Chronic myelogenous leukemia (CML): resistance to tyrosine kinase inhibitors

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Introduction

Chronic myelogenous leukemia (CML) is attributed to the chromosomal translocation t(9;22)(q34;q11), yielding the Philadelphia (Ph) chromosome. This translocation generates a fusion gene that encodes BCR-ABL, a constitutively active protein tyrosine kinase. Signal transduction pathways stimulated by BCR-ABL kinase activity promote cell survival and proliferation while inhibiting apoptosis [1]. The discovery of the BCR-ABL-mediated pathogenesis of CML provided the rationale for the design of an inhibitory agent that targets BCR-ABL kinase activity [2].

Imatinib mesylate (Glivec®, Novartis, Basel, Switzerland) is a selective inhibitor of ABL and its derivative BCR-ABL, as well as other type III tyrosine kinases. It is effective as a single agent in the treatment of CML patients, with the most encouraging results seen in patients in chronic phase (CP) disease. A minority of CML patients in CP and a substantial proportion in advanced disease phases are either initially refractory to imatinib treatment or lose imatinib sensitivity over time and experience relapse. Resistance to imatinib in patients has been associated with a heterogeneous array of mechanisms that range from non-specific multi-drug resistance to BCR-ABL inherent genetic alterations. The most frequently identified mechanism of acquired imatinib resistance is BCR-ABL kinase domain point mutations that impair imatinib binding, either by interfering with an imatinib binding site or by stabilising a BCR-ABL conformation with reduced affinity to imatinib [3].

Resistance to imatinib in CML defined

Resistance to imatinib can be categorised according to the time of onset: primary (intrinsic) resistance is a lack of efficacy from the onset of treatment with imatinib and secondary (acquired) resistance (relapse) is defined as an initial response followed by a loss of efficacy with time of exposure to imatinib.

According to the clinical and laboratory criteria used for detection, resistance should be further subdivided into hematologic, cytogenetic, and molecular resistance, i.e. persistence of residual BCR-ABL transcripts. In CP, hematological resistance is defined as the lack or loss of normalisation of peripheral blood counts, the differential leukocyte count and spleen size. In advanced phases of CML, hematologic resistance means a lack of a return to CP or hematologic relapse after initial response.

Cytogenetic resistance can be defined according to the level of cytogenetic response that is the aim of therapy at certain time points. It can be lack or loss of major cytogenetic response [(MCR) ≤35% Ph-positive metaphases] or complete cytogenetic response [(CCR) 0% Ph-positive metaphases].

Molecular resistance is defined as lack or loss of complete molecular response. In qualitative terms, complete molecular response means undetectable BCR-ABL transcripts by reverse transcriptase polymerase chain reaction (RT-PCR). Positive results must not be judged as relapse or lack of major molecular response (MMR) unless detected in at least two consecutive samples after more than one negative result obtained using nested PCR. Major molecular response is defined quantitatively as a >3 log reduction of BCR-ABL transcripts or a BCR-ABL/ABL ratio of <0.1% [4, 5]. Molecular relapse, in complete cytogenetic responders, is considered to be an increase in BCR-ABL transcript levels by 0.5–1 log.

Resistance to imatinib monotherapy

Chronic phase CML

Early phase II studies involved CP CML patients in whom interferon-alpha (IFN)) therapy had failed. Of 454 patients with CP CML who were treated with imatinib following IFN failure or intolerance, 5% did not achieve a complete hematologic response (CHR) and 40% had less than a MCR after a median follow-up of 18 months, suggesting the presence of primary resistance to imatinib. Twenty-four-month follow-up data demonstrate that the overall rate of failure to achieve CHR was 4% and failure to achieve MCR was 36% among patients in CP previously treated with IFN. Rate of secondary resistance or relapse was approximately 13% [6].

The International Randomized Study of Interferon and STI571 (IRIS) investigated imatinib efficacy in 553 patients newly diagnosed with CP CML and treated with imatinib without prior therapy. The rate of primary resistance to achieving a CHR was ~5% after 18 months. The estimated rate of failure to achieve a MCR was 12% after 24 months of follow-up. The estimated rate of relapse or progression was 10% after 24 months in those treated with imatinib as first-line therapy [7]. Taken together with the phase II study results, the probability of refractoriness or relapse during imatinib therapy is lower among newly diagnosed CML patients.
treated with imatinib without prior therapy than among patients previously treated with IFN.

In the IRIS study, 39% of newly diagnosed CP CML patients achieved a 23-log reduction in the level of BCR-ABL transcripts after 12 months of imatinib therapy as detected by quantitative RT-PCR. Among patients with CCR and a 23-log reduction in BCR-ABL, the probability of progression-free survival at 24 months was 100% compared to 95% in patients with CCR but <3-log reduction of BCR-ABL levels [4].

**advanced phases of CML**

The rates of both primary and secondary resistance to imatinib increase with CML disease progression. In a phase II study of 181 accelerated phase (AP) CML patients, imatinib treatment failed to achieve CHR in 66% of patients and failed to attain MCR in 76% of patients at 12 months [8]. A phase II trial involving 229 myeloid blast crisis (BC) CML patients taking either 400 mg/day or 600 mg/day of imatinib resulted in an ~93% failure rate to achieve a CCR. Eighty-four per cent failed to reach MCR [9]. Together these studies demonstrate that rates of resistance and relapse directly correlate with disease progression.

The trials provide evidence that relapse and resistance rates in CP CML are lower with first-line imatinib therapy compared with treatment after failure with IFN [10]. This may reflect the second observation emerging from these trials, which is that rates of resistance and relapse increase with CML disease progression. Among patients who achieve cytogenetic response, molecular responses are linked with lower rates of resistance and relapse. Early molecular responses correlate with prolonged cytogenetic responses and cytogenetic responses are associated with long-term survival [4, 5].

**assessing resistance**

Periodic (every 6–12 months) cytogenetic monitoring for karyotypic abnormalities is critical throughout imatinib therapy to detect clonal evolution even in cases of early CCR. In several studies the propensity for relapse in the subset of patients with both a cytogenetic and molecular response was lower than for those with only a cytogenetic response.

Once cytogenetic response is achieved, minimal residual disease can be assessed by molecular monitoring. Detecting BCR-ABL transcripts with a degree of sensitivity that defines a MMR requires methodology capable of detecting a single BCR-ABL-positive cell among 10^5–10^6 normal cells. A quantitative PCR reaction including ABL amplification as an internal control can be used to express BCR-ABL as a function of total ABL.

Amplification of the BCR-ABL gene can be determined by interphase fluorescence in situ hybridization (FISH) using fluorescently labeled probes for BCR and ABL genes [11, 12].

**BCR-ABL mutations**

Detection of BCR-ABL mutants prior to and during the course of imatinib therapy may aid in risk stratification as well as in determining therapeutic strategies. A screen for mutations is indicated in patients lacking or losing hematologic response. Mutations are more frequent in relapsed as compared to primary resistant patients. The observation that 89% of patients with mutations eventually relapsed suggests that harbouring any BCR-ABL mutation has prognostic value with respect to disease progression. Thus, search for mutations could be performed even when a 3-log reduction in BCR-ABL transcripts is not achieved or there is a two-fold increase in BCR-ABL transcript levels. Conversely, the likelihood of detecting BCR-ABL mutations increases with CML disease progression. In early CP, mutations are rare. The proportion of patients with mutations in late CP, AP and BC certainly depends on the inclusion criteria for such molecular studies and ranges between 22 and 53%.

Mutations can be reliably and sensitively detected by selection and expansion of specific clones followed by DNA sequencing. However, this process is too cumbersome and labour intensive to be routinely feasible. Alternatively, sequencing of nested-PCR-amplified BCR-ABL products can reveal mutations with a high degree of sensitivity and fidelity. Highly sensitive detection methods can increase the detection rate of point mutations. For example, allele-specific oligonucleotide PCR methods or the analysis of a significant number of clones have permitted the detection of BCR-ABL kinase domain mutations prior to imatinib therapy in patients with CML and acute lymphoblastic leukaemia (ALL). Mutations can also be detected in an automated manner by denaturing high performance liquid chromatography (D-HPLC). D-HPLC compares favourably to DNA sequencing as samples can be rapidly analysed for single nucleotide polymorphisms [13, 14].

Screening for mutations from samples obtained from imatinib-resistant patients has thus far been confined to analysis of products amplified using primers spanning various regions of the BCR-ABL kinase domain (amino acids 240–500). To date, >35 different point mutations that code for distinct single amino acid substitutions in the BCR-ABL kinase domain have been isolated from relapsed CML patients resistant to imatinib treatment as well as from patients with ALL [11, 12, 15–17]. Preclinical studies have demonstrated that mutations outside the kinase domain can also result in molecular conformations of BCR-ABL that impair imatinib binding [18]. Therefore, screening for mutations outside the kinase domain may be necessary in the future to fully account for imatinib resistance in patients. It is also important to note that in certain instances BCR-ABL point mutations may accompany, but cannot explain, resistance to imatinib.

**clinical management**

The goals of CML therapy and the methods used to monitor response can influence clinical management decisions. The following recommendations to avoid or combat imatinib resistance are based on evidence obtained with imatinib in clinical trials combined with laboratory observations on samples from imatinib-treated patients and preclinical studies.

Phylaxis against resistance is favoured with early diagnosis and prompt first-line imatinib therapy using optimal doses. Rational approaches to confront resistance include dose-escalation, combination therapy, and in some cases interruption of therapy depending on the underlying cause of resistance. Several mechanisms of imatinib resistance may operate within...
an individual and these may interact with each other. Therefore, some of these recommendations may necessitate concomitant application. For example, dose escalation in combination with other agents may be a strategy to address resistance arising from multiple mechanisms.

**strategies to prevent resistance**

**optimal dosing at diagnosis**

*Clinical evidence.* It is more likely that maximal depletion of leukemic cells with imatinib will be accomplished by administering full therapeutic doses as early as possible. Clinical data show that early use of imatinib for CML results in rapidly achieved, robust response rates with an encouraging duration. Cytogenetic responses and survival rates are higher with first-line imatinib use in early CP CML rather than in late CP after IFN therapy had failed.

There have been anecdotal reports of resistance developing in patients who received suboptimal doses of imatinib at the outset of treatment or who did not comply with treatment. Starting with at least the approved imatinib dosage—400 mg/day for CML in CP and 600 mg/day for advanced disease—can aid in diminishing relapse risk.

In the phase I dose-escalating trial of imatinib for treatment of CP CML, up to 1000 mg/day was administered without identification of a maximum-tolerated dose; the efficacy results showed a dose–response relationship. While the current standard dose is 400 mg once daily, the safety and toxicity profile of imatinib indicates that considerably higher doses are tolerable [19, 20].

The use of 800 mg/day imatinib as first-line therapy in newly diagnosed early CP CML has been investigated. One-hundred-and-fourteen patients were treated with 400 mg imatinib twice daily. Ninety per cent achieved a CCR, defined as 0% Ph-positive cells. After a median follow-up of 15 months, no patient progressed to advanced disease. Significantly improved rates of CCR and MMR were achieved with 800 mg/day imatinib compared with historical data from patients treated with 400 mg/day [21]. These studies suggest that either in newly diagnosed CML without prior therapy or subsequent to IFN failure, imatinib dose escalation may be the optimal approach to avoid imatinib resistance.

**strategies to treat resistant patients**

**dose escalation**

*Clinical evidence.* To investigate whether imatinib-resistant patients benefit from escalated dosing, 54 CML patients in CP, resistant or refractory to imatinib, were given 300 or 400 mg/day imatinib doses escalated from once to twice daily. CHR were achieved in 65% of patients treated for hematologic resistance. Of patients treated for cytogenetic resistance, 56% achieved CCR, demonstrating that CP CML patients resistant to imatinib can be brought into response by increasing the imatinib dosage [22].

Previously mentioned phase II trials of imatinib demonstrated efficacy and feasibility of increased dosing in advanced phases of CML. Two-year follow-up data of AP patients taking 400 mg/day indicates that 87% failed to reach CCR and 82% failed to reach MCR, whereas among the group taking 600 mg/day, 76% failed to achieve CCR and 67% failed to reach MCR. After 24 months, the estimated progression-free survival is 32% and 49% for the 400 mg/day and 600 mg/day doses of imatinib, respectively [8]. Two-year follow-up results among patients in BC taking 400 mg/day imatinib indicate that 97% failed to reach CCR and 94% failed to reach MCR compared with the group taking 600 mg/day in which 92% failed to achieve CCR and 82% failed to reach MCR, supporting dose escalation to minimise resistance in advanced phases of CML [9].

**Rationale.** Mechanisms of imatinib resistance that have the theoretical potential to respond to increasing concentrations of imatinib have been observed in patients and may explain the encouraging results observed with dose escalation. BCR-ABL kinase domain mutations are the most frequent mechanism associated with relapse during treatment with imatinib.

Approximately 60% of relapsed patients have point mutations detected in one of three main regions of the BCR-ABL kinase domain: (i) the P-loop, (ii) the catalytic domain and intervening sequences containing amino acids that contact imatinib, as well as (iii) the activation loop. Resistance is acquired by the selective expansion of clones bearing BCR-ABL point mutations less sensitive to imatinib compared with wild type.

The relationship between imatinib resistance and the appearance of point mutations in BCR-ABL is not clearly understood because some kinase domain mutations isolated from patients have near wild-type sensitivity to imatinib. This suggests that additional mechanisms of resistance may operate in these cases. For example, BCR-ABL genomic amplification and overexpression of BCR-ABL transcripts have also been reported in resistant patients. As these mechanisms of imatinib resistance retain dependency on BCR-ABL kinase activity, they are likely to respond to increasing concentrations of imatinib.

Multi-drug-resistance mechanisms are potentially involved in resistance to imatinib in patients by virtue of their ability to limit the amount of intracellular drug concentration. Low levels of the multi-drug-resistance protein MRP1 have been shown to predict imatinib responses in patients with myeloid BC CML. Overexpression of P-glycoprotein, a MDRI gene product that functions as a drug efflux pump, has also been suggested to play a role in imatinib resistance [23].

Another mechanism of pharmacological interaction that may influence imatinib responses is the plasma levels of 2′-acid glycoprotein (AGP), which binds imatinib and sequesters it from cells. It is conceivable, however, that increasing imatinib concentrations can assist in overcoming resistance attributable to elevated serum AGP [24].

**interruption or cessation of imatinib therapy**

*Clinical evidence.* Complete cessation or temporary interruption of imatinib therapy can be considered in certain instances of resistance. Discontinuation of imatinib therapy was found to significantly reduce a clone of cells bearing a BCR-ABL Y253H (P-loop) mutation in a resistant patient [25]. Further, a patient taking imatinib in CP progressed to BC and subsequent withdrawal of imatinib resulted in a spontaneous reversion to CP.

**Rationale.** Cessation or interruption of imatinib therapy is most likely to be beneficial where relapse can be attributed to the
expansion of an imatinib-resistant clone owing to BCR-ABL point mutation that severely impairs imatinib binding. Due to the high frequency of binding impairing mutations such as E255K or Y253F and the poor prognosis portended by P-loop mutations, it is conceivable that cessation or interruption of imatinib will become an important tool for clinical management of resistant patients. This approach relies on the reappearance of non-mutant BCR-ABL leukemic clones to suppress the mutant clone by removing its competitive advantage. Y253 was predicted to be an important point of interaction between BCR-ABL and imatinib based on crystal structure analyses of imatinib bound to ABL. Mutation T315I, which is frequently observed in resistant patients, is located in a predicted imatinib-binding site not in the adenosine triphosphate (ATP)-binding domain. This mutation has also been shown to confer complete insensitivity to imatinib. It also has diminished intrinsic kinase activity owing to a decreased affinity for ATP.

**upfront combination therapy**

*Clinical evidence.* Combination therapy is a third option to consider in combating imatinib resistance [26–28]. Studies (e.g. the CML Study IV of the German CML Study Group) are currently underway to directly compare imatinib monotherapy (regular and high dose) versus imatinib plus cytarabine versus imatinib plus IFN as first-line therapy in thousands of randomised newly diagnosed BCR-ABL-positive CML patients [29].

**second-line combination therapy**

*Clinical evidence.* While clinical data for second-line combination therapy are limited, this approach must be considered when imatinib monotherapy fails, despite imatinib dose-escalation, to achieve MCR after 6 months, CCR after 12 months, a 3-log reduction after 18 months or relapse ensues. In a significant proportion of patients, lack of cytogenetic response is associated with the development of cytopenias, which occurs frequently (15–40% of CP patients) [30] during imatinib therapy. Combination therapy with imatinib plus granulocyte colony stimulating factor (G-CSF) resulted in MCR in 7 of 11 CP or AP patients, who had failed to achieve CR after 6 months of imatinib monotherapy.

*Rationale.* Combination therapy with imatinib and other agents best targets BCR-ABL-independent resistance including disease progression arising from clonal evolution or activating mutations downstream of BCR-ABL. In addition, combination therapy may address BCR-ABL-dependent refractoriness or relapse arising from BCR-ABL point mutations or atypical BCR-ABL fusion genes that abrogate sensitivity to imatinib.

Disease progression in CML is associated with non-random, consistently observed, karyotypic abnormalities referred to as clonal evolution. Between 60% and 80% of CML patients who progress to more advanced stages of disease exhibit secondary chromosomal abnormalities in addition to the Ph chromosome [31]. Clonal evolution has been demonstrated to occur during imatinib therapy and to be associated with disease progression [31–34].

Recent reports indicate that chromosomal abnormalities are also emerging in Ph-negative cells in CML patients on imatinib therapy [33, 35, 36]. The underlying mechanism for the appearance of these clones during imatinib treatment is not completely understood. Imatinib may reveal the presence of pre-existing chromosomally abnormal cells. The emergence of cytogenetically unrelated Ph-negative clones with additional aberrations in CML may support the multi-step model of leukemic transformation and the concept of genetic instability inherent in CML. Alternatively, these cells could arise as a consequence of the hematopoietic proliferative pressure applied to normal cells under conditions where Ph-positive cells are eradicated. That the same chromosomal abnormalities in Ph-negative cells have emerged with treatments for CML other than imatinib in patients with cytogenetic responses suggests that it is unlikely that imatinib is responsible for the initial presence of these chromosomal abnormalities. Most patients developing chromosomal abnormalities in Ph-negative cells were not found to progress until now. Whether proliferation of these abnormal Ph-negative clones contributes to relapse or the development of other clonal hematologic disorders, such as myelodysplastic syndromes, during imatinib therapy will require further investigation.

**new BCR-ABL inhibitors**

Two investigational small molecule ABL kinase inhibitors, dasatinib (BMS-354825) and nilotinib (AMN107), have shown efficacy in phase I clinical trials for the treatment of imatinib-resistant CML and are being further evaluated clinically. Long-term efficacy of these new inhibitors remains to be determined.

Dasatinib is a thiazolecarboxamide that is structurally unrelated to imatinib. Co-crystal analysis has shown that the compound binds to the ABL kinase domain in the active (open) conformation and also inhibits SRC family kinases. Preclinical studies have revealed the compound to be ~300-fold more potent than imatinib and to harbour potent inhibitory activity against nearly all imatinib-resistant mutants tested [37].

Nilotinib is an aminopyrimidine that is a structural derivative of imatinib. Like imatinib, nilotinib binds the ABL kinase domain in the inactive conformation, but with ~25-fold increased potency relative to imatinib. Importantly, this compound harbours activity against most imatinib-resistant mutations tested [38].

Preliminary data of phase I and II studies with dasatinib and nilotinib show encouraging hematologic and cytogenetic response rates with good tolerability. The majority of patients with known kinase domain mutations responded. No response was observed in a patient with the T315I mutation.

**treatment of minimal residual disease**

The criterion for complete molecular remission, undetectable BCR-ABL-positive leukemic cells, is associated with continued remission [4]. In the IRIS study, <3% of patients taking imatinib had undetectable BCR-ABL indicating that this standard is rarely attained with imatinib therapy. Current limits of PCR sensitivity imply that even with a complete molecular response a body load of 10^6 leukemic cells potentially remain. The persistence of leukemic cells after imatinib therapy raises questions as to whether and how to treat minimal residual disease.
Analysis of peripheral blood and bone marrow samples from CP CML patients has demonstrated the existence of primitive quiescent Ph-positive stem cells (CD34+) [39] that have been postulated to contribute to residual disease because their proliferation can be induced by exposure to growth factors. BCR-ABL-positive hematopoietic progenitor cells persist in CML patients with growth factors and imatinib. Imatinib appeared to have only an anti-proliferative effect on the quiescent cell subpopulation suggesting that cell-cycle-arrested stem cells are resistant to imatinib. Another study however, found no correlation between the proliferative status of BCR-ABL-positive cell lines and imatinib-induced apoptosis. A plausible explanation for these results is that a molecular mechanism rather than quiescence is responsible for imatinib insensitivity in stem cells [40].

Treatment of minimal residual disease should consist mainly of continued inhibition of BCR-ABL kinase activity with imatinib combined with molecular surveillance. Future trials will determine the duration of treatment with imatinib necessary to sustain molecular response. Combination therapy may prove to be important in addressing the transition from minimal residual disease to resistance and eventual relapse. Trials are underway to evaluate combination therapies with imatinib and chemotherapeutic agents as first-line therapy. Whether resistance and relapse rates are reduced with combination therapy as a consequence of reducing minimal residual disease compared with imatinib monotherapy is currently an open issue.

conclusions and new directions

Molecularly targeted therapy with imatinib has improved treatment of CML, particularly for CP patients. Emergence of resistance and relapse indicates that adaptation nevertheless plays a role in the complex interaction between imatinib and the CML disease process. Accumulating experience has provided insight into the underlying mechanisms of risk factors for imatinib resistance. These have guided recommendations for the management of patients in all phases of CML treated with imatinib. The outlook for long-term treatments is likely to involve combination therapies with cytotoxic agents or agents that target multiple sites along the BCR-ABL signal transduction pathway. The oncogenicity of BCR-ABL will likely mandate that inhibition of its kinase activity will remain as the cornerstone of treatment for CML. Novel inhibitors demonstrating encouraging efficacy with low toxicity are in clinical evaluation. Improvements in the technologies used to characterise disease and monitor response, when integrated with results from clinical trials, will facilitate the design of future strategies aimed at optimising the prognosis for patients with CML.

acknowledgements

The review was supported by the Competence Network ‘Acute and chronic leukemias’, sponsored by the German Bundesministerium für Bildung und Forschung (Projekträger Gesundheitsforschung; DLR e.V.–01 G19980/6) and the European LeukemiaNet.

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Volume 17 | Supplement 10 | September 2006


