makes perfect sense that the stroma contains prognostic indicators.”

Preparing for Prime Time
Several limitations must be mitigated before C-Path becomes clinically available—most important, the use of whole-slide versus TMA images.

“Each TMA image captures only a minute portion of the full tumor volume, which is much smaller than the multiple whole-slide images used in routine diagnostic pathology,” explained study coauthor Ankur R. Sangoi, M.D., a Stanford University School of Medicine pathologist who practices at El Camino Hospital in Mountain View, Calif.

“It is likely that we could have derived a more powerful prognostic model by analyzing whole-slide images, because these might allow the generation of additional higher-level features such as measurements of tumor heterogeneity,” Sangoi explained.

Fortunately, C-Path is not specific to TMA image processing and can be adapted to whole-slide examination. Larger study participation at different institutions is also in the works, explained study coauthor Marc J. van de Vijver, M.D., Ph.D., who heads the NKI division of diagnostic oncology. To train C-Path at each new hospital, the team anticipates pathologists’ recalibrating the system with 50–60 hospital-specific images in about 1 hour. Samples specific to each institution will better reflect handling, sampling, and storage techniques that differ from place to place.

Along with more diverse study groups, the team hopes to improve C-Path’s ability to analyze breast neoplasia earlier than stage I and to integrate other important information with morphological data.

“We are working on several projects to incorporate molecular biomarker and genomics data into C-Path,” Beck explained. “Breast cancer biomarkers include HER2 and triple-negative status; relevant genomics data include copy number abnormalities and gene prognostic signatures.”

The last essential step for translating C-Path to clinical medicine will require a substantial contribution from the pathology community, van de Vijver explained. That step, which will help close the technological gulf between radiologists and pathologists, sounds simple, but it will take time and practice in using digital images.

“Even today, most pathology diagnoses are made from images viewed directly on a light microscope. Digital slide scanners are not routinely used,” van de Vijver said. “Innovative leadership among pathologists will be critical for facilitating widespread implementation of quantitative digital systems in pathology laboratories.”

Greater accuracy and automation can improve clinical pathology, both here and “in parts of the world where expert pathologists may be in short supply,” said study coauthor Torsten O. Nielsen, M.D., Ph.D., codirector of the UBC/VGH Genetic Pathology Evaluation Centre. Nielsen and his coauthors envision building a library of C-Path images from multiple cancer types, optimized to predict clinical outcomes and directly guide treatment decisions.

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Improving the Outlook for Myelodysplastic Syndrome

By Vicki Brower

In his searing memoir, Swimming in a Sea of Death: A Son’s Memoir, David Rieff documents the painful battle his mother, writer Susan Sontag, waged with myelodysplastic syndrome (MDS) in 2004. Sontag’s treatment options were limited to the first and newly approved MDS drug, azacytidine; induction chemotherapy used to treat acute myeloid leukemia; and bone marrow transplantation—none of which worked.

Sontag died the same year in which she was diagnosed—when a wave of research on MDS was just beginning. For decades, doctors had only been able to offer patients supportive care, blood transfusions, and hematopoietic growth factors.

“Perhaps because MDS is neither an orphan disease nor a common cancer, in the past, it did not benefit from much funding, research, or hope,” said Joachim Deeg, M.D., professor of medical oncology at the University of Washington in Seattle. But that began to change about 5 years ago with the approval of azacytidine in 2004, lenalidomide in late 2005, and decitabine in 2006. Now MDS research is booming, with other compounds in early-stage trials, new combinations in testing, and evolving stem cell transplant regimens. Studies on the microenvironment and signaling pathways are also yielding clues about why hematopoietic cells in MDS do not differentiate and mature normally; die prematurely; and, later, resist apoptosis.

But what stands out most is what one researcher called a “veritable explosion” of genetic research published in the past year. Genetic sequencing technology has driven much of this new torrent of genetic studies, which is helping unravel the molecular basis and pathogenesis of MDS, said Benjamin Levine Ebert, M.D., Ph.D., assistant professor of medicine at Harvard Medical School.
“Still, much of the underlying biology of MDS still is not yet known, and a lot of work must be done to understand how these newly discovered mutations are linked to functional properties of the disease,” said Ebert.

MDS is not one disease but rather a collection of at least five related blood disorders. Although this condition manifests in many ways, all myelodysplastic disorders are characterized by low counts of one or more blood cell type due to bone marrow dysfunction—specifically, by abnormal bone marrow stem cells. About 10,000–15,000 new cases of MDS are diagnosed in the U.S. each year, primarily in older adults. The Surveillance, Epidemiology, and End Results (SEER) Program began reporting on MDS only 10 years ago.

For patients like Sontag, who are diagnosed with MDS after previous cancer treatment with alkylating agents and radiation, or topoisomerase inhibitors, the prognosis is bleak. In about 30% of patients, the disease advances to acute myeloid leukemia (AML).

“Correct classification of risk and rapid treatment is vital,” said Elli Papaemmanuil, Ph.D., postdoctoral research fellow at the Wellcome Trust Sanger Institute in Hinxton, UK. Patients are now stratified into high- and low-risk groups on the basis of chromosomal/cytogenetic abnormalities, peripheral blood counts, and myeloblast (immature blood cell) counts, but only about half of patients manifest chromosomal abnormalities. Patient classification systems are constantly being updated as molecular research continues. A revision of the International Prognostic Scoring System (IPSS) appeared in abstract form in Leukemia Research (May 2011), but a full report has not yet been published.

“The IPSS includes cytogenetics, which is effectively a ‘genetic’ criterion, but no molecular markers, such as mutations in specific genes,” said Timothy Graubert, M.D., associate professor of medicine at Washington University in St. Louis. “Even the forthcoming IPSS-R [revised version] does not include molecular markers,” he said.

**Epigenetic Mechanisms**

Two of the three approved MDS drugs, azacitidine and decitabine, target the epigenetic abnormalities affecting DNA methyltransferases, the enzymes involved in methylation. Both drugs produce good response rates in MDS patients and add to the genetic evidence that DNA methylation plays an important role in the disease.

Mutations in several genes that regulate cytosine methylation, including TET2 and IDH1/IDH2, are offering clues to their importance in some new studies, Ebert said. Mutations in genes that affect histone function, including EZH2 and AXL1, are also being studied.

“Many of the mutations we find in MDS are loss-of-function mutations, and it is not clear how to design drugs to target this class of molecular abnormalities. Hypomethylating agents may be synthetic lethal, that is, killing only cells that have particular mutations and not the normal cells without the mutations,” Ebert said.

Point mutations in genes that regulate epigenetic changes, also not detectable using cytogenetics, are now implicated in the pathogenesis of MDS. Graubert identified recurrent mutations in DNMT3A, a methyltransferase gene, in a study of 150 patients published in the July 2011 Leukemia. Patients with this mutation had worse overall and event-free survival than patients without it, and patients with the mutation progressed faster to AML.

To see whether known epigenetic mutations are associated with certain clinical features, such as overall survival and proportion of bone marrow blasts, Ebert and lead author Raphael Bejar, M.D., Ph.D., instructor at Harvard Medical School, and hematologist-oncologist at the Brigham and Women’s Hospital in Boston, used advanced sequencing and mass spectrometry–based genotyping to study 439 bone marrow biopsy samples (June 30, 2011, New England Journal of Medicine). This study focused on known cancer genes in a large sample, whereas other studies involved small sample sizes or analyzed only a few genes.

“We found mutations in 18 MDS patients, including two not previously known to be associated with MDS. Of these genes, five predicted poorer survival than would be expected based on known prognostic clinical features,” Ebert said. “Mutational analysis has not been used clinically for MDS, and we are suggesting it should be part of medical practice,” he said.

**RNA Splicing Mutations**

In the past few months, several groups have, for the first time in cancer, identified mutations in factors that regulate RNA splicing in a total of eight genes. The largest and most comprehensive genetic study was conducted by Seishi Ogawa, M.D., Ph.D., of the Cancer Genomics Project at the University of Tokyo’s Graduate School of Medicine (Oct. 6, 2011, Nature). Ogawa’s group performed whole-exome sequencing of 29 MDS samples, discovering several new pathway mutations in RNA splicing machinery. Expanding the sequencing to 582 samples, they found spliceosome pathway mutations in 40%–90% of samples, depending on the MDS subtype.

“Mutated spliceosome genes have only rarely been identified in any other cancers,” said study researcher Phillip Koeffler, M.D., professor of medicine at the UCLA School of Medicine. How these mutations cause MDS is unclear, he added.

“One possibility is that they alter the intron–exon borders of select transcribed genes by inappropriately including introns or exons into the RNA of that [particular] gene. This mis-splicing seems to alter the ability of the cell to differentiate into fully mature blood cells,” Koeffler said.
Koeffler said that these newly identified mutations could help improve diagnosis and be used as new targets for developing treatments.

“For example, the SF3B1 gene was found to be mutated in 75% in patients with one phenotype, namely, those with refractory anemia with ringed sideroblasts [certain types of cells with appearance of rings inside them], an MDS subtype that rarely progresses to leukemia,” Koeffler said. Using in vitro and preclinical models, researchers are testing several drugs targeting the spliceosome.

Graubert’s team identified another recurrent splicing gene, U2AF1, using whole-genome deep sequencing, in almost 9% of 153 patients (January 2012, Nature Genetics). Patients with the mutation were nearly three times as likely to develop AML, which must be confirmed in a larger patient population. If it is, the mutation could also be used to refine diagnosis and prognosis.

“Deep sequencing implies that each site of a mutation is sampled with a high degree of redundancy, typically over 500-fold,” said Graubert. “This type of measurement yields a quantitative estimate of the proportion of cells containing the mutation, rather than just a qualitative measurement,” he said.

He emphasized that this mutation appears to be an early event, or driver mutation, in the development of MDS. “Founder mutations will be important because hypothetically, the most effective treatments will target early mutations in founder clones,” he said. “We’ve seen this in cancer treatment: that targeting mutations in daughter clones that occur in late-stage disease, like FLT-3 in AML, is not so effective,” Graubert said.

Studies Revitalize MDS Research

Indeed, a few weeks later, in mid-March 2012, Graubert published a new study with surprising evidence about the evolution of MDS to AML and driver mutations (March 14, 2012, New England Journal of Medicine). His team sequenced the DNA of bone marrow and normal skin cells of seven patients who had progressed from MDS to AML; approximately 85% of bone marrow cells in MDS carried clonal mutations. Comparing the changes in each sample enabled Graubert to identify collections of cells that initiated the cancer, as well as those that developed later, the daughter clones.

“It also showed us that MDS and AML are both highly clonal hematopoietic malignancies,” Graubert said. All told, he identified 11 recurrently mutated genes, four of which had not been connected to MDS or AML. Deep sequencing enabled his team to determine whether a mutation was present and gave them a sense of how many cells in a sample carried a particular mutation, as well as an idea of the evolution of mutations.

According to Graubert, these studies have energized the MDS field. Scientists now hope to diagnose more patients earlier with MDS, a disease now believed to be underdiagnosed.

If there were an easier way to diagnose MDS by analyzing mutations, “we would have the potential to improve the care of patients with this disease,” Ebert said.

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