

Chronic Myeloid Leukemia in 2007

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ABSTRACT

BACKGROUND: Chronic myeloid leukemia (CML) is a potentially fatal stem cell cancer that comprises approximately 14% of all leukemias. Although it is estimated that 4,600 people will be diagnosed with CML in the United States in 2007, only 12% of those individuals will die from the disease. That low mortality rate is due to the availability and efficacy of the new kinase inhibitors that target the BCR-ABL oncogene and other targets to hold disease progression in check.

OBJECTIVE: To review the molecular pathogenesis of CML, describe the clinical course of the disease, and explain the current application of cytogenetics and molecular testing for diagnosis and treatment.

SUMMARY: CML is caused by the translocation of chromosomes 9 and 22 to create what is called the Philadelphia chromosome. This translocation removes a critical regulatory domain from the tyrosine kinase, ABL, such that its protein product is constitutively active. This means that the cell escapes the constraints of normal cell growth and proliferates uncontrollably. The modified protein is known as BCR-ABL, and it causes CML by phosphorylating numerous downstream proteins involved in the activation of cell division, among other functions. During the earliest phase of the disease, the chronic phase, kinase inhibitors that target BCR-ABL are effective in stopping disease progression. However, a minority of patients remain unresponsive to this therapy.

Laboratory tests are thus of great importance for this disease. Not only are they required for the diagnosis of CML, but during therapy they can establish the degree of response. That response, in turn, can supply the clinician with a good estimate of the prognosis for the patient. The tests used for CML include complete blood count (CBC) with platelets, cytogenetic analysis, fluorescence in situ hybridization (FISH), and quantitative polymerase chain reaction (PCR). These tests vary in the difficulty of application and in the sensitivity. CBC is commonplace within the average hospital laboratory, whereas cytogenetic analysis, FISH, and PCR require specialized equipment, personnel, and training. Hematologic counts are the least sensitive measures of disease, with a limit of detection of a leukemic burden of 1011 cells. Cytogenetics can detect a burden of 109 cells. Finally, quantitative PCR can detect a burden of as few as 105 leukemic cells. The current costs of these tests range from approximately \$375 to \$1,500 and must be performed every 3 to 6 months to follow the patient's response to therapy.

CONCLUSION: The advent of kinase inhibitor therapy for CML has greatly increased the importance of sensitive analysis of disease burden. Subsequent testing during therapy greatly improves the ability of the clinician to predict the therapeutic outcome. Signs of early treatment failure can give the patient time to switch therapies before the disease progresses to an advanced stage.

KEYWORDS: BCR-ABL, Cytogenetics, Fluorescence in situ hybridization, Polymerase chain reaction, Chronic myeloid leukemia

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The American Cancer Society estimates that in 2007 approximately 4,600 patients are going to be diagnosed with chronic myeloid leukemia (CML) in the United States. CML accounts for approximately 15% to 20% of all adult leukemias, which includes chronic lymphocytic leukemia, acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL), and the incidence is approximately 1 to 1.5 per 100,000 people.¹ Fortunately, only 12% to 15% of these patients are estimated to die from this disease because of the availability of kinase inhibitors and the use of allogeneic hematopoietic stem cell transplants. Kinase inhibitors do not cure the disease; rather, they hold it in check for a while.

Diagnosis of CML

The standard CML work-up will initially involve a history and a physical examination. Blood is drawn to obtain a complete blood count (CBC) with platelets as well as a baseline chemistry to assess their renal function and hepatic function. Other assessments, such as morphological assessments of bone marrow aspirates, cytogenetics, fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR) will be described below. CML can present at any age; however, the median age for diagnosis is 53 years. The disease can be divided into 3 phases, and diagnosis can occur in any of these phases.

Clinical findings associated with CML have been well described.² More than 80% of patients complain of fatigue, regardless of the phase of CML. Interestingly, approximately 40% of these patients go to their primary care physicians for something unrelated, and in the process it is determined that their white blood cell (WBC) count is abnormally high. Otherwise, these patients are asymptomatic regarding their CML. These patients are in what is called the chronic phase (CP) of CML. At least 40% of patients present with splenomegaly. The spleen, and sometimes the liver, become packed with cells. This leads to sensations of early satiety, in addition to weight loss and anorexia. Bone pain is also a feature. CML by definition involves a WBC count >25,000. Erythrocytosis, increased platelet counts, and basophilia are also heralding factors of CML, specifically as the disease moves into the more advanced stages. The bone marrow is hypercellular with evidence of myeloid hyperplasia.

Clinical Phases of CML

CML can be divided into the CP, which is followed by the accelerated phase (AP) and subsequently the blastic phase (BP). AP and BP sometimes are lumped together and considered to be advanced-phase CML. The entire continuum from CP to BP lasts a median of 3 to 5 years. This time period can be broken down into the CP, which, if untreated, lasts for 2 to 5 years; the AP,

which can last up to a year; and finally the fatal BP, which lasts from 3 to 6 months. As stated previously, a patient can present in any of these 3 stages.

The International Randomized Interferon versus STI571 (IRIS) trial was a pivotal study that created some useful definitions.³ CP is defined as having within the peripheral blood and bone marrow less than 15% blasts, less than 20% basophils, and less than 30% blasts plus promyelocytes, with the Philadelphia chromosome (Ph; t9:22 chromosomal translocation) being present in these transformed cells. It is during this phase that kinase inhibitors have had such a marked effect on therapy.

The AP has been defined differently by different medical groups. Sokal et al. originally proposed that AP was achieved when blasts are $\geq 5\%$ of both the periphery and the bone marrow; basophils are $\geq 20\%$ of the periphery; and platelets are $> 1012/L$ or are low; and evidence exists of clonal evolution, progressive splenomegaly, and anemia.⁴ In comparison, the International Bone Marrow Transplant Registry (IBMTR) defines AP as $\geq 10\%$ blasts in the periphery and bone marrow, with $\geq 20\%$ of the periphery being made up of a combination of basophils plus eosinophils; the presence of a persistent thrombocytosis; and again, evidence of clonal evolution, progressive splenomegaly, and anemia.⁵ The M.D. Anderson Cancer Center defines AP as $\geq 15\%$ peripheral blasts with $> 20\%$ peripheral basophils, < 1011 platelets/L, and the presence of clonal evolution.⁶ Finally, the World Health Organization (WHO) defines AP as blasts comprising 10% to 19% in both the periphery and bone marrow, with basophils accounting for $> 20\%$ of the periphery, platelet counts $< 1011/L$ or $> 1012/L$, and evidence of clonal evolution and progressive splenomegaly.⁷ Commonalities between these different scales are an increasing number of peripheral blasts, increased peripheral basophils, and the presence of clonal evolution. Additionally, these patients are beginning to fail in their response to therapy.

Clonal evolution is defined as the appearance of cytogenetic abnormalities in addition to the Ph. Approximately 50% to 80% of patients who progress exhibit these abnormalities. One common finding is the appearance of a second Ph. Trisomy 8, another common cytogenetic abnormality associated with AP CML, consists of 3 copies of chromosome 8. Other abnormalities, such as isochromosome 17q, or deletions of the p53 tumor suppressor gene (chromosome 17p), also are observed.

BP is characterized by a much higher percentage of blasts, both in the periphery and bone marrow. WHO defines BP as $\geq 20\%$ blasts in the periphery or nucleated bone marrow cells, extramedullary blast proliferation, and the presence of large foci or clusters of blasts in bone marrow biopsies.⁸ IBMTR defines the BP as $\geq 30\%$ blasts in the periphery or bone marrow, along with extramedullary infiltrates of leukemic cells.⁸ Perhaps the most important aspect of BP is that it transforms into an acute leukemia and has to be treated as such. Interestingly, 60% to 70% of patients transform into a myeloid leukemia, whereas 20% to 30% of these

patients have the capability of transforming into a lymphoid blast crisis that is treated as an ALL as opposed to an AML.

■ Biology of CML

Examination of the chromosomal structure of cells of lymphoid, eosinophil, or platelet lineage from the patient with CML shows that all these lineages contain the Ph. This indicates that CML is a stem cell disease. To define it more specifically, CML is a myeloproliferative disorder. It is a clonal expansion of the translocation of chromosomes 9 and 22. The consequence of this translocation is the creation of an unregulated tyrosine kinase activity. CML was first described in 1960 and was the first disease state to be associated with a specific cytogenetic abnormality.⁹

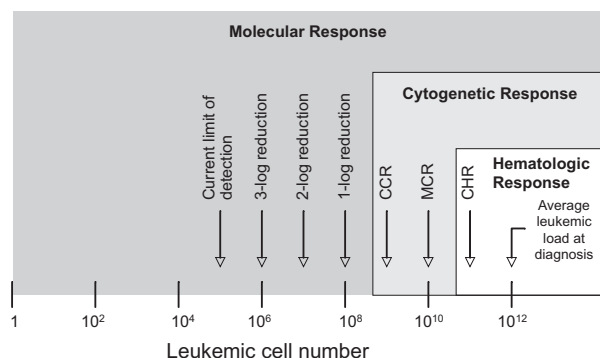
Chromosome 9 contains the tyrosine kinase gene, ABL, named for the Abelson murine leukemia virus. Chromosome 22 contains the BCR (for breakpoint cluster region). In CML a translocation occurs to form a different chromosome, and this chromosome encodes for an oncoprotein that has very strong tyrosine kinase activity. As suggested by its name, the BCR has multiple sites at which the breakpoint can occur. These breakpoints have different implications for the aggressiveness of the disease.¹⁰ The p210 form of BCR-ABL is the most common form observed in patients with CML. The p230 form of BCR-ABL actually is associated with a more indolent disease. Finally, the p190 form of BCR-ABL is also diagnostic for the Ph, but this is most commonly seen in ALL as opposed to CML. The ABL gene region has just 1 breakpoint; thus, there are no variants that can affect disease. The ABL protein normally is a regulated kinase with the usual constraints of a kinase. In contrast, the BCR-ABL kinase continues to phosphorylate downstream protein targets associated with cellular proliferation without constraint. These targets include RAS, RAF, and AKT, among others.² In addition to dysregulating proliferation, these phosphorylation events also create changes in the adherence properties of these cells as well as diminished apoptosis (programmed cell death). All these alterations then lead to the development of CML.

■ Monitoring the Patient With CML

At diagnosis it is estimated that a patient has a load of 1012 leukemic cells (Figure). Assuming that the patient responds to therapy, the first assessment is to determine the number of blasts and basophils in a blood sample. If there are no or few blast cells in the blood and the blood count returns to within normal limits, this is referred to as a hematologic response (HR).⁸ If the assessment is that no leukemic cells can be detected, the HR is complete (CHR); otherwise it is partial (PHR). As shown in the Figure, this CHR assessment has a limit of detecting approximately 1011 leukemic cells or more in the body. An additional aspect of the CHR is the loss of any sign of splenomegaly.

The next level of assessment is cytogenetics. Using chromosomal spreads or FISH, the number of cells containing the Ph is determined. The reduction in leukemic cells observed by these

FIGURE Therapeutic Responses as a Function of the Number of Leukemic Cells Within the Body



CCR = complete cytogenetic response; CHR = complete hematologic response; MCR = major cytogenetic response.

tests is referred to as a cytogenetic response (CR). As shown, a reduction to 1% to 34% of evaluated response is considered a major CR. Less of a response is considered a minor CR. No detectable Philadelphia positive (Ph+) cells would be a complete CR (CCR).

Finally, the most sensitive assessment is quantitative PCR. The result of this assessment is referred to as the molecular response. PCR results are often reported as 10-fold (log) reductions, as shown in the Figure. Currently, our final limit of detection is an approximate load of 105 leukemic cells.

All these assessments are measures of residual disease. They are important, as it has clearly been demonstrated that the lower the leukemic cell burden, the longer the therapeutic response and the better the outcome for the patient.

Cytogenetics (karyotyping) is performed on a bone marrow biopsy. The cells derived from the biopsy are cultured for 1 to 3 days, allowing some of them to enter the metaphase state of mitosis, where the chromosomes are condensed and where cytogenetics can be assessed. At this point the cells are stained and the chromosomes are then identified on the basis of characteristic bands of light and dark staining. The sizes and patterns of these bands allow the identification not only of normal chromosomes but also of translocations, amplifications, and deletions. The Ph is thus identified by its unique pattern of bands.

The cost of this test at Emory Healthcare is approximately \$1,500. It is a crucial test and is always done at diagnosis. Not only does it enable the identification of the Ph but it also shows the presence of other cytogenetic abnormalities. This test will be essential in determining whether clonal evolution has occurred within the bone marrow stem cell pool and thus whether the patient has progressed beyond the CP. Furthermore, it is an essential test to be performed if disease progression is suspected. The 5-year survival data from the IRIS trial show that patients with CML who have no CR at 24 months have an 82% chance

of survival at 5 years.¹¹ In comparison, patients with CML who achieve a CCR at 24 months have a 99% chance of survival at 5 years. If the patient has not begun to respond to therapy within 12 months and possibly as early as 3 months, guidelines recommend that therapy be switched at this point rather than at a later date.

FISH involves the use of fluorescent probes to visually demonstrate the presence of the t(9:22) translocation. The probe that binds to a specific sequence within the ABL gene is attached to a red fluorescent marker. The probe that binds to the DNA sequence within the BCR is then attached to a green fluorescent marker. If the translocation has occurred, the 2 probes will be so close together that their colors fuse to produce yellow. FISH has a superior sensitivity to cytogenetics. In the former case, only cells in metaphase can be assessed, and a minimum of 20 cells must be done. Thus, the limit in sensitivity can be as low as 1 in 20 cells. In comparison, FISH is not performed on metaphase cells, and several hundred can be scanned within a sample assessment. FISH also can be performed on blood or bone marrow tissue. The cost for FISH is approximately \$1,000.

Quantitative PCR is by far the most sensitive assay available. The RNA sample is prepared from the blood or bone marrow sample and reverse transcribed to make complementary DNA (cDNA). The cDNA is then amplified for BCR-ABL transcripts and transcripts for a "housekeeping" gene, such as G6PD, using primers that are specific for the 2 genes. Because the amount of material doubles in each cycle, it is possible, once the sample has reached some predetermined level of amplification, to quantify the amount of starting material by counting cycles. To account for potential differences in starting cell numbers, the starting material is normalized to the amount of housekeeping gene starting material. At present, PCR can detect even 1 Ph+ cell within a sample of 1 million cells.

The cost of the test at Emory Healthcare runs about \$370; however, the test requires specialized equipment and training. Often the test is sent out to be performed, which may increase the cost and the time but decreases the burden on the technical staff. There has been some controversy over using this test at diagnosis; however, the reasons for doing so are becoming more apparent. Most important, the performance of this test at diagnosis gives the clinician a firm baseline measure of disease against which therapeutic response can be measured.

Again, returning to the 5-year survival data from the IRIS trial, patients on imatinib therapy who achieved a CCR and greater than or equal to a 3-log reduction in BCR-ABL transcripts had a 100% survival at 5 years. Patients on imatinib therapy who did not achieve a CCR had an 88% survival rate at 5 years.

The criteria for the various responses just described are as follows: PHR and CHR are differentiated by the presence of immature blasts and splenomegaly. The CR is generally described in terms of percentages of Ph+ metaphases, with the minor CR ranging from 35% to 90% and the major CR ranging from 1% to 34%. A CCR is the absence of detectable Ph+ metaphases. Finally,

TABLE

Genetic Test Indications in CML

Test Period	Source	Cytogenetics	FISH	RT-PCR
At diagnosis	Bone marrow	Yes	No	No*
	Blood	No	Yes	Yes
During therapy	Bone marrow	Q 3-6 mo. until CCR, then replace with blood FISH or RT-PCR		
	Blood	No	Q 3-6 mo. after attaining BM CCR if use FISH for MRD assessment	Q 3-6 mo. after attaining bone marrow CCR
After HSCT	Bone marrow	At 3-6 mo., then as indicated	No	No
	Blood	No	Q 6 mo. for 2 yr. if FISH for MRD assessment	Q 6 mo. for 2 yr.
Transformed to AP or BP	Bone marrow	Yes	No	No
	Blood	No	No	No

*New data from the IRIS trial and others indicate that RT-PCR is necessary at diagnosis.

AP = accelerated phase; BM = bone marrow; BP = blastic phase; CCR = complete cytogenetic response; CML = chronic myeloid leukemia; FISH = fluorescence in situ hybridization; HSCT = hematopoietic stem cell transplantation; IRIS = International Randomized Interferon versus STI571; MRD = minimal residual disease; Q = every; RT-PCR = reverse transcriptase polymerase chain reaction.

Adapted with permission from Tefferi et al.¹²

a major molecular response is defined as at least a 3-log reduction in BCR-ABL transcripts, and a complete molecular response is defined as the complete absence of detectable transcripts.

The Table shows a list of recommended tests during the course of diagnosis and treatment and during the various stages of the disease, derived from the work of Tefferi et al.¹² In general, the tests begin with the least specific, most readily available. Cytogenetics or FISH tests are performed every 3 to 6 months until a CCR is achieved. At that point, the examination switches to PCR, which is repeated every 3 to 6 months throughout the patient's time of therapy. The one point of controversy in this Table is the use of PCR during diagnosis. For the reasons stated above, this author believes strongly that the PCR test should be included at diagnosis.

Conclusions

CML therapy has improved to the point where only a small percentage of patients are expected to die of the disease. However,

survivors are not cured. Rather, their disease is held in check. This has placed even more importance on laboratory tests as means of diagnosis and an assessment of therapeutic response. Standard assays such as CBC and cytogenetics, although less sensitive, are important tools because of their ubiquity and prognostic power. More specialized tests, such as FISH and PCR, require more training; however, their sensitivity allows the clinician to quantify residual disease. PCR is emerging as a test to perform from diagnosis throughout therapy because of its immense power to establish a baseline at diagnosis and to establish the degree of response even at early time points in therapy. These early data will be essential to help identify nonresponders and allow them to switch therapies at an early stage of disease progression.

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DISCLOSURES

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