ABSTRACT
The discovery of the Philadelphia chromosome in 1960, and of the BCR-ABL oncogene in 1984, enabled the development in subsequent years of a targeted therapy that revolutionized the treatment of chronic myeloid leukemia, thus changing its natural history. The use of imatinib resulted in a significant improvement of the prognosis and outcome of patients with chronic myeloid leukemia. However, the occurrence of mechanisms of resistance or intolerance precludes the eradication of the disease in some of the patients. Second-generation tyrosine-kinase inhibitors are efficient in most of these patients, except for those with T315I mutation. We present an overall review of chronic myeloid leukemia, with emphasis on the progress in its treatment.

Keywords: Leukemia, myelogenous, chronic, BCR-ABL positive / therapy; Protein kinase inhibitors/therapeutic use; Drug resistance

INTRODUCTION
Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of an acquired mutation which affects the hematopoietic stem cell.

CML accounts for 20% of leukemias in adults and its frequency is similar worldwide. Its annual incidence is of 1.6 cases/100,000 inhabitants/year, with a slight predominance in males (1.4/1.3), and the median age at presentation is 55 years. Less than 10% of cases occur in patients under 20 years of age(1).

A higher incidence of CML is seen among survivors of the atomic bomb attack during World War II, as well as among patients undergoing radiation therapy for the treatment of malignancies. Despite the likely causal relation between CML and ionizing radiation, most of the cases seem to be sporadic, with no predisposing factor.

PATHOPHYSIOLOGY
In 1960, a minute chromosome was identified in patients with CML(2). For the first time in the history of Medicine, the association between a chromosome abnormality and a malignant disease was described. Later, this chromosome abnormality was proven to result from a reciprocal and balanced translocation between the long arms of chromosomes 9 and 22 t(9;22)(q34;q11), and was called Philadelphia (Ph) chromosome. Present in 95% of patients with CML, the Ph chromosome results from the balanced translocation between the ABL (Abelson Murine Leukemia) gene located on chromosome 9, and the BCR (breakpoint cluster region) gene on chromosome 22(2,3). The resulting hybrid gene – BCR-ABL, codifies an abnormal fusion protein that possesses tyrosine kinase (TK) activity continuously activated in the ABL region, and is responsible for the development of leukemia.

From the identification of the molecular pathogenesis of CML, efforts have been made to identify the signaling pathways that influence the BCR-ABL TK activity, linking these pathways to the characteristic changes of CML. These changes include: increased cell proliferation (RAS pathway...
CML has an initial chronic phase (CP) of variable duration, and then progresses to the blast phase (BP), which is preceded or not by an accelerated phase (AP). Approximately 90% of patients are diagnosed in the CP, and 20% to 45% of them are asymptomatic. They present with leukocytosis with left shift, well-differentiated granulocytic cells and enlarged spleen. When symptomatic, they present with symptoms of hypercatabolism (fatigue, weight loss, night sweats and fever) and abdominal discomfort due to spleen enlargement. Thrombotic complications or hemorrhage occur in less than 5% of the cases in CP.

Patients may suddenly progress from CP to BP or go through a transition period, the accelerated phase (AP) (9).

Several definitions of AP have been described in the past 20 years; those more frequently used are MD Anderson Cancer Center’s (MDACC), International Blood and Marrow Transplantation’s (IBMTR) and the World Health Organization’s (WHO) (6). In all these classifications, there are objective criteria, such as the number of blasts, basophils, and evidence of clonal evolution, in addition to more subjective criteria such as persistent leukocytosis and spleen enlargement unresponsive to treatment (Chart 1). After a period of 1 to 2 years, the AP turns into myeloid BP (70%), lymphoid BP (20% to 30%) or undifferentiated BP, characterized by infections, bleeding, multiple organ failure, with a mean survival of 3 to 6 months if untreated (7).

Transition from CP to more advanced stages of the disease is not well understood, but it is believed to result from genomic instability. BCR-ABL-induced cell proliferation would lead to the acquisition of additional chromosome abnormalities, known as clonal evolution (9).

**PROGNOSTIC FACTORS**

For an early identification of patients in the CP of the disease who could have an unfavorable outcome with conventional therapy, Sokal (10), in 1984, developed a system to sub-classify patients with CP-CML into three groups according to survival and clinical and laboratory characteristics (platelet count, spleen size, age, and percentage of circulating blasts). A similar prognostic score was developed by Hasford et al (11) using the following parameters: age, spleen size, peripheral platelet count, eosinophils, basophils and blasts. Both scores remain highly reproducible today and accepted as prognostic models for CP patients (Chart 2).

**TREATMENT**

The treatment of CML has gone through a real revolution throughout the years. Palliative splenic radiotherapy, started in the early 20th century, remained the standard therapy for more than 50 years. In 1960, busulfan emerged (12), and later, hydroxyurea, which proved superior to busulfan, probably for being better tolerated, and a slight gain in survival was observed (13). However, none of these agents was able to suppress the Ph chromosome, and they were therefore unable to change the natural history of the disease.

In 1980, the efficacy of interferon-alpha (IFN-α) in establishing hematologic and cytogenetic responses, whether partial or complete, was confirmed, and survival was thus prolonged (14). Gradually, IFN-α replaced hydroxyurea and busulfan in the management of patients with newly-diagnosed CP (15).

Also in 1980, the first experiences with allogeneic hematopoietic stem cell transplantation (AHSCT)
in CP-CML were carried out, representing the first curative modality, with a transplant-related mortality of 10% to 20% at one year and five-year survival of approximately 60%\(^{(16)}\), and a high percentage of patients with no evidence of disease. Patients occasionally with relapse were successfully rescued by means of donor lymphocyte infusion, with or without previous chemotherapy\(^{(17)}\). It became evident that the benefit obtained with AH SCT in CML is a result of the graft-versus-leukemia effect, mediated by donor lymphocytes, although the specific target of this effect remains not fully identified\(^{(18)}\). As from 1990, AH SCT became the treatment of choice for CP patients less than 50 years old, and IFN, whether in combination with cytarabine or not, was reserved for patients not eligible for AH SCT\(^{(19)}\). The discovery of the BCR-ABL oncoprotein in 1986 enabled the development, in subsequent years, of a new drug able to inhibit the BCR-ABL oncoprotein activity\(^{(20)}\). Initially denominated STI571, and known today as imatinib, it revolutionized the treatment of CML.

CML then became the first disease model of the so-called targeted therapy. Although imatinib does not act directly in the base of the pathogenesis of CML preventing BCR-ABL codification, it competes for the ATP linking site of tyrosine kinase, thus restoring its cell death mechanism. Druker et al.’s in vivo and in vitro studies showed that this drug reduces by between 92% and 98% the number of BCR-ABL colonies, but without inhibiting normal colony formation\(^{(21)}\).

Imatinib was first used in 1998 to treat IFN-resistant patients\(^{(22)}\). The successful results of this small study led to the development of the IRS study (International Randomized Study of Interferon and STI 571), which demonstrated the superiority of imatinib 400 mg/dl in relation to the IFN and cytarabine combination, regarding the rates of cytogenetic response (CgR), event-free survival (EFS), progression-free survival (PFS) and overall survival (OS)\(^{(23)}\). After the year of 2000, imatinib, at the dose of 400 mg/day, became the first-choice treatment for patients with CP CML. Initial imatinib doses of 800 mg were compared to 400 mg in the TOPS study\(^{(24)}\). In the French study SPIRIT, the 400 mg/day dose was compared to 600 mg/day\(^{(25)}\). Despite the observation that patients receiving higher imatinib doses achieved complete cytogenetic response (CCgR) sooner, no advantage regarding survival has been demonstrated to date.

The combination of imatinib 400 mg/day with pegylated IFN alpha-2b was analyzed in two studies of CP patients and, despite the higher rates of CCgR and major molecular response (MMR), most of the patients discontinued IFN after one year of treatment\(^{(25,26)}\). After the introduction of imatinib, new criteria for response and disease monitoring emerged with the objective of optimizing and standardizing the management of CML. These criteria were created by the Leukemia Net group\(^{(27)}\) by means of a critical review of relevant articles of the literature and consensus meetings.

### DEFINITION OF RESPONSE

Complete hematologic response (CHR) is defined by: platelets \(\leq 450 \times 10^9/L\); leukocytes \(\leq 10 \times 10^9/L\), with normal differential blood count; basophils < 5%; and absence of spleen enlargement.

The CgR may be complete (absence of Ph+ cells), partial (Ph present in 1% to 35% of the cells), minimal (Ph present in 36% to 65% of the cells), or absent (Ph present in more than 95% of the cells).

MMR is defined by a three-log reduction in BCR-ABL transcripts and corresponds to a BCR-ABL/ABL ≤ 0.1%, as standardized by the international scale\(^{(28)}\); complete molecular response (CMR) is defined by the absence of BCR-ABL transcripts by RT-PCR and/or nested PCR in two consecutive samples (Chart 3).

<table>
<thead>
<tr>
<th>Complete hematological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Platelets (\leq 450 \times 10^9/L)</td>
</tr>
<tr>
<td>- Leukocytes (\leq 10 \times 10^9/L), with normal differential count</td>
</tr>
<tr>
<td>- Basophil &lt; 5%</td>
</tr>
<tr>
<td>- With no splenomegaly</td>
</tr>
<tr>
<td>Monitoring: each 2 weeks up to complete response. After, at every 3 months</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytophenic response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete: absence of Ph</td>
</tr>
<tr>
<td>Partial: Ph (+) in 1 - 35 % of cells</td>
</tr>
<tr>
<td>Minor: Ph (+) in 36 - 65% of cells</td>
</tr>
<tr>
<td>Minimum: Ph (+) in 66 - 95% of cells</td>
</tr>
<tr>
<td>No response: &gt; 95% of cells with Ph (+)</td>
</tr>
<tr>
<td>Monitoring: upon diagnosis, 3 months, and every 6 months, up to CCyR; later, once a year, whenever the treatment fails or in case of unexplainable cytopenias</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Molecular response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete: mRNA BCR-ABL transcripts undetectable by RT-PCR and/or nested PCR in 2 consecutive samples</td>
</tr>
<tr>
<td>Major: BCR/ABL rate &lt; 0.1%, standardized by international scale, corresponding to reduction by (\geq 3) logs of BCR-ABL transcripts</td>
</tr>
<tr>
<td>Monitoring: every 3 months up to achieving and confirming MMR; later, at every 6 months</td>
</tr>
<tr>
<td>The mutational analysis should be performed in cases of failure, suboptimal response or increase in number of transcripts, before change of ITK</td>
</tr>
</tbody>
</table>

Based on the degree of hematologic, cytogenetic and molecular response, and on the time needed to achieve response, the response to imatinib may be defined as optimal, sub-optimal or failed (Chart 4).
optimal response means that a change in therapy is not indicated, with a probably increased survival, which is estimated at close to 100% after 6 to 7 years; a suboptimal response means that the patient still benefits from treatment continuation, but could be eligible to an alternative treatment; A failed response means that a favorable outcome is unlikely and the patient should receive a different treatment, provided that it is available and applicable. These definitions of response can also be modulated by prognostic factors that may adversely affect the response to treatment and which thus require a more careful monitoring such as Sokal high-risk patients presenting with clonal evolution to the diagnosis, patients not achieving MMR at 12 months, or also those with increased BCR-ABL transcripts.

With the introduction of second-generation tyrosine-kinase inhibitors (TKI), the definition, albeit preliminary, of new response criteria became necessary (Chart 5).

The 8-year follow-up of the IRIS study showed a CCgR rate in 83% of patients, with an 8-year projected EFS and OS of 81% and 85%, respectively. However, despite these results, approximately 1/3 of patients did not have a favorable outcome: 17% of patients never achieved CCgR; 15% achieved CCgR, but lost it throughout time; and 5% presented with intolerance to imatinib, and new strategies were necessary.

Resistance to imatinib may be primary or secondary. In primary resistance, the patient shows no response since the beginning of treatment, whereas in secondary resistance the patient initially shows response, but then relapses. The resistance mechanisms may be BCR-ABL dependent or not. The BCR-ABL-dependent mechanisms include ABL sequence amplification and point mutations in the ABL molecule, which change its conformation and impede imatinib binding. The ABL-independent resistance mechanisms, which are therefore responsible for primary imatinib resistance, include: drug efflux by means of P-glycoprotein expression, although it is not precisely known whether this mechanism is clinically relevant; low OCT-1 cell transporter activity; imatinib binding to alpha-1 acid glycoprotein (AGP), which reduces its activity; activation of other signaling pathways such as the Ras/Raf/Mek kinase, Src.

Among the resistance mechanisms, point mutations in the BCR-ABL oncogene are the most common cause, occurring in 35% to 70% of patients with secondary resistance. With the purpose of rescuing imatinib-resistant or intolerant patients, second-generation TKI emerged.

Second-generation TKI

Dasatinib

Dasatinib, a piperazinyl derivative, has a potent inhibitory action against the Src and ABL kinases, including the active BCR-ABL conformation, and most of the mutant forms, except for the T315I mutation. In 2006, dasatinib 70 mg twice daily was approved for the treatment of CP, AP and BP CML patients, as well as of imatinib-resistant or intolerant patients.

In the START-R study, patient who had failed with imatinib at 400 and 600 mg doses were randomized 2:1 for dasatinib 70 mg twice daily or imatinib 800 mg. After 2 years, the major CgR was 53% in the dasatinib arm and 33% in the high-dose imatinib arm. Dasatinib also proved superior as regards CCgR (44% versus 18%) and MMR (29% versus 12%) (39).

A prospective study randomized four different dasatinib doses, and found that the 100 mg dose once daily was effective and better tolerated in relation to the other doses for CP patients.
Imatinib-intolerant CP patients showed major CgR and CCgR rates of 76% and 75%, respectively in one study, and 71% and 63%, respectively, in another study(41-42). Imatinib-resistant CP patients had major CgR and CCgR rates of 51% and 40%, respectively, in one study, and 50% and 36%, respectively, in another(40-42).

The median time to achieve response was 5.5 months(43), with response sustained for 2 years in CP patients, with PFS of 80% and OS of 90%.

A multinational study randomized 519 newly-diagnosed CP CML patients to receive dasatinib 100 mg once daily or imatinib 400 mg once daily. After a 12-month follow-up, dasatinib proved superior in relation to the CCgR rate (77% versus 66%, p = 0.007) and in relation to the MMR rate (46% versus 28%, p < 0.0001). Responses were achieved earlier with dasatinib (46% versus 28%, p < 0.0001). The rate of progression to AP was lower in the dasatinib arm (1.9% versus 3.5%). The toxicity profile was similar(44).

Among the adverse events of dasatinib, we should point out grade 3 and 4 myelotoxicity, neutropenia in 21%, thrombocytopenia in 19%, and anemia in 10%. Non-hematologic adverse effects, all grades 1 or 2, include: pleural effusion (10%), diarrhea (17%), rash (11%), and headache (12%)(45). Recently, dasatinib was approved at the dose of 100 mg once daily for imatinib-resistant or intolerant CP patients(46), and 140 mg once daily for AP or BP patients(45). Studies show similar response rates, with a more favorable toxicity profile, especially in relation to pleural effusion(46,47), at the once daily dosage.

Nilotinib
Nilotinib, an amilopyrimidine derivative, inhibits TK activity of BCR-ABL, PDGF, c-kit, and most of the mutant forms of ABL, except for the T315I mutation, and is more potent and selective than imatinib(48-53). It only binds to BCR-ABL in its inactive conformation.

In 2007, nilotinib was approved for the treatment of imatinib-resistant or intolerant CP CML patients and AP patients, at the dose of 400 mg twice daily. It is well tolerated, and the most common grade-3 and 4 laboratory abnormalities are: elevated lipase (17%), hypophosphatemia (16%), hyperglycemia (12%) and elevated total bilirubin (8%). The grade-3 and 4 hematological changes were neutropenia (31%), thrombocytopenia (31%), and anemia (10%). Grade-3 and 4 pleural or pericardial effusion occurred in less than 1%(54).

The CML-CP study showed the effect of nilotinib in 321 imatinib-resistant (30%) or intolerant (70%) CP patients in a follow-up of at least 19 months. Responses obtained showed a major CgR rate in 59% achieved in a median time of 2.8 months, and CCgR in 44% of patients with a median time of 3.3 months. The responses were sustained after 24 months (CgR sustained in 78% and CCgR in 83%). After a 2-year follow-up, 59% of patients discontinued nilotinib due to progression (27%) or adverse effects (15%)(55).

A study included 137 imatinib-resistant (80%) or intolerant (20%) AP patients in a follow-up of at least 11 months. The responses were: CHR in 31% with a median time of 1 month to be achieved; major CgR in 32% with a median time of 2.8 months, and CCgR in 20% of patients, of whom 70% remain in CCgR at 24 months of follow-up, with an OS of 67% after 2 years(55).

The use of nilotinib in newly-diagnosed CP CML patients was tested in a multicenter study which randomized these patients to receive imatinib 400 mg, nilotinib 300 mg bid, or nilotinib 400 mg bid.

After 12 months, the MMR rates for nilotinib (44% at the 300 mg dose and 43% at the 400 mg dose) were practically double the rate for imatinib (22%; p < 0.001). The CCgR rates at 12 months were higher for nilotinib (80% for the 300 mg dose and 78% for the 400 mg dose) in relation to imatinib (65%; p < 0.001). There was a significant reduction of PFS with nilotinib(56).

New agents
Bosutinib (SKI606), an inhibitor 30 times more potent than imatinib, inhibits Src/Abl TK. A phase-I study showed that the 500 mg daily dose was effective. The phase-II study in CP patients who failed imatinib and second-generation TKI is underway.

Preliminary data showed that, among the 69 imatinib-resistant patients, 81% achieved CHR, 45% major CgR, including 32% CCgR. The treatment was well tolerated, and the most common adverse effects were gastrointestinal effects(56).

New TKI are being developed(57) in phase-I studies, with activity on T315I mutation, such as AP24534(58), aurora kinase inhibitors such as PHA-739358 (Nerivano Medical Sciences, Milan, Italy)(58), and KW-2449 (Kyowa Hakko Kirin Pharma, Tokyo, Japan)(58), both with activity against the T315I mutation.

Homoharringtonine (ChemGenex, Victoria, Australia), an apoptosis modulator, was tested in all CML phases in imatinib-resistant patients and second-generation TKI-resistant patients; it acts on the T315I mutation. Preliminary results of phase-II studies showed hematologic and cytogenetic responses with disappearance of the T315I clone in 60% of evaluable patients(58). In a phase-I study, DCC-2036 (Deciphera, Lawrence, Kansas), a non-ATP competitive multi-TK inhibitor that acts on the T315I mutation showed significant activity on Ph + cells(58).
Leukemia Net’s recommendations for CML treatment
Imatinib 400 mg/day is the standard treatment for CP CML. IFN is the drug of choice during pregnancy or in low-risk patients presenting with comorbidities or using other medications that make the use of imatinib inadequate.

In imatinib-intolerant patients, the choices are dasatinib or nilotinib. The choice of the agent should be based on the mutational status of the patient as well as on occasional patient’s comorbidities. Patients who fail imatinib, particularly with loss of hematologic response, should receive nilotinib or dasatinib. For patients with sub-optimal response, there is no solid evidence, to date, that the change in treatment is beneficial, but an increase in the imatinib dose or change for a second-generation TKI may be considered.

Allotransplantation is recommended for patients in AP, BP or with T315I mutation, and for patients who failed second-generation TKI. Occasionally, it may also be considered for patients with sub-optimal response to second-generation TKI, especially if they are high-risk patients.

Naive AP or BP patients should receive allotransplantation, if eligible, after an initial treatment with imatinib 600 to 800 mg/day or second-generation TKI, if resistant to imatinib. Effective treatment with TKI should not be discontinued and doses should not be reduced below standard doses if significant adverse effects are absent.

CONCLUSION
The improved understanding of CML biology enabled the development of a highly effective targeted therapy which revolutionized the treatment of CML, thus changing its natural history. Unlike 10 to 15 years ago, CP patients now have a long expected survival with imatinib.

Second-generation TKI are effective in most of the imatinib-resistant or intolerant patients. However, they are not effective in part of the patients due to other mechanisms, including T315I mutation, which is still a challenge.

A better understanding of resistance mechanisms, as well as the development of new molecules, will contribute to further improvements in the treatment of CML.

REFERENCES


