Chronic myeloid leukemia (CML) has become a model in research and management among malignant disorders. Since the discovery of the presence of a unique and constant chromosomal abnormality slightly more than 40 years ago, substantial progress has been made in the understanding of the biology of the disease. This progress has translated into significant improvement in the long-term prognosis of patients with this disease. This change came first with the use of stem cell transplantation and interferon alfa, but recently it has opened the era of molecularly targeted therapies. Imatinib, a potent and selective tyrosine kinase inhibitor, may be the best example of our attempts to identify molecular abnormalities and develop drugs directed specifically at them. Furthermore, the understanding of at least some of the mechanisms of resistance to imatinib has led to rapid development of new agents that may overcome this resistance. The outlook today for patients with CML is much brighter than just a few years ago. It is our hope that this fascinating journey in CML can be replicated in other malignancies. In this article, we review our current understanding of this disease.


C hronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of a pluripotent stem cell first described by John Hughes Bennett in 1845 at The Royal Infirmary of Edinburgh. It has been classified as a myeloproliferative disorder along with agnogenic myeloid metaplasia, polycythemia vera, and essential thrombocytopenia. Chronic myeloid leukemia was the first malignancy that had a specific chromosomal abnormality uniquely linked to it after the discovery of a minute chromosome now known as the Philadelphia (Ph) chromosome, later defined to result from a t(9;22) reciprocal chromosomal translocation. Of critical importance was the demonstration that this translocation involved the ABL1 (Abelson) protooncogene in chromosome 9 and the BCR (breakpoint cluster region) gene in chromosome 22. Since then, a constant stream of clinical and basic advances has made CML one of the most extensively studied human malignancies. The introduction of rationally designed small molecules that target the tyrosine kinase activity of Bcr-Abl in the treatment of CML has pioneered the development of targeted therapies in cancer medicine. In this review, we summarize the current knowledge of the molecular biology of CML and discuss the current treatment modalities, including the important historical role of recombinant interferon alfa, the development of imatinib mesylate and the new tyrosine kinase inhibitors (TKIs), stem cell transplantation (SCT), and other novel therapeutic approaches still in preclinical or early clinical development.

EPIDEMIOLOGY AND ETIOLOGY

Chronic myeloid leukemia is a rare disease worldwide. Approximately 4600 new cases of CML were diagnosed in 2004 in the United States, among 33,400 new cases of leukemia. Hence, CML represents 14% of all leukemias and 20% of adult leukemias. The annual incidence is 1.6 cases per 100,000 adults, with a slight male preponderance (male-female ratio, 1.4:1). The median age at diagnosis is 65 years. In contrast, CML is exceedingly rare in children, and its incidence increases with age. There are no known hereditary, familial, geographic, ethnic, or economic associations with CML; therefore, the disease is neither preventable nor inherited. In most patients, the factor responsible for the induction of the Ph chromosome is unknown, although CML has been observed with increased frequency in individuals exposed to the atom bomb explosions in Japan in 1945, in radiologists, and in patients with ankylosing spondylitis treated with radiation therapy.

CLINICAL PRESENTATION AND NATURAL HISTORY

Classically, 3 phases of disease progression are recognized in CML: chronic phase (CP), accelerated phase (AP), and blast phase (BP) (Table 1). Currently, nearly 90% of patients with CML have their conditions diagnosed in the CP. Frequently, the diagnosis is made incidentally after a rou-
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tine complete blood cell count is performed for unrelated reasons. This is due to the fact that patients with CML-CP have a competent immune system and may remain asymptomatic for prolonged periods. When symptoms appear, they are generally related to the expansion of CML cells and consist typically of malaise, weight loss, and discomfort caused by splenomegaly. 14 Leukocytosis is a common feature of CP, and the white blood cell (WBC) count can be as high as \(1000 \times 10^9/L\), leading in rare instances to signs and symptoms of hyperviscosity, such as retinal hemorrhage, priapism, cerebrovascular accidents, tinnitus, confusion, and stupor. After a median of 3 to 5 years, untreated patients with CML-CP inevitably progress to CML-BP, an aggressive form of acute leukemia highly refractory to chemotherapy that is rapidly fatal. 15 The risk of transformation to CML-BP is estimated at 3% to 4% per year. 15,16 Chronic myeloid leukemia-AP is characterized by an increasing arrest of maturation that usually heralds transformation to CML-BP.

Different criteria have been used to define CML-AP. One commonly used classification defines AP as the presence of at least 1 of the following hematologic features: 15% or more blasts, 30% or more blasts plus promyelocytes, 20% or more basophils, or platelet counts lower than \(100 \times 10^9/L\) unrelated to therapy. 17 Of note, most classification systems for CML-AP and CML-BP proposed throughout the years in the literature were developed before the introduction of imatinib, and some, such as the recent World Health Organization proposal, 13 have not been validated in clinical studies and therefore may lack clinical importance. 18 Most discrepancies among different classifications relate to the use of slightly different cutoffs for hematologic values and other minor criteria. Although patients may become symptomatic, the transition from CML-CP to CML-AP is usually subclinical, and laboratory monitoring is necessary for detection of disease progression. The median survival for patients with CML-AP is 1 to 2 years. 19 Most patients’ CML will remain in AP for 4 to 6 months before progressing to BP. 20,21 Classic criteria define CML-BP by the demonstration of at least 30% of blasts in the peripheral blood or the bone marrow or the presence of extramedullary blastic foci. The World Health Organization classification proposes a reduction from 30% or more to 20% or more blasts for the diagnosis of CML-BP. Clinically, most patients with CML-BP experience signs and symptoms related to increasing tumor burden, including inability to control WBC counts with previously stable doses of medication, marked constitutional symptoms (fever, night sweats, anorexia, malaise, weight loss), splenic infarcts due to massive splenomegaly, bone pain, and increased risk of infections and bleeding. The presence of cytogenetic abnormalities in addition to the Ph chromosome (ie, clonal evolution) involves most frequently trisomy 8, isochromosome 17, and duplicate Ph chromosome and has been considered a criterion of CML-AP. However, patients who are classified as having CML-AP based solely on the presence of clonal evolution appear to fare better than those whose conditions were diagnosed on the basis of other clinical features. Immunophenotypically, CML-BP can be characterized by myeloid or lymphoid phenotype. In rare instances, blast cells can be biphenotypic with a mixed lymphoblastic-myeloblastic lineage. 18,22 Lymphoid CML-BP occurs in 20% to 30% of patients, myeloid in 50%, and undifferentiated in 25%. 22,23 Median survival of patients with lymphoid CML-BP is 3 to 6 months, with patients with lymphoid CML-BP having a slightly better prognosis than those with myeloid phenotype.

| TABLE 1. Criteria for Accelerated Phase According to 3 Frequently Used Classifications* |
|---------------------------------------------|-----------------|---------------|
| **MDACC** 11 | **IBMTR** 12 | **WHO** 13 |
| Blasts (%) | ≥15 | ≥10 | 10-19† |
| Blasts and | ≥30 | ≥20 | NA |
| promyelocytes (%) | ≥30 | ≥20 | NA |
| Basophils (%) | ≥20 | ≥20 | ≥20 |
| Platelets (× 10^9/L) | <100 | Unresponsive increase or persistent decrease | <100 or >1000 unresponsive to treatment |
| Cytogenetics | CE | CE | CE not at the time of diagnosis |
| White blood cell | NA | Difficult to control or doubling in <5 d | NA |
| Anemia | NA | Unresponsive | NA |
| Splenomegaly | NA | Increasing | NA |
| Other | NA | Chloromas, myelofibrosis | Megakaryocyte proliferation, fibrosis |
| *CE = clonal evolution; IBMTR = International Bone Marrow Transplant Registry; MDACC = M. D. Anderson Cancer Center; NA = not applicable; WHO = World Health Organization. |
| †Blast phase of 20% or more blasts (≥30% for MDACC and IBMTR). |
| §Basophils and eosinophils. |

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LABORATORY AND PATHOLOGICAL FEATURES

Laboratory findings in CML include leukocytosis with a remarkable left shift, basophilia, and eosinophilia. Platelet count may be either high or low, and mild anemia is commonly observed. The leukocyte alkaline phosphatase activity is reduced, although phagocytic function remains essentially normal. Low values may also be observed in agnogenic myeloid metaplasia, whereas activity may increase with infection, clinical remission, or at the onset of BP. The increase in the size of the WBC pool is reflected by marked elevation of serum B₁₂ levels and unsaturated B₁₂-binding capacity. During transformation to CML-BP, circulating basophil levels, histamine levels, or both often increase, but the causative factors and importance of these phenomena remain unknown.

The bone marrow of patients with CML is notoriously hypercellular and devoid of fat. In fact, it can be hypothesized that the primary biologic defect in CML is not unregulated proliferation of leukemic stem cells but rather discordant maturation, wherein a slight delay in cell maturation within the myeloid compartment results in increased myeloid mass. All stages of myeloid maturation are present, with predominance of myelocytes. In CP, the sum of myeloblasts and promyelocytes usually accounts for less than 10% of the marrow cellularity. A decrease in apoptosis of myeloid cells contributes to the expansion of this compartment and defective adherence of immature CML cells to marrow stromal cells in vitro, with subsequent early release into the circulation. Megakaryocytes may be increased, and Gaucher-like cells can be observed in 10% of cases. As CML progresses, varying degrees of reticulin fibrosis may be seen, likely a result of the interaction between the proliferative clone of megakaryocytes and cytokines, such as platelet-derived growth factor, transforming growth factor β, and basic fibroblastic growth factor, whose plasma levels are significantly increased in CML. Of note, these cytokines play an important role modulating angiogenesis, and characteristically the bone marrow from patients with CML shows the highest number of blood vessels and largest vascular area of all leukemias.

Blastic cells from patients with lymphoid CML-BP express terminal deoxynucleotidyl transferase, an enzyme that catalyzes the polymerization of deoxynucleoside tri-phosphates and is mainly found in poorly differentiated B and T cells. In most cases of lymphoid CML-BP, blasts exhibit a B-cell immunophenotype, expressing CD10, CD19, and CD22. In contrast, myeloid CML-BP closely resembles acute myeloid leukemia (AML), and blasts stain with myeloperoxidase and express myeloid markers such as CD13, CD33, and CD117. Frequently, myeloid markers such as CD13 and CD33 may be expressed in patients with lymphoid CML-BP. Rare cases of megakaryoblastic, erythroid, and mastoblastic transformation have been reported.

PROGNOSTIC SYSTEMS

Because of the difference in clinical aggressiveness between the initial CP and advanced phases of CML and because the prognosis in CML can vary significantly even among patients in the same phase of the disease, several risk stratification strategies have been developed in an attempt to prognosticate and guide patient management. The system most extensively used is the Sokal score, which was derived from 813 patients with CML collected from 6 European and American series in the 1960s and 1970s. In this system, the hazard ratio for death is calculated from baseline patient and disease characteristics using the following formula: \( \lambda(t) = \exp(0.0116 \times (\text{age} – 43.4) + 0.0345 \times (\text{spleen} – 7.51) + 0.188 \times \left[ \left( \frac{\text{platelets}}{700} \right)^2 – 0.563 \right] + 0.0887 \times (\text{blasts} – 2.10)) \). This scoring system establishes 3 prognostic groups with hazard ratios of less than 0.8, 0.8 to 1.2, and more than 1.2 and median survivals of approximately 2.5, 3.5, and 4.5 years, respectively. Of note, most of the patients from whom the Sokal score was derived were treated with therapies currently not in use, such as busulfan, intensive chemotherapy, and splenectomy. However, several studies performed in the 1990s have demonstrated the applicability of this system in more modern series. Of 625 patients with CML-CP treated with chemotherapy in one study, 168 (27%) were high risk and had a 2-year actuarial survival of 70% and a subsequent probability of death of approximately 30% per year. In contrast, the low-risk group had a 2-year actuarial survival of 91% and a subsequent risk of death of approximately 17% per year.

The Sokal system has several limitations. First, in asymptomatic patients whose disease is detectable only by molecular testing, lead-time bias can be substantial relative to those whose conditions are diagnosed after presenting with symptoms. Second, the use of more refined and potent therapeutic modalities in CML, such as imatinib, has been associated with the loss of predictive value of some prognostic factors. Still, the Sokal score identifies patients with significantly different probabilities of response to imatinib, although patients with high-risk scores now have a much better outcome.

Other scoring systems have been proposed that may be more applicable to patients treated with interferon alfa. The best-characterized and most widely used is the Hasford score, which considers patient age, platelet count, peripheral blast count, spleen size, eosinophils, and basophils to determine risk. Some analyses have suggested that this scoring system is less predictive among patients treated
with imatinib, but others have found it still useful. Finally, the Gratwohl score system has been developed specifically for patients undergoing SCT.40

CYTOGENETICS

A conclusive diagnosis of CML relies on cytogenetic and molecular testing to identify the (9;22)(q34.1;q11.21) and/or the BCR-ABL1 hybrid gene, which is pathognomonic of this disease.45 The Ph chromosome results from the balanced translocation of cytogenetic material after a break on chromosome 9 at band q34.1 that transposes the 3’ segment of the ABL1 gene to the 5’ segment of the BCR gene on chromosome 22 at band q11.21.3 The Ph chromosome is detected in 95% of patients with CML and in 5% of children and 15% to 30% of adults with acute lymphoblastic leukemia (ALL). In addition, approximately 2% of patients with newly diagnosed AML can present with this cytogenetic abnormality.31 Occasionally, translocations that involve 3 or more chromosomes occur and are termed variant Ph chromosome translocations.42,43 Although initially thought to confer poor prognosis,44 recent data in patients treated with imatinib have demonstrated that variant translocations are associated with a similar prognosis compared with patients with the classic Ph chromosome.45 In 5% of patients with typical CML phenotype, the Ph chromosome is not detectable but harbors the BCR-ABL1 rearrangement.46 These patients have the same biology and outcome as those who express the Ph chromosome. Among the 5% to 10% of patients with CML clinical features who do not express the Ph chromosome, 30% to 50% have molecular evidence of the rearrangement involving BCR and ABL1. Those who do not express this molecular abnormality (ie, true Ph chromosome negative) are called atypical CML and have a different prognosis and treatment.47 The characteristics and management of atypical CML are not covered any further in this article.

Little is known about the mechanisms involved in transformation to CML-BP, but the acquisition of additional cytogenetic abnormalities, referred to as clonal evolution, in Ph chromosome–positive cells has been thought to play a prime role in CML progression. The most frequent secondary cytogenetic abnormalities encountered in patients with clonal evolution are trisomy 8, isochromosome 17, and duplicate Ph chromosome, which have been linked to c-Myc overexpression, loss of 17p, and BCR-ABL1 overexpression, respectively.33-35 Other cytogenetic aberrances, such as trisomy 19, trisomy 21, trisomy 17, and deletion 7, have been implicated in less than 10% of cases of clonal evolution.31,32 These genetic lesions are more frequently associated with myeloid than with lymphoid CML-BP. Nonetheless, a clear correlation between the development of these molecular features and disease progression has not been established yet. In addition, other molecular events, such as deletion or inactivation of p53,53 p16,54 and the retinoblastoma gene product55 have been associated with disease transformation, but like overexpression of EVI-1,56 these are relatively infrequent and therefore not specific to CML-BP.57 Recently, it was suggested that progression of CML to BP probably involves several events in primitive progenitors, including BCR-ABL1 amplification, acquisition of resistance to apoptosis, genomic instability, escape from innate and adaptive immune responses, and activation of β-catenin in granulocyte-macrophage progenitors, resulting in the acquisition of self-renewal capacity.58 Gene methylation has also been associated with progression in CML.59-61 The Pa promoter of ABL1 and the p15 promoter are hypermethylated in 81% and 24% of patients, respectively. Interestingly, hypermethylation of p15, but not of the Pa promoter, is associated with disease progression. Methylation of the cadherin-13 gene occurs at an early stage in CML and is associated with high-risk features and lower probability of response to interferon alfa.62

Cytogenetic aberrancies have been reported occasionally in Ph chromosome–negative cells during treatment with interferon alfa and, more recently, with imatinib. These aberrancies do not represent clonal evolution because they occur in cells without the Ph chromosome and should not be considered as part of the definition of AP. The importance of these events is currently unknown.63 The most common such cytogenetic abnormalities include trisomy 8, monosomy 5 or 7, and deletion 20q.64-66 These abnormalities frequently regress spontaneously, and it has been suggested that patients with these abnormalities have similar long-term outcome as patients without such abnormalities. However, occasional cases of progression to AML or myelodysplastic syndrome have been reported.

MOLECULAR BIOLOGY OF BCR-ABL1

The ABL1 gene is the human homologue of the v-abl oncogene harbored by the Abelson murine leukemia virus,67 and it encodes a nonreceptor tyrosine kinase (Figure 1).68 In turn, BCR encodes a protein with serine–threonine kinase activity. The fusion of these 2 genes results in the activation of the c-ABL protooncogene to its oncogenic form.69,70 The breakpoints within the ABL1 gene at 9q34 map to an area spanning more than 300 kilobase (kb) at its 5’ end occur upstream of exon Ib, downstream of exon Ia, or, more frequently, between the two.71 Alternatively, breakpoints within BCR localize to 3 main breakpoint cluster regions. In most patients with CML and a third of those with Ph chromosome–positive ALL, the break occurs
within a 5.8-kb area spanning BCR exons e12-e16 (formerly called b1-b5), referred to as the major breakpoint cluster region (M-bcr). Because of alternative splicing, a fusion transcript with either b2a2 or b3a2 junctions can result and will give rise to a 210-kd hybrid protein (p210BCR-ABL).\(^72,73\) The clinical features and response to treatment are apparently similar for both transcripts. In two thirds of patients with Ph chromosome–positive ALL and rarely in CML, the breakpoints within BCR localize to an area of 54.4 kb between exons e2′ and e2, termed the minor breakpoint cluster region (m-bcr), giving rise to an e1a2 messenger RNA that is translated to a 190-kd protein (p190BCR-ABL). Patients with CML who carry this transcript can present with marked monocytosis and may have a worse prognosis than those with the typical p210.\(^74\) A third breakpoint cluster region (µ-bcr) downstream of exon 19 has been identified, giving rise to a 230-kd fusion protein (p230BCR-ABL) linked to cases of chronic neutrophilic leukemia.\(^75\) Although all 3 transcripts can induce a CML-like disorder in mice, p230BCR-ABL has reduced tyrosine kinase activity relative to p190BCR-ABL and confers only partial growth factor independence, which parallels the milder clinical course of chronic neutrophilic leukemia.\(^75\) All the isoforms of Bcr-Abl have an active and an inactive configuration. Interestingly, using reverse transcriptase–polymerase chain reaction (RT-PCR) with a sensitivity of approximately 10⁻⁶, the BCR-ABL1 chimerism has been identified in up to 25% to 30% of presumably healthy adults.\(^77,78\) One hypothesis to explain this phenomenon is that BCR-ABL1 might not be the sole genetic lesion necessary for the development of CML. \(\text{JunB}\) is a component of the activator protein 1 family of transcription factors.\(^79\) Modifications of \(\text{JunB}\) expression may have a role in the pathogenesis of CML by modulating the initiation, progression, and maintenance of the myeloid differentiation program.\(^80\) A recent report in which transgenic mice specifically lacking \(\text{JunB}\) in the myeloid lineage (\(\text{JunB}^{+}\)\(\text{Ubi-JunB}\) mice) develop a myeloproliferative disorder that resembles human CML, including progression to BP, seems to support the latter hypothesis.\(^81\)

Several biological models, such as BCR-ABL1–expressing CD34⁺ cells in culture\(^82,83\) or retrovirally transduced BCR-ABL1⁺ mouse cells,\(^84\) have demonstrated that BCR-ABL1 is an oncogene that promotes CML pathogenesis. Numerous pathways are activated in BCR-ABL1–expressing cells,\(^85\) and phosphorylation at the Y177 site of BCR is essential for BCR-ABL1 leukemogenesis.\(^86,87\) This residue constitutes a high-affinity docking site for the SH2 domain of growth factor receptor–bound protein 2 (Grb2). In turn, Grb2 recruits SOS (a guanine-nucleotide exchanger of RAS) and the scaffold adapter Grb2-associated binding protein 2 (GAB2) through its SH3 domain. Phosphorylation of GAB2 leads to recruitment of phosphatidylinositol 3-kinase (PI3K) and SHP2 (also known as PTPN11), whereas SOS activates RAS.\(^88\) Mutation of Y177 to phenylalanine (Y177F) largely abolishes Grb2 binding and abrogates Bcr-Abl–induced RAS activation. Therefore, despite

![FIGURE 1. Activation of signal transduction pathways by BCR/ABL. AKT = protein kinase B; ERK = extracellular signal-regulated kinase; JAK = Janus kinase; MEK = mitogen-activated protein kinase kinase; PI3K = phosphatidylinositol 3-kinase; SAPK = stress-activated protein kinase; STAT = signal transducer and activator of transcription.](image-url)
preservation of the kinase activity of Abl, the Y177F mutant impedes the transformation of primary bone marrow cultures. In an SCT model of CML, BCR-ABL1Y177F has a greatly reduced ability to induce a myeloproliferative disorder in mice. Of note, SHP2 is required for normal activation of the RAS extracellular signal-regulated kinase pathway that most receptor tyrosine kinases signal through. Bcr-Abl also phosphorylates the Src kinases Lyn, Hck, and Fgr. Once phosphorylated, Hck activates signal transducer and activator of transcription (STAT) 5. Importantly, overexpression of Hck/Lyn has been linked with disease progression, particularly with a lymphoid phenotype. Therefore, Bcr-Abl promotes hematopoietic cell transformation mainly through RAS, PI3K, and STAT 5 activation. All these elements have been shown to up-regulate the expression of cyclin D1, thus inducing cell-cycle progression from G1 to S phase. When BCR-ABL1–positive K562 cells were induced to express dominant negative forms of RAS, PI3K, or STAT 5, marked apoptosis was observed in cells coexpressing 2 of 3 dominant negative mutants in any combination. Notably, Bcr-Abl also enhances SET expression, particularly during progression to BP. SET is a nucleus/cyttoplasm-localized phosphoprotein that potently inhibits the tumor suppressor protein phosphatase 2A (PP2A). In turn, PP2A is a phosphatase that regulates cell proliferation, survival, and differentiation. Some of the elements involved in Bcr-Abl signal transduction have been sought as therapeutic targets in CML.

TREATMENT

The treatment of CML has experienced a great degree of refinement during the past few years. Until recently, interferon alfa and SCT were the only therapeutic options for patients with CML. Currently, SCT still remains a valid option, particularly for patients who do not respond appropriately to TKIs, and still remains a potentially curative therapeutic modality. However, the explosion of the field of molecularly targeted TKIs in recent years led by imatinib mesylate has brought about a change in the natural history of the disease and in the therapeutic approach to CML.

INTERFERON

Recombinant human interferon alfa has shown significant antitumor and immunomodulatory activity in the treatment of CML. Major cytogenetic responses (MCGRs) (<35% Ph chromosome–positive cells) have been reported in up to 40% of patients and complete cytogenetic responses (CCGRs) (0% Ph chromosome–positive cells) in up to 25%. The addition of cytarabine resulted in a significantly higher probability of a CCGR in up to 35% of patients.

The achievement of a CCGR is associated with improved survival, with 78% of patients who achieved a CCGR alive at 10 years, establishing the achievement of a CCGR as the therapeutic goal in the interferon alfa era. Furthermore, approximately 30% of patients in CCGR also had undetectable disease by PCR (ie, complete molecular remission); none of them has relapsed after a median follow-up of 10 years and are therefore probably cured. Interestingly, 40% to 60% of those in CCGR with the presence of minimal residual disease at the molecular level have not relapsed after 10 years. This has been attributed to interferon alfa–induced immune modulation, such as the presence of cytotoxic T lymphocytes specific for PR1, a peptide derived from proteinase 3, which is overexpressed in CML cells. This phenomenon has been demonstrated in patients in complete remission after interferon alfa therapy and SCT but not in those who fail to achieve CCGR or those treated with chemotherapy.

STEM CELL TRANSPLANTATION

Stem cell transplantation remains an important treatment option for patients with CML, particularly younger individuals with HLA-identical siblings. Initially, SCT was thought to render the best outcomes when performed within 12 months from CML diagnosis. It has been reported that SCT performed within the first 24 months, and in some reports within the first 36 months, from diagnosis may have similar outcomes. Although it was initially suggested that prior interferon alfa therapy adversely affected the outcome after SCT, subsequent trials demonstrated that this adverse effect was nonexistent and current evidence suggests that prior exposure to imatinib does not affect the outcome either. Current registry data revealed that the post-SCT mortality within the first year is 10% to 20% even in patients who underwent transplantation in optimal conditions (ie, HLA-matched sibling, first CP <1 year from diagnosis, age <<40 years). In addition, only one third to one half of patients may have a suitable HLA-matched sibling, and stringent inclusion criteria, such as age, adequate organ function, and performance status, may preclude the possibility of SCT in a large proportion of patients. A strategy to overcome the lack of suitable donors is the use of matched unrelated donors (MUDs). Unfortunately, the survival rate of patients undergoing MUD SCT is still lower when compared with those receiving grafts from HLA-matched siblings. The outcome of MUD SCT may be improved by careful molecular typing, particularly for HLA-A, HLA-B, and HLA-DRB1. However, the use of stringent typing criteria is inevitably linked to a lower probability of finding an adequate donor. In addition, SCT is fraught with risks such as graft-vs-host disease, veno-occlusive disease, life-
threatening infections, risk of secondary malignancy, and poorer overall quality of life. Also, the risk of late relapse is being increasingly recognized. A recent series analyzed the long-term follow-up of 89 patients who underwent transplantation at a single institution. After more than 10 years of follow-up, 28 patients (31%) were alive. The mean time to hematologic or cytogenetic relapse was 7.7 years, with 5 patients relapsing more than 10 years after SCT. In a recent study by the Seattle group, 131 patients with early CML-CP with a median age of 43 years (range, 14-66 years) received targeted busulfan and cyclophosphamide as a conditioning regimen. At 3 years, the projected non-relapse mortality rate was 14%, and 78% were projected to be alive and free of disease. However, 60% of survivors had extensive chronic graft-vs-host disease 1 year after transplantation, although only 10% had a Karnofsky score less than 80%. Notably, 11% of patients had minimal residual disease documented by PCR but had not relapsed at the time of the report. In contrast, patients with advanced phase CML who undergo SCT have a 5-year survival probability of 40% to 60% in the AP and 10% to 20% in the BP. Notably, the long-term outcome of patients with CML-BP who achieve a second CP and who are undergoing SCT appears to be similar to that of patients with CML-AP who undergo transplantation.

The long-term disease-free survival rate after MUD SCT for young patients in the early CP stage of disease is 57% compared with 67% in patients receiving grafts from HLA-matched siblings. The incorporation of molecular matching in recent years has decreased the rate of graft-vs-host disease and improved the probability of long-term survival in patients undergoing MUD SCT.

Reduced-intensity conditioning regimens have been used as a means to make SCT available to a broader number of patients who otherwise would be ineligible for standard SCT. This approach takes advantage of the ability of the T cells harbored in the graft to eliminate the CML cells (graft-vs-leukemia effect) as opposed to obtaining this effect by using ablative cytotoxic chemotherapy. This strategy increases tolerability and decreases morbidity and mortality significantly. Long-term results with reduced-intensity conditioning regimens are not yet available, but early results in small series (frequently including still mostly young patients) report disease-free survival as high as 85% at 70 months. In contrast, a recent study from the European Bone Marrow Transplant Registry reports a 3-year overall survival rate of 58% and a progression-free survival rate of 37%. Patients with minimal residual disease by PCR 12 months after SCT have a risk of relapse of 30% to 40% compared with less than 5% for those with negative PCR results. However, relapse can be frequently managed successfully with donor lymphocyte infusion in up to 70% of patients who relapse in the CP, particularly when administered at the time of molecular relapse. Alternatively, imatinib can be used in the post-SCT relapse setting and induces CCGR in more than 40% of patients treated in the CP.

**Imatinib Mesylate**

Imatinib mesylate (Gleevec) is an orally bioavailable 2-phenylaminopyrimidine with targeted inhibitory activity against the constitutively active tyrosine kinase of the Bcr-Abl chimeric fusion protein. In addition, imatinib inhibits other kinases, such as c-Kit, platelet-derived growth factor α and β, and Ab1-related gene (ARG). Imatinib has become the standard therapy for CML because of its remarkable activity and mild toxicity profile. In a phase 1 dose-finding study in patients who were intolerant to or in whom interferon alfa-based therapy failed, daily doses of 25 to 1000 mg were investigated. Responses among patients receiving doses lower than 250 mg/d were rare, in contrast to 98% of patients receiving a dose of at least 300 mg/d who achieved a complete hematologic response (CHR), including 54% who attained a cytogenetic response. Of note, no dose-limiting toxicity was identified, and the maximum tolerated dose was not reached. A dosage of 400 mg/d was selected for a subsequent phase 2 study, including 454 patients with late CP disease who were intolerant to or in whom interferon alfa therapy failed. Recently updated data showed that 96% of patients had achieved a CHR and 55% a CCGR. After a median follow-up of 5 years, the overall survival rate was 79%. This rate is in keeping with results from a recent single-institution study in which among 261 patients treated, 73% obtained a MCGR and 63% a CCGR. After a median follow-up of 45 months, 86% of patients are alive, of whom 80% are free of progression. More than 90% of patients who achieved a CCGR maintained a major response. The BCR-ABL1/ABL1 ratio by nested PCR was less than 0.05% in 31% and undetectable in 15% of patients. In the prospective, multicenter phase 3 International Randomized Study of Interferon and STI571, the efficacy of imatinib was compared with the combination of interferon alfa and low-dose cytarabine in patients with newly diagnosed CML-CP. Patients treated with imatinib (n=553) or interferon alfa and low-dose cytarabine (n=553) were allowed to cross over to the alternative group if treatment failure or intolerance was demonstrated. Imatinib therapy was superior to interferon alfa plus low-dose cytarabine regarding hematologic and cytogenetic responses, tolerability, and transformation to CML-AP. After a median follow-up of 54 months, the CHR, MCGR, and CCGR rates were 98%, 92%, and 86%, respectively. Progression-free survival is estimated to be 84%, and only
6% of patients have progressed to AP or BP. The overall annual progression rate has declined to 1.5% in the fourth year of therapy, compared with 4.8% and 7.5% in the previous 2 years, suggesting that disease progression may plateau in the ensuing years. The depth of the response after 12 months of imatinib therapy has important implications regarding disease progression and relapse. In fact, in 97% of patients who had a 3-log or greater reduction in BCR-ABL transcript levels (ie, major molecular response [MMR]) at 12 months, the probability of remaining free of progression to AP or BP was 97% at 54 months compared with 89% for patients in CCGR but not in MMR and 72% for patients who did not achieve a CCGR at 12 months ($P = .001$). In addition, the median BCR-ABL levels continue to decrease, with a mean log reduction at 48 months of 3.4 compared with 3.0 at 12 months. Although this study did not document a survival advantage compared with the interferon alfa arm because of the crossover design, studies comparing survival of patients treated with imatinib with historical cohorts treated with interferon alfa–based therapy demonstrate the anticipated survival advantage. For patients with previously untreated CML-CP. These studies have documented a higher rate of CCGR of up to 95% and higher rates of molecular responses, particularly responses at the level of a 4-log or greater reduction in transcript levels. In addition, responses occur significantly earlier with high-dose imatinib as the starting dose for patients with previously untreated CML-CP. These studies have demonstrated the anticipated survival advantage compared with the interferon alfa arm because of the crossover design, studies comparing survival of patients treated with imatinib with historical cohorts treated with interferon alfa–based therapy demonstrate the anticipated survival advantage.
dosage less than 600 mg had a significantly lower probability both at 12 and at 24 months. Thus, these studies suggest that high-dose imatinib may provide more and better remissions, but the results from ongoing randomized studies will determine whether high-dose imatinib provides only faster responses or whether these translate into prolonged progression-free and/or overall survival.

**Duration of Imatinib Therapy.** Currently, no evidence exists to support the belief that patients taking imatinib can safely discontinue therapy once they achieve a complete molecular response. Most patients who have discontinued imatinib therapy have rapidly experienced both molecular and cytogenetic relapse, even when some had sustained undetectable levels of BCR-ABL for significant periods. Recently, Rousselot et al described 8 patients who discontinued use of imatinib after maintaining undetectable BCR-ABL levels for 24 months, of whom still tested PCR negative after a follow-up of 8, 9, 12, and 13 months, respectively. All these patients had been treated with interferon alfa, suggesting that interferon alfa immunomodulatory effects may account for sustained and prolonged molecular responses observed on therapy discontinuation.

It has been suggested that the most primitive quiescent leukemic progenitors are insensitive to imatinib in vitro and are responsible for CML relapse once the inhibitory pressure of imatinib is removed. Emerging data suggest that resistance of quiescent cells to imatinib is indeed a Bcr-Abl–dependent phenomenon. Other potential mechanisms implicated in quiescent CML progenitors include expression of drug transport proteins such as PgP and Abcg2, atypical kinase domain mutations, and BCR-ABL overexpression. Monitoring for most patients. However, the lack of consistency in reporting BCR-ABL transcript levels has been a source of intense debate. A recent proposal aimed at the standardization of RT-PCR techniques and result reporting will greatly help this objective. According to this proposal, results will be converted by comparing analysis of standardized reference samples with a value of 0.1% corresponding to MMR in all laboratories. After the patient has achieved a complete cytogenetic remission, RT-PCR should be performed every 3 months and a routine cytogenetic analysis every 12 months. Fluorescence in situ hybridization in peripheral blood specimens can be used to monitor the cytogenetic responses among cytogenetic analyses. In addition, approximately 5% of patients who obtain a cytogenetic response develop clonal karyotypic abnormalities in Ph chromosome–negative cells, primarily consistent with those encountered in myelodysplastic syndromes, although only rare cases of myelodysplastic syndrome or AML have been reported. Although the long-term implications of these abnormalities are unknown, the risk of transformation to AML warrants careful follow-up of these patients.

**Imatinib Resistance.** A subset of patients with CML will exhibit either primary or secondary resistance to imatinib. Primary resistance refers to patients never responding to imatinib, whereas secondary resistance occurs when a patient who had an initial response to imatinib eventually loses the response. Among patients treated in CP, the rate of resistance has been estimated to be less than 2% per year. Several mechanisms of resistance have been reported, many of them mostly in vitro or in selected patient samples, and their individual contribution to this phenomenon has not been completely defined. The most frequently identified mechanism of resistance is the development of mutations in the Abl tyrosine kinase domain. More than 40 different BCR-ABL point mutations have been reported thus far, and new ones continue to be described. The region of the Abl kinase where mutations occur is
critical in their ability to confer varying degrees of insensitivity to imatinib. In fact, ranges of 220 to more than 5000 nM/L in the inhibitory concentration that results in a 50% reduction in proliferation (IC50) have been described in the various Bcr-Abl mutants.\textsuperscript{157} The most frequent mutations are those that map to the P-loop region of the kinase domain.\textsuperscript{157-159} The P-loop region is a glycine-rich structure that serves as a docking site for phosphate groups of adenosine triphosphate (ATP). G250E, Q252H, and the highly imatinib-insensitive Y253F and E255K mutations locate in this region. Despite not being directly involved in imatinib binding, these residues have been linked to poor prognosis in some series, with a median survival of 4 to 5 months.\textsuperscript{160-163} Other series have refuted this notion.\textsuperscript{164} The difference in these series may be due to several factors, including patient selection and the criteria used to select patients for screening for mutations. In addition, the first studies that suggested a poor outcome for patients with P-loop mutations did not address the management of patients after developing mutations, and it is now clear that several treatment strategies can overcome resistance through mutations.\textsuperscript{165} Also, considering that individual mutations have different levels of resistance to imatinib and other agents, it is more appropriate to refer to individual mutations rather than grouping them.\textsuperscript{155} The imatinib-insensitive T315I mutation occurs in a highly conserved “gatekeeper” threonine residue near the Bcr-Abl catalytic domain, thus leading to steric interference with imatinib binding.\textsuperscript{161,162,166} Other mutations have been described mapping to the catalytic domain and the activation loop of Abl, impairing the ability of the kinase to adopt a close (inactive) spatial conformation necessary to bind to imatinib.\textsuperscript{167}

Despite the poor prognosis conferred by the T315I mutant, in vitro and clinical data suggest that other mutations may be clinically relevant.\textsuperscript{168} Furthermore, other mechanisms of resistance, such as overexpression or amplification of the BCR-ABL message and/or protein, may influence the outcome of patients treated with imatinib. Recently, it was shown that activation of Src kinases and NF-κB may play a role in imatinib resistance in some patients.\textsuperscript{92,169}

### TREATMENT STRATEGIES FOR THE FUTURE

#### New Bcr-Abl Inhibitors

Several strategies are currently being developed to overcome imatinib resistance (Table 3). New Abl TKIs have been developed with enhanced potency and less stringent binding requirements and, in some cases, with inhibitory activity against other kinases involved in mechanisms of resistance to imatinib. In addition, new agents that block Bcr-Abl in a non–ATP-competitive manner may represent an alternative for patients who develop imatinib-insensitive Bcr-Abl kinase mutations.

**Nilotinib (AMN107)**. Nilotinib is a phenylamino-pyrimidine derivative with a 20- to 30-fold increased potency compared with imatinib against Bcr-Abl. Crystallographic models suggest that this increased potency is a result of
Dasatinib (BMS-354825). Because it is possible that inhibition of Bcr-Abl alone may not be sufficient to eradicate all CML cells, particularly the leukemic imatinib-insensitive quiescent stem cells, agents with additional inhibitory capacity against other kinases have been investigated. Since Src kinases have been implicated in CML pathogenesis and progression, dual Abl/Src inhibitors may have enhanced activity compared with imatinib. Among this new class of compounds, dasatinib has reached the furthest level of clinical development. Dasatinib is an ATP-competitive, dual-specific Src- and Abl-kinase inhibitor with 100-to 300-fold higher potency against Bcr-Abl compared with imatinib and significant activity against c-kit (IC50, 5 nM), platelet-derived growth factor receptor β (IC50, 28 nM), and Hck, Fyn, Src, and Lck kinases (IC50, approximately 0.5 nM).157 However, dasatinib does not inhibit the T315I mutant, even in the presence of micromolar concentrations.157 In phase 2 studies, dasatinib was administered at 70 mg twice daily to patients with CML in all phases after imatinib failure. A CHR was achieved by 87%, 66%, 55%, and 46% of patients in CP, AP, myeloid BP, and lymphoid BP/Ph chromosome–positive ALL, respectively. Among patients in CP, 44% had a CCGR, whereas 46% to 66% of patients with advanced-phase CML had MCGRs.173-176 In a dose-escalating study, dasatinib rendered 2-log or greater reductions in Bcr-Abl transcripts in 43% of patients in advanced phase, and 37% of patients in CP achieved 2-log or greater reductions, including 4 patients in each group who had an MMR.178 Overall, dasatinib is well tolerated, with myelosuppression and gastrointestinal toxic effects seen in some patients. Pleural effusions have also been reported in 10% to 15% of patients, particularly those in BP, and it is usually grade 1 or 2. Despite the impressive results achieved with dasatinib, it may not eradicate all quiescent leukemic stem cells, although it may be more effective at this level than imatinib.149 Another dual Abl/Src kinase inhibitor termed SKI-606279 is currently being evaluated in phase 1 studies, and others, such as NS-187 (INNO406),180 AZD0530,181 PD166326,182 and PD180970,183 may soon follow.

Non–ATP-Competitive Bcr-Abl Inhibitors. Imatinib, nilotinib, and dasatinib are ATP-competitive inhibitors and therefore amenable to being affected in their ability to bind to the Bcr-Abl kinase by the T315I mutation, which is considered the “gatekeeper” of the kinase domain. Compounds that target binding sites unrelated to the ATP-kinase domain may overcome this problem. ONO12380 targets the substrate-binding site of Bcr-Abl, competing with natural substrates such as Crkl but not with ATP.184 In fact, ONO12380 causes regression of leukemia induced by intravenous injection of 32DcI3 cells that express the Bcr-Abl mutant T315I.184 BIRB796, a p38 mitogen-activated protein kinase inhibitor, binds with excellent affinity to the Bcr-Abl T315I mutant. Homoharringtonine is a taxus alkaloid that represented the best therapeutic option before the introduction of imatinib in clinical practice.189 Its potential as salvage therapy in imatinib-resistant CML is being evaluated in phase 1 studies, and others, such as PD180970,183 may soon follow.

Other Therapeutic Options. Other approaches are being developed for patients who develop resistance to imatinib. Farnesyl transferase inhibitors, such as lonafarnib and tipifarnib, have demonstrated activity both as single agents and in combination with imatinib.185-188 This approach has rendered anecdotal responses in patients harboring the T315I mutant. Homoharringtonine is a Cephalotaxus alkaloid that represented the best therapeutic option in patients in whom interferon alfa–based therapies failed before the introduction of imatinib in clinical practice.189 Its potential as salvage therapy in imatinib-resistant CML has rekindled interest in this drug. Another avenue that has been pursued in recent years is the use of hypomethylating agents190,191 and histone deacetylase inhibitors.192-194 In a phase 2 study, decitabine administered at 15 mg/m2, 5 days a week for 2 consecutive weeks, rendered a CHR in 34% and a CCGR in 46% of patients.190,191 These and other agents with different targets are currently being developed in an attempt to prevent or overcome resistance to imatinib.

Immune-mediated events play an important role in the suppression of the CML clone, as evidenced by a subset of
patients who maintained a durable CHR after discontinuation of interferon alfa therapy, and after allogeneic-SCT due to a graft-vs-leukemia effect. These observations have sparked interest in developing immunologic approaches to treating CML. One such approach is the use of vaccines to elicit specific immune responses directed toward CML-restricted tumor antigens. Several vaccines are currently being developed in clinical studies, which encompass the use of BCR-ABL junction-spanning sequences, the nonapeptide PR1 derived from proteinase 3, and granulocyte-macrophage colony-stimulating factor secreting vaccines. The Ber-Abl breakpoint fusion peptide vaccine has been already tested in phase 2 clinical studies, and Bocchia et al have reported on 16 patients with stable residual disease after more than 12 months of imatinib therapy (n=10) or 24 months of interferon alfa therapy (n=6) who received 6 vaccinations with a peptide vaccine derived from the sequence p210-b3a2. Five of 9 patients taking imatinib obtained a CCGR after vaccination, and 3 had undetectable levels of b3a2 transcripts by RT-PCR. Among the patients treated with interferon alfa, 2 achieved a CCGR, and 3 had improvement in the percentage of Ph chromosome-positive metaphases. Overall, these data suggest that this peptide vaccine may have a role in the management of patients with CML and minimal residual disease.

SUMMARY
Consensus exists that imatinib therapy is the standard approach to the treatment of CML, and this modality will be measured in the future against all new therapies. The recent addition of the highly potent TKIs nilotinib and dasatinib has further improved the armamentarium against CML but also posed new challenges. First, the role of these new compounds in modern algorithms of CML therapy is still unknown and must be defined. Despite having shown impressive response rates in patients after imatinib failure or intolerance, the duration of these responses will be determined with longer follow-up. Their role in frontline treatment for CML will also have to be defined. Second, as our resources for controlling CML improve, the refinement in molecular monitoring must improve in parallel. In this regard, the lack of consistency among different laboratories in BCR-ABL transcript results by current PCR technologies is still an ongoing problem. Future improvements in molecular techniques and their standardization by using a laboratory-specific conversion factor will be crucial to further our understanding of the dynamics of molecular response to TKIs, providing a uniform method and definition of MMR and defining the concept of molecular relapse. Other challenges, such as defining the best treatment for patients who develop the Bcr-Abl T315I mutant, understanding the mechanism of resistance of patients without detectable mutations, the emergence of new mechanisms of resistance such as new mutations to the new TKI and other agents, developing novel strategies for the management of minimal residual disease, and delineating the current role of SCT, will be the subject of arduous investigation in future years. Despite all these uncertainties, the treatment prospects of patients with CML have never been brighter.

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