Cleveland Clinic
Pathology and Laboratory Medicine Institute

Pathology Innovations

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Routine Molecular Testing in AML:
Current Practice and Emerging Markers

By James R. Cook, MD, PhD

The most common form of acute leukemia in adults, acute myeloid leukemia (AML) represents a group of clonal hematopoietic disorders in which both failure to differentiate and uncontrolled proliferation in the stem cell compartment result in the accumulation of myeloid blasts.[1,2] A diagnosis of AML is established through the identification of greater than 20% myeloid blasts in the bone marrow or peripheral blood. AML can be further subclassified into specific clinicopathologic entities that are characterized by distinct clinical features, differences in prognosis, and, in some cases, differences in terms of the optimal therapeutic management.

Metaphase cytogenetic studies have been a critical part of AML diagnosis and classification for many years. Several distinct subtypes of AML are recognized on the basis of specific recurring cytogenetic abnormalities, such as balanced translocations leading to production of oncogenic fusion transcripts. For example, AML with t(8;21)(q22;q22) *RUNX1-RUNX1T1* or inv(16)(p13.1q22) *CBFB-MYH11* (sometimes referred to collectively as "core binding factor leukemias"), each show characteristic morphologic findings and are associated with

a relatively good prognosis. In contrast, cases of AML with inv(3)(q21q26.2) *RPN1-EVI1* are associated with a very aggressive clinical course and poor outcome. For this reason, metaphase cytogenetic analysis has long been considered part of the routine workup and classification of new AML.

In more recent years, it has become clear that other distinct subtypes of AML can be identified on the basis of recurrent molecular abnormalities. The 2008 revision of the WHO classification (Table 1) of hematolymphoid tumors included two new provisional entities defined by the presence of specific mutations: AML with mutated *NPM1* and AML with mutated *CEBPA*.[1,2] Additional studies since 2008 have confirmed that these disorders represent distinct clinicopathologic entities. In addition, other molecular abnormalities have been described that do not define specific subtypes, but are important prognostic markers in certain subtypes of AML. The most important of these prognostic markers to date are *FLT3* and *KIT*. Molecular studies for mutations in *NPM1*, *FLT3*, *CEBPA* and *KIT* now represent part of the routine workup of AML,[3,4] and these genetic markers are included in current NCCN and European LeukemiaNet guidelines for diagnosis, prognostic assessment, and therapeutic decision-making (Table 2).

The *NPM1* gene at chromosome 5q35 encodes nucleophosmin, a nucleolar protein that appears to play a role as a histone chaperone and in ribosome biogenesis. Mutations in *NPM1* are found in approximately one-third of AML, making this one of the most common abnormalities in AML. More than 40 different mutations in *NPM1* have been described, but all represent small insertions (usually 4 nucleotides) in exon 12. Mutations in *NPM1* can be identified by PCR of the relevant region of exon 12 followed by capillary electrophoresis to assess for the characteristic 4bp insertion. Cases of AML with mutated *NPM1* often show monocytic

Table 1. AML with recurrent genetic abnormalities, as defined in the 2008 WHO classification
AML with t(8;21)(q22;q22) *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) *CBFB-MYH11*
APL with t(15;17)(q22;q12) *PML-RARA*
AML with t(9;11)(p22;q23) *MLLT3-MLL*
AML with t(6;9)(p23;q34) *DEK-NUP214*
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) *RPN1-EVI1*
AML with t(1;22)(p13;q13) *RBM15-MKL1*
AML with mutated *NPM1**
AML with mutated *CEBPA**
*provision entities in 2008 WHO edition

Table 2. Risk status based on cytogenetic and molecular abnormalities in current NCCN guidelines.[3]

RISK STATUS	CYTOGENETIC ABNORMALITIES	MOLECULAR ABNORMALITIES
Better Risk	inv(16) or t(16;16) t(8;21) t(15;17)	Normal cytogenetics with *NPM1* mutation or isolated *CEBPA* mutation without *FLT3-ITD*
Intermediate Risk	Normal cytogenetics +8 t(9;11) Other non-defined	t(8;21), t(16;16) or inv(16) with *KIT* mutation
Poor Risk	Complex (≥3 abnormalities) -5,5q-,-7,7q- 11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)	Normal cytogenetics with *FLT3-ITD*

differentiation, and 85-95% of cases show a normal karyotype by metaphase cytogenetics. The prognosis of *NPM1*-mutated AML varies depending on whether or not a *FLT3* mutation is also present, as discussed below.

FLT3 is a receptor tyrosine kinase normally expressed at the cell surface of hematopoietic precursors. Several types of mutations in *FLT3* occur in AML, including internal tandem duplications (ITD), tyrosine kinase domain (TKD) mutations, and rarely juxtamembrane region mutations. The most clinically important of these mutations is the ITD, a variably sized duplication that occurs in exons 14-15. PCR for this region and subsequent fragment length analysis by capillary electrophoresis allows for the detection of this abnormality. *FLT3-ITD* mutations do not define a specific subtype of AML, but rather can be found in many different forms of AML. The *FLT3-ITD* is associated with unfavorable prognosis in AML. *FLT3-TKD* mutations also appear to be associated with a less favorable prognosis in many studies, although this remains somewhat controversial.

The combination of *NPM1* and *FLT3-ITD* status can be used to stratify prognosis in AML, especially in cases with normal karyotype by metaphase cytogenetics. An *NPM+/FLT3-ITD-* genotype is associated with a favorable clinical course, while a *NPM1-/FLT3-ITD+* genotype is associated with poor outcome and other permutations are associated with intermediate prognosis. This information can be used to guide the choice of therapy, as *NPM1+/FLT3-ITD-* cases may not benefit from

undergoing allogeneic bone marrow transplant, while other cases with other genotypic profiles may.

The *CEBPA* gene, located at chromosome 19q13.1, encodes a transcription factor that is involved in differentiation of granulocytes. Mutations in *CEBPA* occur in 5-15% of AML and are extremely diverse. More than 100 different *CEBPA* mutations have been described, including point mutations, insertions and deletions. Due to this marked diversity, mutations in *CEBPA* are best detected by Sanger sequencing of the entire coding region. The majority of mutated cases show biallelic mutations in *CEBPA*, usually with one located in the N-terminal region and one located in the C-terminal region. Cases with mutated *CEBPA*, especially those with biallelic mutations, show a favorable prognosis similar to cases with an *NPM1+/FLT3-ITD-* genotype, and often show monocytic differentiation. Approximately 70% of cases are associated with a normal karyotype by metaphase cytogenetics. Gene expression profiling studies have shown that *CEBPA* mutated AML has a distinct genetic signature, providing further evidence that this represents a distinct subtype of AML.

Mutations in the *KIT* gene on chromosome 4q12 can be found in approximately 17% of AML cases, including a variety of cytogenetic subtypes. *KIT* encodes a receptor tyrosine kinase that signals through several cellular pathways, and mutations in this gene lead to increased proliferation. *KIT* mutations are very heterogeneous, but the vast majority of mutations in AML are located in exons 8 and 17. These diverse mutations

are best identified by Sanger sequencing. *KIT* abnormalities appear to be especially important in AML with t(8;21) or inv(16). These core binding factor leukemias are normally considered to be associated with good prognosis. The presence of a concurrent *KIT* mutation, however, is associated with intermediate risk disease, at least in t(8;21) positive cases. The effect on prognosis in inv(16) AML is more controversial and may be weaker. The significance of *KIT* mutations in other forms of AML remains uncertain. The NCCN and European LeukemiaNet guidelines currently include *KIT* mutation analysis only for cases carrying t(8;21) and inv(16).

While *NPM1*, *FLT3*, *CEBPA* and *KIT* mutation studies are now widely accepted as standard practice in AML diagnosis and prognostic assessment, studies over the last several years have identified a diverse array of other recurrently mutated genes that are also associated with prognostic differences in AML. Some of the more widely studied abnormalities in this category include duplications in *MLL* and mutations in *DNMT3A*, *RUNX1*, *TET2*, *EZH2*, *ASXL1*, *IDH1*, *IDH2* and *TP53*.[5,6] AML researchers now face the challenge of determining which mutations, or combinations of these mutations, represent the most important abnormalities to identify, and how to use this information to guide selection of therapeutic regimens. For example, a recent landmark *New England Journal of Medicine* study by Patel *et al*[7] examined mutations in 17 genes, and identified an 11-gene panel of abnormalities that greatly refines prognostic evaluation of AML. It is likely that the next few years will see numerous similar studies in an effort to define an optimal consensus panel. The major challenge for molecular diagnostic laboratories in this period will be to identify techniques to evaluate such genetic panels in a timely, cost-effective manner. The Department of Molecular Pathology at PLMI is currently exploring options for the use of next-generation sequencing technologies for such applications.

Cost-effective utilization of molecular testing is possible using an algorithmic approach. Samples for metaphase cytogenetics and DNA extraction should be obtained in any bone marrow biopsy for suspected AML. Extracted DNA may then simply be held in the laboratory, pending results of metaphase cytogenetic studies. In the case of normal karyotype, reflex testing for

NPM1, *FLT3*, and *CEBPA* mutations is recommended. In the case of AML with t(8;21) or inv(16), *KIT* mutation studies may be performed. In contrast, in cases where metaphase cytogenetic results clearly identify a poor risk genotype, further molecular testing may not be necessary. This type of integrated, algorithmic approach is likely to become even more important as the number of mutational studies performed continues to grow in the future.

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Dr. Goldblum Receives CAP Excellence in Teaching Award

John R. Goldblum, MD, FACP, chair of the Department of Anatomic Pathology, received the CAP Excellence in Teaching Award from the College of American Pathologists (CAP) at the organization's annual meeting in San Diego September 9.

Dr. Goldblum was recognized for his expertise in anatomic and surgical pathology, particularly soft tissue pathology, and his effectiveness as a three-time faculty member at the CAP's annual meetings. The College established the CAP Excellence in Teaching Award to individuals who contribute outstanding contributions as faculty for one or more education activities resulting in exceptional professional development opportunities for pathologists.

"It is my sincere honor to receive this award," says Dr. Goldblum. "Soft tissue pathology is among the more difficult areas of surgical pathology, and one of the most rewarding aspects of my professional career is serving as faculty at the CAP's annual meetings."

Dr. Goldblum specializes in the interpretation of biopsy and resection specimens in the fields of soft tissue pathology and gastrointestinal pathology. Co-author of the world's largest selling textbooks on soft tissue tumors and gastrointestinal pathology, he has lectured extensively and published more than 200 peer-reviewed articles.

As an active member of the CAP, Dr. Goldblum served on the Surgical Pathology Committee. He also is a member of numerous other medical professional societies, including the Arthur Purdy Stout Society of Surgical Pathologists, the Gastrointestinal Pathology Society, the United States & Canadian Academy of Pathology, and the American Society for Clinical Pathology.

After growing up in Pittsburgh, Dr. Goldblum received his bachelor of science degree in biology and his medical degree from the University of Michigan. He went on to complete his residency in anatomic pathology serving as chief resident, and later he served as a fellow in surgical pathology under Dr. Sharon W. Weiss. He is certified in anatomic pathology by the American Board of Pathology.

The College of American Pathologists is currently celebrating 50 years as the gold standard in laboratory accreditation, serving more than 18,000 physician members and the global laboratory community. It is the world's largest association composed exclusively of board-certified pathologists and is the worldwide leader in laboratory quality assurance.

Shelley Odronic Receives CAP Leadership Development Award

The College of American Pathologists Foundation presented Shelley Odronic, MD, a chief resident in Anatomic Pathology, with its 2012 CAP Foundation Leadership Development Award during the CAP House of Delegates/Residents Forum Joint Session on September 8. The award is a travel stipend to help defray travel expenses for attending two consecutive CAP meetings. The Joint Session preceded CAP '12 – The Pathologists' Meeting in San Diego.

New PLMI Building Awarded LEED® Gold Certification for Energy and Environmental Design
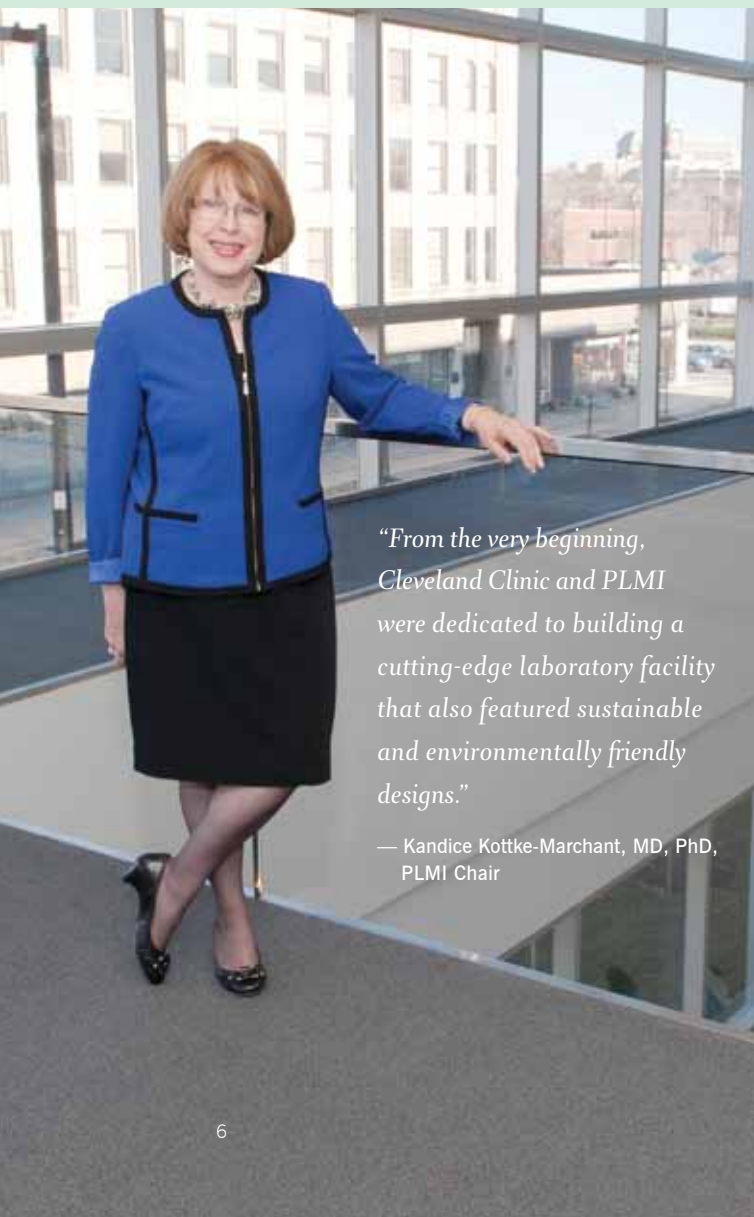
The Cleveland Clinic Pathology and Laboratory Medicine Institute's new LL Building was recently awarded LEED® Gold certification, established by the U.S. Green Building Council and verified by the Green Building Certification Institute (GBCI). LEED stands for "Leadership in Energy and Environmental Design" and is the nation's preeminent program for the design, construction and operation of high performance green buildings.

"From the very beginning, Cleveland Clinic and PLMI were dedicated to building a cutting-edge laboratory facility that also featured sustainable and environmentally friendly designs," says Kandice Kottke-Marchant, MD, PhD, PLMI Chair. "This achievement is certainly a testament to hours of hard work and dedication. A special thank you to Perspectus Architecture, Donley's Inc. Construction, Cleveland Clinic Construction Management, and everyone else who had a hand in this accomplishment."

In order to obtain this certification, the team committed to green goals and enhanced sustainable performance in the following categories: sustainable sites, water efficiency, energy and atmosphere, materials and resources, indoor environmental quality, and innovation and design process.

For example, energy efficiencies are achieved through natural lighting, daylight responsive lighting controls, LED lighting and exhaust air energy recovery, helping to achieve a 41.6% improvement in building energy performance compared to the baseline model. In addition, the use of water efficient irrigation systems, automated weather monitoring and drought-tolerant landscape materials reduces potable water use by 83%.

Opened in January, the new building doubles the size of Cleveland Clinic Laboratories and is designed to support testing needs in microbiology, special chemistry, immunopathology and molecular pathology.

"From the very beginning, Cleveland Clinic and PLMI were dedicated to building a cutting-edge laboratory facility that also featured sustainable and environmentally friendly designs."

— Kandice Kottke-Marchant, MD, PhD, PLMI Chair

Updates in Chronic Myeloid Leukemia Diagnostics

By David Bosler, MD

Molecular diagnostics testing plays an important role in many facets of diagnosis and management of chronic myelogenous leukemia (CML), including diagnosis, disease monitoring and prognosis, and response to therapy and resistance.

The impact of molecular diagnostics on the care of patients with CML has only grown with time, with integral components of contemporary management including disease-defining diagnostic tests, guiding choice of therapy, monitoring of response to therapy through minimal residual disease testing, and testing for development of resistance to therapy. In addition, recent updates, such as the 2012 National Comprehensive Cancer Network clinical practice guidelines and International Scale for standardization of reporting between laboratories, continue to advance the appropriate use of molecular diagnostics to optimize patient care.

The development of tyrosine kinase inhibitors (TKIs) such as imatinib revolutionized therapy for CML and has served as a model for other malignancies. After demonstration of its clinical utility, imatinib was approved for use in 2001 for the treatment of CML and was the first molecular-targeted therapy approved for use in human cancer. Other therapies, such as nilotinib, dasatinib and bosutinib have followed, with FDA approval of panotinib also anticipated. Since the introduction of imatinib, long-term survival rates for CML have increased to 95%, allowing better survival and quality of life when compared to previous therapeutic regimens.

The transformation of CML treatment and course that was provided by tyrosine kinase inhibitors (TKIs) was largely made possible by early knowledge of the molecular genetic events that cause CML. CML has been known for decades as the myeloproliferative disease associated with the "Philadelphia chromosome," an abnormally small chromosome 22 created by a reciprocal translocation of chromosomes 9 and 22. The *BCR-ABL* fusion gene created on this newly derived chromosome 22 gives rise to a chimeric fusion protein with abnormally enhanced and unregulated tyrosine kinase activity. Unchecked, this tyrosine kinase activity sends signals downstream for myeloid precursors to proliferate that result in CML.

Testing at Diagnosis

Since CML is a disease defined by the presence of the *BCR-ABL* fusion, a sensitive method of detecting the fusion is critical to accurate diagnosis. Fluorescence *in situ* hybridization (FISH) for *BCR-ABL* is a sensitive and specific method that is most widely used for diagnosis. Dual color, dual fusion probe strategies employ fluorescently labeled probes that span and flank the full range of breakpoints for both *BCR* and *ABL*, allowing a fusion signal to be detected regardless of which break point is present. Since the probe sets flank the breakpoints, the reciprocal translocation also creates two fusion signals, significantly enhancing specificity over single fusion strategies. FISH can be performed on interphase cells, so results are not dependent on cell culture, resulting in the ability to perform testing on a wider range of samples as well as a more rapid turnaround time than can be achieved by the culture-dependent cytogenetic karyotyping. Additionally, *BCR-ABL* fusions can be detected by FISH in rare Philadelphia chromosome-negative cases of CML.

Comprehensive multiplex PCR strategies have also been employed as a reliable and sensitive means of detecting *BCR-ABL* fusions. This method provides the flexibility and sensitivity of FISH testing because it can be designed to detect all possible break points. It also provides information at diagnosis about which break points are present, which cannot be determined by FISH.

Although cytogenetic karyotyping detects t(9:22) and complex translocations involving these chromosomes in most cases, a small percentage of cases is not detectable by this method and would be missed if cytogenetics were used alone. Cytogenetic karyotyping is also time consuming and may result in delayed diagnosis compared to other methods. However, cytogenetic karyotyping maintains value as a diagnostic tool because it provides information about abnormalities that may be present elsewhere within the karyotype, such as a second Philadelphia chromosome, trisomy 8 or isochromosome 17q. Since none of the other more targeted methods used in CML diagnosis detect these abnormalities, cytogenetic karyotyping provides information that is complementary to these other techniques.

RT-PCR designed to detect the *BCR-ABL* transcript for the p210 fusion is a rapid and very analytically sensitive technique, but its use at initial diagnosis is limited since it detects only the p210 fusion. A negative result does not exclude CML since a small percentage of cases have alternate fusion transcripts that would be missed by a primer set designed to detect only

the p210 fusion. Additionally, rare deletions occurring in or near the primers may cause false-negative results by PCR even when the correct breakpoints are targeted.

In practice, molecular diagnostic testing for CML at diagnosis uses a combination of each of these methods. While FISH or multiplex PCR are best used to make the diagnosis, cytogenetic karyotyping provides complementary information regarding the presence of any other genetic abnormalities, and *BCR-ABL* RT-PCR is useful to provide a baseline level of fusion transcript for subsequent monitoring during therapy.

Response to Therapy

Although of limited use at initial diagnosis, RT-PCR for *BCR-ABL* p210 fusion is the gold standard for monitoring disease burden during therapy. *BCR-ABL* fusion transcripts are measured quantitatively over time to track response to therapy. The results are expressed in normalized copy number (NCN), the ratio of *BCR-ABL* copy number to the copy number of a control gene (often normal *ABL*), and compared to either the patient's baseline NCN at diagnosis or a standardized baseline value. In tracking response to therapy, a major molecular response is defined as a 3 log reduction (or 0.1%) from this baseline value. One recent improvement to *BCR-ABL* quantitative testing is the use of standards and the International Scale (IS) to provide results that are comparable across different laboratories. Without the use of the International Scale, results can vary greatly between laboratories due to the many different methods used to obtain results. The International Scale uses calibrators of known concentrations derived from World Health Organization-certified NIBSC reference material for both *BCR-ABL* and *ABL*. By plotting patient results against the results of the calibration curves, the patient result can be converted to an International Scale value that is standardized across all different labs that use the IS. These standards have significantly advanced the efforts at inter-laboratory standardization and have improved the care of patients who are seen at more than one medical center.

National Comprehensive Cancer Network (NCCN) Guidelines provide guidance for monitoring response to therapy in CML. Different levels and types of responses (hematologic, cytogenetic, molecular) are expected at different time points in order for a response to be considered optimal. For example, optimal responses based on the 2009 European LeukemiaNet (ELN) recommendations include complete hematologic response at 3 months, complete cytogenetic response at 12 months, and major molecular response at 18 months. Patient responses are categorized as optimal, sub-optimal or failure by NCCN

based on outcomes studies, and the criteria used for measuring response drive the specific methods used to monitor response at each time point. Although hematologic response and *BCR-ABL* fusion transcript levels can be determined from peripheral blood, cytogenetic karyotyping requires bone marrow sampling.

2012 National Comprehensive Cancer Network (NCCN) Guidelines

The 2012 NCCN CML Guidelines update the recommendations for disease monitoring and therapy based on newly available outcomes studies. The updated guidelines generally seek to provide greater clarity surrounding when to alter the current course of therapy by changing to a different TKI based on sub-optimal and/or failure level responses, providing clinicians with a proposed course of action using the best tools and knowledge available.

One significant change in the 2012 guidelines compared to the 2009 European LeukemiaNet (ELN) Recommendations is the emphasis on early response. At the three-month evaluation, continuation of current therapy requires that the patient is demonstrating optimal response (ie. complete hematologic and partial cytogenetic responses), and also places increased emphasis on early molecular response by adding a one log reduction of *BCR-ABL* fusion transcripts to the criteria for optimal response. This added criterion is based on studies showing improved long-term overall survival in patients that achieve a one log reduction after 3 months of imatinib therapy. For anything less than optimal response at 3 months, the NCCN guidelines recommend evaluation of patient compliance and possible drug-drug interactions, as well as *ABL* kinase mutation analysis for the purposes of switching to the appropriate second generation TKI.

Another significant change in the 2012 update of NCCN guidelines is the de- emphasized importance of achieving a major molecular response (MMR) at 18 months. MMR was previously the sole criterion for optimal response in the 2009 ELN Recommendations. However many studies show that, among patients achieving complete cytogenetic response, the long-term survival does not vary significantly between those achieving MMR and those without MMR. The 2012 NCCN Guidelines therefore essentially lump optimal (MMR and complete cytogenetic response) and sub-optimal (complete cytogenetic response but no MMR) ELN responses together in order to make the more clinically important distinction between complete cytogenetic response and lack of complete cytogenetic response at 18 months. The lack of complete

cytogenetic response is what drives evaluation for mutations and switching to a second generation TKI at 18 months under the new guidelines. Failure to meet milestones at any time or loss of response in therapy adherent patients should prompt *ABL* kinase mutation analysis. A complete list of the 2012 NCCN guidelines for CML can be found at: National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: chronic myelogenous leukemia. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.

Table 1. Guideline Recommendations for Treating Patients with Mutations	
Mutation	**Treatment**
T315I	HSCT or clinical trial (ponatinib)
V299L, T315A, F317L/V/I/C	Consider nilotinib rather than dasatinib
Y253H, E255K/V, F359V/C/I	Consider dasatinib rather than nilotinib
Any other mutations	Consider high-dose imatinib or dasatinib or nilotinib

Therapy Resistance Testing

Over 100 mutations at a variety of locations within the ABL kinase domain can confer resistance to various TKIs. Although the TKIs themselves are not mutagenic, use of a TKI creates an environment that can allow a mutated clone to emerge, somewhat analogous to the emergence of antibiotic resistant bacterial strains under the pressure of antibiotic use. These mutations may interfere with the binding or function of TKIs differentially, so that a given mutation may confer resistance to one TKI and not others. Failure to respond to TKI therapy in CML or loss of response is often due to the emergence of a clone bearing a resistance-conferring *ABL* kinase mutation, and given the differential effects of these various mutation, analysis of which mutation is present provides an important contribution to guiding choice of therapy. Gene sequencing is one of the most appropriate methods employed for *ABL* kinase mutation analysis given the number of possible mutations, although multiplex PCR approaches have also been used to effectively interrogate a more limited number of mutation hot spots.

One of the challenges surrounding interpretation of *ABL* kinase mutation analysis is understanding the clinical implications of each particular mutation. A number of mutations that can be encountered in the *ABL* kinase domain have either no known effect or incompletely understood effect on TKI resistance. However, sufficient clarity has emerged for a subset of these mutations for them to be included into recommendations regarding therapy. In fact, for a few of these mutations, the differential effects on different second generation TKIs allow recommendation of one TKI over another (see Table 1 above).

The T315I mutation is both the most common mutation detected after imatinib failure and the most TKI resistant of ABL kinase mutations. Cases of CML that have the T315I mutation are resistant to all approved TKI therapies to date, making hematopoietic stem cell transplantation (HSCT) or clinical trial participation the current recommended options. A new drug called ponatinib, currently in clinical trials, has shown significant activity against CML with T315I mutations, and may prove a viable new option for this currently refractory group. If ponatinib receives FDA approval and proves effective against T315I mutated cases, the role of testing for the T315I will likely expand significantly.

Summary

At diagnosis, reliable and sensitive methods for detection of *BCR-ABL* fusion include fluorescence *in situ* hybridization and some comprehensive multiplex RT-PCR assays. Cytogenetic karyotyping and PCR for the p210 fusion also detect the vast majority of CML cases, but they miss cases that are cytogenetically cryptic or that involve variant breakpoints, respectively. Nonetheless, it remains important to perform these tests at diagnosis in order to get baseline information that will become important in disease monitoring. Assuming that a p210 *BCR-ABL* fusion is present at diagnosis, quantitative RT-PCR designed to detect the p210 fusion is the most sensitive method of monitoring residual disease. Obtaining a baseline result for the RT-PCR p210 assay is helpful in order to ensure that cases are subsequently negative for the *BCR-ABL* fusion during disease monitoring because they have achieved complete molecular response rather than because they have a variant rearrangement that is not detected by the assay. Additionally, cytogenetic karyotyping remains an important method of detecting additional cytogenetic abnormalities, such as a second Philadelphia chromosome, trisomy 8 or isochromosome 17q, which may be harbingers of disease progression and are not detected by RT-PCR or FISH. When molecular disease monitoring, hematologic findings or clinical course suggests loss of response to therapy, analysis of the *ABL* kinase for mutations

Relevant CCL Tests:

Test Name	Ordering Code
• Cytogenetic karyotyping	CHRBMH, CHRBLL
• *BCR-ABL* FISH	BCRFSH
• RT-PCR for *BCR-ABL* p210 transcript	BCRPCR
• RT-PCR for *BCR-ABL* p190 transcript	190BCR
• *ABL* kinase mutation analysis	KINASE
• *BCR-ABL* multiplex PCR for diagnosis	*(in development)*

that confer resistance to imatinib and/or other tyrosine kinase inhibitors helps to guide the choice of subsequent therapy.

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About the Authors

James Cook, MD, PhD, is an associate professor of Pathology at the Cleveland Clinic Lerner College of Medicine. He serves as Section Head of Molecular Hematopathology, Medical Director of the Manual Hematology Laboratory and Co-director of the Cell Culture Core Facility for the Pathology and Laboratory Medicine Institute.

Dr. Cook's clinical interests focus on diagnostic hematopathology and molecular diagnostics. His research interests include diagnostic and prognostic markers in non-Hodgkin lymphoma and plasma cell disorders, and molecular diagnosis of leukemia and lymphoma. He is a member of the Lymphoma Translational Medicine Committee for the Southwest Oncology Group, and he regularly serves as faculty for post-graduate educational courses at national meetings.

Dr. Cook received his undergraduate degree from Pennsylvania State University, and his medical and doctorate degrees from Washington University School of Medicine in St. Louis. He served his residency in anatomic pathology at Barnes-Jewish Hospital in St. Louis and completed a fellowship in hematopathology at the University of Pittsburgh School of Medicine. Dr. Cook is certified in anatomic pathology and hematopathology. He is a past recipient of the Pathologist in-Training Award from the Society for Hematopathology and the John Beach Hazard Distinguished Teaching Award in the Cleveland Clinic Institute of Pathology and Laboratory Medicine.

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David Bosler, MD, is Head of Cleveland Clinic Laboratories within the Pathology & Laboratory Medicine Institute at Cleveland Clinic. He is responsible for the strategic execution of Cleveland Clinic Laboratories' business plan for local, regional and national outreach of reference laboratory testing. Dr. Bosler also is Staff in Hematopathology in the Department of Clinical Pathology and Assistant Professor, Cleveland Clinic Lerner College of Medicine. He is involved in bone marrow sign-out and assay development in molecular hematology, as well as serving as Co-Chair of the Point-of-Care Testing Compliance Council.

Dr. Bosler received his undergraduate degree from Miami University and his medical degree from the University of Cincinnati. He completed fellowships in hematopathology and molecular genetic pathology at Mayo Clinic, served his residency at William Beaumont Hospital in Royal Oak, Mich., and obtained his medical degree from the University of Cincinnati College of Medicine. He has been on the Cleveland Clinic staff since 2009.

Dr. Bosler is a frequent speaker at national meetings and has authored numerous peer-reviewed journal articles and book chapters. He is certified as a diplomate of the American Board of Pathology in Anatomic and Clinical Pathology, Hematology and Molecular Genetic Pathology. He currently serves as a member of the Point-of-Care Testing Committee for the College of American Pathologists (CAP).

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