CLINICAL INVESTIGATION

COMMENTARY

Clin. Invest. (2013) 3(9), 817–821

Overcoming resistance in chronic myeloid leukemia

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"As the number of drugs available for the treatment of chronic myeloid leukemia has expanded, so have the strategies for overcoming or avoiding resistance."

Chronic myeloid leukemia (CML) is characterized by the BCR–ABL fusion protein produced as a result of the reciprocal translocation between a portion of the *ABL* tyrosine kinase gene on chromosome 9 and a region within the *BCR* gene on chromosome 22. The BCR–ABL oncoprotein displays constitutively elevated tyrosine kinase activity that drives the pathogenesis of CML by activating multiple signaling pathways including the RAS/MAPK, PI3K/AKT and JAK2/STAT5 pathways.

The development of tyrosine kinase inhibitors (TKIs) aimed at BCR–ABL revolutionized the treatment of CML. Imatinib was the first of the TKIs to become available after it was approved by the US FDA in 2002 based upon data from the IRIS. Indeed, imatinib was associated with unprecedented rates of hematologic, cytogenetic and molecular responses in comparison with IFN-α with complete hematologic response (CHR) rates of 97%, complete cytogenetic response (CCyR) rates of 82% and major molecular response (MMR) rates on the international scale (IS) of 86% [1]. Treatment with imatinib also resulted in significantly improved rates of freedom from progression in comparison with IFN-α with an 8-year freedom from progression to accelerated or blast phase of 92% and afforded patients who were able to remain on treatment an 8-year overall survival (OS) of 85% [1].

However, imatinib use has been complicated by the development of resistance. Resistance led to 24% of patients in IRIS failing to achieve a CCyR at 18 months, which represented treatment failure [2]. Resistance also led 17% of patients on imatinib in IRIS to develop relapsed disease and 7% to develop progressive disease [2]. These numbers are, in fact, likely to be underestimates given the number of patients in the IRIS study who came off the trial and were not ultimately included in analysis. Treatment failure and disease progression due to resistance is also seen in patients treated with the subsequently approved second-generation TKIs, dasatinib, nilotinib and bosutinib.

The etiology of resistance to TKIs is multifactorial and involves BCR–ABL independent and dependent mechanisms. Insufficient plasma levels of imatinib due to noncompliance, drug–drug interactions or binding to acute-phase inflammatory proteins can all result in resistance. It is known that trough plasma levels of imatinib are important for good clinical outcomes, and it has been demonstrated that patients with high imatinib exposure have better rates of CCyR, MMR and event-free survival [3]. Imatinib and all three of the second-generation TKIs are substrates for, and are extensively metabolized by, the cytochrome P450 enzymes including CYP3A4. As there are multiple drugs, food products and supplements that either induce or inhibit CYP3A4 activity, the potential for drug interactions is high. Alpha 1 acid glycoprotein has been identified as an acute-phase inflammatory product that binds directly to imatinib and is frequently

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Keywords: chronic myeloid leukemia • dasatinib • imatinib • mechanism • nilotinib • ponatinib • resistance • strategy

ISSN 2041-6792

elevated in advanced-phase CML [4]. There are data that show alpha 1 acid glycoprotein impairs imatinib-induced apoptosis and results in increased clearance of imatinib [5].

In addition to the aforementioned pharmacokinetic variables that can give rise to TKI resistance, other BCR–ABL independent factors, such as the proteins involved in TKI transport, can also play a role. Imatinib transport involves hOCT1 for drug influx and the ABC transporter ABCB1 (or MDR-1) for drug efflux. It has been found that hOCT1 expression correlates with response rates, progression-free survival and OS. Higher hOCT1 activity has been associated with significantly improved response rates and OS, while lower hOCT1 activity has been linked to increased likelihood of developing kinase domain mutations and leukemic transformation [6]. At least one study revealed that ABCB1 was overexpressed in patients who failed to attain a major cytogenetic response (MCyR) or progressed while on imatinib [7].

Activation of pathways involving genes that are independent of BCR–ABL could potentially also play an important role in TKI resistance and progression of disease. The SRC pathway has been implicated in this capacity, as highly activated LYN kinase and HCK kinase (both members of the SRC family kinases) have been detected in patients with imatinib-resistant CML without a BCR–ABL mutation [8].

Other mechanisms of resistance are BCR–ABL-related. For example, overexpression of BCR–ABL can lead to resistance by increasing the amount of target protein needed to be inhibited by imatinib [9]. It has been shown that CML cells that express higher amounts of BCR–ABL are less sensitive to imatinib and take less time to yield mutant subclones that are resistant to imatinib, than cells with lower expression levels [10].

It is felt that a major mechanism of resistance in CML arises from mutations in the tyrosine kinase domain of BCR–ABL. These mutations can be found in 36–90% of patients with imatinib resistance and may arise spontaneously or as a result of selective pressure by imatinib [11]. More than 100 distinct point mutations responsible for single amino acid substitutions in the BCR–ABL kinase domain have been identified to date. The most frequently occurring mutations fall within the ATP-binding region (P-loop, residues 244–255) of the kinase domain. These mutations alter the protein conformation and hydrogen bonds important for interaction with TKIs and are associated with a 70–100-fold decrease in sensitivity to imatinib compared to native BCR–ABL [12]. A subset of mutations also occur at the activation (A) loop (residues 381–402) and prevent the kinase from adopting the inactive conformation to which imatinib binds. BCR–ABL mutations have also been described in the catalytic (C) domain (residues 350–363). One of the most common mutations, present in up to 15% of CML patients with resistance to TKIs, involves Thr315, which is also known as the gatekeeper residue based on its location at the periphery of the nucleotide binding site of ABL1 and the key H-bond interaction it has with imatinib [12]. The T315I mutation disrupts this H-bond interaction, which, in addition to the bulk of the isoleucine side-chain, sterically impairs TKI binding, resulting in complete insensitivity to imatinib and the second-generation TKIs dasatinib, nilotinib and bosutinib [12].

It is also important to acknowledge the existence of CML stem cells, not as a mechanism for the development of resistance, but rather as a reservoir of cells responsible for disease persistence. This $CD34^+$ $CD38^-$ CML progenitor cell population is suspected to be liable for the continued evidence of disease at a molecular level in some patients even when they have achieved a CCyR on a TKI and is felt to explain why chronic-phase CML can reemerge after discontinuation of imatinib therapy [13]. In addition to cell-intrinsic mechanisms being responsible for the CML stem cell's innate resistance to TKIs, there is evidence that the stroma plays an important role by releasing cytokines such as IL-6, IL-8, GRO1, MCP-1, MCP-3, G-CSF and GM-CSF, which provide critical survival cues to the stem cell [14].

A number of strategies have been and are being used to overcome TKI resistance. For example, though there are no data to support a role for front-line high-dose imatinib, a subset of patients with suboptimal responses or loss of previous response do appear to benefit from dose escalation [15,16]. In a study of 84 patients with chronic phase CML who had failed imatinib (hematologic or cytogenetic failure), imatinib dose escalation resulted in 40% achieving a CCyR that was durable [16].

Alternative strategies to overcoming TKI resistance include switching a patient to a different TKI at the time of treatment failure or disease progression, use of a second- or third-generation TKI over imatinib as front-line therapy or switching to an alternative TKI based upon failure to achieve early milestones.

There are ample data to support regaining control of disease by switching a patient to a different TKI at the time of treatment failure or disease progression. In the Phase II START-R trial, patients with imatinib-resistant chronic-phase CML were randomized to either dasatinib or high-dose imatinib, and at 2 years, those receiving dasatinib had superior CHR (93 vs 82%) and CCyR (44 vs 18%) [17]. As with dasatinib, a number of trials have demonstrated that nilotinib has significant clinical activity in patients with imatinib-resistant disease. In a retrospective summary of 1422 patients with imatinib-resistant or -intolerant chronic-phase CML treated with nilotinib, the CCyR rate and progression-free survival

at 2 years were reported to be 34 and 81%, respectively [18]. Furthermore, nilotinib has been effective in patients with chronic- or accelerated phase CML who have failed both imatinib and dasatinib. In 37 patients with chronic-phase CML in the aforementioned situation, nilotinib resulted in a CHR of 79% and CCyR of 9% [19].

If resistance is due to a kinase domain mutation and an alternative TKI is required, it is critical to note the particular mutation(s) present as some mutations confer less sensitivity to dasatinib while others result in reduced sensitivity to nilotinib. For example, the V299L and F317I/L kinase domain mutations are associated with resistance to dasatinib while the L248L, Y253F/H, E255K/V and F359C/V mutations are associated with resistance to nilotinib. As previously stated, the T315I mutation confers resistance to imatinib and the second-generation TKIs, dasatinib, nilotinib and bosutinib.

Ponatinib, the newest of the TKIs, was specifically designed to overcome the T315I mutation while also exerting potent activity against the full range of other BCR–ABL kinase domain mutations, as well as the native unmutated enzyme. A key structural feature of ponatinib is a carbon–carbon triple bond that makes productive hydrophobic contact with the side chain of I315, allowing for inhibition of the T315I mutant enzyme. Another important attribute of ponatinib is the incorporation of multiple contact points with the ABL kinase domain, which allows for high binding affinity and high potency, and renders binding less susceptible to disruption by any single amino acid mutation. *In vitro*, ponatinib potently inhibited viability of cell lines expressing native BCR–ABL and 14 major clinically observed imatinib-resistant BCR–ABL mutants [20]. In addition, cell-based mutagenesis screening assays showed that at a concentration of 40 nM, ponatinib suppressed the outgrowth of BCR–ABL mutant subclones, suggesting that it might be effective in preventing the emergence of resistance if used early in the course of disease [20].

Ponatinib has now been tested in CML patients with relapsed or refractory disease in two clinical trials. In the Phase I dose-escalation trial, 65 patients with relapsed or refractory Philadelphia chromosome positive (Ph^+) leukemia were treated with once-daily ponatinib at doses ranging from 2 to 60 mg. Of the 43 chronic-phase CML patients in the study, 98% had previously received two or more approved TKIs and 49% had received imatinib, dasatinib and nilotinib in the past. In these 43 patients, at a median follow up of 56 weeks, 98% had a CHR, 72% had a MCyR, 63% had a CCyR and 44% had a MMR [21]. Of the 12 chronic-phase CML patients with a T315I mutation, 100% had a CHR, 92% had a MCyR, 75% had a CCyR and 67% had a MMR [20]. In addition, of the 22 patients with accelerated- or blast-phase CML or Ph^+ lymphoblastic leukemia, 36% had a major hematologic response and 32% had a MCyR [21]. Common adverse events included rash, myelosuppression and constitutional symptoms [21]. Dose-limiting toxicities were reversible and included elevated lipase or amylase and pancreatitis, which resulted in a maximum-tolerated dose and suggested dose for the Phase II trial of 45 mg once daily [21]. Thus, the Phase I data demonstrated that ponatinib has substantial activity in relapsed or refractory CML in all phases, including patients with the gatekeeper T315I mutation.

The Phase II trial of ponatinib includes 449 patients with chronic-phase CML or Ph^+ acute lymphoblastic leukemia resistant or intolerant to dasatinib or nilotinib or with the T315I mutation. The median number of TKIs patients had previously received was three. At a median follow up of 11 months, of the 203 patients with chronic-phase CML without a T315I mutation, 46% had a CCyR and 32% had a MMR with an additional 20% achieving a deeper molecular response with 4-log reduction in BCR–ABL transcript levels (MR^4) and an additional 12% achieving a molecular response with 4.5-log reduction in BCR–ABL transcript levels ($MR^{4.5}$) [22]. Of the 64 patients with chronic-phase CML with a T315I mutation, 70% achieved a MCyR [22]. Thus, ponatinib appears to be able to overcome the mechanism(s) of resistance including, but not limited to, the development of a T315I kinase domain mutation that give rise to persistent or progressive disease.

Ponatinib is now being studied in newly diagnosed patients with chronic-phase CML in a randomized Phase III clinical trial against imatinib. The rationale behind this trial is to determine if upfront treatment with a TKI other than imatinib will result in superior overall response rates, perhaps at an earlier timepoint, that will then translate into a reduction in cases of resistance. This was the same question investigators hoped to answer with the Phase III ENESTnd trial comparing upfront treatment of chronic phase CML with nilotinib versus imatinib, and the Phase III DASISION trial that compared upfront treatment of chronic-phase CML patients with dasatinib versus imatinib. In the ENESTnd trial, at 3 years, the rates of MMR were significantly higher in patients treated with nilotinib [23]. These patients also had a higher likelihood of achieving a deeper molecular response (MR^4 and $MR^{4.5}$) in comparison with those patients treated with imatinib [23]. In addition, nilotinib was associated with a significantly lower probability of progression to accelerated or blast phase (0.7 vs 4.2%) [23]. Similarly, in the DASISION trial, at 2 years, patients with chronic-phase CML treated with dasatinib had significantly higher rates of MMR and $MR^{4.5}$ and a lower likelihood of progressing to accelerated or blast phase (2.3 vs 5%) [24]. Thus, it appears that the second-generation TKIs result in

deeper molecular responses and, given the decreased incidence of progression to a more aggressive phase seen with these agents in comparison to imatinib, upfront use of a second-generation TKI in the treatment of chronic-phase CML seems to be a reasonable strategy to overcome development of resistance. It should be noted that neither nilotinib nor dasatinib have demonstrated a significant improvement in OS over imatinib when used in the front-line setting. It is possible that this will be revealed with longer follow up, but only time will tell. It is also necessary to await the results of the upfront ponatinib trial prior to drawing any firm conclusions about the superiority of using this third-generation TKI over the first- and second-generation agents in newly diagnosed patients.

As opposed to using the second- or third-generation agents upfront as a means of preventing development of resistance or switching to one of these agents at the time of treatment failure as measured by the traditional landmarks, an alternative would be to switch to these agents if patients failed to achieve early deep responses with imatinib. Data show that in newly diagnosed chronic phase CML patients treated with imatinib, those who achieved a BCR–ABL transcript level of ≤10% (international scale [IS]) or ≤35% Ph^+ cells by cytogenetics at 3 months and a BCR–ABL transcript level of ≤1% (IS) or 0% Ph^+ cells at 6 months had a significantly higher 5-year OS [25]. A similar trend was seen in the ENESTnd trial, in that patients who had BCR–ABL transcript levels of ≤10% (IS) at 3 and 6 months had a better 3-year progression-free survival and OS in comparison with patients who did not achieve these early molecular and cytogenetic landmarks. Though a greater proportion of patients receiving nilotinib achieved these early goals, the patients treated with imatinib who did achieve these early deep responses, had the same improved outcomes [26]. This perhaps argues against the need to treat newly diagnosed chronic-phase CML patients with second- or third-generation TKIs upfront, since they seem to do just as well as long as they have a robust and speedy response to imatinib. This strategy becomes especially attractive when one takes the higher cost of the second- and third-generation TKIs in comparison with imatinib into consideration and will become even more pertinent when imatinib comes off patent in 2015.

As the number of drugs available for the treatment of CML has expanded, so have the strategies for overcoming or avoiding resistance. Given the variety of mechanisms by which resistance occurs, the data suggest that not all CML patients necessarily need treatment with the more recently approved TKIs, the newest of which is ponatinib. Among all the currently available TKIs, however, ponatinib certainly does have many attractive and superior properties, including its potency, its ability to inhibit both native BCR–ABL along with all of the most common mutant variants including the previously completely resistant T315I mutant, and its ability, *in vitro*, to suppress the emergence of any resistant BCR–ABL mutations. Though it is unlikely to be able to overcome or prevent all mechanisms of resistance and disease persistence, it may very well prove to be the best available compound for the treatment of CML developed to date.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Deininger M, O'Brien SG, Guilhot F *et al.* International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. *Blood* 114, 1126 (2009).

2 Druker BJ, Guilhot F, O'Brien SG *et al.* Five-year follow up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* 355, 2408–2417 (2006).

3 Larson RA, Druker BJ, Guilhot F *et al.* Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. *Blood* 111, 4022–4028 (2008).

4 Larghero J, Leguay T, Mourah S *et al.* Relationship between elevated levels of the alpha 1 acid glycoprotein in chronic myelogenous leukemia in blast crisis and pharmacological resistance to imatinib (Gleevec) *in vitro* and *in vivo*. *Biochem. Pharmacol.* 66, 1907–1913 (2003).

5 Delbaldo C, Chatelut E, Re M *et al.* Pharmacokinetic-pharmacodynamic relationships of imatinib and its main metabolite in patients with advanced gastrointestinal stromal tumors. *Clin. Cancer Res.* 12, 6073–6078 (2006).

6 Wang L, Giannoudis A, Lane S *et al.* Expression of the uptake drug transporter hOCT1 is an important clinical determinant of the response to imatinib in chronic myeloid leukemia. *Clin. Pharmacol. Ther.* 83, 258–264 (2008).

7 Galimberti S, Cervetti G, Guerrini F *et al.* Quantitative molecular monitoring of BCR–ABL and MDR1 transcripts in patients with chronic myeloid leukemia during imatinib treatment. *Cancer Genet. Cytogenet.* 162(1), 57–62 (2005).

8 Wu J, Meng F, Kong L-Y *et al.* Association between imatinib-resistant BCR–ABL mutation-negative leukemia and persistent activation of LYN kinase. *J. Natl Cancer Inst.* 100, 926–939 (2008).

9 Cortes J, Goldman JM, Hughes T. Current issues in chronic myeloid leukemia: Monitoring, resistance, and functional cure. *J. Natl Compr. Canc. Netw.* 10(Suppl. 3), 1–13 (2012).

10 Barnes DJ, Palaiologou D, Panousopoulou E *et al.* Bcr-Abl expression levels determine the rate of development of resistance to imatinib mesylate in chronic myeloid leukemia. *Cancer Res.* 65(19), 8912–8919 (2005).

11 Jabbour E, Kantarjian H, Jones D *et al.* Frequency and clinical significance of BCR–ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia* 20(10), 1767–1773 (2006).

12 Gorre ME, Mohammed M, Ellwood K *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* 293, 876–880 (2001).

13 Kavalerchik E, Goff D, Jamieson CH. Chronic myeloid leukemia stem cells. *J. Clin. Oncol.* 26, 2911–2915 (2008).

14 Traer E, MacKenzie R, Snead J *et al.* Blockade of JAK2-mediated extrinsic survival signals restores sensitivity of CML cells to ABL inhibitors. *Leukemia* 26, 1140–1143 (2012).

15 Cortes JE, Baccarani M, Guilhot F *et al.* Phase III randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: tyrosine kinase inhibitor optimization and selectivity study. *J. Clin. Oncol.* 28, 424–430 (2010).

16 Jabbour E, Kantarjian HM, Jones D *et al.* Imatinib mesylate dose escalation is associated with durable responses in patients with chronic myeloid leukemia after cytogenetic failure on standard-dose imatinib therapy. *Blood* 113, 2154–2160 (2009).

17 Kantarjian H, Pasquini R, Levy V *et al.* Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia resistant to imatinib at a dose of 400 to 600 milligrams daily: two-year follow-up of a randomized Phase 2 study (START-R). *Cancer* 115, 4136–4147 (2009).

18 Nicolini FE, Kim D-W, Ceglarek B *et al.* Impact of prior therapy and suboptimal response to imatinib on the efficacy and safety of nilotinib among 1,422 patients with imatinib-resistant or -intolerant chronic myeloid leukemia (CML) in chronic phase (CP): sub-analyses of the ENACT (Expanding Nilotinib Access in Clinical Trials) Study. Presented at: *ASH Annual Meeting.* New Orleans, LA, USA, 5–8 December 2009.

19 Giles FJ, Abruzzese E, Rosti G *et al.* Nilotinib is active in chronic and accelerated phase chronic myeloid leukemia following failure of imatinib and dasatinib therapy. *Leukemia* 24, 1299–1301 (2010).

20 O'Hare T, Shakespeare WC, Zhu X *et al.* AP24534, a Pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell* 16, 401–412 (2009).

21 Cortes JE, Kantarjian H, Shah NP *et al.* Ponatinib in refractory Philadelphia-chromosome-positive leukemias. *N. Engl. J. Med.* 367(22), 2075–2088 (2012).

22 Cortes JE, Kim D-W, Pinilla-Ibarz J *et al.* A pivotal Phase 2 trial of ponatinib in patients with chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) resistant or intolerant to dasatinib or nilotinib, or with the T315I BCR-ABL mutation: 12-month follow-up of the PACE trial. *Blood* 120(21), Abstract 163 (2012).

23 Larson RA, Hochhaus A, Hughes TP *et al.* Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. *Leukemia* 26, 2197–2203 (2012).

24 Kantarjian HM, Shah NP, Cortes JE *et al.* Dasatinib or imatinib in newly diagnosed chronic-phase myeloid leukemia: 2-year follow-up from a randomized Phase 3 trial (DASISION). *Blood* 119(5), 1123–1129 (2012).

25 Hehlmann R, Hanfstein B, Muller MC *et al.* The prognostic significance of early molecular and cytogenetic response for long-term progression-free and overall survival in imatinib-treated chronic myeloid leukemia (CML). Presented at: *2012 ASCO Annual Meeting.* Chicago, IL, USA, 1–5 June 2012 (Abstract 6510).

26 Hochhaus A, Hughes TP, Saglio G *et al.* Outcome of patients with chronic myeloid leukemia in chronic phase (CML-CP) based on early molecular response and factors associated with early response: 4-year follow-up data from ENESTnd (Evaluating nilotinib efficacy and safety in clinical trials newly diagnosed patients). *Blood* 120(21), Abstract 167 (2012).