Management of imatinib-resistant patients with chronic myeloid leukemia

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Abstract: Since its approval in 2001 for frontline management of chronic myelogenous leukemia (CML), imatinib has proven to be very effective in achieving high remission rates and improving prognosis. However, up to 33% of patients will not achieve optimal response. This has led researchers to develop new second- and third-generation tyrosine kinase inhibitors. In this article, we review the mechanisms of resistance, recommendations for monitoring, assessment of milestones, and management options for patients with CML who are resistant to imatinib therapy. We further explain the potential pitfalls that can lead to unnecessary discontinuation, the prognosis of patients whose condition fails to respond to treatment, and the upcoming therapies.

Keywords: CML, imatinib resistance, treatment, tyrosine kinase inhibitor

Introduction

Imatinib mesylate (Gleevec, Novartis Pharma, New Jersey, USA) is a first-generation tyrosine kinase inhibitor (TKI) that was approved for frontline therapy in patients with chronic myeloid leukemia (CML) by the US Food and Drug Administration (FDA) in 2002. It is dosed at 400 mg daily in patients with CML in the chronic phase (CML-CP) and 600 mg daily in patients with CML in the accelerated phase (CML-AP) and for those in the blast phase (CML-BP). Imatinib mesylate is a BCR-ABL-targeted therapy and considered the standard of care in CML management. It has shown to produce superior results in terms of response rates, prognosis, and side-effect profile compared with the previously accepted standard, combination therapy with interferon and cytarabine.

The pathogenesis of CML involves a characteristic genetic abnormality: the fusion of the Abelson murine leukemia (*ABL*) gene in chromosome 9 with the breakpoint cluster region (*BCR*) gene in chromosome 22. This balanced translocation between chromosomes 9 and 22 [t(9;22)(q34;q11)], termed the Philadelphia (Ph) chromosome, is the hallmark of CML. The fused *BCR-ABL* gene encodes the 210 kDa BCR-ABL fusion oncoprotein, which contains the activated tyrosine kinase region of ABL. This *p210BCR-ABL* is a deregulated, constitutively active tyrosine kinase that promotes growth and replication by causing aberrancies in proliferation, apoptosis resistance and adhesion through interference with β-integrin signaling and with multiple downstream pathways, such as Janus kinase signal transducer and activator of transcription (Jak-STAT), Phosphatidylinositol 3-kinases – protein kinase B (PI3K/Akt), JUN kinase, MYC, Wnt-β-catenin and Ras-Raf-MAPK signaling routes.

Imatinib works through competitive inhibition at the adenosine triphosphate (ATP) binding site of the BCR-ABL protein, which results in the inhibition of phosphorylation of proteins involved in BCR-ABL signal transduction. It shows specificity for BCR-ABL but also the receptor for platelet-derived growth factor (PDGF), and c-kit tyrosine kinases [Druker and Lydon, 2000]. The BCR-ABL inhibition results in apoptosis of the hematopoietic cells that express BCR-ABL without affecting the normal cells [Deininger *et al.* 1997; Druker and Lydon, 2000].

Despite the positive results obtained in previous studies, approximately 33% of patients with CML treated with imatinib do not achieve a complete cytogenetic response (CCyR), while others have drug resistance or cannot tolerate drug-related toxicities [Bixby and Talpaz, 2009; Hochhaus *et al.* 2009b; Kantarjian *et al* 2011.]

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Causes for imatinib treatment failure

Mechanisms of resistance

There are two categories of resistance: primary resistance is the failure to achieve any of the landmark responses established by the European LeukemiaNet (ELN) or National Comprehensive Cancer Network (NCCN) guidelines. Primary resistance can be further divided into primary hematologic resistance, which occurs in 2–4% of cases who fail to normalize peripheral counts within 3–6 months of initiation of treatment; or primary cytogenetic resistance, which is more common, and occurs in approximately 15–25% of patients who fail to achieve any level of cytogenetic response (CyR) at 6 months, a major CyR (MCyR) at 12 months or a CCyR at 18 months [Shah, 2007]. Secondary resistance occurs in those who have previously achieved and subsequently lost their response in accordance with those guidelines. The mechanisms of resistance to imatinib can be either *BCR-ABL* dependent (gene amplification or point mutations) or *BCR-ABL* independent.

BCR-ABL-dependent mutations

Point mutations in the *BCR-ABL* kinase domain (KD) can lead to imatinib resistance, particularly secondary resistance, and are responsible for treatment failure in many cases [Bixby and Talpaz, 2009; Branford *et al*. 2003; Hochhaus *et al*. 2002; Jabbour *et al*. 2006; Lee *et al*. 2008; O'Hare *et al*. 2005]. Numerous mutations have been characterized throughout the *ABL* sequence, including the ATP phosphate-binding loop (P-loop), and substrate-binding site mutations. Point mutations can change the conformation of the BCR-ABL oncoprotein to the active form, causing a conformational change in the imatinib binding site, prohibiting imatinib binding [Lee *et al*. 2008], and can also eliminate critical molecules required for bonding, thus mitigating its efficacy [Bixby and Talpaz, 2009; Deininger *et al*. 2005; O'Hare *et al*. 2005; Shah, 2005].

Over 100 different point mutations have been identified so far and important examples include T315I, Y253H and F255K, among others. T315I and certain mutations occurring in the P-loop are the most frequently identified mutations [Ravandi, 2011]. The T315I mutation (also known as the gatekeeper mutation) is seen in 4–15% of patients with imatinib resistance, and results when a single C to T nucleotide substitution occurs at position 944 of the *ABL* gene, resulting in a threonine to isoleucine substitution at amino acid 315 (Th315 to Ile315). This eliminates a critical oxygen molecule needed for hydrogen bonding between imatinib and the ABL kinase. It confers resistance not only to imatinib but also to nilotinib (Tasigna, Novartis Pharma) and dasatinib (Sprycel, Bristol-Myers Squibb Pharma, Princeton, NJ, USA) and has been shown to impact long-term outcome [Jabbour *et al*. 2006; Nicolini *et al*. 2006]. The clinical significance of other mutations, such as the P-loop mutations, is still controversial [Jabbour *et al*. 2006; Khorashad *et al*. 2008].

Amplification of the *ABL* kinase oncogene has been observed in several studies [Bixby and Talpaz, 2009; Gorre *et al*. 2001; Le Coutre *et al*. 2000; Mahon *et al*. 2000; Weisberg and Griffin, 2000]. However, in the majority of patients, it was not shown to be a primary mode of treatment failure. Reactivation of *BCR-ABL* signal transduction is another mechanism of resistance and has been associated with both *BCR-ABL* point mutations and gene amplification [Gorre *et al*. 2001].

To validate the ELN guidelines regarding when to perform mutational analysis, Soverini and colleagues, from GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto), performed mutational analyses on their database of 1301 patients. These data were presented at the American Society of Hematology (ASH) 2011 annual meeting [Soverini *et al*. 2011]. Imatinib-resistant mutations were detected in zero and two imatinib-naïve patients with CML-CP and CML-BP at initial diagnosis respectively. Among those treated with imatinib who had suboptimal response and those with imatinib failure, only 11/233 (4.7%) and 45/166 (27.1%) were found to be positive for one or more *BCR-ABL* KD mutations respectively. Among those who achieved CCyR but were molecular suboptimal responders, 0/52 with less than major molecular response (MMR) and 4/95(4.2%) who lost MMR were found positive for KD mutations. Interestingly, newly acquired mutations were detected in 93/131 (71%) patients who lost a previously achieved hematologic response or CyR. The authors concluded that patients with KD mutations are more likely to have suboptimal CyR than suboptimal molecular responders and any *BCR-ABL* transcript increase that is not associated with MMR loss should not trigger a mutation analysis. This later statement actually

supported the report by Kantarjian and colleagues [Kantarjian *et al.* 2009c].

BCR-ABL-independent mechanisms

BCR-ABL-independent mechanisms of resistance to imatinib include increased efflux of the drug by increased expression of P-glycoprotein efflux pumps [Bixby and Talpaz, 2009; Che *et al.* 2002; Jabbour *et al.* 2011b; Kotaki *et al.* 2003; Rumpold *et al.* 2005], overexpression of the P-170 glycoprotein that enhances drug efflux and reduces intracellular drug accumulation [Chu and DeVita, 2010], decreased drug uptake secondary to decreased expression of the drug uptake transporter human organic cation transporter 1 (hOCT1) [Bixby and Talpaz, 2009; Hughes *et al.* 2006; Thomas *et al.* 2004; Wang *et al.* 2008; White *et al.* 2006], sequestration of imatinib by increased serum protein α1 acid glycoprotein, which binds imatinib and impairs subsequent binding to *ABL* kinase [Gambacorti-Passerini *et al.* 2000; Jabbour *et al.* 2011b; Widmer *et al.* 2006], low serum drug concentration, and alternative signaling pathway activation through Ras/Raf/MEK kinase, STAT, Erk2, or SFK phosphorylation of BCR-ABL [Bixby and Talpaz, 2009]. Elevated transcript levels of prostaglandin-endoperoxide synthase 1/cyclooxygenase 1, which encodes an enzyme that metabolizes imatinib, has also been associated with primary resistance [Zhang *et al.* 2009].

In a recent study published by Ng and associates a novel mechanism was identified among East Asians that explains the primary resistance and suboptimal response to TKIs compared with other ethnic groups [Ng *et al.* 2012]. The TKI resistance observed was secondary to a germline deletion polymorphism of the BH3 domain of BIM (BCL2L11) that is needed for TKIs to induce apoptosis in malignancies driven by kinases. This allows the researchers to develop BH3 mimetics that can be used conjunction with TKIs to overcome the resistance and thus to personalize the therapy.

Patient compliance

Rates of imatinib adherence have been estimated to range from 75% to 90%, and lower adherence rates correlate with worse outcome [Darkow *et al.* 2007; Marin *et al.* 2010; Noens *et al.* 2009]. In a study of 87 patients with CML-CP treated with imatinib 400 mg daily, adherence rates of 90% or less resulted in MMR rates of 28.4% compared with 94.5% in patients with greater than 90% adherence rates ($p < 0.001$) [Marin *et al.* 2010]. Complete molecular response (CMR) rates were 0% *versus* 43.8% respectively ($p = 0.002$), and no molecular responses were observed when adherence rates were 80% or lower. Lower adherence rates have been described in younger patients, those who experience adverse events (AEs) related to therapy, and those who require dose escalations.

In conclusion, patient compliance should be evaluated in patients with TKI resistance. Mutation analysis should be considered in someone who has a suboptimal response or has had a loss of cytogenetic or molecular response at any time [O'Brien S *et al.* 2011].

Measuring response in chronic myeloid leukemia in the chronic phase

Important components of treatment response

Based on available evidence from several independent series [Alvarado *et al.* 2009; Kantarjian *et al.* 2009b] and the outcomes of four expert consensus conferences, the ELN has published management recommendations, including definitions for treatment failure, suboptimal response and optimal response, in patients with CML-CP treated with imatinib. The criteria defining treatment response are summarized in Table 1.

Defining imatinib treatment failure

Criteria for failure (Table 2) with imatinib therapy are judged relative to the duration of the therapy. This includes the lack of a complete hematologic response (CHR) at 3 months, lack of a CyR at 6 months (Ph > 95%), lack of MCyR at 12 months (Ph > 35%) irrespective of hematologic response, and lack of CCyR at 18 months of therapy. It is also considered failure if there is a loss of response to imatinib therapy at any time, including cytogenetic or hematologic relapse [Baccarani and Dreyling, 2009]. The risk of loss of MCyR or CHR, or progression to accelerated or blast phase, is highest in the first 2–3 years of therapy, with decreases in failure rates as therapy continues [Alvarado *et al.* 2009; Baccarani *et al.* 2009; Deininger *et al.* 2009; Druker *et al.* 2006]. In the second revision of 2013 guidelines update by NCCN, hematologic response is no longer considered to make treatment response/failure decision at 3 months. Instead, quantitative real-time polymerase chain reaction (RT-PCR) using

Table 1. Monitoring treatment response in chronic myelogenous leukemia (CML).

Complete hematologic remission	Cytogenetic remission				Molecular response	
1. Normalization of peripheral counts and differential*	Karyotype analysis of metaphases from bone marrow				Quantitative PCR (RT-PCR)	
2. Disappearance of all signs and symptoms of CML$		Major cytogenetic response (0–34% Ph+)			Complete molecular response	Major molecular response
Optimal response (3 months)	Complete cytogenetic response	Partial cytogenetic response	Minor cytogenetic response	No cytogenetic response	Undetectable *BCR–ABL* transcripts	*BCR–ABL/ABL* ratio of <0.1% (international scale)
	(0% Ph+)	1–35% Ph+	36–95% Ph+	>95% Ph+		
Optimal response-3m	12 months	6 months	3 months	–		18 months

*Normalization of peripheral counts is defined through platelet count less than 450 × 10^9, white blood cell count less than 10 × 10^9, the absence of immature granulocytes and less than 5% basophils on differential.
$Includes resolution of splenomegaly.
Ph, Philadelphia; RT-PCR, real-time polymerase chain reaction.

Table 2. Treatment response criteria.

Time on therapy (months)	Treatment failure	Suboptimal response	Optimal response
3*	No CHR	No CG response	CHR with <95% Ph+
6*	>95% Ph+ (no CyR)	36–95% Ph+ (less partial CyR)	≤35% Ph+ (partial CyR)
12	≥35% Ph+	1–35% Ph+ (partial CyR)	0% Ph+
18	≥1% Ph+	<MMR	MMR
Any	Loss of CHR Loss of CCyR Mutation, CE	Loss of MMR Imatinib-sensitive mutation	Stable or improving MMR

European LeukemiaNet's (ELN) criteria for patient failure and suboptimal response to imatinib therapy.
*These criteria were modified in the 2013 update of the National Comprehensive Cancer Network guidelines.
CCyR, complete cytogenetic response; CE, clonal evolution; CG, cytogenetic; CHR, complete hematologic response; MMR, major molecular response; Ph, Philadelphia chromosome.

the international scale to monitor the *BCR-ABL* transcript levels is used. A *BCR-ABL* transcript level of up to 10% by quantitative RT-PCR is considered treatment response and 10% and over is considered failure. If a quantitative RT-PCR is unavailable, a partial CyR (PCyR) by bone marrow cytogenetics at 3 months is considered treatment response and anything less than PCyR is considered failure. Also, the evaluation of treatment response at 6 months was removed in this update. Instead, these guidelines suggest a 3-monthly quantitative RT-PCR for 3 years and every 3–6 months thereafter if a response is achieved at 3 months. If no response is achieved in the first 3 months, patient compliance should be evaluated and mutational analysis performed. At 12 months, if a patient has not achieved a CCyR, it is considered as treatment failure.

Importance of suboptimal response

Suboptimal responses are those that do not meet criteria for response, or those for failure, but represent a slow or inadequate response. Suboptimal response early in therapy is more prognostic than at later time points. Patients classified as suboptimal responders at 6 months have similar outcomes in terms of overall survival (OS), progression-free survival (PFS) and event-free survival (EFS) as those whose condition fails to respond to therapy.

In contrast, those with suboptimal response at 18 months of therapy have outcomes not statistically different than those classified as having an optimal response [Alvarado *et al.* 2009; Hughes *et al.* 2003; Marin *et al.* 2008]. Patients with suboptimal responses have greater risk of disease progression compared with optimal responders [Druker *et al.* 2006; Hochhaus *et al.* 2008b]. For this reason, NCCN guidelines recommend therapy change as if in treatment failure.

The primary goal of therapy for patients with CML is still the achievement of CCyR. Those who achieve this goal, have a low probability of eventually progressing. Also, achieving a MMR early is desirable, as it further improves the long-term outcome. Thus, evaluating response by quantitative real-time PCR (RT-PCR) for this molecular response at critical time points, as stated by the ELN, is important. In the International Randomized study of Interferon vs STI571 (IRIS) study, patients who achieved MMR by 12 months had estimated EFS of 99% at 7 years and MMR by 18 months had 100% freedom from progression to AP/BP and 95% EFS at 7 years compared with those with CCyR without MMR by 18 months [Hughes *et al.* 2010]. However, according to the study published by Kantarjian and colleagues, an increase in RT-PCR among patients in CCyR is not a criterion for treatment failure. Most patients with increases in RT-PCR remain in CCyR. Patients who lose a MMR or never achieve a MMR and have more than 1 log increase of RT-PCR should be monitored more closely, and may be evaluated for mutations of the *BCR-ABL* KD [Kantarjian *et al.* 2009c].

As the IRIS trial and several independent retrospective reviews have confirmed [Deininger *et al.* 2009; Druker *et al.* 2006; Hughes *et al.* 2003; O'Brien *et al.* 2003] CyR is the gold standard for assessing optimal response and predicting long-term outcome. Thus, the bone marrow must be reassessed at 3/6 months and at 12 months if CCyR was not achieved. As per international guidelines, NCCN and ELN, fluorescence in situ hybridization (FISH) and RT-PCR serve as important monitoring tools after CCyR is achieved. FISH is highly sensitive in assessing CyR, particularly when no analyzable metaphases are obtained from bone marrow. Thus, molecular response should be assessed by RT-PCR every 3 months in the peripheral blood until a MMR is achieved and then every 3–6 months [Alvarado *et al.* 2009; Baccarani *et al.* 2006; Marin *et al.* 2008; O'Brien *et al.* 2011]. The NCCN guidelines state that patients with a stable MMR can be monitored every 6 months [O'Brien *et al.* 2011].

It is worth mentioning that the endpoints to assess treatment response proposed by ELN are based on clinical trials with imatinib. Also, it is important to note that the CyR and survival rates obtained in the IRIS trial are not completely reproducible in the community and imatinib was shown to be less effective in obtaining the same results outside a clinical trial. Lucas and colleagues showed that, by 24 months, in 49% of patients, their condition failed to respond to imatinib treatment [Lucas *et al.* 2008]. Furthermore, these same endpoints may not be applicable for patients who are treated upfront with dasatinib or nilotinib. We have recently reported that among patients with newly diagnosed CML-CP treated with second-generation TKIs, the achievement of an early CCyR (e.g. at 3 or 6 months) correlated with an optimal outcome.

Potential pitfalls

Imatinib intolerance

Intolerance to imatinib therapy is one of the prime reasons for discontinuation of therapy. In general, a patient should be categorized as being intolerant to TKI therapy if the patient meets one or more of the following criteria: any life-threatening grade 4 nonhematologic toxicity; any grade 3 or 4 nonhematologic toxicity that has recurred despite dose reduction and treatment of symptoms; any grade 2 nonhematologic toxicity that persists for more than a month despite optimal supportive measures; a grade 3 or 4 hematologic toxicity that does not respond to supportive measures and requires dose reductions below the accepted minimal effective dose [Jabbour *et al.* 2011a]. Common side effects of imatinib in order of incidence include neutropenia, thrombocytopenia, anemia, elevated liver enzymes, edema, nausea, muscle cramps, musculoskeletal pains, rash, fatigue, diarrhea and headache [O'Brien *et al.* 2003]. Common toxicity criteria may be used to grade AEs and identify acute toxicities, and likewise, may help determine TKI intolerance. With long-term therapies such as TKIs, a patient's quality of life may be a better tool to gauge therapy intolerance [Pinilla-Ibarz *et al.* 2011]. This is a particularly salient factor in CML since patient adherence is critical to successful long-term disease management.

Rising BCR-ABL transcript levels: when to respond

A small increase in *BCR-ABL* transcript levels does not necessarily indicate treatment failure or loss of response. This is supported by the 3-year follow up of the IRIS trial in which CCyR, regardless of MMR, was associated with improved OS relative to interferon and cytarabine [Kantarjian *et al*. 2006b]. In the setting of a previously achieved MMR, a new 1 log, or fivefold, increase in *BCR-ABL* transcripts by RT-PCR should be evaluated by repeating the RT-PCR in 1–3 months, and if the increase is confirmed, conventional cytogenetics on a bone marrow sample should be obtained to evaluate for loss of CyR [Branford *et al*. 2003; Ross *et al*. 2006]. Furthermore, the limitation of using molecular response as an endpoint is the lack of standardization in laboratories that perform this test [Hughes *et al*. 2006]. Variability among centers can be introduced because of methodological differences. Molecular studies including a combination of FISH and RT-PCR may be required to ensure concordance and high-quality stability of response. Importantly, both tests can return false-positive or false-negative results. Bone marrow cytogenetic studies are warranted every 2–3 years or more if abnormalities are found in Ph-negative diploid cells (e.g. abnormalities in chromosomes 5 or 7).

As per ELN recommendations, mutation analysis to identify any KD mutations is beneficial in cases of imatinib failure, in cases of increased *BCR-ABL* transcript levels leading to MMR loss, in any other case of suboptimal response (for imatinib patients), and in cases of hematologic or cytogenetic failure during second-line dasatinib or nilotinib therapy. Furthermore it was also suggested that performing regular mutational analysis is worthless given the stable nature of CML-CP on imatinib.

Chromosomal abnormalities in normal metaphases

Chromosomal abnormality (CA) in Ph-negative cells occurs in 5% of cases of CML responding to imatinib. Most disappear spontaneously with continued therapy. In a study conducted in 258 patients with newly diagnosed CML-CP at MD Anderson Cancer Center treated with imatinib, CA occurred in a small subset and was transient with no significant impact on outcome. Thus, no changes in treatment strategy are usually warranted in the absence of additional complications. The progression of CML to myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML) in those with cytogenetic abnormalities with normal peripheral counts or morphology is low. Cytogenetic analysis should suffice to monitor these patients with a 'wait and watch' approach, as was reiterated by Loriaux and colleagues in their 2004 study [Loriaux and Deininger, 2004]. Additional studies in larger cohorts are needed to identify the best treatment strategy for these patients [Jabbour *et al*. 2007].

Management of imatinib failure

Imatinib dose escalation

In a retrospective review of patients who underwent imatinib dose escalations as part of the IRIS trial according to IRIS protocol guidelines from a standard dose of 400 mg daily to doses of 600 or 800 mg daily, statistically significant improvements in responses were noted. In those who had not achieved a CHR by 3 months, 86% achieved a CHR 3 months after dose escalation and 29% went on to achieve a CCyR by 12 months. In patients who had not achieved a minor CyR by 12 months, 25% achieved a MCyR by 12 months after imatinib dose escalation and 50% by 24 months. Of these patients, 50% went on to achieve a CCyR by 48 months. In patients with a loss of MCyR, 50% regained their MCyR within 12.5 months, 33% of whom went on to achieve a CCyR. In patients who exhibited progression of disease, 67% experienced normalization of their white blood cell count. Of note, dose escalations were not attempted for those who lost CCyR [Kantarjian *et al*. 2009a].

In another study assessing 84 patients over 61 months whose condition failed to respond to standard-dose imatinib, doses were escalated to 600–800 mg daily. In 21 patients with hematologic failure, 48% achieved a CHR, with only 14% achieving a CyR. In 63 patients with cytogenetic failure, 75% responded with CCyR; 2- and 3-year EFS rates were 57% and 47% respectively, with OS rates of 84% and 76% respectively [Jabbour *et al*. 2009a]. Interestingly, among those with cytogenetic failures, a second CCyR was achieved in 52% with dose escalation and these responses were durable in 88% after 2 years. In contrast, less than 5% of those who had lost their hematologic response (25%) achieved a CyR, and even then, it was transient [Jabbour *et al*. 2009a].

Thus, while dose escalation after failure of standard-dose imatinib is an important and practical treatment option, it is likely to be effective only in the subset of patients with previous CyRs and is not indicated for patients with intolerance to the drug, particularly at the higher doses. Clinical consideration should be given to second-generation TKIs in this setting [Kantarjian *et al.* 2009b]. Current treatment guidelines recommend dose escalation of imatinib to 600 or 800 mg/day in cases of suboptimal response [O'Hare *et al.* 2005]. However, it should be acknowledged that there are minimal data available regarding the effectiveness of this approach [Jabbour *et al.* 2009a; Kantarjian *et al.* 2009a]. For patients with clear failure to imatinib therapy, dose escalation from an initial dose of 400 mg is no longer recommended in the 2013 update of the NCCN guidelines. The current approach is to change therapy to a second-generation TKI, unless the patient is intolerant to dasatinib or nilotinib, although allogeneic hematopoeitic stem cell transplantation (allo-HSCT) is also an option following treatment failure [Deininger, 2008; Kantarjian *et al.* 2006a].

Second-generation tyrosine kinase inhibitors

In an international, multicenter, phase II trial assessing 387 patients with CML-CP who had demonstrated imatinib intolerance or resistance (START-C trial), patients were treated with dasatinib 70 mg twice daily. After 2 years, data showed rates of CHR of 91%, CCyR of 53%, and MMR of 47%. Two-year PFS and OS were 80% and 94% respectively [Mauro *et al.* 2009]. These data were supported by another study in which dasatinib produced MCyR rates of 45% and CCyR rates of 33% in patients with CML-CP who were imatinib resistant or intolerant [Brave *et al.* 2008].

The dose optimization study CA180034 randomized 622 patients with imatinib-resistant or -intolerant CML-CP to four dasatinib treatment arms, including 100 mg daily, 50 mg twice daily, 140 mg daily and 70 mg twice daily. Dasatinib dosed at 100 mg daily produced similar CyR and PFS rates as other dosing schedules, with significantly fewer occurrences of grade 3–4 neutropenia, thrombocytopenia, anemia and pleural effusions. There were also fewer treatment interruptions, reductions and discontinuations at 100 mg daily compared with the other treatment groups.

Nilotinib has also been investigated as a therapeutic strategy in patients with CML-CP who have exhibited imatinib failure. One phase II study included 321 patients receiving nilotinib 400 mg twice daily after previous imatinib failure. The median length of therapy was 18.7 months, with 62% of patients receiving treatment for at least 12 months, and 42% receiving treatment for 24 months or more. The median dose delivered was 788.5 mg/day. In this population, CCyR was achieved in 46%, and of these, 56% achieved a MMR. The overall MMR rate was 28% for the entire study population. The 24-month rates of PFS and OS were 64% and 87% respectively [Kantarjian *et al.* 2010]. Unlike imatinib, nilotinib activity is not affected by hOCT1 [White *et al.* 2006].

Bosutinib was recently approved by FDA on 4 September 2012 for this indication. A phase I/II study evaluating the efficacy and safety of bosutinib (500 mg once daily) in 288 patients with imatinib-resistant or -intolerant CML-CP demonstrated that after a median follow up of 24.2 months, 86% of patients achieved CHR, 53% had a MCyR (41% with a CCyR). At 2 years, PFS was 79% and OS was 92%. Responses were seen across *BCR-ABL* mutants, except T315I [Gambacorti-Passerini, 2010]. These data suggest that bosutinib is effective with good tolerability in patients with imatinib-resistant or -intolerant CML-CP. Table 3 summarizes the response to second-generation TKIs dasatinib, nilotinib and bosutinib in patients who are imatinib resistant or intolerant in CML-CP, -AP and -BP.

Choosing the right second-generation tyrosine kinase inhibitor

Safety and tolerability are important considerations in choosing a TKI. The potential impact of the drug's AE profile on any of the patient's preexisting conditions should be considered in choosing between second-generation BCR-ABL inhibitors. Pleural effusion is more common for patients receiving dasatinib therapy. Therefore patients with risk factors for pleural effusion such as a prior cardiac history, chronic obstructive pulmonary disease and hypertension are at greater risk for developing these complications on dasatinib therapy [Quintas-Cardama *et al.* 2007]. Risk factors for developing pleural effusions while taking dasatinib also include disease stage (BP > AP > CP) and previous lung problems, such as smoking or infections. The AE profile for nilotinib includes an

Table 3. Response to second-generation tyrosine kinase inhibitors (dasatinib, nilotinib and bosutinib) in patients whose condition is imatinib resistant or who are intolerant in chronic myelogenous leukemia CP, AP and BP.

	Dasatinib				Nilotinib				Bosutinib		
	CP $n = 387$	AP $n = 174$	MyBP $n = 109$	LyBP $n = 48$	CP $n = 321$	AP $n = 137$	MyBP $n = 105$	LyBP $n = 31$	CP $n = 146$	AP $n = 51$	BP $n = 38$
Median follow up (months)	15	14	12+	12+	24	9	3	3	7	6	3
Resistant to imatinib (%)	74	93	91	88	70	80	82	82	69	NR	NR
Hematologic response (%)	–	79	50	40	94	56	22	19	85	54	36
CHR (%)	91	45	27	29	76	31	11	13	81	54	36
NEL (%)	–	19	7	6	–	12	1	0	–	0	0
Cytogenetic response (%)	NR	44	36	52	NR	NR	NR	NR	–	NR	NR
Complete (%)	49	32	26	46	46	20	29	32	34	27	35
Partial (%)	11	7	7	6	15	12	10	16	13	20	18
Survival (%) (at 12 months)	96 (15)	82 (12)	50 (12)	50 (5)	87 (24)	67 (24)	42 (12)	42 (12)	98 (12)	60 (12)	50 (10)

[Brave *et al*. 2008; Hochhaus *et al*. 2009a].
AP, accelerated phase; CHR, complete hematologic response; CP, chronic phase; LyBP, lymphoid blast phase; MyBP, myeloid blast phase; NEL, no evidence of leukemia; NR, not reported.

increased risk of pancreatitis. Therefore, in patients with a prior history of severe pancreatitis, nilotinib should be used with caution and patients should be monitored closely for recurrence.

Furthermore, mutational data can help in choosing the right second-generation TKI, as certain mutations are resistant to specific drugs [Branford *et al*. 2009]. A study of specific *BCR-ABL* KD mutations identified after TKI therapy by Hochhaus and colleagues reported that failure of dasatinib therapy was more commonly associated with mutations at V299 and F317, while nilotinib resistance was associated with mutation in the P-loop, especially at Y253 and E255, or at the F311 or F359 residue [Hochhaus *et al*. 2008a].

Defining adequate response to second-generation tyrosine kinase inhibitors after imatinib failure

To better define adequate response to secondary TKI therapy, Jabbour and colleagues analyzed outcomes in 113 patients receiving nilotinib ($N = 43$) or dasatinib ($N = 70$) after imatinib failure. They reported that after 12 months of therapy, patients achieving a MCyR had a significant projected 1-year survival advantage of 97% *versus* 84% in those with a minor CyR or CHR ($p = 0.02$) [Tam *et al*. 2008]. Furthermore, in patients with CML being treated with second-generation TKIs after imatinib failure, the absence of CyR to previous imatinib therapy and a performance status of 1 or higher were found to be predictive of poor response to second-generation TKI therapy, and poor long-term outcome, warranting an alternative approach such as allo-HSCT [Jabbour *et al*. 2009b].

When to choose allogeneic hematopoeitic stem cell transplantation

The number of patients undergoing allo-HSCT for CML-CP has dropped significantly since TKIs were introduced. However, many patients may eventually require transplantation due to the development of TKI resistance associated with very resistant mutations or when patients otherwise progress and evolve into AP/BP. Allo-HSCT remains an important therapeutic option for CML-CP when patients are harboring the T315I mutation, in those whose condition fails to respond to second-generation TKIs [Baccarani *et al*. 2009], and in young patients with CML-CP who have closely matched donors available, especially if they harbor high half maximal inhibitory concentration (IC50) mutations and have not achieved a MCyR after 12 months of therapy with a second-generation TKI. Patients who do not

exhibit these factors could reasonably continue TKI therapy until failure. Age may also be a factor in considering allo-HSCT. Older patients (those over the age of 70) or those unable to find a well matched stem cell donor may forgo curative transplantation for several years of controlled CML disease.

Imatinib is less effective in advanced stage chronic myelogenous leukemia

Though imatinib has revolutionized the management of CML-CP, it is less effective in the advanced phases of CML. As mentioned above, mutations, which occur more frequently in the advanced phases of disease, decrease the response rate to TKI-based therapies, leading to therapy failure [Soverini *et al.* 2006]. In patients with imatinib failure in CML-AP/CML-BP, a second-generation TKI with or without combination chemotherapy can be used as a bridge therapy, after which an allo-HSCT should be performed. Similarly, evidence of the T315I mutation in any phase of CML should warrant the use of a specific T315I inhibitor as a bridge therapy that would be followed by an allo-HSCT.

Upcoming therapeutic options targeting tyrosine kinase inhibitor resistance

Despite extraordinary progress, a true cure for CML is not generally achieved by *ABL* kinase inhibitors. TKIs are potent inhibitors of *BCR-ABL* kinases (among others), resulting in rapid reduction of the majority of cells carrying the Ph chromosomal marker. However, suppression of *ABL*-driven hematopoiesis may be insufficient to eradicate quiescent stem cells. Studies assessing the combination of TKIs with promising agents are ongoing. These combinations include TKI and hedgehog inhibitors, omacetaxine, vaccines and hypomethylatings agents. If successful, this strategy could lead to a safe and permanent discontinuation of therapy in patients with a good response. The future of CML therapy may include early use of these potent agents, perhaps in combination with new molecules, to help more patients achieve CMR, which could lead to therapy discontinuation and cure.

Ponatinib

Ponatinib (Ariad Pharma, Cambridge, MA, USA) is a potent, oral pan-BCR-ABL TKI which is under active investigation and is promising for patients with CML and Ph+ acute lymphoblastic leukemia (ALL) whose condition fails to respond to imatinib, dasatinib and nilotinib. Importantly, it is active against T315I and other imatinib-resistant mutations. In addition to inhibiting BCR-ABL, ponatinib also inhibits FLT3, FGFR, VEGFR, PDGFR and c-Kit, some of which have been implicated in the pathogenesis of AML [O'Hare *et al.* 2009]. In March 2010, ponatinib was granted Orphan status in the European Union for CML and Ph+ ALL.

In vitro, ponatinib potently inhibited native ABL and also several clinically relevant mutations, including T315I. In a mouse model of human CML, treatment with ponatinib resulted in complete tumor regression. Ponatinib is currently being investigated in a phase II clinical trial in patients with relapsed or refractory CML. Early results from these trials have been promising. A phase I study of oral ponatinib in patients with refractory CML/ALL or other hematologic malignancies recently reported that 66% and 53% of patients with CML-CP achieved MCyR and CCyR respectively [Cortes *et al.* 2010a]. Dose-limiting toxicities (DLTs) reported were elevated pancreatic enzymes, pancreatitis and rash. The study was an open-label dose-escalation study that evaluated safety and clinical responses in patients with refractory CML (CML-CP, CML-AP and CML-BP), Ph+ ALL, AML and other hematologic malignancies. Of 67 patients enrolled, 48 Ph+ patients were evaluable for response. These included 32 patients in CML-CP and 16 other patients in CML-AP and CML-BP, or Ph+ ALL. Ninety-four percent of the patients in CML-CP had CHR and 63% had a MCyR (12 complete and 8 partial). Among 11 patients in CML-CP with T315I mutations, all had a CHR and 82% had a MCyR. Among the evaluable patients with CML-AP, CML-BP and Ph+ ALL, 31% had a major hematologic response, 19% had a MCyR and 6% had a minor CyR. Of nine patients in CML-AP, CML-BP, or Ph+ ALL with T315I mutations, 33% had a major hematologic response and 20% had a MCyR. Responses were also observed in patients with highly refractory disease with either no mutations or other mutations resistant to approved TKIs (e.g. M351T, F359C, F317L, M244V and G250E). Early MMR occurred in 12 patients who were on treatment for up to 4 months, and 4 patients achieved MMR within 2 months or less. MMR was also achieved in patients with M351T, F359C, F317L, M244V, G250E mutations, and 1 patient with no mutation.

Ponatinib was generally well tolerated. In studies in which patients were treated at doses as high as 60 mg, frequently occurring drug-related AEs (≥10% any grade) included thrombocytopenia (24%), headache (14%), nausea (14%), arthralgia (13%) and fatigue (13%). Other AEs were anemia (11%), elevated lipase (11%), pancreatitis (10%), muscle spasms (11%), rash (11%) and myalgias (10%). DLT in 4/14 patients treated at 60 mg were pancreatic enzyme elevations and pancreatitis. One of 22 patients treated at the 45 mg dose had a DLT of grade 3 rash. All DLTs were reversible [Cortes *et al*. 2010a].

These results led to the phase II PACE (Ponatinib Ph+ALL and CML Evaluation) trial reported by Cortes and colleagues. The PACE trial enrolled 449 patients with all stages of CML or Ph+ ALL who were resistant or intolerant to dasatinib or nilotinib, or developed the T315I mutation after therapy with any TKI. Among these patients, 315 were either resistant or intolerant to imatinib therapy and 64 patients had the T315I mutation. A large majority of the patients with each stage of disease, approximately 80–90%, were resistant to dasatinib or nilotinib. The primary endpoint was a MCyR for CML-CP and a major hematologic response for patients with advanced stage disease. At a median follow up of 6.6 months, among patients in CML-CP, 49%, 41% and 26% achieved MCyR, CCyR and MMR respectively. Among patients in CML-AP, 67%, 38% and 17% attained MHR, MCyR and CCyR respectively and among those in CML-BP, 37%, 34% and 27% attained MHR, MCyR and CCyR respectively [Cortes, 2011].

Omacetaxine

Omacetaxine, a subcutaneously administered first-in-class cetaxine agent that has a mechanism of action independent of tyrosine kinase inhibition has also been evaluated in patients with Ph+ CML who have the T315I mutation and resistance to imatinib therapy. It acts through reversible, transient inhibition of protein elongation that does not depend on BCR-ABL binding. By blocking ribosomal function, the drug decreases intracellular levels of several antiapoptotic regulatory proteins, inducing antitumor activity via apoptosis [Perez-Galan *et al*. 2007].

More phase II/III studies investigating the benefit of omacetaxine in patients with CML post multiple TKI failure, with a significant proportion of these patients with baseline mutations, are ongoing. Preliminary data in patients with CML-CP with T315I mutations who are resistant to imatinib show achievement of an 85% CHR rate, 28% CyR rate, 15% MCyR rate, 15% MMR rate and 57% reduction in the T315I clone. OS was not met in this group of patients. In CML-AP, 37.5% showed hematologic response with OS of 18.8 months. In CML-BP, hematologic response was demonstrated in 30% with OS of 1.8 months [Cortes-Franco *et al*. 2009]. An update combining both of the previous study populations with and without T315I mutations treated with omacetaxine was presented at the ASH 2010 annual meeting [Cortes *et al*. 2010b].

An update combining the two studies that included omacetaxine in patients with the T315I mutation whose condition had failed to respond to prior imatinib and in patients with resistance or intolerance to at least two TKIs was recently presented. In the CML-CP group, 16 patients (20%) achieved a MCyR and 4 (5%) had a minor CyR; the median duration of MCyR was 18 months. A total of 56 (69%) achieved a CHR with a median response duration of 12.2 months (range 8–26); the median OS was 34 months.

In the CML-AP group, 11 patients (27%) had a MHR, including 10 (24%) with CHR and 1 (2%) with no evidence of leukemia; the median duration of MHR was 9 months (range 4–14). In addition, 2 (5%) achieved a return to chronic phase and 3 (7%) had hematologic improvement. Three (7%) patients had a minor and 3 (7%) had a minimal CyR. The median OS was 16 months. Omacetaxine was well tolerated in this population. This study suggests that patients with CML whose condition has failed to respond to previous treatment with at least two TKIs may attain a clinically meaningful response to omacetaxine therapy.

Conclusion

The introduction of TKIs and their implementation in the treatment of CML have changed the management and outcome of this disease dramatically. They have transformed this disease from an immediately life-threatening leukemia, with a 10–20% mortality rate per year, to a chronic disease, managed with oral medications, and with 1–2% mortality per year. Although good results were obtained with the first-generation TKI, imatinib, the results were not consistent among some patients who developed imatinib resistance.

As the resistance mechanisms become more evident, newer drugs are being developed to overcome this problem. Mutational analysis should be performed in those with imatinib failure, escalating *BCR-ABL* transcript levels and those with suboptimal response. Mutational data also help in choosing the right second-generation TKI based on the point mutations that led to imatinib failure. Of particular importance is the development of ponatinib, a pan-BCR-ABL TKI that targets multiple point mutations, including T315I, which is notoriously associated with imatinib and multiple second-generation TKI failure. The introduction of novel therapies may further improve outcomes and address the common mechanisms of resistance in the treatment of CML.

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