Diagnosis and Treatment of Chronic Myeloid Leukemia in 2015

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Learning Objectives: On completion of this article, you should be able to (1) identify clinical and laboratory features consistent with a diagnosis of chronic myeloid leukemia (CML) and diagnose the disease based on laboratory testing; (2) identify factors associated with poor treatment outcomes and how these are identified and managed; and (3) describe standard first and second line therapeutic options for CML and their adverse effects.

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Abstract

Few neoplastic diseases have undergone a transformation in a relatively short period like chronic myeloid leukemia (CML) has in the last few years. In 1960, CML was the first cancer in which a unique chromosomal abnormality was identified and a pathophysiologic correlation suggested. Landmark work followed, recognizing the underlying translocation between chromosomes 9 and 22 that gave rise to this abnormality and, shortly afterward, the specific genes involved and the pathophysiologic implications of this novel rearrangement. Fast forward a few years and this knowledge has given us the most remarkable example of a specific therapy that targets the dysregulated kinase activity represented by this molecular change. The broad use of tyrosine kinase inhibitors has resulted in an improvement in the overall survival to the point where the life expectancy of patients today is nearly equal to that of the general population. Still, there are challenges and unanswered questions that define the reasons why the progress still escapes many patients, and the details that separate patients from ultimate cure. In this article, we review our current understanding of CML in 2015, present recommendations for optimal management, and discuss the unanswered questions and what could be done to answer them in the near future.

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Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm, characterized by the unrestrained expansion of pluripotent bone marrow stem cells.[1] The hallmark of the disease is the presence of a reciprocal t(9;22)(q34;q11.2), resulting in a derivative 9q+ and a small 22q−. The latter, known as the Philadelphia (Ph) chromosome, results in a *BCR-ABL* fusion gene and production of a BCR-ABL fusion protein[2]; BCR-ABL has constitutive tyrosine kinase activity[3] and is necessary and sufficient for production of

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the disease.[4] In a few cases (5%-10%), the Ph chromosome is cytogenetically cryptic, often due to a complex translocation, and the diagnosis requires fluorescence in situ hybridization (FISH) to reveal the *BCR-ABL* fusion gene or polymerase chain reaction (PCR) to reveal the BCR-ABL messenger RNA transcript.[5] A 210-kDa BCR-ABL transcript (p210) transcribed from the most common rearrangements between exons 13 or 14 of BCR and exon 2 of ABL (known as e13a2 [or b2a2] and e14a2 [or b3a2], respectively) is most common, but rare cases will have alternative *BCR-ABL* breakpoints, leading to a p190 transcript (from the e1a2 rearrangement, most typically seen in Ph-positive acute lymphoblastic leukemia [ALL]) or a p230 transcript.[5] Indication of the typical hematopathologic features and the t(9;22)(q34;q11.2) by conventional cytogenetics or FISH and/or BCR-ABL by PCR is required for diagnosis.[5]

CLINICAL FEATURES

Up to 50% of patients are asymptomatic and have their disease diagnosed incidentally after routine laboratory evaluation.[6] Clinical features, when present, are generally nonspecific. Splenomegaly is present in 46% to 76%[6,7] and may cause left upper quadrant pain or early satiety, fatigue, night sweats, symptoms of anemia, and bleeding due to platelet dysfunction may occur, the last occurring most commonly in patients with marked thrombocytosis. Less than 5% of patients present with symptoms of hyperviscosity, including priapism, which are generally seen when the presenting white cell count exceeds 250,000/μL.[7]

The disease is classically staged into chronic phase (CP, most patients at presentation), accelerated phase (AP), and blast phase (BP).[5] Many definitions have been used for these stages, but all the data generated from the tyrosine kinase inhibitor (TKI) studies have used the historically standard definition in which AP is defined by the presence of one or more of the following: 15% or more blasts in peripheral blood and bone marrow, 20% or more basophils in peripheral blood, and platelet counts less than 100,000/μL unrelated to treatment or the development of cytogenetic evolution. The blast phase is defined by the presence of 30% or more blasts in the peripheral blood or bone marrow, the presence of clusters of blasts in marrow, or the presence of extramedullary disease with immature cells (ie, a myeloid sarcoma).[8] Progression to BP occurs at a median of 3 to 5 years from diagnosis in untreated patients, with or without an intervening identifiable AP.[6]

Presenting Hematologic Parameters

Characteristic complete blood cell count features are as follows: absolute leukocytosis (median leukocyte count of 100,000/μL) with a left shift and classic "myelocyte bulge" (more myelocytes than the more mature metamyelocytes seen on the blood smear); usual blast counts of less than 2%; nearly universal absolute basophilia, with absolute eosinophilia in 90% of cases[5]; monocytosis but generally not an increased monocyte percentage; absolute monocytosis in the unusual cases with a p190 BCR-ABL[9]; normal or elevated platelet count; and thrombocytopenia, which suggests an alternative diagnosis or the presence of AP, rather than CP, disease.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for chronic phase CML (CP-CML) includes the following Ph-negative conditions.

Chronic Myelomonocytic Leukemia

Chronic myelomonocytic leukemia is a myelodysplastic/myeloproliferative neoplasm that can be distinguished from CML by the presence of dysplastic features, more prominent cytopenias, more prominent monocytosis, and lack of basophilia. Chronic myelomonocytic leukemia is Ph negative and may have other cytogenetic abnormalities.[5]

Atypical CML

Atypical CML is a Ph-negative myelodysplastic/myeloproliferative neoplasm.

Chronic Neutrophilic Leukemia

Rare cases of CML with a p230 BCR-ABL transcript may be mistaken for chronic neutrophil leukemia (CNL) because of the predominant neutrophilia associated with this version of CML, but cytogenetics revealing the Ph chromosome will easily distinguish them. Importantly, this and other atypical rearrangements might not be detected by some standard PCR methods. The presence of these abnormalities should be suspected in instances where the Ph

TABLE 1. Definitions of Response

Response	Definition
CHR	Leukocyte count $<10 \times 10^9$/L, basophils <5%, platelets $<450 \times 10^9$/L, the absence of immature granulocytes, impalpable spleen
Minor CyR	36%-95% Ph+ metaphases in bone marrow
Major CyR	1%-35% Ph+ metaphases in bone marrow
CCyR	0% Ph+ metaphases in bone marrow
MMR	BCR-ABL International Scale ≤0.1%
CMR	Undetectable BCR-ABL with assay sensitivity ≥4.5 or 5.0 logs

CHR = complete hematologic response; CMR = complete molecular response; CyR = cytogenetic response; MMR = major molecular response; Ph = Philadelphia chromosome.

chromosome is detected by routine karyotype but with PCR "negative" for BCR-ABL, hence the importance of cytogenetic evaluation in all patients at baseline.

Essential Thrombocythemia

Rare cases of CML may present with isolated thrombocytosis, without leukocytosis. Basophilia is often present as a diagnostic clue. These cases will be distinguished by cytogenetics and molecular studies revealing Ph positivity and BCR-ABL positivity.[10]

Diagnostic Workup

The diagnosis will usually be suspected from the complete blood cell count and blood smear. FISH for t(9;22)(q34;q11.2) and quantitative reverse transcriptase PCR (qRT-PCR) for BCR-ABL can be performed on peripheral blood. However, bone marrow aspirate and unilateral biopsy with conventional cytogenetics and flow cytometry are essential at the time of diagnosis to exclude unrecognized advanced-stage disease and to detect rare cases with an alternative BCR-ABL transcript not detected by routine BCR-ABL PCR. Flow cytometry will identify cases with unrecognized progression to lymphoid blast crisis by their phenotypic features, whereas conventional karyotyping may identify additional cytogenetic abnormalities (cytogenetic clonal evolution).

DETERMINING PROGNOSIS IN CP-CML AT BASELINE

The prognosis of CP-CML has markedly improved since the development of TKIs. The stage of disease is the most important prognostic feature. Most patients presenting with CP-CML achieve long-term control, and stem cell transplant is only needed in a few. Several prognostic scoring systems have been developed to assess the risk of poor outcome at presentation: the Sokal score[11] and Hasford score were developed in the pre-imatinib era[12] but retain prognostic significance in imatinib-treated patients. An online calculator is available to compute both these scores at www.leukemia-net.org/content/leukemias/cml/cml_score/index_eng.html. Approximately 25% of high-risk patients fail to achieve complete cytogenetic response (CCyR) with imatinib-based treatment by 18 months; this and other important therapeutic milestones are discussed in detail subsequently. A simpler system based on basophil percentage in peripheral blood and spleen size, the European Treatment and Outcome Study (EUTOS) system, found that 34% of high-risk patients fail to achieve CCyR by 18 months.[13] Notwithstanding the appeal of the simplicity of the EUTOS score, its predictive value has not been universally confirmed.[14,15] The prognostic relevance of these classifications is ameliorated but not completely eliminated among patients treated with second-generation TKIs. Currently, we do not make treatment decisions based solely on these risk scores.

Other proposed pretreatment predictors include the level of CML cell membrane expression of the organic cation transporter-1 (OCT-1). OCT-1 is required for entry of imatinib into the cell; this protein (and its corresponding RNA) can be measured, and higher levels of expression and/or activity are associated with superior survival in imatinib-treated patients.[16] Importantly, patients with lesser OCT-1 activity may benefit more from higher starting doses of imatinib.[16] OCT-1 activity is not important for nilotinib-[17] or dasatinib-treated patients because these drugs are not OCT-1 substrates.[18]

RESPONSE DEFINITIONS

Dynamic response assessment is essential to identify patients at high risk of disease progression, who may benefit from a change of therapy. Response definitions are given in Table 1.[19] A complete hematologic response (CHR) is defined by clinical and peripheral blood criteria. Cytogenetic response is classified according to the percentage of Ph-positive metaphases by routine karyotype on bone marrow aspiration. A CCyR has also been defined in some instances

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by interphase FISH on peripheral blood as the absence of detectable *BCR-ABL* fusion in at least 200 examined nuclei.[20] Molecular testing for BCR-ABL transcripts using qRT-PCR is more sensitive for low-level residual disease than cytogenetics or FISH (sensitivity of 10^{-4} to 10^{-5}). Levels of response in this qRT-PCR assay are clinically relevant and reported as a percentage of the transcript levels of a normal housekeeper gene, such as *ABL1* or *BCR*. Given that assays used by different laboratories have significantly different sensitivities, attempts have been made to harmonize reporting by developing the international scale. By parallel testing of samples with a reference laboratory, laboratory-specific conversion factors are produced to correct for differing sensitivities and allow a laboratory to report BCR-ABL transcript levels in a more uniform way.[21] A World Health Organization standard material has also been developed for assay calibration.[22] Major molecular response (MMR or $MR^{3.0}$) corresponds to less than 0.1% BCR-ABL on the international scale, which represents a 3-log reduction from the standardized baseline rather than a 3-log reduction from the individual patient's baseline BCR-ABL transcript level (which can vary significantly).[23] $MR^{4.0}$ is less than 0.01% bcr-abl on the international scale, and $MR^{4.5}$ is 0.0032% or less on the international squale (equivalent to a $\geq$4.5-log reduction), which is the limit of sensitivity of many assays. There is also a fair correlation between transcript levels and depth of cytogenetic response such that transcript levels of 1% on the international scale are grossly equivalent to a CCyR.

ROUTINE MONITORING SCHEDULE

Different monitoring schedules have been proposed, with the aim of early identification of patients who are not achieving therapeutic milestones and are therefore at higher risk of disease progression. Our own practice is to monitor the complete blood cell count every 1 to 2 weeks for the first 2 to 3 months to identify treatment-related cytopenias and the achievement of complete hematologic response. Most instances of grade 3 to 4 myelosuppression occur in the first few months. Thus, once the peripheral blood cell counts become stable, monitoring with complete blood cell count can be reduced to every 4 to 6 weeks and eventually every 3 to 6 months. In addition, BCR-ABL qRT-PCR is performed from peripheral blood every 3 months until the achievement of MMR then every 6 months thereafter. We perform a bone marrow aspiration with cytogenetics every 6 months until achievement of stable CCyR. This allows not only for the confirmation of CCyR but also for the discovery of chromosomal abnormalities in the emerging Ph-negative metaphases, a phenomenon that occurs in 10% to 15% of patients and may be associated with eventual development of myelodysplastic syndrome or acute myeloid leukemia.[24-26] Subsequently, bone marrow examination only need be performed in the following circumstances: failure to achieve therapeutic milestones; evaluation of a significant, unexplained increase in BCR-ABL after initial response, not attributable to lack of adherence (see later sections for evaluation of suboptimal response or loss of response); to monitor known chromosomal abnormalities in Ph-negative metaphases; and to investigate unexplained cytopenia(s).

INITIAL TREATMENT OF CP-CML

The TKIs have transformed outcomes in CML. The pivotal International Randomized Study of Interferon and STI571 (IRIS) study found far superior rates of CHR, CCyR, and MMR in imatinib-compared with interferon-treated patients and a superior progression-free survival (PFS).[27,28] Before IRIS, other than interferon-based therapy, allogeneic stem cell transplant (alloSCT) was the treatment of choice for eligible patients and achieved long-term disease-free survival (DFS) in approximately 50% to 85% of patients[29-33] due to a graft-vs-leukemia effect.[34] AlloSCT is associated with a unique toxicity profile, particularly opportunistic infections and graft-vs-host disease, resulting in treatment-related mortality of 5% to 20% and significant morbidity in many long-term survivors. Combined with the marked success of TKIs, alloSCT is now reserved for patients with advanced-stage disease or treatment failure; this is discussed in more detail in later sections.

Which TKI and Dose?

Three TKIs are now approved by the Food and Drug Administration for initial treatment of CP-CML: imatinib, nilotinib and dasatinib. Debate continues regarding the optimal initial TKI and dose, with compelling arguments supporting each. A number of studies have

attempted to improve on results achieved with 400 mg/d of imatinib.

Shortly after imatinib was introduced as frontline therapy for CML, studies focused on use of higher doses to improve outcome.[35] The single-arm TIDEL (Therapeutic Intensification in De Novo Leukemia) study, in which patients were treated with 600 mg/d of imatinib, found superior rates of MMR at 12 and 24 months in those patients able to maintain a daily mean of 600 mg of imatinib for the first 6 months[36]; in our experience, 400 mg of imatinib twice daily was associated with superior cumulative rates of CCyR and MMR relative to a historical control cohort and was generally well tolerated, with 82% of patients continuing to take at least 600 mg/d.[37,38] In a confirmatory randomized study, the German CML study group reported that an initial imatinib dose of 800 mg was associated with higher rates of MMR at 12 months than 400 mg of imatinib or 400 mg of imatinib plus interferon alfa (59% vs 44% vs 46%). The mean daily dose tolerated in the group assigned to 800 mg of imatinib was 628 mg because of the higher adverse event profile of higher doses.[39] The higher initial dose was also associated with more rapid achievement of $MR^{4.5}$. There was, however, no event-free survival (EFS) or survival benefit, relative to 400 mg/d of imatinib.

Combinations of imatinib and interferon have been reported in several randomized trials, with mixed results. The French SPIRIT (STI571 Prospective International Randomized Trial) study and a Nordic group found higher rates of MMR at 12 months for patients receiving imatinib plus pegylated interferon alfa 2a or pegylated interferon alfa 2b, respectively, but no difference in CCyR.[40,41] In contrast, the German CML study group found no difference in MMR at 12 months between 400 mg of imatinib with or without nonpegylated interferon alfa,[39] and there was no difference in the rates of CCyR or MMR when pegylated interferon alfa 2b was combined with 800 mg/d of imatinib compared with imatinib alone.[42] All studies have found poor tolerability of interferons with high rates of discontinuation, and none have found PFS or survival benefit.

Ten years ago, the first studies using second-generation TKI as initial therapy for CML were initiated, which found very high rates of CCyR and MMR using first-line dasatinib, 100 mg/d or 50 mg twice daily,[43] and nilotinib, 400 mg twice daily.[44,45] Two major company-sponsored randomized studies later confirmed these results, comparing second-generation TKIs to imatinib, 400 mg/d. The Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients study (ENEST-nd) compared imatinib, 400 mg, to nilotinib, 300 mg, twice daily and nilotinib, 400 mg, twice daily. Nilotinib at both doses was associated with more, faster, and deeper responses and higher freedom from progression. The 400-mg twice-daily dose was associated with a small, but statistically significant, improvement in overall survival (OS) compared with 400 mg of imatinib; however, the results were also notable for a significantly higher incidence of major arteriothrombotic events, including ischemic heart disease, cerebrovascular accidents, and peripheral arterial disease (especially at 400 mg twice daily).[46] The Dasatinib versus Imatinib Study in Treatment-Naive CML Patients (DASASION) compared 400 mg/d of imatinib with 100 mg/d of dasatinib. More, faster, and deeper responses were seen, with fewer transformations to AP and BP, but at 5 years of follow-up (the end of the study), there was still no PFS or OS benefit.[47] A frequent (although usually grade 1 or 2) adverse effect of dasatinib is the development of pleural effusions, which may require dose adjustments and occasionally thoracentesis. Of some concern, also, was the development of pulmonary hypertension, which was diagnosed in 8 patients by echocardiographic criteria; however, only one patient had right-heart catheterization, which did not confirm pulmonary hypertension.

Imatinib, 400 mg, has also been compared with bosutinib, 500 mg. Faster and deeper responses, with a higher rate of MMR (but not CCyR, the primary end point) at 12 months, were seen in the bosutinib group, leading to fewer transformations. There was a higher rate of treatment discontinuation in the bosutinib arm, particularly due to diarrhea and liver function test abnormalities.[48] A second randomized trial, using a lower starting dose of bosutinib (400 mg/d), has been initiated, seeking regulatory approval for this indication.

Ponatinib is a highly potent TKI and is the only TKI with activity in patients with the T315I mutation in *ABL1*. Because of the high level of preclinical and clinical activity of

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ponatinib in the salvage setting,[49] it was also investigated as frontline therapy. Both a single-arm, phase 2 study[50] and a randomized phase 3 study were conducted, the latter comparing 400 mg of imatinib with 45 mg of ponatinib.[51] Ponatinib treatment resulted in faster and deeper responses, including very high rates of early $MR^{4.5}$. Both studies reported a 3-month rate of BCR-ABL/ABL less than 10% of 94%, the highest of any study with TKI. Unfortunately, the high rate of major arterial thrombotic events (7% in the ponatinib arm vs 1% in the imatinib arm) and pancreatitis led to the 2 studies being terminated early at a median follow-up of 23 and 5.1 months, respectively.[51]

We believe that imatinib, dasatinib, and nilotinib all constitute adequate treatment options for patients with CML at the time of diagnosis. Outside clinical trials, the decision regarding which TKI to use should be tailored to an individual patient and depends on an assessment of factors such as the relative risk of the disease, risk factors for specific adverse events (eg, arteriothrombotic events, pleural effusion, pulmonary hypertension, poorly controlled diabetes, and pancreatitis), possible effect of the dose administration schedule, and cost. In patients with a poorer likelihood of responding to 400 mg of imatinib (eg, those with high Sokal scores or those with e1a2 CML), a second-generation TKI might be preferred. Patients with low OCT-1 activity may also benefit from high-dose imatinib or a second-generation TKI, but this test is not clinically available in the United States. In contrast, in patients with lower-risk disease or those with a higher risk for arteriothrombotic events, imatinib might be preferred. Higher doses of imatinib might offer similar efficacy benefits as dasatinib or nilotinib (eg, similar rates of early responses and transformation to AP and BP).[50] Although higher-dose imatinib is associated with increased incidence of some adverse events, these usually consist of peripheral edema, muscle cramps, and gastrointestinal toxicity, but not arteriothrombotic events. Specific agents may be avoided because of their particular toxicity profiles; for example, it may be preferable not to use nilotinib in a patient with a history of coronary artery disease or with several coronary risk factors, and dasatinib may be avoided in patients who have tenuous respiratory function because of the risk of pleural effusions. Increasingly, pharmacoeconomic concerns may drive therapeutic decision making; generic imatinib will soon be available, and there will be a substantial cost differential between imatinib and second-generation TKIs, which no doubt will be a factor in the decision-making process.

TREATMENT OBJECTIVES

Response definitions according to hematologic, cytogenetic, and molecular criteria are given in Table 1.[19,52] It is important to remember that different laboratories have different BCR-ABL qRT-PCR sensitivity, and quantitative results may differ markedly.[53] If the laboratory does not report results on the international scale, BCR-ABL should be monitored in the same laboratory for consistency. In addition, MMR cannot be adequately identified if a laboratory does not report on the international scale, increasing the importance of cytogenetic analysis for response assessment.

Achievement of CCyR and $MMR/MR^{3.0}$

The European LeukemiaNet (ELN) 2013 guidelines (Table 2) place a strong emphasis on the importance of achieving MMR, ideally by the 12-month time point. This is achieved by 1 year in 18% to 58% of patients taking 400 mg of imatinib and 43% to 77% taking 600 to 800 mg.[19] This is based on data from long-term follow-up of IRIS,[54] which found that, of patients in MMR at 18 months, only 3% lost CCyR, compared with 26% of patients with BCR-ABL levels of greater than 0.1% to less than 1.0%. The key transcript levels at the 6-, 12-, and 18-month landmarks found to be associated with favorable EFS were 10% or less, 1% or less, and 0.1% or less, respectively.[54,55] Despite the importance of achieving MMR with imatinib, however, there are no data to indicate that switching therapy in a patient in the ELN warning (formerly suboptimal response) category improves outcome.[56] In addition, our own data suggest that achievement of MMR offers no EFS or survival advantage over the achievement of CCyR by 12 or 18 months during frontline treatment with second-generation TKIs; achievement of CCyR by 3 months should be considered optimal response in this setting, with PCyR considered suboptimal.[57] In a combined analysis of patients receiving either imatinib or second-generation TKIs, patients achieving CCyR by 6

TABLE 2. European LeukemiaNet (ELN) Response Criteria[a]

	Optimal	Warning	Failure
Baseline	NA	High risk or CCA/Ph+, major route	NA
3 mo	BCR-ABL1 ≤10% and/or Ph+ ≤35%	BCR-ABL1 >10% and/or Ph+ 36%-95%	Non-CHR and/or Ph+ >95%
6 mo	BCR-ABL1 <1% and/or Ph+ 0	BCR-ABL1 1%-0% and/or Ph+ 1%-35%	BCR-ABL1 >10% and/or Ph+ >35%
12 mo	BCR-ABL1 ≤0.1% (ie, MMR)	BCR-ABL1 >0.1%-1% (ie, lack of MMR)	BCR-ABL1 >1% and/or Ph+ >0% (ie, lack of CCyR)
Then and at any time	BCR-ABL1 ≤0.1%	CCA/Ph− (−7, or 7q−)	Loss of CHR Loss of CCyR Confirmed loss of MMR[b] Mutations CCA/Ph+

[a]CCA/Ph+ = clonal cytogenetic abnormalities in Ph-positive cells; CCA/Ph− = clonal cytogenetic abnormalities in Ph-negative cells; CCyR = complete cytogenetic response; MMR = major molecular response; NA = not applicable; Ph = Philadelphia chromosome.
[b]In 2 consecutive tests, at least one of which has BCR-ABL transcripts of 1% or greater.
This table was originally published in Baccarani et al.[19] © The American Society of Hematology.[19]

months have a 97% 3-year EFS on landmark analysis, which was the major point of difference, and this did not differ according to subsequent achievement of MMR or not.[58]

Several studies[38,47,55,56,59] also support achievement of BCR-ABL of 10% or less at 3 months as an important goal. Patients with this level of response at 3 months have an improved long-term outcome (EFS and OS) compared with those who have more than 10% transcripts. Although this has triggered recommendations for change in therapy for patients without this depth of response, no data suggest that the change in therapy alters the long-term outcome. Furthermore, even when those with slower responses have a worse outcome, the EFS at 5 years is approximately 80% in all series. Changing therapy for all represents an overreaction for most patients who will still have a favorable outcome. In fact, with additional assessment at 6 months, 30% to 50% will catch up in their response, and these patients have a similarly favorable outcome as if they had achieved the less than 10% BCR-ABL/ABL at 3 months.[60] Adding more than one time point thus improves the prognostication abilities of early response. The rate of change of BCR-ABL transcripts in the first 3 months of therapy may also be important; patients with BCR-ABL greater than 10% at 3 months had superior outcomes if they had a halving time of less than 76 days.[61] Patients who receive less than 80% of the target dose of imatinib, either because of dose reductions or because of missed doses, have a significantly lower probability of achieving the optimal response. Thus, at the moment, it is most prudent to minimize unnecessary treatment interruptions and dose reductions and to monitor patients carefully at early time points. No change in therapy is indicated until there is clear evidence of failure as defined by the ELN.

Definitions of Treatment Failure

Primary treatment failure can be defined as failure to achieve CHR and less than 95% Ph positive at 3 months, less than 10% BCR-ABL and Ph less than 35% at 6 months, or less than 1% BCR-ABL and CCyR at 12 months. This occurs in approximately 25% of imatinib-treated patients.[19] Progression to AP and BP defines treatment failure at any point. Secondary treatment failure is loss of response after initially meeting treatment targets. Loss of response is defined as loss of CCyR, loss of CHR, or progression to AP and BP. Loss of response should not be defined on the basis of a single qRT-PCR result due to potential fluctuations inherent in testing method. Increasing molecular markers on 2 occasions should prompt further investigation.[62,63] However, only 11% of patients in CCyR who have increasing molecular markers develop clinical events (loss of CCyR, loss of

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CHR, development of AP and BC), and switching TKIs has not been found to benefit patients with only molecular relapse but without loss of CCyR.[64] Similarly, although ELN recommends the appearance of mutations to be considered treatment failure, it is not advised to investigate the presence of mutations unless there is clinical evidence of treatment failure. Furthermore, if a mutation were to be identified in a patient with adequate response, there is no evidence suggesting that change of therapy at that time improves outcome compared with change when clinical failure becomes evident, further supporting the recommendation to only test for mutations in instances of clinical failure.

Causes of treatment failure are diverse.[65] Poor adherence is the most frequent cause of treatment failure and must be carefully evaluated. *BCR-ABL* mutations, which alter drug binding by directly altering an amino acid at the drug-binding site (eg, T315I, F317L, F359C/V) or indirectly by altering protein conformation (eg, G250E, Q252H, E255K/V), are crucial to identify because they determine sensitivity to salvage therapy and the subsequent choice of TKI. Other potential causes include pharmacokinetic interactions, such as accelerated TKI metabolism due to use of CYP3A4 hepatic enzyme inducers, or the use of proton pump inhibitors, which inhibit drug absorption. Diverse mechanisms may result in lower drug concentration within the cell despite adequate plasma levels, such as p-glycoprotein or ABCG2 drug efflux protein overexpression (affecting imatinib, nilotinib, and dasatinb), or low OCT1 activity, which is required for imatinib transportation into the cell (see earlier). Finally, overexpression of the Src kinase Lyn[66] has been reported in some instances of resistance, but the incidence of this phenomenon is unknown.

Changes in BCR-ABL transcript levels may be associated with disease progression or development of resistance. However, identification of a sustained increase and an increasing trend are more important than a single increase, given the fluctuation that can occur in the assay results. In addition, the kinetics of change in BCR-ABL may vary according to the type of loss of response: patients with a rapid increase in BCR-ABL generally have disease progression to AP and BP or are nonadherent with therapy; in contrast, patients who have developed *BCR-ABL1* mutations generally have a more gradual increase in BCR-ABL transcripts.[63] An increase in BCR-ABL on a single occasion, particularly if the increase is greater than 5-fold or if MMR is lost, should prompt questioning regarding adherence to therapy and an early additional BCR-ABL qRT-PCR. If the rise is confirmed and adherence is not thought responsible, bone marrow aspiration should be performed to assess for the presence of disease progression, cytogenetic evolution, and *BCR-ABL1* mutations. As mentioned previously, routine testing for mutations in patients with adequate response is not warranted. Even in the instance of suboptimal response or warning, mutations are identified in less than 5% of instances.

TREATMENT OF PATIENTS WITH PRIMARY OR SECONDARY TREATMENT FAILURE WHO REMAIN IN CP

Treatment of patients with refractory disease still in CP depends on several factors, particularly the type of initial therapy, the presence of *BCR-ABL1* mutations, adherence, comorbidities, and eligibility for alloSCT.[67] Patients who meet the definition of failure per the ELN have a shortened survival, with a median of approximately 5 years, and thus need a change in therapy.[68] No randomized comparisons of switching to a second TKI compared with performing alloSCT exist, but our practice is to treat with at least a second TKI; patients are closely monitored. Although eligible patients for alloSCT should be considered for this approach if meeting failure criteria after a second TKI, in practice, most patients prefer to try a third TKI; still, a discussion about alloSCT should be held after initial failure.

Switching to a Second TKI

Six-year results of switching to dasatinib after imatinib failure or intolerance have been reported and reveal PFS and OS of 49% and 71% at 6 years, respectively. The CCyR rates were less than 50%, and the MMR rate was approximately 40% in long-term follow-up[69]; importantly, early responses (BCR-ABL $<10\%$ at 3 months) predicted longer-term outcomes. Comparable results have been reported with nilotinib, 400 mg twice daily, with the option to escalate to 600 mg twice daily, with a 4-year OS of 78%, PFS of 57%, and CCyR rate of 45%.[70] Finally, bosutinib is active in imatinib-resistant patients, including all those

with ABL1 mutations except T315I, at a dose of 500 mg/d; CCyR was achieved in 41% of patients with a 2-year PFS of 73% in imatinib-refractory patients and 95% in imatinib-intolerant patients. Bosutinib has an adverse effect profile that does not overlap substantially with the other TKIs, with the most frequent adverse events being diarrhea, rash, and biochemical liver function abnormalities.[48] The drug is approved by the Food and Drug Administration for patients in whom at least one TKI has previously failed. Higher response rates to second-line TKI after imatinib failure are seen in patients with a low baseline Sokal risk score, greater depth of initial cytogenetic response with imatinib (particularly if CCyR was achieved), lack of recurrent neutropenia during imatinib therapy, and a good performance status.[71,72]

Identification of specific *BCR-ABL1* mutations is critical to subsequent TKI choice. Patients with a T315I mutation are resistant to all TKIs except ponatinib. Patients with the F317L mutation are resistant to dasatinib but responsive to nilotinib. Y253H, E255K/V, and F359V/C mutations are resistant to nilotinib but sensitive to dasatinib. There are no randomized studies to guide choice of subsequent TKI; however, changing to dasatinib is superior to increasing imatinib dose.[73] Although the three second-generation TKIs have never been compared head to head, it appears that they have somewhat equivalent efficacy and can be selected based on known mutations, risk factors for toxicity, and schedule preferences. Still, despite the overall good results, less than 50% of patients achieve a CCyR with either of these drugs. Thus, better second-line treatment options are needed. In addition, for patients treated with second-generation TKI as frontline therapy, the results with any of these agents as second-line therapy are not known but are expected to be inferior to what is achieved when used after imatinib failure. Ponatinib is a logical candidate to fill this void, but unfortunately there is limited experience in this setting. Still, in instances of resistance to a second-generation TKI used as initial therapy, we usually select ponatinib as second line provided the patient does not have excessive risk factors for arteriothrombotic events. It is clear then that, despite the many good treatment options available in CML, new drugs or new approaches would still be welcome for the relatively small percentage of patients facing this clinical scenario.

Patients in whom 2 TKIs failed have more limited options, and treatment should be individualized. In the absence of *BCR-ABL1* mutations predicted to produce resistance, nilotinib or dasatinib could be used, although there is limited, mostly retrospective, data with these agents. Bosutinib was prospectively investigated and is active in patients with failure of 2 previous TKIs, with a CCyR rate of 22% to 40% and 2-year PFS of 73%.[74,75] The PACE (Ponatinib Ph ALL and CML Evaluation) study found that 45 mg/d of ponatinib is highly active, achieving a 63% CCyR rate in a heavily pretreated population (>90% of patients had previously received at least 2 TKIs, and nearly 60% had previously received at least 3 TKIs). A subgroup analysis of the PACE study found equivalent efficacy for patients with the T315I mutation, who are resistant to all other TKIs.[76] Ponatinib has therefore been approved for patients with the T315I mutation or for whom no other TKI is indicated, under a risk evaluation and mitigation strategy, due to the risk of arterial thrombotic events.[77] Omacetaxine is a non-TKI protein synthesis inhibitor, given by subcutaneous injection for 14 days in a 28-day cycle, that is approved by the Food and Drug Administration for patients in whom 2 or more TKIs have failed.[78] In a phase 2 study in patients in whom 2 TKIs had previously failed, the rates of CHR, minor cytogenic response, and CCyR were 67%, 22%, and 4%, respectively[79]; in addition, the drug is active in patients with T315I mutation. In a separate phase 2 study, the rates of CHR and CCyR were 77% and 16%, respectively. However, PFS was only 7.7 months.[80] The drug is associated with substantial myelosuppression.[79,80] Although these results are more modest than those seen with TKIs, we use omacetaxine in instances where TKIs have failed or may not be indicated because of unacceptably high risk of specific adverse events.

AlloSCT should be considered for patients with CP-CML in whom 2 TKIs have failed. There are no data to guide the choice between third-line TKI or alloSCT, and this decision must therefore be individualized. However, the relatively low rates of CCyR and 2-year PFS with bosutinib and the risk of cardiovascular toxicity of ponatinib suggest that alloSCT should be considered in eligible patients; conversely, there are limited data on transplant outcomes

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in these heavily pretreated patients. A recent German CML study group study found that, provided they remain in CP, patients who undergo transplant after imatinib failure have excellent results post-alloSCT, with an 89% achievement of CMR after transplant, a treatment-related mortality of 6%, and a 3-year survival of 94%.[81] Whether these impressive results can be replicated in patients who have experienced resistance to 2 or more TKIs remains to be seen.

STOPPING TREATMENT IN PATIENTS WITH PROLONGED CMR

Overall, 41% to 47% of patients who have been in continuous CMR for at least 24 months may remain with stable undetectable transcripts after ceasing imatinib.[82-84] If recurrence up to the level of MMR is tolerated, the success rate increases to approximately 60%. Predictors of increased relapse likelihood in this setting include a high baseline Sokal risk score and a duration of imatinib therapy of less than 5 years.[82] Continuous CMR for more than 64 months and treatment with a second-generation TKI may be associated with lower incidence of relapse after TKI cessation.[84] Relapses occur most frequently within approximately 6 months; notably, most patients remain imatinib sensitive and regain CMR when use of the drug is recommenced.[85] However, the follow-up is still relatively short. Therefore, one needs to consider that late relapses after interferon therapy or alloSCT occurring more than 10 years after cessation of therapy may occur, and these are often in the lymphoid BP. Thus, continued monitoring is required, perhaps indefinitely, through peripheral blood PCR. Most patients who stop taking imatinib and maintain undetectable transcripts by standard qRT-PCR still have evidence of low-level disease when more sensitive, patient-specific DNA-based PCR assays are used.[83] In addition, some patients have low-level fluctuation of BCR-ABL levels detected by standard RNA-based qRT-PCR, without experiencing true molecular relapse.[85] The reasons for the lack of relapses in these patients are unclear, but it has been suggested that these patients may have an increased number of natural killer cells that may contribute to keeping the disease at bay.[86]

Although reported to be safe in relatively small numbers of patients in the clinical trial setting, this approach should only be undertaken in a clinical study or where a protocol for prospective, very close monitoring of patients is implemented to allow detection of early relapses and intervene promptly.

TREATMENT OF AP-CML

Criteria for the diagnosis of AP-CML have been outlined earlier. *ABL1* mutations increase in frequency in advanced-stage disease; mutational evaluation should therefore be performed and TKI choice based on this.[62,67] The optimal therapeutic approach in AP-CML differs according to whether the patient is TKI naive or has progressed from CP while taking a TKI. Eighty to ninety percent of treatment-naive patients will achieve CCyR with TKI[87,88] and have a similar EFS and OS to patients presenting in CP, particularly when treated with second-generation TKI. Those patients with cytogenetic clonal evolution as the only criterion for AP also have superior outcomes to those with hematologic and clinical features of AP.[89] In contrast, much lower response rates and inferior EFS, with continued relapses, have been seen in studies of second-generation TKIs in patients with imatinib failure and AP disease.[90,91]

Treatment options include a TKI or alloSCT (either de novo or after initial TKI therapy). There are no randomized data to guide the choice or dose of TKI. However, there is a suggestion from nonrandomized studies that second-generation TKIs have superior response rates to imatinib,[87] and ponatinib provides perhaps the best outcome.

There are also no randomized data to guide the decision to perform alloSCT for patients with AP-CML. In the pre-imatinib era, patients transplanted in AP had 30% to 40% DFS at 4 years compared with 70% to 80% for CP.[92,93] Nonrandomized data suggest superior outcomes in patients treated with imatinib followed by alloSCT compared with imatinib alone, but there is the standard selection bias in this study.[94]

In summary, patients with de novo AP-CML may have good outcomes, particularly if treated with a second-generation TKI. We treat these patients following the same guidelines we use for CP patients, and alloSCT is only considered on failure of 2 TKIs. However, patients with AP developing after imatinib failure have significantly poorer outcomes and may be best treated more aggressively with a second-generation TKI followed by alloSCT when eligible. Patients

with excellent, rapid responses to the second TKI may be followed up closely and alloSCT considered only if showing recurrence. Another important question for which there are no data to guide decisions is the role of maintenance TKI after transplant. Our practice is to continue prescribing TKI after transplant after count recovery for patients who previously progressed to AP or BP.

TREATMENT OF BP-CML

Criteria for BP progression were outlined above. Approximately 50% to 60% of patients have myeloid blast phase (MBP) and 20% to 30% lymphoid blast phase (LBP). The remaining 10% to 30% are mixed.[5] The aim of treatment is to achieve reversion to CP, then perform alloSCT with or without posttransplant TKI maintenance.

Treatment of LBP

Induction chemotherapy is given as per de novo ALL, with the addition of a TKI. Chemotherapy with hyperfractionated cyclophosphamide, vincristine, doxorubicin (adriamycin), and dexamethasone (hyper-CVAD) with a TKI can achieve CHR in approximately 90% of patients.[95] Most patients will have previously received a TKI. However, in patients presenting with de novo transformation, it is important (although sometimes difficult) to distinguish CML in LBP from Ph-positive ALL. Morphologic criteria to suggest preexisting CML, such as monolobated megakaryocytes and basophilia, may be useful, as is the BCR-ABL transcript type; p210 BCR-ABL is present in most CML-LBP, whereas most Ph-positive ALL has the p190 transcript. Mutations in *BCR-ABL1* in patients in whom imatinib therapy has failed are more frequent in BP (73%) relative to C and/AP[96]; the use of *ABL1* mutational analysis to guide treatment is therefore essential. T315I is very frequent and, in contrast to CP, may be identified even before exposure to a TKI. These patients require treatment with ponatinib, usually combined with chemotherapy (hyper-CVAD, in our hands). Additional chromosomal abnormalities are frequent (particularly monosomy 7),[95] and outcomes are generally poor. AlloSCT after initial response appears to improve outcomes, but selection bias in such studies is inevitable.[96]

Treatment of MBP

CML-MBP has a poor response to standard acute myeloid leukemia induction regimens.[97] Patients with de novo MBP may respond to TKI monotherapy, but responses are shallow and transient.[98,99] There are few studies of AML induction chemotherapy or low-dose cytarabine combined with TKI.[100,101] Our general approach is to give standard acute myeloid leukemia induction chemotherapy with the addition of a TKI and perform alloSCT in responding patients.[102] Although outcome for patients with prior BP is better when there is only minimal residual disease or no detectable disease even by PCR, we recommend alloSCT as soon as a patient is back to CP or has CHR because continued chemotherapy is no guarantee of improved response and may cause complications that can disqualify the patient for a later transplant.

Which TKI Should Be Used in BP-CML?

There are no head-to-head data in this area, and much existing data concern use of single-agent TKIs, which are rarely used in practice. Imatinib, 600 mg, results in shallow and transient single-agent responses.[98,99] Imatinib does not cross the blood brain barrier and so is inadequate when central nervous system involvement exists.[103,104] Dasatinib, 140 mg/d, achieves a significantly higher rate of CCyR (26% and 46% in MBP and LBP, respectively), but responses are again transient, with a median survival of less than 12 months for MBP and less than 6 months for LBP.[105] Although dasatinib crosses the blood brain barrier, we do not rely on this for prophylaxis or management of central nervous system disease and give standard treatment with intrathecal chemotherapy, high-dose systemic chemotherapy, and occasionally radiotherapy to approach this issue. Nilotinib, 400 mg twice daily, is associated with no better results compared with dasatinib and is not approved for this indication.[70] Bosutinib is also approved for BP and may induce hematologic response in 28% and minor cytogenic response in 37%.[106] Ponatinib has resulted in favorable response in heavily pretreated patients and patients with T315I mutations. Approximately 50% patients had a hematologic response after failure of dasatinib or nilotinib in MBP or LBP,[107] and 18%

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achieved CCyR. The 1-year survival was an impressive 55%. Whenever possible, we use ponatinib because this might be the most effective agent and covers all mutations. Dasatinib and bostunib are suitable alternatives.

Treatment of Refractory and Relapsed BP

Novel agents for ALL, such as the CD22-immunoconjugate inotuzumab ozogamicin and the CD3/19 bispecific antibody blinatumomab, are yet to be evaluated because major studies have excluded Ph-positive patients. However, these could potentially be effective, as could CAR T cells directed against CD19, although there would likely be potential for antigen-negative escape or development of frank myeloid reversion, and any response would require consolidation with alloSCT.

CONCLUSION

Although CML remains one of the great success stories in modern oncology treatment, a number of challenges remain. Pre-treatment identification of patients likely to have poor outcomes is crude at best, and predictive tools to guide the optimal choice of TKI at baseline are not widely available, making treatment decisions largely empiric. Patients with failure of more than 1 TKI have relatively poor outcomes, and no data exist for second-line therapy for patients treated initially with a second-generation TKI. The mechanisms underlying the risk of arteriothrombotic events seen with several of the TKIs need to be better understood so that prevention and management can be approached more rationally. Finally, most patients require indefinite suppressive therapy, with an associated cumulative risk of potential toxic effects, particularly cardiovascular disease, as well as chronic, low-grade toxic effects that affect quality of life. Strategies to produce eradication of MRD, with minimal toxic effects, are essential to address these issues and to reduce the long-term pharmacoeconomic burden of indefinite TKI therapy.

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The Symposium on Neoplastic Hematology and Medical Oncology will continue in an upcoming issue.

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