Chronic myelogenous leukemia (CML) is the prototype of the myeloproliferative disorders (See table below). The myeloproliferative disorders are characterized by an overproduction of at least 1 cell lineage (ie, granulocytic, megakaryocytic, erythroid, and/or monocytic), a subacute to chronic course, and a tendency to evolve into a more aggressive lethal form. The chronic myeloproliferative disorders differ from acute leukemias clinically by a less acute course and morphologically by maturation of the neoplastic clone. Numerous blasts typical of acute leukemia are not seen in the myeloproliferative syndromes unless transformation has occurred.

The myelodysplastic syndromes are distinguished by ineffective hematopoiesis leading to cytopenias rather than the overproduction of cells seen in the myeloproliferative syndromes. Myelodysplasias are also associated with different cytogenetic abnormalities. CML is the most common of the myeloproliferative syndromes and the best characterized with regard to molecular abnormalities, resulting from a characteristic Philadelphia chromosomal abnormality, t(9;22)(q34q11). The molecular consequence of this chromosomal translocation is production of a hybrid BCR-ABL (breakpoint cluster region–Abelson leukemia virus) fusion protein product (p210) with tyrosine kinase activity and transforming properties. Morphologic, cytogenetic, and molecular studies are essential in the diagnosis and monitoring of patients with CML. Newer chemotherapeutic agents and hematopoietic stem cell transplantation have substantially improved the prognosis for patients with CML.

This is the first article in a 3-part series on hematology. After reading this article, the reader should be able to describe the common clinical and laboratory features of CML, recognize the typical peripheral blood and bone marrow morphologic features, describe the most common techniques for identifying the diagnostic molecular alterations of CML, and describe some of the strengths and weaknesses of these methods.

**World Health Organization Classification of Myeloproliferative Diseases**

- Chronic myelogenous leukemia, Ph chromosome positive [t(9;22)(q34q11), BCR/ABL]
- Chronic neutrophilic leukemia
- Chronic eosinophilic leukemia
- Chronic idiopathic myelofibrosis
- Polycythemia vera
- Essential thrombocythemia
- Myeloproliferative disease, unclassifiable

**Abstract**

Chronic myelogenous leukemia (CML) is the most common and best studied of the chronic myeloproliferative disorders, resulting from a characteristic Philadelphia chromosomal abnormality, t(9;22)(q34q11). The molecular consequence of this chromosomal translocation is production of a hybrid BCR-ABL (breakpoint cluster region–Abelson leukemia virus) fusion protein product (p210) with tyrosine kinase activity and transforming properties. Morphologic, cytogenetic, and molecular studies are essential in the diagnosis and monitoring of patients with CML. Newer chemotherapeutic agents and hematopoietic stem cell transplantation have substantially improved the prognosis for patients with CML.

**Clinical Features**

CML may occur at any age but most frequently affects adults, with a slight male predominance. The patients most frequently present with nonspecific symptoms of fatigue, weight loss, and anorexia, which are gradual in onset. Occasionally,
patients may have symptoms related to leukostasis from extremely high peripheral WBC counts or pain secondary to splenic infarction. Many have no symptoms at diagnosis. More than half of the patients have palpable splenomegaly, a condition much more commonly found in the chronic myeloproliferative disorders than in myelodysplasia or acute leukemia.

Most patients present in the chronic phase of disease, which may persist for several years with standard therapy. Ultimately, the chronic phase progresses to a lethal blast crisis resembling that of acute leukemia. An accelerated phase often precedes and heralds an impending blast crisis. Criteria for accelerated phase vary somewhat but usually include increased peripheral blood or bone marrow blasts (>10%), clonal evolution by cytogenetics, and increasing WBC count, splenomegaly, or persistent thrombocytopenia unresponsive to therapy. Treatment regimens aim to either prolong the chronic phase or achieve a true cure with eradication of the neoplastic clone.

Glossary

**BCR-ABL product**—The chimeric messenger RNA transcription product or fusion protein translation product resulting from t(9;22)(q34;q11).

**Chromosomal translocation**—The relocation of a portion of 1 chromosome to a different chromosome.

**Probe**—A small molecule that is complementary and will bind to a sequence of interest. Specifically labeled probes are commonly used in molecular genetic testing.

**t(9;22)(q34;q11)**—A balanced chromosomal translocation involving the long (q) arms of chromosomes 9 and 22 with breakpoints at bands q34 and q11, respectively.

**Transformation**—Regarding stage of disease in CML, transformation refers to the progression from chronic phase to the more aggressive accelerated phase or blast crisis. Regarding individual cells, transformation refers to the process whereby normal benign cells become malignant, losing normal growth controls.

**Tyrosine kinase**—An enzyme capable of phosphorylating tyrosine residues. Tyrosine kinases are frequently involved in signal transduction and cellular growth control.

**Laboratory Findings**

The diagnosis of CML rests primarily on simple laboratory evaluation of the peripheral blood and documentation of the t(9;22) by cytogenetics or molecular genetic analyses. The peripheral WBC count is almost always elevated to at least 25,000/µL (25 x 10^9/L), with half of cases exceeding 100,000/µL (100 x 10^9/L). Granulocytes predominate with a spectrum of “left-shifted,” immature forms including bands, metamyelocytes, myelocytes, and promyelocytes (Fig 1). Circulating blasts may also be seen in chronic phase CML but are usually few (<5%).

Virtually all patients have basophilia, which may be useful in differentiating CML from other myeloproliferative disorders or reactive leukocytes. Thrombocytosis occurs in many patients but is usually less dramatic than that seen with essential thrombocythemia (eg, <1,000,000/µL [1,000 x 10^9/L]). Anemia is also common at presentation. Adjunctive laboratory findings in CML include a decreased leukocyte alkaline phosphatase (LAP) score, in contrast to reactive leukocytes, which manifest increased LAP scores. The LAP test, however, is not very reproducible and is less commonly used than in the past.

**Bone Marrow**

Though less essential than the peripheral blood findings for diagnosis, examination of the bone marrow may provide useful information, particularly for staging chronic phase vs accelerated phase or blast crisis. Chronic phase CML shows a striking myeloid hyperplasia, producing a markedly hypercellular marrow (often >95% cells) replacing the marrow fat, which normally occupies approximately half of the marrow space in an adult (Fig 2). The granulocytic precursors are left-shifted toward immature forms, a finding that is accentuated near bony trabeculae. Whereas in a normal marrow specimen the ratio of myeloid to erythroid precursors is approximately 3:1, in CML the ratio is usually more than 10:1. Megakaryocytes are typically increased in number and often exhibit clustering and atypical small forms with hypolobated nuclei. Reticulin fibrosis may be increased and, if severe, may herald an accelerated phase. The aspirate smears reflect a left-shifted myeloid hyperplasia.
Blasts in the marrow are usually less than 5% in the chronic phase, with higher levels (≥10%) indicating transformation to a more aggressive course. Thirty percent or more blasts in the blood or marrow defines blast crisis of CML, though newer proposals may lower the cutoff to 20%, as has been proposed for acute leukemia. The blasts demonstrate a myeloid phenotype in approximately two thirds of cases, with the remainder lymphoblastic or mixed.2 An intermediate number of blasts in the bone marrow or blood is 1 criterion for accelerated phase.

**Molecular Features**

A unique cytogenetic abnormality, a shortened chromosome 22, later called the Philadelphia (Ph) chromosome, was first described in CML in 1960.3 This karyotypic hallmark of CML results from a translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11), and is found in more than 90% of patients (Fig 3).4 The molecular consequence of the juxtaposition of the BCR gene on chromosome 22 with the ABL gene on chromosome 9 is the formation of a BCR-ABL fusion protein with enhanced tyrosine kinase activity, thought to be important in leukemogenesis.5 Molecular studies have shown that the CML cases that do not show the classic karyotype display equivalent genetic alterations leading to production of the BCR-ABL protein. The Ph chromosome, or BCR-ABL product, is found not only in the granulocytic precursors, but in the erythroid, megakaryocytic, and B-lymphoid lineages.

The Ph chromosome and BCR-ABL protein are not restricted to patients with CML, but can be found in a subset of cases of acute lymphoblastic leukemia (ALL). The precise breakpoints in the gene vary somewhat, however (Fig 4). In usual chronic CML, the translocation results in a 210-kd BCR-ABL fusion protein product, whereas in most (80%) childhood Ph-positive ALL and 50% of adult Ph-positive ALL cases, the breakpoint on chromosome 22 is further upstream, resulting in a smaller, 190-kd, BCR-ABL protein product.6 A third BCR-ABL variant, 230-kd, has been described in some...
patients with neutrophilic CML, a less aggressive variant of CML with a predominance of mature segmented neutrophils.7

**Laboratory Detection of Ph Chromosome**

Classic karyotypic analysis, which will identify the Ph chromosome in more than 90% of cases, is a standard first-line diagnostic procedure for patients with suspected CML. An additional 5% of patients have variant Ph chromosomes with translocations involving 22q and a chromosome other than 9, or multiple chromosomes.4 Masked translocations can also occur that do not lead to a shortened chromosome 22 (Ph) as detected by routine karyotypic analysis. Molecular studies (eg, fluorescence in situ hybridization [FISH] or reverse transcriptase–polymerase chain reaction [RT-PCR]) can detect evidence of t(9;22) (q34q11) in cases missed by classic cytogenetics. Ph-negative CML by both karyotypic and molecular analysis is extremely rare; in such cases, other diagnoses should be considered. Karyotype abnormalities other than the Ph chromosome frequently occur with transformation.

**Fluorescence In Situ Hybridization**

FISH utilizes specifically colored fluorescent probes to regions of the BCR and ABL genes to detect the t(9;22)(q34q11). For example, in normal specimens, one would expect 2 green signals and 2 red signals separate from one another corresponding to the 2 alleles of the normal BCR gene on chromosome 22q11 and ABL gene on chromosome 9q34, respectively. When a translocation occurs, one of the red-labeled probes will be juxtaposed to a green-labeled probe, often producing a yellow fluorescent signal (Fig 5). An advantage of FISH over classic karyotypic analysis is that nondividing interphase cells can be analyzed. Another important advantage is that many more cells can be analyzed efficiently, often 200 to 500 cells in bone marrow samples, compared with only 20 to 25 metaphase cells with cytogenetics. Classic karyotypic analysis, however, enables detection of additional chromosomal changes (eg, trisomy 8, isochromosome i(17q), or trisomy 19) sometimes associated with disease progression, which would not be detected using the specific FISH probes. For this reason, karyotypic studies are often used in conjunction with FISH or other molecular techniques in monitoring treatment responses and disease course.

**Reverse Transcriptase Polymerase Chain Reaction**

The sensitivity of PCR for minimal residual disease is unparalleled, identifying 1 Ph-positive cell among 10⁴ to 10⁵ normal cells. In RT-PCR, the BCR-ABL messenger RNA transcript is converted to complementary DNA by the enzyme reverse transcriptase and then amplified using the cyclical PCR reaction. Without the translocation, the PCR primers for BCR and ABL are too far apart to produce an amplifiable

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**Fig 4. Schematic of various BCR-ABL Fusion Proteins.**

The various BCR-ABL protein products result from fusion of the ABL sequence containing the tyrosine kinase (TK) domain with different lengths of the BCR product. Breakpoints are indicated by arrows. In CML the product is 210 kd whereas in acute leukemia it can be either 190 kd or 210 kd. The 230 kd product is found in some cases of neutrophilic CML.
product in the reaction. Because RT-PCR examines at the messenger RNA level, it will identify all types of translocations that result in a BCR-ABL fusion transcription product, even variant and cryptic translocations. Different primer sets are used for detecting the p190, p210, or p230 fusion transcription products. A labeled probe against BCR or ABL is used to confirm the specificity of the RT-PCR reaction product.

The meaning of a positive qualitative RT-PCR result, which could represent as little as 1 Ph-positive cell in 100,000 cells, is not clear and does not appear to be a good predictor of disease relapse or overall survival. Some proposed explanations for this phenomenon include presence of the Ph chromosome in certain marrow populations (eg, histiocytes) without true neoplastic potential and the ability of the immune system to keep a minimal Ph-positive malignant cell population dormant for prolonged periods. Quantitative RT-PCR assays appear to be more clinically useful predictors of relapse, particularly in patients who have undergone stem cell transplantation.

**Treatment**

**Standard Chemotherapy**

Hydroxyurea and busulfan are the most commonly used and widely available chemotherapeutic agents for CML. Both reduce the WBC count (hematologic remission), with more complete remissions and fewer toxic effects obtained with hydroxyurea. Hematologic remission does not mean that the Ph-positive neoplastic clone is abolished, however. Neither hydroxyurea nor busulfan produces regular cytogenetic remissions or affects eventual disease progression. Median survival is 3 to 6 years with termination in blast phase.

**Allogeneic Hematopoietic Stem Cell Transplantation**

Allogeneic stem cell transplantation has potential curative effects (hematologic, cytogenetic, and molecular remission) but has limited applicability due to age restrictions and need for HLA-matched donors. The best results are achieved in younger patients in chronic-phase CML using HLA-matched sibling donors. Before transplantation, the patients receive high-dose chemotherapy with or without total-body irradiation to cleanse their marrow, followed by transplantation with donor stem cells collected from peripheral blood or bone marrow. Older patients fare less well primarily because of increased mortality related to the intensive transplant regimen. Disease-free survival is 60% to 70% in favorable groups with relapse rates of 15% to 30%. Quantitative RT-PCR appears to be a clinically useful predictor of relapse in this population. Many patients with relapse after transplantation benefit from infusion of donor T lymphocytes, due to an immune-mediated check of the malignant clone.
Interferon Alfa

Interferon alfa can produce both hematologic and cytogenetic remissions in patients with chronic-phase CML. Because of its superior activity compared to traditional chemotherapy, interferon alpha is the preferred initial therapy for patients ineligible for bone marrow transplantation. Combination therapy with cytarabine appears to have some added benefit. Although much safer, the rates of sustained complete cytogenetic response are less with interferon therapy (only 5%-10%) than bone marrow transplantation. Roughly 60 to 70% of groups favorable to bone marrow transplantation show long-term disease-free survival.

New Therapies: Tyrosine Kinase Inhibitors

A new type of drug designed specifically for CML, ABL tyrosine kinase inhibitors, has shown promise in early clinical trials inducing both hematologic and frequent cytogenetic responses. These inhibitors were developed based on the understanding that the increased tyrosine kinase activity of the fusion BCR-ABL protein is essential in the transformation of the leukemic cells. The promising early success of these drugs serves as an example of the potential practical benefit of basic research in the molecular pathogenesis of neoplasia. Other potentially effective new therapies are being actively investigated, including matched unrelated donor transplantation, homoharringtonine, decitabine, polyethylene glycol interferon, antisense oligonucleotides, and adoptive immunotherapy.

Conclusion

CML is the prototype of the chronic myeloproliferative disorders; it results from expansion of a neoplastic clone exhibiting a characteristic t(9;22)(q34q11) chromosomal abnormality resulting in a BCR-ABL fusion transcription product with enhanced tyrosine kinase activity. Laboratory morphologic evaluation of peripheral blood and bone marrow is essential in diagnosis and in directing confirmatory cytogenetic or molecular studies. Assessment of residual disease by karyotype, RT-PCR, and/or FISH analysis, along with morphologic evaluation, has become standard for monitoring response to treatment. Potentially curative stem cell transplantation, interferon alfa, and newer chemotherapeutic agents based on an understanding of the molecular pathogenesis of CML are improving patient prognosis. Clinical and basic laboratory research in this field continue to make significant inroads and assure exciting progress in our understanding of CML and treatment capabilities.

References