Monitoring response and resistance to treatment in chronic myeloid leukemia

S. Assouline MD MSc BSc* and J.H. Lipton MD PhD†

ABSTRACT
Chronic myeloid leukemia (CML) results from expression of the constitutive tyrosine kinase activity of the Bcr-Abl oncprotein. Imatinib, a tyrosine kinase inhibitor (TKI), is highly effective in the treatment of CML. However, some patients treated with imatinib will fail to respond, will respond suboptimally, or will relapse because of primary or acquired resistance or intolerance. Research activities focusing on the mechanisms that underlie imatinib resistance have identified mutations in the BCR-ABL gene, clonal evolution, and amplification of the BCR-ABL gene as common causes. Cytogenetic and molecular techniques are currently used to monitor CML therapy for both response and relapse. With multiple and more potent therapeutic options now available, monitoring techniques can permit treatment to be tailored to the individual patient based on disease characteristics—for example, according to BCR-ABL mutation profile or to patient characteristics such as certain comorbid conditions. This approach should benefit patients by increasing the potential for better long-term outcomes.

KEY WORDS
Chronic myeloid leukemia, protein kinase inhibitors, imatinib, drug resistance, drug monitoring

1. INTRODUCTION
Chronic myeloid leukemia (CML) is normally a triphasic disease. It starts with a relatively indolent chronic phase (CP) that can last for a number of years. If untreated, CML inevitably progresses to either or both of an accelerated phase (AP) and a blast (acute) phase (BP), the latter being associated with a poor prognosis and a median survival time measured in months 1.

The current first-line treatment for CML is imatinib mesylate (formerly called STI571). In the phase III International Randomized Study of Interferon and Cytarabine Versus STI571 (IRIS) in newly diagnosed patients with CML in CP, treatment with imatinib, compared with the previous standard treatment of interferon alfa (IFNα) in combination with cytarabine, resulted in superior outcomes, with only an estimated 7% of patients progressing to AP or BP during 5 years of follow-up 2. Highly effective second-line treatments (that is, dasatinib and nilotinib) are now commercially available, and patients that do not respond well or that are intolerant to imatinib are more likely to achieve a better long-term outcome if they switch treatment. Based on current guidelines for response milestones, about one third of patients with CP-CML experience an unsatisfactory therapeutic effect with imatinib because of failure to respond, relapse, or intolerance 2,3.

The present review describes practical methods for assessing response and resistance to imatinib, the mechanisms behind resistance, and the therapeutic options to consider after failure on imatinib.

2. DISCUSSION
2.1 What Is the Molecular Basis of CML?
Chronic myeloid leukemia is associated with the acquisition of a cytogenetic abnormality known as the Philadelphia (Ph) chromosome, resulting from a reciprocal translocation that fuses the ABL1 gene on chromosome 9 to the BCR gene on chromosome 22. Variant rearrangements involving other chromosomes may also occur. The resultant oncogene encodes a fusion protein (Bcr-Abl) with constitutively upregulated tyrosine kinase activity. By phosphorylating substrates such as Ras and phosphoinositide 3 kinase, Bcr-Abl dysregulates the proliferation, transformation, and apoptotic behaviour of hematopoietic cells (reviewed in Deininger et al. 4).

2.2 Which Tests Should Be Performed After Diagnosis?
Patients are typically diagnosed in CP (90%) 5. In most cases, the diagnosis is based on a characteristic blood count and differential (left-shifted leukocytosis). The most common physical sign, if
present, is splenomegalic; however, 40% of patients are asymptomatic6. To confirm the diagnosis, the Ph chromosome is identified by karyotyping metaphase chromosomes. However, in approximately 5% of cases, a Ph chromosome cannot be detected, and confirmation requires fluorescence in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR) to detect the BCR-ABL gene. In cases in which neither the Ph chromosome nor the BCR-ABL gene is detected, a diagnosis of CML is unlikely, and alternative diagnoses such as chronic myelomonocytic leukemia, myelofibrosis, or myelodysplastic and myeloproliferative disorders should be considered.

Cytogenetic response (CyR) to treatment for CML can be monitored using either conventional cytogenetic assessment (bone marrow metaphase cells) or FISH (peripheral blood or bone marrow, metaphase or interphase cells). Detection of BCR-ABL-positive (BCR-ABL+) cells by FISH is based on co-localization of two differentially labelled fluorochrome probes (for BCR and ABL) at the site of translocation, producing a single fused signal. However, because of false positive (random co-localization of BCR and ABL signals) and false negative (CML cells scored as normal) results, which can be as high as 10%–20%, interpretation is difficult. Automated scoring systems have been developed in an attempt to improve accuracy, but these are not widely used8.

Significant differences between FISH and conventional cytogenetics have been reported. In a study comparing peripheral blood FISH with bone marrow FISH and with conventional cytogenetics, a good correlation between procedures was observed when monitoring changes in the level of Ph-positive (Ph+) cells after therapy. However, compared with both peripheral blood and bone marrow FISH, cytogenetic analysis identified significantly higher levels of BCR-ABL+ cells. Observed differences were hypothesized to relate to the detection by FISH of non-dividing cells, including T lymphocytes in peripheral blood, which are less likely to be Ph+. A further limitation of FISH compared with conventional cytogenetics is that secondary chromosomal abnormalities that may arise at later stages of response (for example, trisomy 8, trisomy 19, or isochromosome 17q)—will not be detected using the BCR/ABL dual probe alone. As a result, periodic conventional cytogenetic analysis is required even if FISH is used for regular monitoring.

As a more sensitive alternative to FISH, quantitative RT-PCR (qRT-PCR) quantifies the level of BCR-ABL messenger RNA (mRNA) in peripheral blood by comparing transcript levels to one of several specific control genes, namely ABL, BCR, or β-glucuronidase (GUSB), among others. The results for an individual patient, expressed as a ratio of BCR-ABL transcript copies to control gene copies, can be converted to an international standard using established conversion factors. Although there is no evidence to suggest that the level of BCR-ABL in blood at diagnosis will predict how a patient will respond to treatment, continual assessment of BCR-ABL transcript levels can be used as an alternative to cytogenetic assessment for frequent monitoring.

Classical prognostic indicators such as the Sokal and Hasford scores have been used to estimate the relative risk of outcome in CP-CML, based on age, spleen status, platelet count, and the proportion of blood myeloblasts noted at diagnosis. Prognostic relevance is also attributed to cytogenetic abnormalities, the number of CD34+ cells at diagnosis, and the degree and timing of hematologic, cytogenetic, and molecular responses to treatment. Although the introduction of imatinib has to some extent attenuated the predictive value of these indices, the Sokal and Hasford scores remain the only validated predictors of response in newly diagnosed patients. Because of the prognostic value of early response to treatment and level of response achieved, cytogenetic and molecular testing to monitor both therapeutic response and level of residual disease have become crucial elements of clinical decision-making for patients with CML. Ongoing assessments allow patients who are not responding optimally to be considered for alternative treatment strategies.

2.3 How Are Treatment Responses Categorized Using Various Monitoring Methods?

The aim of current CML therapies is to inhibit Bcr-Abl activity and to lower the number of Ph+ cells. Treatment responses have been categorized in the European LeukemiaNet (ELN) and U.S. National Comprehensive Cancer Network (NCCN) guidelines. A hematologic response (HR) indicates improvement in peripheral blood cell counts and may be complete [CHR (normalized peripheral blood counts, white blood cell count below 10×10⁹/L, platelets below 450×10⁹/L, immature cells absent or normalized differential, no signs or symptoms of disease)] or partial (persistent of immature cells, platelets below 50% of pre-treatment levels but above 450×10⁹/L). A CyR defines the proportion of Ph+ cells identified in bone marrow or peripheral blood and may be complete [cCyR (a complete absence of Ph+ cells)], partial [pCyR (1%–35% Ph+ cells)], minor (36%–65% Ph+ cells), or minimal (66%–95% Ph+ cells). A major CyR (mCyR) is defined as cCyR or pCyR. Loss of CyR is considered when an increase in Ph+ metaphases of 30% or more is observed.

Molecular response defines the level of BCR-ABL gene transcripts relative to an established baseline level, determined by measuring the BCR-ABL or BCR transcript levels in blood pooled from patients with CP-CML before they start treatment. The transcript level is then standardized according to the international scale where possible. A complete absence of transcripts is defined as a complete molecular response (CMR); a
3-log decrease or a reduction to 0.1% compared with the baseline level of BCR-ABL transcripts is defined as a major molecular response (MMR) 11. Results from qRT-PCR and cytogenetic analysis correlate, with a 2-log reduction in transcripts (to 1% from baseline) roughly equating to a ccyr, and a 1-log reduction (to 10% from baseline) equating to a mcyr 17. Classification of a cmr has different implications depending on the sensitivity of the particular laboratory’s assessment. An increase in BCR-ABL transcript levels can be variable, any change should be confirmed before a subsequent treatment decision is made. Although some laboratories show very high sensitivities, a confirmed increase of at least 0.5 log is felt to be significant.

2.4 Which Response Milestones Are Most Important in Patients with CP-CML?

Based on the times taken to reach various levels of response, the eln provided guidelines for defining optimal response, failure, suboptimal response, and warning signs in patients with cp-cml 16. Although time to response does not always affect prognosis, patients who do not achieve a timely response are at increased risk of a worse long-term outcome because of intervening disease progression, and the guidelines recommend the time points that should be used to guide treatment decisions. In this context, “failure” means that continuing imatinib treatment at the current dose is no longer appropriate, and a “suboptimal response” signifies that, although these patients may still benefit from continuing imatinib, the long-term outcome of treatment is less likely to be favourable. “Suboptimal response” was defined as no cyr at 3 months, less than pcyr at 6 months, less than ccyr at 12 months, less than mmr at 18 months or loss of mmr at any time (Table i). “Failure” was defined as less than chr at 3 months, absence of cyr at 6 months, less than pcyr at 12 months, less than ccyr at 18 months, or loss of chr or ccyr at any time.

The eln definitions of suboptimal response and failure have also been cited in the European Society for Medical Oncology recommendations for cml 6. However, other guidelines, such as those provided by the nccn 13 and the Canadian Consensus Group on the Management of CML 19, proposed different milestones in some cases (Table i). It should be remembered that these guidelines and recommendations were based on responses to imatinib. For newer drugs, whose response rates may be faster, landmarks may need to be reassessed, and other standards for success and failure considered.

Preliminary data have confirmed that prognosis in patients with a suboptimal response according to eln definitions is inferior to that in patients who respond optimally. In a study of 224 patients with early cp-cml, suboptimal responders at 6 and 12 months had a significantly poorer progression-free survival and a lower probability of ccyr, and suboptimal responders

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chr = complete hematologic response (platelet count < 450×10^9/L; white blood cell count < 10×10^9/L; differential without immature granulocytes and with <5% basophils; nonpalpable spleen); hr = hematologic response; pcyr = partial cytogenetic response [1%–35% Philadelphia chromosome–positive (Ph+) cells]; ccyr = complete cytogenetic response (0% Ph+ cells); cyr = cytogenetic response; mmr = major molecular response (BCR-ABL transcript level ≤0.1 compared with a standardized control gene—that is, a 3-log lower level).
at 12 months also had a significantly lower overall survival. However, validation of the concept of suboptimal response has been hindered by low accrual in clinical trials aimed at enrolling these patients. As a result, few clinical data support treatment selection after a suboptimal response to imatinib, and only landmark analyses indicating failure are routinely used to guide patient management.

2.5 What Are the Responses Achieved with Imatinib Therapy?

Results from the IRIS trial in newly diagnosed CP-CML showed that, cumulatively, 98% of patients who received imatinib as initial therapy achieved a CHR, and 87% achieved a CCyR. The median reduction of BCR-ABL transcripts was 3.08 log at 1 year and 3.78 log at 4 years. In a separate study performed in the United Kingdom, the 5-year cumulative MMR rate in 204 CP-CML patients treated with imatinib was 50.1%, and the CMR rate (BCR-ABL undetectable) was 5%.

In the IRIS study, no patient who had achieved a CCyR and MMR at 12 or 18 months after starting imatinib therapy had progressed by 60 months. Interestingly, only 2% of patients who had achieved a CCyR but no MMR at 18 months progressed to AP or BP at 60 months, suggesting that achieving a MMR is perhaps a less important milestone once CCyR has been achieved. At 60 months, the estimated overall survival was 89%.

Some newly diagnosed patients do not achieve a CCyR, however. In the IRIS trial, an estimated 24% of patients showed primary or intrinsic resistance to imatinib and failed to achieve a CCyR at 18 months. Of the patients who achieved a CCyR, approximately 10% subsequently experienced treatment failure. In the U.K. study, the 5-year probability of patients with newly diagnosed CP-CML being in cytogenetic remission with imatinib was 62.7%.

2.6 What Are the Causes of Imatinib Failure and How Can This Be Assessed?

Mechanisms that may contribute to lack of response or relapse on imatinib include mutations in the Bcr-Abl kinase domain that prevent imatinib binding, clonal evolution, pharmacokinetic variability, amplification of the BCR-ABL fusion gene, overexpression of drug transporter genes, and overexpression of tyrosine kinases such as the Src family kinases (SFKs), and toxicities resulting in dose interruptions or reductions.

Activity of Bcr-Abl depends on the conformation of a highly conserved series of amino-acid residues comprising four regions:

- The contact binding site
- The SH2 domain
- The activation loop (A-loop) that has distinctive active and inactive conformations (imatinib competitively inhibits ATP binding by occupying the ATP binding site when the A-loop is in the inactive conformation)

Mutations in the Bcr-Abl kinase domain have been detected, on average, in approximately 50% of patients with CML and imatinib resistance. Mutations can affect residues that make direct contact with imatinib (for example, amino acid T315), rendering the active site inaccessible through steric hindrance; prevent the structural rearrangements required for imatinib binding (for example, P-loop mutations that destabilize the inactive conformation); or stabilize the active conformation of Bcr-Abl (for example, M351 and A-loop mutations)—reviewed by Apperley.

The contribution of mutations to the resistant phenotype is much lower in CP than in AP or BP, and is lower in patients with primary as compared with acquired resistance.

Current recommendations for identifying signs of primary and secondary resistance resulting from mutations were outlined in the recently updated NCCN guidelines. These recommendations suggest that screening for mutations is appropriate in patients with CP-CML who experience inadequate initial responses to imatinib therapy or who experience any loss of response.

A number of methods are available for the detection of mutations. The most common involve amplification and sequencing of the kinase domain, including direct sequencing, sequencing after subcloning of PCR products or after denaturing high-performance liquid chromatography (d-HPLC), allele-specific oligonucleotide PCR, assays based on restriction-fragment-length polymorphism, peptide nucleic acid–based clamping techniques, and pyrosequencing. The sensitivity of these tests and the range of mutations detected varies depending on the method used. For example, direct sequencing of the Bcr–Abl kinase domain will reveal emerging mutant clones once they represent more than 10%–20% of the leukemic clones, but d-HPLC has lower detection limits of 1%–10%. Results should therefore be interpreted with caution. A mutation detected in 0.5% of leukemic cells is less likely than a mutation detected in 20% of cells to be responsible for a loss of response, although recent studies have indicated that mutations that may eventually cause resistance can be detected at low levels several months before loss of response and are predictive for relapse and progression.
Clonal evolution is defined as the presence within CML cells of additional translocations that are thought to drive disease progression. Some of the most common translocations in CML are isochromosome 17q and additional Ph chromosomes that increase the expression of Bcr-Abl (reviewed by Sessions). In the pre-imatinib era, clonal evolution occurred in approximately 30%–50% of patients. Today, the true incidence of clonal evolution is not clear, but appears to be 2%–17% in imatinib-treated patients, correlating with a decreased response. Annual karyotyping of bone marrow aspirates assesses clonal evolution and, increasingly, the development of new cytogenetic abnormalities in Ph-negative (Ph−) cells. But because neither FISH nor ORT-PCR detects new chromosome abnormalities in Ph+ or Ph−cells, those techniques are not useful in screening for either event.

Decreased responses to imatinib therapy may relate to pharmacokinetic variability. Drug exposure below the target level could lead to imatinib levels that are insufficient to inhibit BCR-ABL and to achieve CCYR or MMR. However, because exposure levels have not been examined in patients on long-term therapy, results must be interpreted with caution. Reasons for low drug levels in plasma potentially include poor compliance to daily oral therapy, variations in metabolizing enzyme activity, drug–drug interactions, or food interactions. The isoenzyme chiefly responsible for imatinib metabolism is CYP3A4, whose activity can vary from patient to patient and be inhibited or induced by drugs such as rifampicin, ketoconazole, and St. John’s wort, altering imatinib whose activity can vary from patient to patient and be inhibited or induced by drugs such as rifampicin, ketoconazole, and St. John’s wort, altering imatinib.

Resistance may also be mediated in part through overexpression of other tyrosine kinases such as the SFKs. Increased expression or activity of the SFKs Lyn and Hck are seen in BCR-ABL + CML cells cultured in the presence of imatinib or obtained from patients with imatinib-resistant CML. The SFKs are involved in regulation of cell survival and proliferation, and their activation can support the antiapoptotic functions of Bcr-Abl, even in conditions in which the activity of Bcr-Abl is diminished by imatinib. In a recent study, expression of Lyn and Hck was evaluated in CML cells derived from 6 imatinib-intolerant patients and 12 imatinib-resistant patients who expressed either unmuted Bcr-Abl kinase or a mutated Bcr-Abl kinase that had negligible impact on imatinib sensitivity. Highly activated Lyn and Hck kinases detected in the imatinib-resistant CML patients were not suppressed by imatinib treatment; however, Lyn and Hck phosphorylation was suppressed in CML cells from imatinib-intolerant patients, supporting the idea that SFK activation is associated with the failure of some CML patients to respond to imatinib.

2.7 What Are the Available Treatment Options After Imatinib Resistance?

Reactivation of Bcr-Abl at the time of relapse means that imatinib at the current dose no longer represents an effective therapy. Second-line treatment options include higher doses of imatinib, a second-generation TKI, or allogeneic stem cell transplant (allo-SCT) (Figure 1). Administration of the selected second-line therapies should occur before the disease transforms into AP-CML or BP-CML.

2.7.1 Imatinib Dose Escalation

The effect of dose escalation has been investigated in a number of studies. Of the 553 patients initially randomized to receive imatinib in the IRIS trial, 106 received imatinib dose escalation to 600 mg or 800 mg daily. Approximately half the patients showed improved response within 12 months of the dose increase, and after 3 years, the overall
rate of freedom from progression to AP and BP was 89% \(^{62}\). In a study of 84 patients with hematologic or cytogenetic resistance or relapse, 40% of patients who underwent dose escalation achieved a CCyR \(^{63}\). Some reports suggest that patients who respond to increased doses of imatinib do so transiently \(^{64}\), but other studies have demonstrated durable responses of up to 5 years \(^{63}\).

### 2.7.2 Second-Generation TKIs

Second-generation TKIs, which have increased potency relative to imatinib and activity against many Bcr-Abl kinase domain mutations, have been developed as alternative therapeutic agents (reviewed in Jabbour et al. \(^{65}\)). To date, dasatinib and nilotinib have been approved for the treatment of CML in adults with resistance or intolerance to previous imatinib therapy. Dasatinib is approved for all phase of CML, and nilotinib is available for patients with CP-CML or AP-CML. Other agents are in clinical development.

**Dasatinib:** *In vitro*, dasatinib inhibits unmutated Bcr-Abl 325 times more potently than does imatinib, and it inhibits all imatinib-induced mutations investigated except T315I \(^{66,67}\). Dasatinib has a lower potency against mutations occurring in amino acids F317, V299, and E255 \(^{68,69}\). In addition to inhibiting Bcr-Abl, dasatinib has potent activity against SFKs.

The efficacy of dasatinib across all phases of CML was demonstrated in five phase II studies [Src/Abl Tyrosine Kinase Inhibition Activity: Research Trials of Dasatinib (START)] \(^{70-73}\). Initial results after 8 months of follow-up from the START-C study (CP-CML treated with dasatinib 70 mg twice daily) showed 90% of patients achieving CHR and 52% achieving MCyR. Dasatinib also induced molecular responses, reducing the median BCR-ABL/ABL transcript ratio from 66% at baseline to 2.6% at 9 months \(^{72}\). Subsequent follow-up data, reported after 15 and 24 months, showed response rates increasing with continuing treatment (MCyR 59% and 62%, and CCyR 49% and 53% respectively). The MCyR were durable, with 88% of patients maintaining response at 24 months. At 24 months, progression-free survival was 80% and overall survival (OS) was 94% \(^{74,75}\).

In the START-R trial of dasatinib in patients with CP-CML resistant to imatinib 400–600 mg daily, dasatinib treatment resulted in responses superior to those with imatinib dose escalation to 800 mg daily. After 12 weeks of treatment (primary endpoint), dasatinib treatment resulted in higher rates of MCyR (36% vs. 29%, \(p = 0.40\)) and CCyR (22% vs. 8%, \(p = 0.041\)) \(^{73}\).
After a minimum follow-up of 2 years, the ccpyr rate was 44% for dasatinib as compared with 18% for high-dose imatinib, and MMR was also more frequent with dasatinib (29% vs. 12%) 76.

In a phase iii dose-optimization trial in patients with imatinib-resistant or -intolerant cp-cml, dasatinib 100 mg once daily was found to have efficacy similar to that of the then-approved 70 mg twice-daily dose, but with less toxicity. As a result, 100 mg once daily is now the approved dose in patients with cp-cml and imatinib resistance or intolerance.

Nilotinib: Nilotinib is an analog of imatinib that, because of its better topographical fit with Bcr-Abl, is 20–30 times more potent than imatinib 66. In vitro, nilotinib inhibited all Bcr-Abl mutants tested except T315I, but it had lower potency against certain mutations occurring in the P-loop region (Y253F/H, E255K/V) and in amino acid F359 H68,69. After 6 months of follow-up in a phase ii study in which nilotinib 400 mg was administered twice daily to 280 patients with cp-cml, MMR was observed in 48% of patients and ccpyr in 31% 78. In the most recent analysis of 321 patients with a follow-up of at least 24 months, the ccpyr rate was 46%, and most responders (84%) were maintaining their ccpyr at 24 months. The estimated os rate at 24 months was 87% 79.

Bosutinib and INNO-406: Bosutinib and INNO-406, in clinical development, are dual inhibitors of the Src and Abl kinases, with greater potency than imatinib and activity against a number of mutations except for T315I 80,81. A phase i/ii study of bosutinib in patients with cp-cml after imatinib failure is ongoing. After a median duration of approximately 8 months’ treatment, 34 of 84 evaluable patients (40%) achieved MMR, including 24 (29%) who achieved ccpyr, and 20 of 60 (33%) achieved MMR 82. A phase i dose-finding study of INNO-406 in 56 patients with advanced Ph+ leukemias and resistance or intolerance to imatinib, 46 of whom had previously received second-generation TKIs, has been completed: ccyrs were seen in 3 patients with cp-cml, including one patient with cp-cml intolerant to both imatinib and dasatinib 83.

MK-0457: The small-molecule aurora kinase and Janus kinase 2 (Jak2) inhibitor MK-0457 (VX-680) has in vitro activity against cells expressing unmuted and mutated Bcr-Abl, including the T315I Bcr-Abl mutation 84. Enrolment in clinical trials involving MK-0457 was suspended after preliminary safety data indicated QTc prolongation in 1 patient 85; drug development subsequently stopped.

AP24534: The pan–Bcr-Abl inhibitor AP24534 potently inhibits unmuted and mutated variants of Bcr-Abl, including the T315I mutation. A phase i study of AP24534 in patients with hematologic malignancies is ongoing. After a median treatment duration of 3.4 months, 16 of 18 patients with cp-cml (88%) achieved chr. Of 12 patients with the T315 mutation, 9 remain on study without progression. Two patients with cp-cml and a T315I mutation achieved MCYR 86.

Interferon: Pre-imatinib, interferon alfa (IFNα) was the mainstay of cml therapy, producing a substantially better 5-year survival rate than the standard chemotherapy regimens of busulfan or hydroxyurea 87. Post-imatinib, a distinct mode of action for IFNα has provided the basis for investigating its potential role in the treatment of imatinib resistance or intolerance. Pegylated IFNα, a modification of IFNα, has an improved pharmacokinetic profile and fewer side effects. In phase i/ii studies, pegylated IFNα demonstrated significant advantages over standard IFNα, producing higher hr and cpyr rates, and greater overall survival 88,89.

Other Novel Agents: Several novel Bcr-Abl inhibitors—including SGX-393, and XL-228, which inhibit the T315I mutation—are currently in development (reviewed in O’Hare et al. 90). In addition, promising results have been observed with omacetaxine mepacrine, a semi-synthetic formulation of homoharringtonine, an alkaloid plant extract with activity independent of mutation status. In a phase i/ii study, chr was obtained in 5 evaluable patients with AP- or BP-cml who had failed prior therapy; in addition, mutations became undetectable in 2 patients who had had a Bcr-Abl kinase domain mutation at the start of therapy 91. In a phase i trial of homoharringtonine plus cytarabine in previously untreated patients with cp-cml, 36 of 44 patients (82%) achieved chr. However, the rate of MMR was much lower than that associated with imatinib 92.

2.8 Which Factors Should Be Considered When Choosing Between Second-Line Treatment Options?

At present, there are no clinical data to suggest that any second-generation TKI is better than another after imatinib failure because no head-to-head comparisons have been undertaken. However, the methods used to monitor a patient’s response to imatinib therapy could potentially be used to indicate whether a particular second-line therapy is more appropriate than another at any given time.

Mutational analyses in patients who have lost a response or who have failed to achieve a response could be used to determine the TKI best suited to overcome the mutation. For example, although allo-sct or clinical trials of novel agents might be most appropriate for patients harbouring the T315I mutation 37, patients who harbour P-loop mutations (amino acids 248–256) or other mutations with a high level of imatinib resistance would be more likely to benefit from dasatinib or nilotinib. Table ii presents
in vitro data from mutational studies with imatinib, nilotinib, and dasatinib. More recent clinical studies have shown that, although certain mutations in the P-loop (Y253F/H, E255K/V) and amino acids F311 and F359 may respond less favourably to nilotinib \(^93,94\), mutations at residue F317 may respond less well to dasatinib \(^93,95,96,97\).

Using mutational analysis to sequence \(\text{TKI}\) therapies has been considered. In a study by Shah et al., 2 patients who developed V299L mutations on dasatinib, after previously relapsing on imatinib, responded to retreatment with imatinib or nilotinib \(^98\). In a second study, mutational analysis of a patient with imatinib resistance identified multiple mutations. Dasatinib administration resulted in a \(\text{CCYR}\) that was subsequently lost after 11 months. Further screening detected F486S and V299L mutations, and dasatinib therapy was terminated. The patient did not respond to bosutinib, but when nilotinib therapy was initiated, the patient achieved \(\text{CCYR}\), and \(\text{MMR}\) \(^99\).

In a case report, sequencing of the Bcr-Abl kinase domain in a patient who had not responded within 12 months to imatinib treatment revealed an F359I point mutation. After 1 month of nilotinib therapy, the patient developed rapidly progressing clinical symptoms, and treatment was changed to dasatinib, resulting in clinical improvement \(^100\). It should be noted that sequential \(\text{TKI}\) treatment could lead to the emergence of compound drug-resistant mutations with enhanced Bcr-Abl oncogenicity \(^98\), which provides an argument for the use of \(\text{TKIs}\) in combination to lower the potential for resistance or to potentiate kinase inhibition \(^101,102\). Concerns regarding the additive toxicity associated with combination therapy have limited its implementation, however.

Selecting between treatment options may also be influenced by patient comorbidities. Dasatinib and nilotinib are both generally well tolerated, and in most cases, adverse events are manageable and resolve with drug interruption or dose reduction (or both). Pleural effusion is a rare complication of imatinib or nilotinib therapy, but has been associated with dasatinib treatment \(^103,104\). However, in the recent phase III dose-optimization study, dasatinib 100 mg once daily resulted in significantly lower rates of pleural effusion than were seen with the previously approved 70-mg twice-daily regimen (any grade: 7% vs. 16%; grades 3 and 4: 1%–2%; reported in each treatment group) and in lower rates of grades 3 and 4 thrombocytopenia (22% vs. 37%), with equivalent drug efficacy \(^77\). Despite this change, dasatinib may not be suitable for patients with pulmonary disease. Nilotinib is associated with biochemical abnormalities: serum lipase, glucose, and bilirubin elevations and magnesium and phosphate reductions have been reported \(^78,79\). Patients with a history of pancreatitis should therefore not be given nilotinib. In addition, product labelling indicates that patients with hypokalemia, hypomagnesemia, or long QT syndrome should not receive nilotinib. Because of increased bioavailability, nilotinib-treated patients should avoid food 2 hours before and 1 hour after taking their tablets \(^105\), which may affect patient compliance.

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<th>Nilotinib</th>
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Table II: Half maximal inhibitory concentration (IC\(_{50}\)) values required to inhibit cellular proliferation in Ba/F3 cells expressing unmutated Abl or common mutated Bcr-Abl proteins \(\text{in vitro}\) \(^70\).
2.9 Which Response Milestones Might Be Important During Second-Line Treatment?

Approximately half the patients on second-line TKI therapy will have incomplete suppression of the Ph+ clone in the marrow, usually without evidence of overt disease progression. Monitoring response to second-line TKI therapy requires the same tests that imatinib monitoring requires, but because responses are more rapid, testing at more frequent intervals may be appropriate. The ELN guidelines provide provisional response milestones for second-line TKIs, whereby a suboptimal response is defined as less than a CYR at 3 months, less than cCR at 6 months, or less than a MMR at 12 months, and failure is defined as no CHR at 3 months, no CYR at 6 months, less than a pCR at 12 months, or the development of new BCR-ABL mutations at any time. A prudent approach to monitoring response in a patient on a second-generation TKI would therefore be to perform a cytogenetic analysis every 3 months until cCR is attained, and every 6 months thereafter. In one study, landmark analyses were performed on data from patients receiving second-line TKI therapy (nilotinib, n = 43; dasatinib, n = 70) after imatinib failure. Patients achieving MCR after 12 months of therapy had less chance of progression to AP or BP and had a significant survival advantage over patients who achieved a minor CR or CHR only. An early CYR was strongly predictive of achieving MCR by 12 months, with fewer than 10% of patients who failed to achieve CYR at 3–6 months going on to attain MCR at 12 months. The results of that study support ELN recommendations that patients that fail to respond with dasatinib or nilotinib at 3–6 months should be considered for allo-SCT if eligible.

2.10 When Should Allo-SCT Be Considered?

The timing of a decision to consider allo-SCT for patients with CML is a matter of debate. Although allo-SCT remains the only curative therapy for CML, the results obtained using second-line TKIs have displaced allo-SCT to third-line treatment or later. When determining the optimal timing of allo-SCT, regular monitoring may help to identify patients who should receive early allo-SCT (younger patients with an available donor) and those who should receive a second-generation TKI. If a second-generation TKI is used for young patients with an available donor, the window allowed for response should be short (for example, 3–6 months). The NCCN guidelines suggest that allo-SCT should be considered for eligible patients who are not in hematologic remission or are in hematologic relapse 3 months after primary imatinib treatment; in patients with no CYR or in cytogenetic relapse at 6, 12, and 18 months after an initial response; in patients with a T315I mutation; and in patients presenting with or progressing to BP or AP on treatment with a TKI. In such cases, the decision to proceed with allo-SCT will depend on donor availability, patient age, and patient compliance.

2.11 Is There a Point at Which Therapy Can Be Safely Stopped?

If durable CYR is maintained, or BCR-ABL becomes undetectable, one question that may arise is whether therapy can be safely stopped. Despite the increasing sensitivity of available monitoring methods, residual leukemic cells capable of expansion in the absence of therapy are likely to persist. A few cases of patients successfully stopping therapy after treatment with imatinib have been reported (reviewed in le Coutre et al.), and prospective trials are investigating imatinib discontinuation in patients with at least 2 years of undetectable Bcr-Abl transcripts. However, until more is known about the long-term stability of responses off-therapy, patients should continue to receive treatment and stop only if under the supervision of a clinical study.

3. CONCLUSIONS

Although imatinib is a highly effective treatment for CML, resistance and intolerance remain major clinical concerns. Regular monitoring will identify patients who fail to reach response milestones and may help to identify the factors associated with or contributing to imatinib resistance. Practical monitoring of response, resistance, and intolerance can be used to guide treatment choices over time so that patients have the chance of a significantly better long-term outcome.

4. CONFLICT OF INTEREST DISCLOSURE

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5. REFERENCES


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Correspondence to: Sarit Assouline, Division of Hematology, Jewish General Hospital, 3755 Cote Ste Catherine, Suite E-725, Montreal, Quebec H3T 1E2.

E-mail: sarit.assouline@mcgill.ca

* Department of Medicine and Oncology, McGill University, Jewish General Hospital, Montreal, QC.
† Department of Medical Oncology and Hematology, Princess Margaret Hospital, University of Toronto, Toronto, ON.