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Guidelines in focus

Chronic myeloid leukemia

Leucemia mieloide crônica

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Description of the evidence collection method

These guidelines were drafted after the preparation of 19 questions relevant to the diagnosis and treatment of chronic myeloid leukemia. The questions were structured using the PICO (Patient-Intervention-Comparison-Outcome) methodology, thus enabling the creation of strategies to search for evidence (Appendix 1) in the main scientific electronic databases (MEDLINE/PubMed, Embase, LILACS/SciELO, Cochrane Library, PreMEDLINE via OVID). A manual search for evidence in dissertations and theses was also conducted (Digital Library of Theses and Dissertations [BDTD] of Instituto Brasileiro de Informação em Ciência e Tecnologia [IBICT]). Evidence was selected by critical evaluation using discriminatory instruments (scores) according to the category of question: diagnosis (QADAS) or therapy (Jadad for randomized clinical trials and the Newcastle-Ottawa scale for non-randomized studies). After identifying potential studies to support recommendations, they were selected based on the

strength of evidence and grade of recommendation calculated using the Oxford classification (available at www.cebm.net).

Degree of recommendation and strength of evidence

- A: Experimental or observational studies of higher consistency.
- B: Experimental or observational studies of lesser consistency.
- C: Case reports (non-controlled studies).
- D: Opinions without critical evaluation based on consensus, physiological studies, or animal models.

Objectives

To set parameters for clinical diagnosis, to evaluate severity, and to standardize treatment, maintenance, and monitoring options for chronic myeloid leukemia (CML) patients. The target audience of these guidelines is hematologists, aiming at contributing to decision-making during the diagnosis and treatment of CML.

Introduction

The Guidelines Project is a joint initiative of the Brazilian Medical Association and the Federal Medical Council that aims to reconcile medical information to standardize practices in order to help reasoning and decision-making during treatment.

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Data contained in this manuscript were prepared and are recommended by the Brazilian Association of Hematology, Hemotherapy, and Cell Therapy. Even so, all possible practices should be evaluated by the physician responsible for treatment depending on the patient's setting and clinical status.

1. What are the diagnostic criteria for chronic myeloid leukemia?

The diagnosis of CML is based on blood count (leukocytosis and often also thrombocytosis), and on differential blood count (immature granulocytes, metamyelocytes, myeloblasts, and basophilia). Diagnosis depends on the identification of the Philadelphia chromosome (22q-), resulting from the translocation t(9;22)(q34;q11) and/or BCR-ABL gene rearrangement in peripheral blood or bone marrow cells. In some cases, the Philadelphia chromosome cannot be detected and the diagnosis is made by molecular methods. Most diagnoses are made in the chronic phase, and the clinical course has three stages: the chronic phase, the accelerated phase, and the blast crisis. The accelerated phase is defined as the presence of 1% to 19% blasts in the blood or bone marrow, basophils > 20%, thrombocytosis or thrombocytopenia not related to therapy, and clonal evolution in cytogenetic evaluation. The blast crisis is characterized by blasts > 20% or extramedullary blast proliferation¹⁻³ (D).

Recommendation

The diagnosis of CML depends on the identification of the Philadelphia chromosome and/or the BCR-ABL rearrangement.

2. Is there any difference in the prognosis of CML patients with p210 e13a2(b2a2) and e14a2(b3a2) or p190(e1a2) rearrangements?

The prevalence of the BCR-ABL p190(e1a2) transcript in CML patients is 1%. This rearrangement is associated with decreased therapeutic response to tyrosine kinase inhibitors, with complete hematologic response attained in 30% of cases, complete cytogenetic response in 20% of cases (three to 18 months), and major molecular response in 10% of cases. Progression to other phases (accelerated or blast crisis) occurs in 60% of chronic phase patients⁴ (C).

The response of treatment-naïve CML patients to treatment with imatinib is different for the b3a2(e14a2) and b2a2(e13a2) transcripts. In 12 months of treatment, patients with the b3a2(e14a2) transcript have a 29% increase in complete cytogenetic response, which is faster, and a longer disease-free survival⁵ (B).

In CML patients on imatinib treatment for six months, the number of b2a2(e13a2) transcripts is lower when compared to the number of b3a2(e14a2) transcripts, suggesting greater sensitivity of the b2a2(e13a2) transcripts to Imatinib and consequently better prognosis⁶ (B).

Imatinib treatment in chronic-phase CML patients with the BCR-ABL b2a2(e13a2) transcript has better results compared to those with the b3a2(e14a2) transcript, with a 31% increase in the major cytogenetic response and a smaller number of BCR-ABL transcripts⁷ (B).

Recommendation

The p190(e1a2) transcript is associated with a reduced therapeutic response; there is controversy as to whether there is difference in response between the p210 e13a2(b2a2) and p210 e14a2(b3a2) transcripts.

3. Upon diagnosis, do the Philadelphia chromosome and 9q deletion have a prognostic significance?

There is no difference in survival between CML patients with the chromosome 9q deletion on interferon alpha treatment and those without this deletion. However, there is a reduction in the survival of patients with the deletion spanning the BCR-ABL junction compared to those without this deletion. The survival rate is 44% higher in chronic phase patients submitted to bone marrow transplantation who do not have the deletion (number needed to treat [NNT]: 2)⁸ (B). There is evidence that the disease-free survival, overall survival, and cytogenetic response is reduced in CML patients with the chromosome 9q34 deletion under treatment with interferon alpha^{9,10} (B).

A comparison of first-generation (imatinib) or second-generation (nilotinib or dasatinib) tyrosine kinase inhibitors in the treatment of CML patients with chromosome 9 deletion shows that there is no difference in overall survival, disease-free survival, or in cytogenetic response between patients with and without the chromosome 9 deletion over a two-year follow-up^{11,12} (B). There is, however, evidence that there is a reduction in survival of patients with derivative chromosome 9 deletions¹³ (B).

The ABL deletion on the derivative chromosome 9 (15.1%) in CML patients reduces disease-free survival; the BCR deletion reduces overall survival, and combined ABL and BCR deletions reduce the overall and disease-free survival¹⁴ (B). There is evidence that only the ABL deletion reduces the survival time and the duration of the chronic phase¹⁵ (B).

Over a five-year follow up, imatinib-treated CML patients with variant Philadelphia chromosome translocations do not demonstrate differences in overall survival, disease-free survival, progression-free survival, complete hematological response, cytogenetic response, or molecular response compared to patients without variant Philadelphia chromosome translocations^{16,17} (B). Other studies have shown that Philadelphia chromosome mosaicism increases mortality by 3.3 years in 21% (number needed to harm [NNH]: 5), and that translocation variations reduce the cytogenetic response^{18,19} (B) in CML patients.

Recommendation

Despite controversy on whether chromosome 9q, BCR deletions, and variant Philadelphia chromosome result in worse prognoses, there is evidence of reduction in overall and disease-free survival, as well as in therapeutic response of CML patients treated with interferon alpha or first- and second-generation tyrosine kinase inhibitors. ABL deletion reduces the overall and disease-free survival of these patients. The presence of variant Philadelphia chromosome and mosaicism also appear to worsen the prognosis in CML.

Table 1 – Classifications and definitions of the accelerated phase of chronic myeloid leukemia.

Characteristic	MDACC	IBMTR	WHO
Blasts (%)	> 15	> 10	10-19
Platelets	< 100	No response	< 100 or > 1000

IBMTR, International Bone Marrow Transplant Registry; MDACC, M.D. Anderson Cancer Center; WHO, World Health Organization.

4. Do cytogenetic abnormalities in addition to the Philadelphia chromosome (Ph) at diagnosis have a prognostic significance?

In CML patients under treatment with second-generation (dasatinib or nilotinib) tyrosine kinase inhibitors or imatinib, the presence of additional chromosomal abnormalities reduces disease-free and overall survival by five years²⁰ (B).

The presence of additional chromosomal aberrations in CML patients under treatment with nilotinib increases mortality by 28% due to disease progression (NNH: 4). Additionally, mortality is increased by 38% at two years in chronic phase patients with additional chromosomal aberrations (NNH: 3)²¹ (B).

Aberrations reduce the survival time of these patients^{22,23} (B). The presence of additional chromosomal aberrations increases mortality by 36% (NNH: 3) and reduces the mean overall survival of CML patients submitted to stem cell transplantation²⁴ (B).

CML-related disease-free and overall survival at five years is different in patients with cytogenetic changes compared to those without them. The presence of major cytogenetic aberrations (major route), such as a second Philadelphia chromosome, trisomy 8, isochromosome 17q, or trisomy 19, reduces disease-free and overall survival at five years by 40%²⁵ (B).

Recommendation

The presence of additional chromosomal aberrations at diagnosis (major route) reduces the overall and disease-free survival, and increases mortality by 36% to 40%.

5. Are the criteria of the World Health Organization comparable to other criteria to classify chronic myeloid leukemia phases (chronic, accelerated, and blast crisis phases)?

The use of the World Health Organization (WHO) classification of CML stratifies patients into chronic, accelerated, and blast crisis phases at ratios of 77.8%, 15.5%, and 6.7%, respectively²⁶ (C). Appropriate classification allows for the establishment of adequate response estimates²⁷ (D).

In the treatment of CML patients with imatinib, there is no difference in the overall classification of patients in the chronic, accelerated, and blast crisis phases between the standard method and the WHO criteria. The distribution of patients

according to the standard classification is approximately 60% in the chronic phase, 28% in the accelerated phase, and 12% in blast crisis. Although there is no significant difference between the classifications, 6% of patients classified in the chronic phase by the standard classification were reclassified into the accelerated phase (WHO). Similarly, 9% of patients classified in the accelerated phase and 7% in the chronic phase were reclassified as blast crisis according to the WHO criteria²⁸ (B).

There are little differences between the M. D. Anderson Cancer Center (MDACC), International Bone Marrow Transplant Registry (IBMTR), and WHO classifications and definitions of the accelerated phase of CML, particularly with respect to the percentages of blasts and platelets (Table 1)²⁹ (D):

Recommendation

The WHO classification for the chronic, accelerated, and blast crisis phases of CML is similar to the IBMTR and MDACC classifications.

6. Is it important to define risk in chronic myeloid leukemia patients using the Sokal and Hasford scores?

The Sokal score can be determined using an online calculator (www.pharmacoepi.de). The score takes into account the size of the spleen (in centimeters) palpable below the left costal border (LCB), the platelet count, the percentage of blasts, and the age. A result < 0.8 corresponds to low risk; from 0.8 to 1.2, intermediate risk; and > 1.2, high risk. The Sokal score has a predictive value in CML patients treated with imatinib, where molecular and cytogenetic responses are higher in low-risk patients. High-risk, intermediate-risk and low-risk patients who achieve cytogenetic response within 12 months have probabilities of survival of 90%, 94%, and 97%, respectively. The Hasford score considers the age, the percentage of eosinophils, basophils, platelet count, spleen size in centimeters, and percentage of blasts; the patient has low risk when the result is < 780, intermediate risk when the result is between 780 and 1,480, and high risk when > 1,480. The five-year survival rate corresponding to each risk group is 76%, 55%, and 25%, respectively³⁰ (A) ³¹ (D).

The Sokal score predicts response to treatment of CML patients with interferon alpha therapy; the high-risk, intermediate-risk, and low-risk groups comprise 48%, 29%, and 23% of the cases with mean survival times of 45, 76, and 105 months, respectively. The ten-year survival is 8%, 28% and 34%, respectively³² (B).

After the introduction of imatinib treatment, the Sokal score identified an increase in the five-year survival rate of low-risk CML patients of 11%, of 40% in intermediate-risk patients, and of 38% in high-risk patients³³ (B). Moreover, it is known that high-risk patients are more likely to evolve to the accelerated phase or blast crisis on imatinib therapy³⁴ (A). The Sokal score is also inversely related to cytogenetic response in high-risk patients³⁵ (B), as there is a 30.4% reduction in the cytogenetic response³⁶ (B).

The Hasford score identifies patients at low risk with a probability of survival at nine years of 41%; intermediate risk,

with probability of 0.16%; and high-risk, with a zero probability at nine years. The Sokal and Hasford scores classify 23% and 9% of all patients as high-risk, respectively. Patients with low or intermediate risk who achieve complete hematologic response have probabilities of survival of 51% and 23%, respectively; those without complete hematologic response have probabilities of 26% and 12%, respectively. High-risk patients who achieve cytogenetic response have prognoses similar to those at low risk³⁷ (B). Of the different groups as classified by Hasford, 57% of low-risk patients present complete cytogenetic response and 27% of intermediate-risk and high-risk patients achieve complete cytogenetic response³⁸ (B).

The Hasford and Sokal scores predict complete hematologic responses mainly in low-risk patients³⁹ (B).

Recommendation

The Sokal and Hasford scores are prognostic predictors of CML patients.

7. Is imatinib better than second-generation tyrosine kinase inhibitors as a first-line treatment of chronic phase chronic myeloid leukemia?

A comparison between dasatinib (100 mg) and imatinib (400 mg) as a first-line treatment in chronic-phase CML patients demonstrates that complete hematologic response is 11% higher, cytogenetic response is 11% higher, and molecular response is 18% higher with dasatinib (NNT: 9)⁴⁰ (B). The two-year follow-up of these patients upholds the higher beneficial effect of dasatinib compared to imatinib⁴¹ (B).

Initial treatment of chronic-phase CML patients using nilotinib (300 mg or 400 mg twice daily) compared to imatinib (400 mg once daily) increases the molecular response at 12 months by 22% (NNT: 5), increases the cytogenetic response by 15% (NNT: 7), and reduces the likelihood of progression to the accelerated phase and blast crisis⁴² (A). In the two-year follow up, the effect of nilotinib increases the molecular response by 27% (NNT: 4), the cytogenetic response is 10% higher than imatinib (NNT: 10); this difference is 5% lower than the evaluation at 12 months. The reduction in progression is maintained⁴³ (A).

Recommendation

Dasatinib and nilotinib provide greater benefits than imatinib in the first-line treatment of chronic-phase CML patients regarding molecular, cytogenetic, and hematologic responses, as well as disease progression.

8. Does the time between diagnosis and start of treatment with imatinib have prognostic significance?

In chronic-phase CML patients, imatinib treatment may be started after diagnosis (early), or may be started after 24 months

of treatment with interferon (late), leading to different results regarding toxicity and effectiveness. Early treatment reduces the risk of grade I and II adverse effects by 52% (NNT: 2), and grade III and IV adverse effects by 81% (NNT: 1), although it increases the risk of neutropenia and thrombocytopenia by 5% (NNH: 20). After one year of follow-up in patients who have not achieved complete cytogenetic response, early treatment produces a reduction in the risk of grade I adverse events by 3% (NNT: 33); grade II, by 8% (NNT: 12); and grades III and IV, by 7% (NNT: 14)⁴⁴ (B).

In early treatment, there is a 16% increase in complete cytogenetic response (NNT: 7), a 2% reduction in the risk of relapse (NNT: 50), and a 15% increase in disease-free survival (NNT: 7)⁴⁴ (B).

There is reduction in the risk of non-hematological adverse events with early treatment, including weight gain (11%), periorbital edema (12%), rash (9%), diarrhea (11%), and infections (19%), but there is increased risk of hemorrhage (5%), and bone pain (8%)⁴⁴ (B).

Imatinib treatment after diagnosis of chronic phase CML (early treatment) increases the likelihood of major molecular response by 20% (NNT: 5), and increases the likelihood of response maintenance at 30 months by 36% (NNT: 3), compared to beginning treatment one year after diagnosis (late treatment). After one year of imatinib treatment, the likelihood of loss of or not achieving molecular response is 58% lower in early treated patients (NNT: 2)⁴⁵ (B).

Treatment with 400 mg of imatinib produced higher major and complete cytogenetic response rates compared to the interferon and cytarabine combination in initial chronic-phase CML patients (87.1% vs. 34.7%) and higher survival free of progression to the accelerated phase and blast crisis (96.7% vs. 91.5%; p-value < 0.001)³⁰ (A).

Recommendation

Imatinib treatment of chronic-phase CML patients should be started as early as possible after diagnosis.

9. Does the cytogenetic evaluation have an impact on prognosis?

The identification of CML patients on imatinib treatment with cytogenetic clonal evolution provides some information on the prognosis, which depends on the disease phase. The presence of this change in the chronic and accelerated phases is not associated with a different cytogenetic response; however, it reduces the survival rate. Cytogenetic response after three months of treatment is an independent prognostic factor. The absence of complete or partial response is associated with lower survival rates⁴⁶ (B).

In CML patients on imatinib treatment, the presence of a cytogenetic response increases four-year survival by 23% (NNT: 4) and disease-free survival by 38% (NNT: 3)⁴⁷ (B).

After six years of treatment with imatinib, 77% of the patients were still with stable complete cytogenetic response, with a survival rate of 91%; 44% of the patients progressed to the accelerated phase or blast crisis⁴⁸ (B).

The expected loss of cytogenetic response in the first year of imatinib treatment is 0.6%, and the mortality rate at two years of patients who achieved response is reduced. The estimated eight-year mortality rate of these patients is 4.8%⁴⁹ (B).

For CML patients unresponsive to imatinib and thus treated with second-generation tyrosine kinase inhibitors (dasatinib and nilotinib), the cytogenetic response confers 20% greater survival (NNT: 5); when associated with hematologic response, the increase in the survival rate is of 42% (NNT: 2)⁵⁰ (B).

The presence of minor or major cytogenetic response in chronic-phase CML patients under treatment with second-generation tyrosine kinase inhibitors increases event-free survival, overall survival, and disease-free survival by approximately 25% (NNT: 4)⁵¹ (B).

Recommendation

The cytogenetic evaluation of patients under tyrosine kinase inhibitors treatment can predict the prognosis by complete or partial response, whether or not associated to other factors.

10. Does molecular evaluation by quantitative real-time polymerase chain reaction have an impact on prognosis?

The BCR-ABL/ABL ratio is almost always below 2% in chronic-phase CML patients who attain a cytogenetic response on imatinib treatment. Patients with the BCR-ABL/ABL ratio below 0.0001% are regarded as having complete molecular response. For patients who lose the cytogenetic response within 24 months (2.5%), the mean value of the ratio is 0.12%. Some relapsed patients evolve with disease progression (15.4%) with BCR-ABL/ABL ratios that vary from 0.3% to 0.0075%, which, within the usefulness of quantitative real-time PCR in molecular evaluation, defines the extremes of positive or negative residual disease, but with a great variability in the mean⁵² (B).

In CML patients investigated using quantitative PCR, the estimated major molecular response rate at 60 months is 67.1% and the cytogenetic response is 81.7%. Regarding the outcomes event-free survival, including transformation to accelerated phase and blast crisis; death from any cause; loss of adherence to treatment; or loss of cytogenetic response, there are more patients who attain molecular response than those who do not. Patients with major molecular response have better survival than patients with complete cytogenetic response who do not achieve major molecular response⁵³ (B).

The estimated molecular response obtained by PCR analysis in CML patients treated with imatinib also allows for a comparison with hematologic and cytogenetic responses over time. Thus, in an 18-month follow-up, the molecular, cytogenetic and hematologic responses were 79%, 83%, and 93%, respectively⁵⁴ (B).

Cytogenetic progression (loss of response, clonal evolution, 20% increase in the Philadelphia clone) may occur in 13% of CML patients on imatinib treatment in two years of follow-up. At the time of progression, none of these patients presented major molecular response (reduction > 3-log in BCR-ABL).

Thus, there is a suggestion that the cytogenetic analysis should be restricted to cases that do not attain or that lose molecular response as measured by quantitative real-time PCR⁵⁵ (B).

To evaluate changes in the levels of BCR-ABL transcripts as prognostic markers by quantitative real-time PCR, monitoring during four years demonstrates major molecular response (> 3-log reduction) and predicts higher disease-free survival rates. A minimal increase of 0.5-log predicts shorter relapse-free survival. Loss of molecular response (< 2.5-log reduction) also defines reduction in disease-free survival. A complete molecular response (PCR undetectable) corresponds to an increased disease-free survival⁵⁶ (B).

Recommendation

The prognosis (survival, relapse, progression) of CML patients on imatinib treatment can be predicted using quantitative real-time PCR.

11. Can cytogenetics be replaced by quantitative PCR to monitor chronic myeloid leukemia patients taking tyrosine kinase inhibitors who attain complete cytogenetic response?

There is a correlation between the levels of transcripts in the bone marrow and peripheral blood at three months of treatment and the success in obtaining a molecular response at six months⁵⁷ (B).

The comparison among quantitative real-time PCR (qPCR), cytogenetics, and fluorescence *in situ* hybridization (FISH) to monitor response to treatment using tyrosine kinase inhibitors in CML patients demonstrates the following correlations and/or concordances: qPCR in the bone marrow and peripheral blood; cytogenetics in the bone marrow, FISH in peripheral blood, and qPCR in peripheral blood⁵⁸ (B).

Despite the correlation between qPCR and cytogenetic analysis, other prognostic factors may be associated with molecular or cytogenetic responses, affecting the outcomes during tyrosine kinase inhibitors treatment of chronic-phase CML patients. This allows multivariate analyses that estimate the impact of the interaction of prognostic factors present in the medical practice, but in multivariate analysis, only the three-month cytogenetic response is predictive of the response at six months and disease-free survival at two years⁵⁷ (B).

Relapse occurs at 24 months in 2.5% of patients who have obtained cytogenetic response, and these patients may experience disease progression to the accelerated phase and blast crisis. The correlation between PCR analysis and cytogenetic response may contain a range of values that hamper interpretation and thus do not favor the substitution of methods⁵² (B).

Three-monthly monitoring using qPCR may provide the prognostic data needed for decision-making in CML patients, thereby reducing the need of bone marrow aspirations. The reasons why PCR monitoring is sufficient include: the level of log reduction in the BCR-ABL/ABL ratio correlates with cytogenetic response; in the 12-month follow-up, no patient

has disease progression without an indication of risk by qPCR (half-log increase or five-fold increase in the previous value of the BCR-ABL/ABL ratio); and no patient has cytogenetic progression in the presence of molecular response⁵⁵ (B).

Recommendation

qPCR in peripheral blood can be used as the examination of choice to monitor chronic-phase CML patients on imatinib treatment. Cytogenetics is a fundamental option for monitoring that may be used in association with qPCR or that may be reserved for cases where either there is no molecular response or the molecular response was lost.

12. What is the treatment of choice for chronic-phase chronic myeloid leukemia patients resistant to imatinib 400 mg?

In chronic-phase CML patients resistant to imatinib 400 mg (lack of complete hematological response at three months, lack of cytogenetic response at six months, or lack of major cytogenetic response at 12 months of treatment), a comparison of treatment with dasatinib 140 mg and an increase in the dose of imatinib (800 mg) demonstrated the following results for dasatinib: complete hematologic response increases in 11% of patients (NNT: 9), complete cytogenetic response increases by 23% (NNT: 4), and major molecular response increases by 12% (NNT: 8). Moreover, there were 27% and 15% reductions in the risk of swelling and water retention, respectively, with dasatinib 140 mg. However, the risk of neutropenia and thrombocytopenia increases by 22% (NNH: 5) and 42% (NNH: 2), respectively⁵⁹ (B). These results persisted at 18 months of follow-up, with an increase in disease-free survival⁶⁰ (B).

The treatment of these patients (chronic phase CML resistant to imatinib) with dasatinib 100 mg/day compared to 140 mg/day leads to a similar clinical response in six months and two years of follow-up (complete hematologic response, cytogenetic response, and disease-free survival); however, the risk of pleural effusion is reduced by 9% (NNT: 11), of thrombocytopenia, by 15% (NNT: 7) and of discontinuity of treatment^{61,62} (A).

The response rate of chronic-phase CML patients on nilotinib treatment (400 mg twice a day) is no different to patients resistant or intolerant to imatinib (600 mg/day). The lack of response to imatinib (hematologic or cytogenetic) predicts absence of response to nilotinib⁶³ (B). Patients who attain a response with nilotinib remain with 96% to 98% of response (hematologic or cytogenetic) and disease-free survival at six months of follow-up⁶⁴ (B). The mean time to obtain a complete hematologic response is 2.8 months and complete cytogenetic response is 3.2 months; disease-free survival and overall survival at 24 months are estimated at 64% and 87%, respectively⁶⁵ (B). Patients resistant to imatinib or dasatinib treatment attain 79% complete hematologic response and 24% complete cytogenetic response at 12 months⁶⁶ (C).

In chronic-phase CML patients resistant to imatinib and dasatinib, treatment with bosutinib (500 mg/day) produces complete hematological and cytogenetic responses in 62% and 31% of the cases, respectively. Patients resistant to imatinib and nilotinib treatment achieve complete hematological and

cytogenetic responses in 75% and 35% of cases, respectively. In cases of resistance to imatinib or dasatinib, the likelihood of maintaining response, disease-free survival, and overall survival from 12 months onwards are 27%, 32.4%, and 72.9%, respectively. In patients resistant to imatinib and nilotinib treated with bosutinib, the odds of maintaining response, disease-free survival, and overall survival from 12 months onwards are 22.2%, 44.4%, and 77.7%, respectively⁶⁷ (B).

Recommendation

Chronic-phase CML patients, who are resistant to imatinib at a dose of 400 mg, should be treated with dasatinib (100 mg/day), nilotinib (800 mg/day), or bosutinib (500 mg/day).

13. Are there differences in the toxicity profiles of second-generation tyrosine kinase inhibitors (dasatinib and nilotinib)?

The difference in adverse effects between imatinib with nilotinib or dasatinib is expressed as NNT when these latter two drugs produce a reduction in the risk of adverse effects, and as NNH when the risk of a particular adverse effect increases.

The use of nilotinib (at any dose) as first-line therapy of patients with newly diagnosed CML reduces the rates of nausea (NNT: 8), diarrhea (NNT: 7), vomiting (NNT: 6), muscle spasm (NNT: 6), edema (NNH: 11), and neutropenia (NNT: 3) when compared to imatinib. However, the rates of rash (NNH: 4), headache (NNH: 8), pruritus (NNH: 8), and alopecia (NNH: 11) increase, and there are also increases in liver enzymes (NNH: 2), total bilirubin (NNH: 2), and glucose (NNH: 5)⁴² (A).

When nilotinib is given as second-line therapy to chronic-phase CML patients, cardiotoxicity can occur, with increases in the QTc (1% of cases) and thrombocytopenia (29% of cases)⁶⁴ (B).

In a comparison of dasatinib and imatinib as first-line therapy for CML, the main non-hematological adverse effects including nausea (NNT: 9), myositis (NNT: 8), and water retention (NNT: 4) are reduced with dasatinib. However, there are increases in pleural effusion in 10% (NNH: 10), thrombocytopenia in 9% (NNH: 11), and cardiotoxicity in 0.4%⁴⁰ (B).

As second-line therapy in chronic-phase CML patients, dasatinib causes an increase in the rates of pleural effusion (NNH: 6), neutropenia (NNH: 5), thrombocytopenia (NNH: 2), dyspnea (NNH: 6), and headache (NNH: 7)⁵⁹ (B).

Recommendation

Regarding most expected adverse effects using this class of medication, dasatinib and nilotinib have similar results but with slight differences in degree. However, nilotinib appears to cause more hepatotoxicity and dasatinib causes more water retention (pleural effusion).

14. Does adherence to imatinib treatment have prognostic impact?

CML patients on imatinib treatment who have suboptimal response are less adherent to treatment (do not take the

medication) than patients with optimal response. Patients treated for more than 12 months who have complete cytogenetic response also have better compliance than those with partial cytogenetic response. There is no difference in the hematologic response between adherent and non-adherent patients⁶⁸ (B).

There is a direct correlation between adherence (< 90% or > 90%) of CML patients to imatinib treatment and the likelihood of higher molecular response at six years (an increase in 66.1% of response in adherence > 90%). When adherence is less than 80%, there is no molecular response. Patients who need to increase the dose of imatinib have a 12.8% reduction in adherence⁶⁹ (B).

In the treatment of CML with imatinib, adherence < 85% increases the risk of loss of complete cytogenetic response by 34.9% (NNH: 3). None of the patients with adherence > 95% lost cytogenetic response. Patients with adherence level < 85% who never attained molecular response, have low adherence as a predictor of loss of cytogenetic response. Adherence of < 85% reduces the disease-free survival by 37% (NNH: 3). Adherence of more than 85% confer prognoses similar to those for major molecular response patients⁷⁰ (B).

The five-year disease-free survival in chronic-phase CML patients who adhere to imatinib treatment is 16.9% higher than for non-adherent patients. Non-compliance reduces the possibility of complete cytogenetic response by 18% (NNH: 6). The greatest cause of imatinib treatment cessation (29.6% of the cases) is related to nonadherence. Complete cytogenetic response is correlated to adherence to treatment, with a reduction in the response in noncompliant patients by 20%⁷¹ (B).

Recommendation

Adherence to imatinib treatment is directly correlated to the probability of molecular and cytogenetic responses and disease-free survival.

15. Are prior cytogenetic response to imatinib and performance status prognostic factors for response to second-line inhibitors in imatinib-resistant patients?

The best cytogenetic response (0% positive Philadelphia chromosome) during treatment with imatinib is predictive of response to dasatinib and nilotinib, with an increase in cytogenetic response by 21% when compared to the maximum range of Philadelphia chromosome rate between 1% and 94%, and 66.8% when compared to no cytogenetic response during treatment with imatinib, i.e. Philadelphia > 95%⁵¹ (B).

The response to second-line tyrosine kinase inhibitors of imatinib-resistant CML patients is associated with some other prognostic factors, which are: 1. low-risk Sokal: 25.5% increase in cytogenetic response and 27.0% in disease-free survival; 2. percentage of positive Philadelphia chromosome at the beginning of treatment < 95%: 43.8% increase in the cytogenetic response and 27.3% in disease-free survival; 3. Time to therapeutic failure of imatinib < six months: 37.2% increase in

cytogenetic response, 24.3% increase in overall survival rate, and 13.8% increase in progression-free survival⁵¹ (B).

The prognosis of treatment using second-line tyrosine kinase inhibitors (nilotinib or dasatinib) in imatinib-resistant CML patients can be predicted by prior cytogenetic response (imatinib), giving an estimated 37% increase in disease-free survival at three years and in the cytogenetic response at one year. A performance status (European Cooperative Oncology Group [ECOG]) of "0" at the beginning of treatment with second-line inhibitors predicts an 18% increase in disease-free survival and a 32% increase in overall survival at three years⁷² (B).

Other prognostic factors may be associated with response to treatment with nilotinib or dasatinib, such as: age higher than 55 years old with a 24% reduction in cytogenetic response at one year, a 20% reduction in disease-free survival at three years, and a 6% reduction in overall survival at three years; > 90% Philadelphia chromosome-positive metaphases at start of treatment with second-line inhibitor with a 30% reduction in the cytogenetic response and a 21% reduction in disease-free survival⁷² (B).

Recommendation

Information related to cytogenetic response and performance status (ECOG) should be used to assess prognosis on starting second-line treatment with nilotinib or dasatinib in previously imatinib-resistant CML patients. Additionally, age and cytogenetic response prior to treatment with second-generation tyrosine kinase inhibitors should be taken into account.

16. When is it necessary to make an analysis of BCR-ABL mutations in chronic myeloid leukemia patients on treatment with tyrosine kinase inhibitors?

BCR-ABL mutations are associated with 100% resistance to Imatinib treatment in accelerated phase CML patients, and in 79% of chronic-phase patients⁷³ (B).

The presence of BCR-ABL mutations increases the risk by 52% of chronic-phase CML patients evolving to the accelerated phase or blast crisis within nine months (NNH: 2). These mutations, especially P-loop mutations, also reduce the time free of disease progression and survival of these patients⁷⁴ (B).

In the follow-up of CML patients, BCR-ABL mutations occur at different times in patients on treatment with imatinib, and are correlated with lower survival rates. For patients in the early phase of the disease, mutations are associated with increases in transformation to the accelerated phase (32%) and blast crisis (16%), and with a reduction in the complete cytogenetic response (24%). Regardless of the stage of the disease, mutations reduce hematologic response⁷⁵ (B).

BCR-ABL mutations in CML patients on imatinib treatment predict, within approximately 20 months, loss of complete cytogenetic response and progression to the advanced stages of the disease⁷⁶ (B).

Hematologic and cytogenetic responses are similar in patients with and without BCR-ABL mutations on treatment with tyrosine kinase inhibitors (dasatinib and nilotinib). Moreover, disease-free survival and overall survival are not significantly different between these two groups of patients⁷⁷ (B).

In the four-year follow-up of chronic-phase CML patients, the time from the beginning of imatinib treatment to the progression of the disease to the accelerated phase or blast crisis is shorter in patients with mutations than those without mutations. The overall survival of patients with mutations and those without mutations is 10 and 51 months respectively, but this varies according to the type of mutation: P-loop (13 months), T315I (nine months), and without mutation (51 months)⁷⁸ (B).

Among chronic-phase CML patients under nilotinib treatment, the two-year overall survival is reduced by 38% in the presence of BCR-ABL mutations. In addition, the presence of mutations is associated with a 34% reduction in the cytogenetic response²¹ (B).

T315I mutations occur more often in patients treated with dasatinib. The presence of mutations during nilotinib or dasatinib treatment is predictive of a worse prognosis in these patients²⁰ (B).

Recommendation

BCR-ABL mutations should be investigated in CML patients resistant to tyrosine kinase inhibitors (suboptimal response or failure) regardless of the stage, since their presence predicts the greater risk of resistance and shorter survival.

17. Does the diagnosis of mutations guide the choice of treatment in Imatinib-resistant patients?

In imatinib-resistant CML patients, mutations can assist in the choice of second-generation inhibitors, nilotinib or dasatinib. An evaluation of the sensitivity of mutations to inhibitors in *in vitro* studies (IC_{50}) defines three groups of sensitivity (low, intermediate, and high concentrations) of the mutation to: dasatinib ($IC_{50} < 3$ nM, 3-60 nM, and > 60 nM) and nilotinib ($IC_{50} < 50$ nM, 50-500 nM and > 500 nM) with the worst case scenario (resistance) corresponding to high concentrations⁷⁷ (B).

Hematologic and cytogenetic responses at one year are significantly lower in patients with mutations and in the chronic phase, particularly for mutations with intermediate IC_{50} (25% and 25%, respectively) compared to low IC_{50} (96% and 54%, respectively). In the accelerated phase there is also a reduction in the cytogenetic response for mutations, with 10% reduction in intermediate IC_{50} and 31% reduction in low IC_{50} ⁷⁷ (B). In the chronic phase, disease-free survival and overall survival are lower in patients with mutations with high IC_{50} (0% and 75%, respectively), when compared to mutations with low IC_{50} (78% and 100%, respectively)⁷⁷ (B). T315I mutation is associated with high IC_{50} (resistance), but there is no difference in its distribution comparing dasatinib and nilotinib⁷⁷ (B).

Other specific mutations associated with high IC_{50} in the chronic phase of CML treated with dasatinib are: T315I/A, F317L/I/V/C, and V299L⁷⁹⁻⁸¹ (B), and with nilotinib: T315I, Y253H, E255K/V, and F359V/C⁸² (B). The G250E mutation also has an impact on resistance common to both forms of treatment⁸¹ (B).

Mutations associated with resistance to dasatinib, such as V299L, T315A, and F317I, may be sensitive to nilotinib, while mutation V299L may be resistant to bosutinib^{82,83} (B).

Complete cytogenetic response subsequent to treatment using dasatinib or nilotinib is lower in patients with resistant mutations (0%) compared to patients with other mutations or without mutations (41% and 49%, respectively). The survival of chronic-phase CML patients when resistant mutations are detected is 0% compared with 51% and 45% in patients with other mutations or without mutations, respectively⁸⁴ (B).

Recommendation

The identification of mutations, especially resistant mutations, can assist in the choice of the tyrosine kinase inhibitor, allowing for the selection of the therapeutic option that will provide the best response.

18. How should monitoring of chronic myeloid leukemia patients taking tyrosine kinase inhibitors be performed?

Wang et al. stated that monitoring of chronic-phase CML patients for BCR-ABL during imatinib treatment can be achieved with PCR in peripheral blood (BCR-ABL/ABL ratio), correlating this with the result obtained through the usual cytogenetic study of bone marrow, which identifies responsive, partially responsive, and unresponsive patients in respect to BCR-ABL/gene control of up to 0.08%, of 0.08% to 10%, and of above 11%, respectively⁸⁵ (B).

In a randomized study, 1,106 CML patients were treated with interferon and imatinib as initial treatment. All patients who achieved cytogenetic remission performed qPCR for BCR-ABL. The results were expressed in terms of the logarithmic reduction in relation to the median level of transcripts in 30 newly-diagnosed patients. Patients who achieved complete cytogenetic remission and at least a 3-log reduction in the level of transcripts had progression-free survival of 100% at 24 months, compared to 95% for those with complete cytogenetic remission and less than a 3-log reduction in the level of transcripts, and 85% for patients without complete cytogenetic response⁸⁶ (B).

Thus, this form of monitoring also allows for the identification of the two-year progression-free survival with the low values of transcripts^{86,87} (B).

Using samples from 38 international centers, a study validated the use of an international scale of BCR-ABL values that established 0.1% as a 3-log reduction⁸⁷ (B).

It is possible to stratify patients through PCR during the three-year follow-up of patients whose indexes reflect increases, stability, reduction, or even loss of cytogenetic response⁵² (B).

Plasma imatinib levels are significantly higher in patients with molecular and cytogenetic responses compared to patients without response. The level that differentiates molecular response and lack of response with the greatest accuracy (77% sensitivity and 71% specificity) is 1,002 ng/mL⁸⁸ (B).

The use of FISH to monitor CML patients on imatinib treatment enables the use of peripheral blood to identify cytogenetic response. A positive result points to the absence of cytogenetic response, and a negative result identifies its presence. The association with PCR allows the molecular response to be monitored. In the study by Reinhold et al., the estimated cytogenetic and molecular responses at five years were 81.7% and 67.1%, respectively⁵³ (B). However, the comparison between the results of FISH using peripheral blood leukocytes and the cytogenetics of the bone marrow may not establish an appropriate correlation in⁸⁹ (B).

Identifying the existence and occurrence of mutations in tyrosine kinase inhibitors treatment of CML patients allows for the estimate of the prognosis and guides the treatment. High-performance liquid chromatography is a practical and sensitive method that identifies mutations, which can be used for clinical monitoring of patients⁹⁰ (B).

Some mutations can be identified by direct sequencing during the follow-up of patients including: T315T, T315I, F317L, V339L, M351T, E355G, Y253F, F359V, among others; these are associated with different responses to available inhibitors. A 31% reduction in the overall survival of patients with mutations was identified in the three-year follow-up⁷³ (B).

Recommendation

The monitoring of CML patients treated with tyrosine kinase inhibitors can be accomplished by bone marrow cytogenetics and qPCR for the BCR-ABL gene in peripheral blood, thereby allowing an estimation of prognosis and the definition of therapeutic strategies. Mutational analysis should be performed in patients with suboptimal response or loss of response to tyrosine kinase inhibitors.

19. When should bone marrow transplantation be indicated for chronic myeloid leukemia patients?

Imatinib may be used as treatment for relapse after allogeneic hematopoietic stem cell transplantation, the prevalence of which ranges from 40% to 70% at five months. In the chronic phase, the cytogenetic and hematologic response rates obtained and survival at nine months are 58%, 84%, and 100%, respectively^{91,92} (B). Imatinib has become first-line treatment in the chronic phase of CML, demonstrating increased survival when used before bone marrow transplant⁹³ (B).

Due to the lower cost, resistance to imatinib, or advanced stages of the disease (accelerated phase and blast crisis), some case series have been reported with comparative results or in association to imatinib, demonstrating similar disease-free survival, overall survival, and cardiotoxicity⁹⁴ (C). The previous use (before transplantation) of imatinib in patients in advanced stages of CML produces hematological response in 73% and

cytogenetic response in 40% of patients, and three years after the transplant, 66.7% of patients have complete molecular response⁹⁵ (C).

In a prospective study, Jiang et al. compared accelerated phase patients treated either with imatinib (n = 87) or allogeneic transplantations (n = 54). In their study, a multivariate analysis established hemoglobin < 10.0, blasts in peripheral blood < 5%, and disease duration of less than 12 months as independent risk factors for survival. High-risk (two risk factors or more) or intermediate-risk patients (one risk factor) had better overall survival and progression-free survival with allogeneic transplant. No difference was observed in low-risk patients⁹⁶ (B).

The mortality of CML patients on imatinib treatment associated with hematopoietic stem cell transplant is 9.7%, and the relapse rate is 5.0% at one year⁹⁷ (C).

Despite the new options in imatinib-resistant patients, such as dasatinib or nilotinib, non-comparative case series that associate tyrosine kinase inhibitors and transplant are still being performed⁹⁸ (C).

Data are still limited for the pediatric population, but the results with imatinib are similar to those for adults. Millot et al. published their experience with 44 children with newly-diagnosed CML treated with imatinib. With a median follow-up of 31 months, the estimated progression-free survival at 36 months was 98%. The rates of complete cytogenetic response and major molecular response at 12 months were 61% and 31%, respectively. About 30% of the children discontinued the use of medication, mainly due to lack of effectiveness. Tyrosine kinase inhibitors adversely affect the growth of children, and this aspect should be monitored⁹⁹ (B).

Regarding transplantation, researchers reported the results of a prospective study involving 200 CML children and teenagers treated by allogeneic transplantation according to donor availability. The probability of survival at five years was 87 ± 11% for matched related donors, 52 ± 9% for matched unrelated donors, and 45 ± 16% for unmatched donors. The likelihood of relapse at five years was 20 ± 12%¹⁰⁰ (B).

Recommendation

Bone marrow transplantation is a therapeutic option to treat CML, but should be reserved for cases resistant to tyrosine kinase inhibitor treatment and patients in the advanced stages of the disease after an initial course of tyrosine kinase inhibitors.

Conflicts of interest

Bendit, I: Received honoraries for lectures on “Molecular Monitoring in Chronic Myeloid Leukemia” sponsored by the companies Novartis Brasil and BMS do Brasil.

Freitas, CMBM: Received honoraries for presentations in lectures sponsored by the companies Novartis and BMS do Brasil.

Coelho, AM: Received honoraries for presentations in lectures sponsored by the company BMS do Brasil.

R E F E R E N C E S

1. Baccarani M, Dreyling M; ESMO Guidelines Working Group. Chronic myelogenous leukemia: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol*. 2009;20 Suppl 4:105-7.
2. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114:937-51.
3. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*. 2008;22:14-22.
4. Verma D, Kantarjian HM, Jones D, Luthra R, Borthakur G, Verstovsek S, et al. Chronic myeloid leukemia (CML) with P190 BCR-ABL: analysis of characteristics, outcomes, and prognostic significance. *Blood*. 2009;114:2232-5.
5. Lucas CM, Harris RJ, Giannoudis A, Davies A, Knight K, Watmough SJ, et al. Chronic myeloid leukemia patients with the e13a2 BCR-ABL fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript. *Haematologica*. 2009;94:1362-7.
6. de Lemos JA, de Oliveira CM, Scerni AC, Bentes AQ, Beltrão AC, Bentes IR, et al. Differential molecular response of the transcripts B2A2 and B3A2 to imatinib mesylate in chronic myeloid leukemia. *Genet Mol Res*. 2005;4:803-11.
7. Sharma P, Kumar L, Mohanty S, Kochupillai V. Response to imatinib mesylate in chronic myeloid leukemia patients with variant BCR-ABL fusion transcripts. *Ann Hematol*. 2010;89:241-7.
8. Kreil S, Pffirmann M, Haferlach C, Waghorn K, Chase A, Hehlmann R, et al. Heterogeneous prognostic impact of derivative chromosome 9 deletions in chronic myelogenous leukemia. *Blood*. 2007; 110:1283-90.
9. Ghaith F, Abdou S, El-Bendary A, Shahin D, Eid M, Megeed WA, et al. Prognostic relevance of 9q34 deletion and the suppressor of cytokine signalling-1 in CML patients. *Int J Lab Hematol*. 2010;32:103-12.
10. Cohen N, Rozenfeld-Granot G, Hardan I, Brok-Simoni F, Amariglio N, Rechavi G, et al. Subgroup of patients with Philadelphia-positive chronic myelogenous leukemia characterized by a deletion of 9q proximal to ABL gene: expression profiling, resistance to interferon therapy, and poor prognosis. *Cancer Genet Cytogenet*. 2001;128:114-9.
11. Quintás-Cardama A, Kantarjian H, Shan J, Jabbour E, Abruzzo LV, Verstovsek S, et al. Prognostic impact of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia treated with nilotinib or dasatinib. *Cancer*. 2011;117:5085-93.20
12. Quintas-Cardama A, Kantarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, et al. Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. *Blood*. 2005;105:2281-6.
13. Huntly BJ, Reid AG, Bench AJ, Campbell LJ, Telford N, Shepherd P, et al. Deletions of the derivative chromosome 9 occur at the time of the Philadelphia translocation and provide a powerful and independent prognostic indicator in chronic myeloid leukemia. *Blood*. 2001;98:1732-8.
14. Fourouclas N, Campbell PJ, Bench AJ, Swanton S, Baxter EJ, Huntly BJ, et al. Size matters: the prognostic implications of large and small deletions of the derivative 9 chromosome in chronic myeloid leukemia. *Haematologica*. 2006;91:952-5.
15. Vaz de Campos MG, Montesano FT, Rodrigues MM, Chauffaille M de L. Clinical implications of der(9q) deletions detected through dual-fusion fluorescence in situ hybridization in patients with chronic myeloid leukemia. *Cancer Genet Cytogenet*. 2007;178:49-56.
16. Marzocchi G, Castagnetti F, Luatti S, Baldazzi C, Stacchini M, Gugliotta G, et al. Variant Philadelphia translocations: molecular-cytogenetic characterization and prognostic influence on frontline imatinib therapy, a GIMEMA Working Party on CML analysis. *Blood*. 2011;117:6793-800.
17. Castagnetti F, Testoni N, Luatti S, Marzocchi G, Mancini M, Kerim S, et al. Deletions of the derivative chromosome 9 do not influence the response and the outcome of chronic myeloid leukemia in early chronic phase treated with imatinib mesylate: GIMEMA CML Working Party analysis. *J Clin Oncol*. 2010;28:2748-54.
18. Landstrom AP, Knudson RA, Dewald GW, Ketterling RP, Tefferi A. Philadelphia chromosome mosaicism at diagnosis in chronic myeloid leukemia: clinical correlates and effect on imatinib mesylate treatment outcome. *Leuk Lymphoma*. 2007;48:2137-40.
19. Gorusu M, Benn P, Li Z, Fang M. On the genesis and prognosis of variant translocations in chronic myeloid leukemia. *Cancer Genet Cytogenet*. 2007;173:97-106.
20. Meggyesi N, Kozma A, Halm G, Nahajevszky S, Bátaí A, Fekete S, et al. Additional chromosome abnormalities, BCR-ABL tyrosine kinase domain mutations and clinical outcome in Hungarian tyrosine kinase inhibitor-resistant chronic myelogenous leukemia patients. *Acta Haematol*. 2012;127:34-42.
21. Kim TD, Türkmen S, Schwarz M, Koca G, Nogai H, Bommer C, et al. Impact of additional chromosomal aberrations and BCR-ABL kinase domain mutations on the response to nilotinib in Philadelphia chromosome-positive chronic myeloid leukemia. *Hematologica*. 2010;95:582-8.
22. Haus O, Noworolska A, Laskowski M, Kuliszkiwicz-Janus M, Kozłowska J, Harłozinska-Szmyrka A, et al. Prognostic significance of secondary cytogenetic changes and nonspecific cross-reacting antigen (NCA) in patients with Ph-positive chronic myeloid leukemia. *Exp Mol Pathol*. 1990;52:235-42.
23. Hsiao HH, Liu YC, Tsai HJ, Hsu JF, Yang WC, Chang CS, et al. Additional chromosome abnormalities in chronic myeloid leukemia. *Kaohsiung J Med Sci*. 2011;27:49-54.
24. Vranová V, Katina S, Kirschnerová G, Mistrík M, Lakota J, Horáková J, et al. A significance of additional chromosomal aberrations and other variables on post transplantation outcome of patients with CML. *Neoplasma*. 2005;52:381-7.
25. Fabarius A, Leitner A, Hochhaus A, Müller MC, Hanfstein B, Haferlach C, et al. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML Study IV. *Blood*. 2011;118:6760-8.
26. Ahmed R, Naqi N, Hussain I, Khattak BK, Nadeem M, Iqbal J. Presenting phases of chronic myeloid leukaemia. *J Coll Physicians Surg Pak*. 2009;19:469-72.
27. Cortes J, Kantarjian H. Advanced-phase chronic myeloid leukemia. *Semin Hematol*. 2003;40:79-86.
28. Cortes JE, Talpaz M, O'Brien S, Faderl S, Garcia-Manero G, Ferrajoli A, et al. Staging of chronic myeloid leukemia in the imatinib era: an evaluation of the World Health Organization proposal. *Cancer*. 2006;106:1306-15.
29. Cortes J. Natural history and staging of chronic myelogenous leukemia. *Hematol Oncol Clin North Am*. 2004;18:569-84.

30. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2003;348:994-1004.
31. Aguayo A, Couban S. State-of-the-art in the management of chronic myelogenous leukemia in the era of the tyrosine kinase inhibitors: evolutionary trends in diagnosis, monitoring and treatment. *Leuk Lymphoma.* 2009; 50 Suppl2:1-8.
32. Bonifazi F, De Vivo A, Rosti G, Tiribelli M, Russo D, Trabacchi E, et al. Testing Sokal's and the new prognostic score for chronic myeloid leukaemia treated with alpha-interferon. Italian Cooperative Study Group on Chronic Myeloid Leukaemia. *Br J Haematol.* 2000;111:587-95.
33. Corm S, Roche L, Micol JB, Coiteux V, Bossard N, Nicolini FE, et al. Changes in the dynamics of the excess mortality rate in chronic phase-chronic myeloid leukemia over 1990-2007: a population study. *Blood.* 2011;118:4331-7.
34. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving 22 imatinib for chronic myeloid leukemia. *N Engl J Med.* 2006; 355:2408-17.
35. Deenik W, Janssen JJ, van der Holt B, Verhoef GE, Smit WM, Kersten MJ, et al. Efficacy of escalated imatinib combined with cytarabine in newly diagnosed patients with chronic myeloid leukemia. *Haematologica.* 2010;95:914-21.
36. Forrest DL, Trainor S, Brinkman RR, Barnett MJ, Hogge DE, Nevill TJ, et al. Cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia are correlated with Sokal risk scores and duration of therapy but not trough imatinib plasma levels. *Leuk Res.* 2009;33:271-5.
37. Hasford J, Pfirrmann M, Hehlmann R, Baccarani M, Guilhot F, Mahon FX, et al. Prognosis and prognostic factors for patients with chronic myeloid leukemia: nontransplant therapy. *Semin Hematol.* 2003;40:4-12.
38. Lee JP, Birnstein E, Masiello D, Yang D, Yang AS. Gender and ethnic differences in chronic myelogenous leukemia prognosis and treatment response: a single-institution retrospective study. *J Hematol Oncol.* 2009;2:30.
39. Rajappa S, Varadpande L, Paul T, Jacob R, Digumarti R. Imatinib mesylate in early chronic phase chronic myeloid leukemia: Experience from a developing country. *Leuk Lymphoma.* 2008; 49:554-8.
40. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2010; 362:2260-70.
41. Kantarjian HM, Shah NP, Cortes JE, Baccarani M, Agarwal MB, Undurraga MS, et al. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). *Blood.* 2012;119:1123-9.
42. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2010 17;362:2251-9.
43. Kantarjian HM, Hochhaus A, Saglio G, De Souza C, Flinn IW, Stenke L, et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. *Lancet Oncol.* 2011;12:841-51.
44. Breccia M, Stefanizzi C, Cannella L, Latagliata R, Frustaci AM, Carosino I, et al. Differences in hematological and nonhematological toxicity during treatment with imatinib in patients with early and late chronic phase chronic myeloid leukemia. *Leuk Lymphoma.* 2008;49:2328-32.
45. Scerni AC, Alvares LA, Beltrão AC, Bentes IR, Azevedo TC, Bentes AQ, et al. Influence of late treatment on how chronic myeloid leukemia responds to imatinib. *Clinics (Sao Paulo).* 2009;64:731-4.
46. Cortes JE, Talpaz M, Giles F, O'Brien S, Rios MB, Shan J, et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. *Blood.* 2003;101:3794-800.
47. Kantarjian HM, Cortes JE, O'Brien S, Luthra R, Giles F, Verstovsek S, et al. Long-term survival benefit and improved complete cytogenetic and molecular response rates with imatinib mesylate in Philadelphia chromosome-positive chronic-phase chronic myeloid leukemia after failure of interferon-alpha. *Blood.* 2004;104:1979-88.
48. Palandri F, Iacobucci I, Martinelli G, Amabile M, Poerio A, Testoni N, et al. Party on CML. Long-term outcome of complete cytogenetic responders after imatinib 400 mg in late chronic phase, philadelphia-positive chronic myeloid leukemia: the GIMEMA Working Party on CML. *J Clin Oncol.* 2008;26:106-11.
49. Gambacorti-Passerini C, Antolini L, Mahon FX, Guilhot F, Deininger M, Fava C, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. *J Natl Cancer Inst.* 2011;103:553-61.
50. Fava C, Kantarjian HM, Jabbour E, O'Brien S, Jain N, Rios MB, et al. Failure to achieve a complete hematologic response at the time of a major cytogenetic response with second-generation tyrosine kinase inhibitors is associated with a poor prognosis among patients with chronic myeloid leukemia in accelerated or blast phase. *Blood.* 2009;113:5058-63.
51. Milojkovic D, Nicholson E, Apperley JF, Holyoake TL, Shepherd P, Drummond MW, et al. Early prediction of success or failure of treatment with second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukemia. *Haematologica.* 2010;95:224-31.
52. Marin D, Kaeda J, Szydlo R, Saunders S, Fleming A, Howard J, et al. Monitoring patients in complete cytogenetic remission after treatment of CML in chronic phase with imatinib: patterns of residual leukaemia and prognostic factors for cytogenetic relapse. *Leukemia.* 2005;19:507-12.
53. Furukawa T, Narita M, Koike T, Takai K, Nagai K, Kobayashi M, et al. Clinical value of assessing the response to imatinib monitored by interphase FISH and RQ-PCR for BCR-ABL in peripheral blood for long-term survival of chronic phase CML patients: results of the Niigata CML-multi-institutional co-operative clinical study. *Int J Hematol.* 2011;93:336-43.
54. Cortes JE, Kantarjian HM, Goldberg SL, Powell BL, Giles FJ, Wetzler M, et al. High-dose imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: high rates of rapid cytogenetic and molecular responses. *J Clin Oncol.* 2009;27: 4754-9.
55. Ross DM, Branford S, Moore S, Hughes TP. Limited clinical value of regular bone marrow cytogenetic analysis in imatinib-treated chronic phase CML patients monitored by RQ-PCR for BCR-ABL. *Leukemia.* 2006;20:664-70.
56. Press RD, Galderisi C, Yang R, Rempfer C, Willis SG, Mauro MJ, et al. A half-log increase in BCR-ABL RNA predicts a higher risk of relapse in patients with chronic myeloid leukemia with an imatinib-induced complete cytogenetic response. *Clin Cancer Res.* 2007;13:6136-43
57. Lange T, Bumm T, Otto S, Al-Ali HK, Kovacs I, Krug D, et al. Quantitative reverse transcription polymerase chain reaction should not replace conventional cytogenetics for monitoring patients with chronic myeloid leukemia during early phase of imatinib therapy. *Haematologica.* 2004;89:49-57.

58. Lima L, Bernal-Mizrachi L, Saxe D, Mann KP, Tighiouart M, Arellano M, et al. Peripheral blood monitoring of chronic myeloid leukemia during treatment with imatinib, second-line agents, and beyond. *Cancer*. 2011;117:1245-52.
59. Kantarjian H, Pasquini R, Hamerschlag N, Rousselot P, Holowiecki J, Jootar S, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia after failure of first-line imatinib: a randomized phase 2 trial. *Blood*. 2007;109:5143-50.
60. Kantarjian H, Pasquini R, Lévy V, Jootar S, Holowiecki J, Hamerschlag N, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia resistant to imatinib at a dose of 400 to 600 milligrams daily: two-year follow-up of a randomized phase 2 study (START-R). *Cancer*. 2009;115:4136-47.
61. Shah NP, Kantarjian HM, Kim DW, Réa D, Dorlhiac-Llacer PE, Milone JH, et al. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. *J Clin Oncol*. 2008;26:3204-12.
62. Shah NP, Kim DW, Kantarjian H, Rousselot P, Llacer PE, Enrico A, et al. Potent, transient inhibition of BCR-ABL with dasatinib 100 mg daily achieves rapid and durable cytogenetic responses and high transformation-free survival rates in chronic phase chronic myeloid leukemia patients with resistance, suboptimal response or intolerance to imatinib. *Haematologica*. 2010;95:232-40.
63. Koren-Michowitz M, le Coutre P, Duyster J, Scheid C, Panayiotidis P, Prejzner W, et al. Activity and tolerability of nilotinib: a retrospective multicenter analysis of chronic myeloid leukemia patients who are imatinib resistant or intolerant. *Cancer*. 2010;116:4564-72.
64. Kantarjian HM, Giles F, Gattermann N, Bhalla K, Alimena G, Palandri F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. *Blood*. 2007;110:3540-6.
65. Kantarjian HM, Giles FJ, Bhalla KN, Pinilla-Ibarz J, Larson RA, Gattermann N, et al. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. *Blood*. 2011;117:1141-5.
66. Giles FJ, Abruzzese E, Rosti G, Kim DW, Bhatia R, Bosly A, et al. Nilotinib is active in chronic and accelerated phase chronic myeloid leukemia following failure of imatinib and dasatinib therapy. *Leukemia*. 2010;24:1299-301.
67. Khoury HJ, Cortes JE, Kantarjian HM, Gambacorti-Passerini C, Baccarani M, Kim DW, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. *Blood*. 2012;119:3403-12.
68. Noens L, van Lierde MA, De Bock R, Verhoef G, Zachée P, Berneman Z, et al. Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. *Blood*. 2009;113:5401-11.
69. Marin D, Bazeos A, Mahon FX, Eliasson L, Milojkovic D, Bua M, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *J Clin Oncol*. 2010;28:2381-8.
70. Ibrahim AR, Eliasson L, Apperley JF, Milojkovic D, Bua M, Szydlo R, et al. Poor adherence is the main reason for loss of CCyR and imatinib failure in chronic myeloid leukemia patients on long-term therapy. *Blood*. 2011;117:3733-6.
71. Ganesan P, Sagar TG, Dubashi B, Rajendranath R, Kannan K, Cyriac S, et al. Nonadherence to imatinib adversely affects event free survival in chronic phase chronic myeloid leukemia. *Am J Hematol*. 2011;86:471-4.
72. Jabbour E, Kantarjian H, O'Brien S, Shan J, Garcia-Manero G, Wierda W, et al. Predictive factors for outcome and response in patients treated with second-generation tyrosine kinase inhibitors for chronic myeloid leukemia in chronic phase after imatinib failure. *Blood*. 2011;117:1822-7.
73. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCRABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood*. 2003;102:276-83.
74. Soverini S, Martinelli G, Rosti G, Bassi S, Amabile M, Poerio A, et al. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. *J Clin Oncol*. 2005;23:4100-9.
75. Jabbour E, Kantarjian H, Jones D, Talpaz M, Bekele N, O'Brien S, et al. Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia*. 2006;20:1767-73.
76. Khorashad JS, de Lavallade H, Apperley JF, Milojkovic D, Reid AG, Bua M, et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. *J Clin Oncol*. 2008;26:4806-13.
77. Jabbour E, Jones D, Kantarjian HM, O'Brien S, Tam C, Koller C, et al. Long-term outcome of patients with chronic myeloid leukemia treated with second-generation tyrosine kinase inhibitors after imatinib failure is predicted by the in vitro sensitivity of BCR-ABL kinase domain mutations. *Blood*. 2009;114:2037-43.
78. Ono T, Miyawaki S, Kimura F, Kanamori H, Ohtake S, Kitamura K, et al. BCRABL1 mutations in patients with imatinib-resistant Philadelphia chromosome-positive leukemia by use of the PCR-Invader assay. *Leuk Res*. 2011;35:598-603.
79. Müller MC, Cortes JE, Kim DW, Druker BJ, Erben P, Pasquini R, et al. Dasatinib treatment of chronic-phase chronic myeloid leukemia: analysis of responses according to preexisting BCR-ABL mutations. *Blood*. 2009;114:4944-53.
80. O'Hare T, Eide CA, Deininger MWN. BcrAbl kinase domain mutations, drug resistance, and the road to a cure for chronic myeloid leukemia. *Blood*. 2007;110:2242-9.
81. Redaelli S, Piazza R, Rostagno R, Magistri V, Perini P, Marega M, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. *J Clin Oncol*. 2009;27:469-71.
82. Hughes T, Saglio G, Branford S, Soverini S, Kim DW, Müller MC, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. *J Clin Oncol*. 2009;27:4204-10.
83. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients after imatinib failure: does the BCR-ABL mutation status really matter?. *Blood*. 2009;114:5426-35.
84. Parker WT, Lawrence RM, Ho M, Irwin DL, Scott HS, Hughes TP, et al. Sensitive detection of BCR-ABL1 mutations in patients with chronic myeloid leukemia after imatinib resistance is predictive of outcome during subsequent therapy. *J Clin Oncol*. 2011;29:4250-9.

85. Wang L, Pearson K, Pillitteri L, Ferguson JE, Clark RE. Serial monitoring of BCR-ABL by peripheral blood real-time polymerase chain reaction predicts the marrow cytogenetic response to imatinib mesylate in chronic myeloid leukaemia. *Br J Haematol.* 2002;118:771-7.
86. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2003;349:1423-32.
87. Branford S, Fletcher L, Cross NC, Müller MC, Hochhaus A, Kim DW, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. *Blood.* 2008;112:3330-8.
88. Picard S, Titier K, Etienne G, Teilhet E, Ducint D, Bernard MA, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood.* 2007;109:3496-9.
89. Reinhold U, Hennig E, Leiblein S, Niederwieser D, Deininger MW. FISH for BCR-ABL on interphases of peripheral blood neutrophils but not of unselected white cells correlates with bone marrow cytogenetics in CML patients treated with imatinib. *Leukemia.* 2003;17:1925-9.
90. Mascarenhas CC, Cunha AF, Miranda EC, Zulli R, Silveira RA, Costa FF, et al. New mutations detected by denaturing high performance liquid chromatography during screening of exon 6 bcrabl mutations in patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. *Leuk Lymphoma.* 2009;50:1148-54.
91. Olavarria E, Ottmann OG, Deininger M, Clark RE, Bandini G, Byrne J, et al. Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Leukemia.* 2003;17:1707-12.
92. Crawley C, Szydlo R, Lalancette M, Bacigalupo A, Lange A, Brune M, et al. Outcomes of reduced-intensity transplantation for chronic myeloid leukemia: an analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. *Blood.* 2005;106:2969-76.28
93. Lee SJ, Kukreja M, Wang T, Giral SA, Szer J, Arora M, et al. Impact of prior imatinib mesylate on the outcome of hematopoietic cell transplantation for chronic myeloid leukemia. *Blood.* 2008;112:3500-7.
94. Burke MJ, Trotz B, Luo X, Weisdorf DJ, Baker KS, Wagner JE, et al. Imatinib use either pre- or post-allogeneic hematopoietic cell transplantation (allo-HCT) does not increase cardiac toxicity in chronic myelogenous leukemia patients. *Bone Marrow Transplant.* 2009;44:169-74.
95. Luo Y, Zhao Y, Tan Y, Shi J, Han X, Zheng Y, et al. Imatinib combined with myeloablative allogeneic hematopoietic stem cell transplantation for advanced phases of chronic myeloid leukemia. *Leuk Res.* 2011;35:1307-11.
96. Jiang Q, Xu L, Liu D, Liu K, Chen S, Jiang B, et al. Imatinib mesylate versus allogeneic hematopoietic stem cell transplantation for patients with chronic myelogenous leukemia in the accelerated phase. *Blood.* 2011;117(11): 3032-40.
97. Boehm A, Walcherberger B, Sperr WR, Wöhrer S, Dieckmann K, Rosenmayr A, et al. Improved outcome in patients with chronic myelogenous leukemia after allogeneic hematopoietic stem cell transplantation over the past 25 years: a single-center experience. *Biol Blood Marrow Transplant.* 2011;17:133-40.
98. Breccia M, Palandri F, Iori AP, Colaci E, Latagliata R, Castagnetti F, et al. Second generation tyrosine kinase inhibitors before allogeneic stem cell transplantation in patients with chronic myeloid leukemia resistant to imatinib. *Leuk Res.* 2010;34:143-7.
99. Millot F, Baruchel A, Guilhot J, Petit A, Leblanc T, Bertrand Y, et al. Imatinib is effective in children with previously untreated chronic myelogenous leukemia in early chronic phase: results of the French national phase IV trial. *J Clin Oncol.* 2011;29:2827-32.
100. Suttorp M, Claviez A, Bader P, Peters C, Gadner H, Ebell W, et al. Allogeneic stem cell transplantation for pediatric and adolescent patients with CML: results from the prospective trial CML-paed I. *Klin Padiatr.* 2009;221:351-7.