference, and direct tumor-directed apoptosis. To date, very few studies have identified pathways upon which this class of agents acts or if the different agents have divergent mechanisms of action. In this paper, Drs. Xu and Xie and their colleagues from Celgene, demonstrated that pomalidomide activates monocytes by activating specific small-molecular-weight G-proteins, RhoA and Rac1, with subsequent enhancement of F-actin formation, stabilization of microtubules, and increase in cell migration. When examined in T cells, similar findings were observed with both pomalidomide and lenalidomide. RhoA activation with lenalidomide was tied to T-cell production of the “general officer” cytokine IL-2 that, in many prior publications, was shown to be required for lenalidomide-induced activation of other immune effector cells. This activation was not direct, thereby providing a lead for other investigators to pursue just how this is occurring in future investigations of this novel class of drugs.

While the mechanisms of action of many therapeutic agents for cancer remain unknown, understanding how an agent works is an important step in including a therapy in rational combinations, and also in fully appreciating its potential. Lenalidomide is one such agent that we know for certain is effective for the treatment of a host of hematologic malignancies, and yet our understanding of its mechanism(s) of action is quite limited. This study, therefore, is important to the field in that it directs other researchers to pursue further the ability of members of this class of drug to enhance cell membrane-based signaling and activation of RhoA along with related family members.

Figure

VWbp proT VWbp•proT VWbp•proT+ S VWbp•proT+S

Hysteretic behavior of the VWbp-proT complex

PETE LOLLAR, MD

Dr. Lollar indicated no relevant conflicts of interest.

JOHN C. BYRD, MD

Dr. Byrd indicated no relevant conflicts of interest.
The Ultimate Mentorship

Q&A With Dr. John Byrd, Co-Director of the 2009 Clinical Research Training Institute

JOHN C. BYRD, MD

Dr. Byrd is D. Warren Brown Professor of Leukemia Research, Professor of Medicine and Medicinal Chemistry at The Ohio State University Comprehensive Cancer Center, Arthur G. James Cancer Hospital.

The Clinical Research Training Institute prepares hematologists for careers in patient-oriented clinical research. The year-long education and mentoring program focuses on the foundation, methodologies, and applications of clinical research. The program begins with an intensive week-long summer workshop in California, where participants work from their own proposed clinical research projects and refine and revise their plans through formal and informal interaction with faculty.

The Hematologist: Why is the Clinical Research Training Institute important?

Dr. Byrd: Doing clinical research in the field of hematology has become increasingly complicated given the complex scientific, economic, and regulatory environment we are now in. Outstanding training and teamwork among clinical, translational, and basic scientists will be essential to fully attain the clinical potential of scientific advances made in the field of hematology. The Clinical Research Training Institute extends from the recognition of the ASH leadership that specialized training in clinical research is necessary to be successful in academic medicine. An essential component of this, as one begins his or her academic career as a hematologist, is the identification and completion of high-impact projects under the guidance of an experienced, caring mentor. The program expands this opportunity to allow external review of these research ideas with respect to both protocol development and career-development grants by a cadre of experienced hematology faculty members with diverse backgrounds. Concomitant with this, an intense interactive didactic lecture series is focused on aspects of clinical research, manuscript preparation, grant application strategies, career development, and issues relevant to senior fellows and junior faculty members focused on clinical and translational research. Unlike other research courses, the Clinical Research Training Institute does not end after the week of training but continues throughout the year with interaction between the students and faculty as they develop their projects.

The Hematologist: What do you hope the participants gained from this year’s program?

Dr. Byrd: I hope that, with their local mentor’s guidance, they will be able to launch a successful academic career as clinical-translational investigators in hematology. I also hope that they gained insight that will help them balance different components of their careers with other personal life goals.

The Hematologist: Who is eligible to attend the Clinical Research Training Institute?

Dr. Byrd: Individuals who are in their second year of fellowship through three years post fellowship are eligible.

The Hematologist: Is the application process difficult?

Dr. Byrd: The application process is relatively simple. It includes information about your short- and long-term goals, letters of support, a summary of the clinical project you intend to develop during the week-long program, and bibliographic sketches of you and your mentor. Your project should have a high probability of moving forward to a true clinical protocol. As with all other grant applications, it is important to review the application carefully and follow the rules put forth in the application. Additionally, it is important to include information about your mentor’s involvement and commitment.

The Hematologist: I’m in my institution’s CTSA K12 program; why should I apply for the Clinical Research Training Institute?

Dr. Byrd: The Clinical Research Training Institute provides contacts and input into your career development from more than 20 hematology faculty outside of your institution. All of these individuals have "made it" in academic hematology and are vested in seeing the next generation of hematologists be successful. During the close interactions, these faculty members will share formally through the didactic lectures and informally through discussions/activities how they have navigated difficult issues most relevant to hematologists. These faculty members will not only do their best to help you during the week-long program, but often they will write letters of support for applications, promotions, etc. that are important in your long-term career progression. Also, the experience of refining your protocol, career-development plan, and specific aims for a possible NIH K23 or K08 application during the week you are at the Institute will certainly help you in the submission of career-development grants that often provide for extended salary support (3-5 years). Many program officers from hematology-relevant funding agencies, including NIH Centers and Institutes, will be at the Institute.

The Hematologist: How is the Clinical Research Training Institute assessed for effectiveness?

Dr. Byrd: The Clinical Research Training Institute is best assessed by how students graduating from prior classes have ultimately done with respect to publications, competition for peer-reviewed grants, and promotions at their institutions as clinical-translational academic hematologists. Many of the students have met these goals with numerous high-impact papers coming from previous classes, and many have gone on to receive career-development grants from NCI, NHLBI, and private foundations. These data are tracked. The Clinical Research Training Institute is also evaluated by different committees in ASH based on feedback from the students and faculty. This is coordinated in great part through ASH staff (Joe Basso and others) and the senior co-chairperson; this year it is Michael DeBaun, MD, MPH, from Washington University. Dr. DeBaun has done an incredible job of planning this year’s program, and it has been an honor to learn from him for the upcoming year, in which I will serve as senior co-chairperson.
This year marks the 150th anniversary of the publication of Charles Darwin’s book, *On the Origin of Species*, in which he proposes his theory of evolution. In celebration of this scientific milestone, it seems appropriate that we would also celebrate the launch of a newly redesigned ASH Web site, which has evolved quite a bit since it was first established in 1997. You may have already noticed the incremental changes being made to the site over the last few years, leading up to this full-scale redesign.

The new Hematology.org includes a vibrant, dynamic design and new features that allow users to easily find what they need in as few clicks as possible. The site is now powered by a Web content management system that helps streamline the Web content development and delivery process and allows for automation of certain functions that were not possible under the previous static structure of the site.

We encourage you to check out (and bookmark) the new Hematology.org site; it features special top navigation and distinct landing areas for three of the Society’s main audiences: Practice, Research, and Training. In addition, the right column highlights programs and items of interest to users, and the improved left-side navigation automatically expands within each section to unveil additional content. The site also allows visitors to easily filter news items by topic or specific areas of interest.

Additional enhancements are already being planned for the next phase of the site redesign, including more seamless integration with the ASH Store and Members Only section. We are interested in hearing from you about how we can continue to improve Hematology.org. If you have any thoughts or suggestions about how the site could better meet your needs, please share them with us by e-mailing the ASH Webmaster at webmaster@hematology.org.
Lessons From an ASH Pioneer and Extraordinary Mentor: Theodore H. Spaet

Theodore Spaet, MD, graduated from New York Medical College in 1946. After serving in the military in Japan, he trained in Boston with Dr. William Dameshek and then spent four years at Stanford University where he was closely associated with Dr. Paul Aggeler, co-discoverer of coagulation factor IX.1 This stimulated Spaet’s interest in blood coagulation. He also collaborated with Dr. Robert Evans and became interested in hemolytic anemia and thrombocytopenia.

In 1955, Spaet was recruited by Dr. Martin Cherkasky to establish a hematology division at Montefiore Hospital, in the Bronx. Montefiore Hospital was then a home for the elderly and chronically ill, but Cherkasky developed it into a world-class academic institution—an environment in which Spaet would flourish. In a tiny laboratory with a kitchen counter bench, he began training residents and postdoctoral fellows, ultimately educating more than a hundred hematologists, 60 percent of whom remained in academic medicine. He served on the NIH Hematology Study Section and was one of the original editors of the journal Thrombosis and Hemostasis. His article titled “The Platelet in Hemostasis,” published in 1964, still provides relevant insights 45 years after publication. 2

Spaet’s first postdoctoral fellow, Dr. Aaron J. Marcus, was charged with the isolation and purification of phospholipids from brain and platelets and determining whether they could replace whole platelets in the thromboplastin generation test (TGT). Marcus was initially unfamiliar with laboratory procedures and methods for lipid separation. “You will do it,” Spaet simply said. This typified Spaet’s rigorous but effective philosophy of training research fellows. The efforts culminated in a paper in the Journal of Clinical Investigation3 and launched the scientific career of an ASH pioneer now in his sixth decade of productivity. Marcus, setting the precedent for future trainees, had many clinical responsibilities. On a daily basis, hematology technicians would show him blood smears they considered abnormal. These had to be reviewed and relevant patients had to be seen. He directed the performance of many further tests as appropriate, including the Rumpel-Leede test, the Duke and Ivy bleeding times and TGT, and hemoglobin electrophoresis.

A subsequent postdoctoral fellow, Dr. Parviz Lalezari, had a similar experience. He was told to study leukocyte antibodies. This suggestion was based on the observation by Dr. Jean Dausset that multi-transfused patients could develop leukocyte agglutinins. It was also noted that multiparous women also developed such antibodies. Lalezari was given a copy of Dausset’s textbook and told, “From now on, you are on your own.” Lalezari’s research on leukocyte antibodies led to the discovery that fetal/maternal leukocyte incompatibility could cause neonatal neutropenia.4 Subsequently, he identified neutrophil-specific antigens and described the autoimmune neutropenia of infancy. While at Stanford, Spaet suggested that Rose Payne, then a research associate, study the development of leukocyte antibodies in multiparous women. Payne’s investigation, together with the work of others, led to the description of the HLA system.

Dr. Dorothea Zucker-Franklin overcame even greater obstacles: “You’ll have to work at the bedside.” There was a very narrow staircase leading to it and no ventilation. (Little did she surmise that the heavy starch blocks used for hemoglobin electrophoresis and separation of intermediate coagulation products would have to be carried up those stairs daily!) Zucker-Franklin learned the technique of protein electrophoresis from her husband, Dr. Edward C. Franklin, a postdoctoral fellow at the Rockefeller Institute. Eventually, this led to isolation and purification of Product I, an intermediate in the coagulation cascade.5

In the 1960s, Spaet and Lalezari, then Blood Bank director at Montefiore, announced that blood for transfusion would be available only for life-threatening blood loss, treatment of aplastic anemia, or surgical procedures, but not for wound healing or other ill-defined conditions. Spaet’s irresistible power of persuasion was the major factor in the success of this paradigm-shifting program.

Many additional examples could be cited to convey the essence of Spaet’s impact on academic hematology. He died at a relatively young age, but left a legacy of a remarkable number of productive academic hematologists who were spawned directly from his laboratory or from those of his trainees. Importantly, all his trainees were taught to be productive at the bench and caring physicians at the bedside.

The ASH Web site 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.

Refer your patients to a resource they can trust: BLOOD: THE VITAL CONNECTION (www.bloodthevitalconnection.org). The site includes:

- Hematologist-approved information on various blood disorders and issues
- Tips for how patients can communicate effectively with their doctors
- A guide to clinical trials
- Major medical advances in hematology
- Detailed blood disease animations and short films

Additionally, the site includes a career resources section for medical students who want to learn more about hematology. Please help ASH promote this valuable resource by directing your patients to www.bloodthevital-connection.org.

Attending the 51st ASH ANNUAL MEETING in New Orleans? Get information about the meeting at www.hematology.org/meetings/annual-meeting. Keep in mind that in order to qualify for the advance registration fees, registration forms and full payment must be received by November 5.

Read THE HEMATOLOGIST ONLINE at www.hematology.org/hematologist, and catch up on the latest news in the field of hematology right at your desktop.

Peruse the ASH JOB BANK at https://jobbank.hematology.org. This is a free resource designed to help you connect with open hematology jobs. Review the latest job openings in the field of hematology, and discover an opportunity that’s right for you.

### SEPTEMBER

**9 – 12**

38th Annual Scientific Meeting of the Society for Hematology and Stem Cells

Athens, Greece

[www.iseh.org](http://www.iseh.org)

**11 – 12**

2009 ASH State-of-the-Art Symposium

Chicago, IL

[www.hematology.org](http://www.hematology.org)

**11 – 13**

22nd Annual European Haemophilia Consortium Conference

Vilnius, Lithuania


**13 – 16**

Oncology: Clinical Issues and Trends

Washington, DC

[www.contemporaryforums.com/m414009/bene.asp](http://www.contemporaryforums.com/m414009/bene.asp)

**21 – 22**

Autoimmune Lymphoproliferative Syndrome (ALPS) 2009 Workshop

Rockville, MD

[www3.niaid.nih.gov/topics/ALPS](http://www3.niaid.nih.gov/topics/ALPS)

**24 – 25**

World Federation of Hemophilia Global Forum 2009

Montreal, Canada

[www.wfh.org](http://www.wfh.org)

**24 – 26**

5th International Symposium on Acute Promyelocytic Leukemia 2009

Rome, Italy

[www.spl2009.com](http://www.spl2009.com)

### OCTOBER

**5 – 9**

41st Congress of the International Society of Paediatric Oncology

São Paulo, Brazil

[www.siop.nl](http://www.siop.nl)

**9 – 11**

Cutaneous Lymphoma Summit

New York, NY

[www.cfoundation.org/summit](http://www.cfoundation.org/summit)

**14 – 16**

International Association for Comparative Research on Leukemia and Related Diseases (IACRLRD) Symposium XXIV: Featuring a Comparative Animal Models of Leukemia Mini-Symposium

Columbus, OH

[www.osucc.osu.edu/iacrlrd](http://www.osucc.osu.edu/iacrlrd)

**15 – 16**

Two-Day Communication Skills Training Program in Oncology

New York, NY

[www.mskcc.org](http://www.mskcc.org)

**21 – 24**

Ninth Cooley’s Anemia Symposium

New York, NY

[www.nyas.org](http://www.nyas.org)

**28 – 31**

Annual Meeting of the International Society for Biological Therapy of Cancer

Washington, DC

[www.isbtc.org](http://www.isbtc.org)