

Ponatinib—A Step Forward in Overcoming Resistance in Chronic Myeloid Leukemia

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Abstract

With the current therapy, the improvement in survival of patient with early chronic phase chronic myelogenous leukemia (CML) is unrivaled by that of any other leukemia. In fact, extrapolation of the survival curves may suggest that life expectancy of patients who achieve and maintain predetermined milestones may not differ from that of the age-matched healthy adults. The main reasons for such success are the presence of a well-defined molecular target, the *BCR-ABL* oncogene, necessary and sufficient for the initiation and propagation of CML, and the powerful and selective agents that inhibit it. Five U.S. Food and Drug Administration (FDA)-approved tyrosine kinase inhibitors (TKI), each with unique activities and toxicity profiles, allow for individualized patient care. Despite the remarkable responses of most patients, a small but significant fraction of patients develops clinical resistance to the TKIs, some of which is attributed to the *BCR-ABL* kinase domain mutations affecting TKI binding and activity. The recently approved third-generation TKI ponatinib showed remarkable activity in the patients with multi-TKI-resistant disease. Particularly impressive was its efficacy in patients with T315I mutation that is resistant to all other TKIs. In lieu of the current emphasis on achieving earlier and more profound responses and excellent activity of ponatinib in the refractory setting, its optimal position among the available armamentarium of agents is being established. *Clin Cancer Res*; 19(21); 5828–34. ©2013 AACR.

Introduction

Chronic myelogenous leukemia (CML) is an uncommon hematologic disorder, representing 15% to 20% of adult leukemia cases. In the United States in 2012, an estimated 5,430 patients were diagnosed with CML and 610 patients died from CML (1). The pathogenesis and clinical manifestation of the disorder is driven by the presence of a reciprocal chromosomal translocation $t(9;22)(q34;11.2)$ between the Abelson leukemia virus oncogen (*C-abl*) on the chromosome 9 and the breakpoint cluster region (*BCR*) on chromosome 22 (2). The resultant hybrid abnormal chromosome, called Philadelphia chromosome in honor of the city in which it was discovered, leads to a chimeric fusion protein, typically p210kDa *BCR-ABL*, although p185-p230 kDa proteins also exist in CML, depending on the breakpoints (3, 4). These proteins are deregulated constitutively active tyrosine kinases that drive proliferation and survival of CML clone by activating various downstream intracellular signaling transduction pathways such as *RAS/RAF/MAPK*, *PI3K-akt*, *STAT5*, *JUN*, *MYC* (5–7). This leads to cytokine-independent cell cycle and creates genetic as well

as epigenetic instability, allowing for acquisition of additional genetic alterations, which is reflected clinically by the inevitable progression from the chronic (CP) to accelerate (AP) to blast phases (BP) of CML. Targeting this tyrosine kinase activity resulted in successful CML therapy, initially with imatinib followed by the other tyrosine kinase inhibitors (TKI).

In recent years, five TKIs and one protein synthesis inhibitor received U.S. Food and Drug Administration (FDA) approval for CML therapy, imatinib, dasatinib, and nilotinib as the first-line therapeutic options; bosutinib, ponatinib, and omacetaxine, in a setting of resistant or intolerant disease (Fig. 1).

TKI resistance in CML

Overall, the clinical outcomes of patients receiving imatinib are remarkable. At the 8-year follow-up of the International Randomized Study of Interferon and STI571 (IRIS), the overall survival (OS) was reported at 85% and CML-related death at 7%. However, only 55% of the original cohort remained on the trial; others discontinued therapy due to drug intolerance or disease progression. From this and other first-line clinical trials, it is estimated that 20% to 30% of the patients will develop either primary (failure to achieve a predetermined milestones) or secondary (loss of the response after initial achievement of predetermined milestones) imatinib resistance (8, 9). Both patient- and disease-specific factors are implicated in the development of resistance. Patient-dependent characteristics include inadequate compliance, poor intestinal

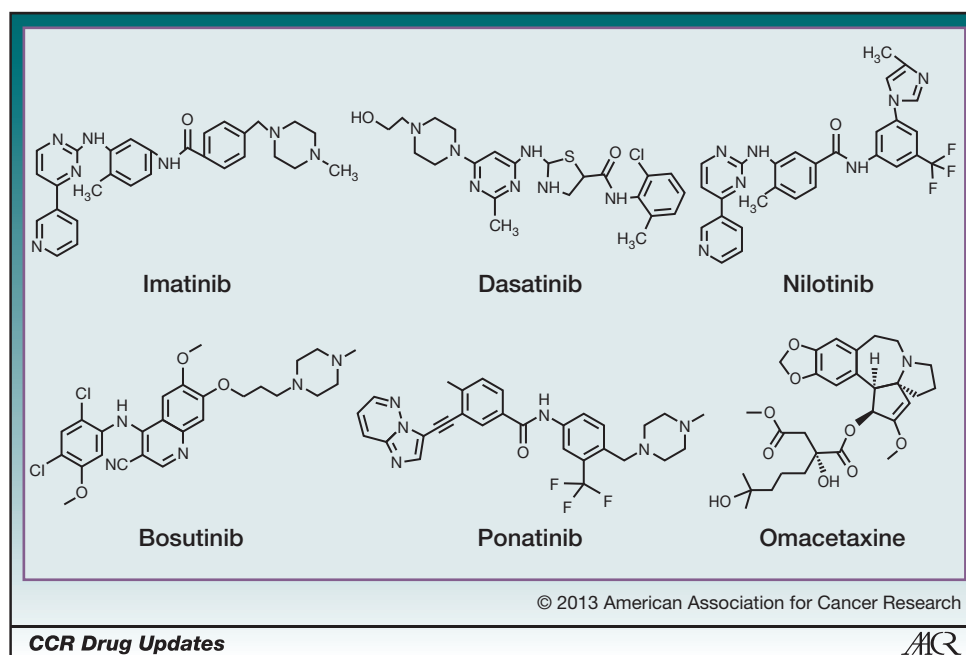
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Figure 1. Chemical structure of ponatinib and other drugs approved by the FDA for CML therapy.



absorption, drug interactions, and efficiency of drug transporters (particularly relevant for imatinib). Among the leukemia-specific mechanisms of resistance, including the persistence of the quiescent BCR-ABL-independent stem cells, BCR-ABL amplification, and activation of alternative signaling pathways, the development of BCR-ABL kinase domain mutations emerged to play a significant role. Well over 100 distinct point mutations of BCR-ABL kinase domain have been documented, some affecting amino acid that directly interact with imatinib, whereas others prevent the BCR-ABL to acquire the inactive conformation required for imatinib binding.

From several clinical studies, it seems that approximately 50% of the patients who show clinical evidence of resistance found to have kinase domain mutations (8, 9). Although more commonly seen in patients with advanced forms of CML, mutations were also identified in about 40% of those with chronic phase (CP)-CML. Presence of the mutations may not necessarily be the direct cause of clinical resistance (though to be responsible for 25%–60% of cases of resistance), but a hallmark of genomic instability, identifying patients at high risk for the disease progression (10). Second-generation TKIs including dasatinib, nilotinib, and bosutinib are active in patients with imatinib resistance leading to the major cytogenetic response (MCyR) in 35% to 63% of cases (refs. 11, 12; Table 1). There are mutations within BCR-ABL, however, that are resistant to second-generation TKIs. In fact, it was suggested that occurrence of multiple mutations within the kinase domain, so-called compound mutations, conferring high-level resistance, may be fostered by the sequential administration of various TKIs (13, 14). Several compound mutations have been shown to confer resistance even to ponatinib (14).

The outcomes of patients with advanced stages of CML are poor regardless of the mutational status. For example, in patients with AP- and BP-CML, OS after progression on imatinib is 16 and 5 months, whereas event-free survival after failure of two TKIs is only 5 and 3 months, respectively (15, 16).

Specifics and clinical significance of the T315I mutation

The highly resistant T315I kinase domain mutation was initially reported in 2001 in a case of transformed Ph⁺ leukemia after imatinib exposure (17, 18). This so-called "gatekeeper" mutation results in the replacement of the wild-type threonine (Thr) at Abl residue 315 with isoleucine (Ile). In order for the first- and second-generation TKIs to bind the ATP-binding pocket, they must form a crucial hydrogen bond with the side chain hydroxyl group of Thr315 (19–21). A mutation of the Thr to Ile precludes hydrogen bond formation and causes steric hindrance between the large hydrophobic Ile residue and the TKIs, thus blocking their access to the hydrophobic pocket.

The incidence of T315I mutation increases in parallel with disease progression and development of clinical resistance to sequential TKIs, with an incidence as high as 40% in patients who failed second-line TKI therapy. The survival of patient with T315I mutation is reflective of the disease phase at the time of mutation detection, reported to be 22, 28, 4, and 4.9 months in patients with CP-, AP-, BP-CML, and Ph⁺ ALL, respectively (22). Despite the remarkable activity of the second-generation TKIs in patients with CML who are refractory to imatinib therapy, none of these agents are active in patients with T315I mutation (23, 24).

Structure and Mechanism of Action of Ponatinib

A decade after T315I mutation was identified, the solution of the crystal structures of ABL kinase domain in

Table 1. Summary of the efficacy data for refractory/intolerant CP-CML

	Dasatinib (32)	Nilotinib (24, 33)	Bosutinib (34)	Ponatinib (refs. 25, 26; phase I trial)	Ponatinib (27) PACE trial	Omacetaxine (35)
^c Patients, <i>n</i>	101 (100%-R)	321 (70%-R)	288 (69%-R)	43	203	46
Prior TKIs	1	1	1	≥2%–98% ≥3%–63%	≥2%–98% ≥3%–67%	≥2–85% ≥3–59%
Follow-up, y	2	2	2	1.9	1.3	1.6
CHR (%)	93	74	86	98	N/R	61
MCyR (%)	53	59	53	72	51	22
CCyR (%)	44	44	41	65	46	4
MMR (%)	29	28	41 ^c	51	32	None
2-year PFS	>80%	64%	79%	83% MCyR ^a 55% MMR	91% MCyR ^b	7 months
2-year OS	90%	87%	92%	N/R	N/R	30.1 months

Abbreviations: CCyR, complete cytologic response; CHR, complete hematologic response; MMR, major molecular response; PFS, progression-free survival.

^aEstimated 83% of MCyR and 55% if MMR will maintain their responses at 2 years.

^bEstimated 91% of MCyR will maintain their responses at 1 year.

^cReported in all treated patients regardless of the CCyR status; not measured in 4 countries participating in the trial.

complex with imatinib clarified the nature of the resistance and led to the design of ponatinib. Ponatinib (Iclusig, ARIAD Pharmaceuticals) is a third-generation structure-guided, rationally designed TKI aimed to overcome the resistance of the T315I mutation by avoiding the interaction with the side chain of 315I. Ponatinib, similarly to imatinib, targets the inactive conformation of Abl kinase. Its essential structural characteristic is a triple carbon-carbon bond (ethynyl linkage) between the purine and methylphenyl groups, able to accommodate the Isl side chain without steric hindrance. Ponatinib derivatives with altered ethynyl linkers exhibit markedly diminished activity against T315I in cellular essays, highlighting the significance of this region. Importantly, the hydrogen bond is maintained when Thr is mutated to Isl.

Ponatinib potently inhibits native [50% inhibitory concentration (IC₅₀, 0.37 nmol/L)] and T315I (IC₅₀ 2.0 nmol/L) ABL kinases, as well as other mutant forms of BCR-ABL. Preclinical data from a cell-based mutagenesis model showed that at the clinically relevant concentrations, ponatinib abrogates development of all the mutant clones (14). The mechanism of action of ponatinib against other mutations is thought to be its ability to make multiple points of contact so than an individual mutation has less effect on the overall binding affinity of the drug. In addition to BCR-ABL, ponatinib targets VEGF receptor, platelet-derived growth factor receptor (PDGFR), EGF receptor, SRC kinase, KIT, RET, FLT3, but not aurora kinases.

Clinical Development/Toxicity

Ponatinib has shown remarkable clinical efficacy in patients with multi-TKI-resistant CML, leading to accelerated FDA approval for all phases of Ph⁺ leukemia resistant

or intolerant to prior TKIs. Ponatinib was granted an orphan products designation, and Ariad Pharmaceuticals is required by the FDA to conduct additional trials to confirm drug clinical efficacy and safety.

In the original phase I clinical dose-escalation trial of 65 patients with refractory Ph⁺ leukemia (43 CP-CML, 9 AP-CML, 8 BC-CML, 5 Ph⁺ ALL), 94% of the patients received at least 2 and 63% of the patients received at least 3 prior TKIs. At the study entry BCR-ABL mutations were detected in the majority of patients (65%), with the most common ones being T315I (29%) and F317L (11%). At a median follow-up time of 19.4 months (0.5–38), half of the patients remained on study, whereas 17% discontinued because of the disease progression and 14% secondary to development of the adverse events (AE), most common being rash (42%), thrombocytopenia (32%), arthralgia (20%), elevated serum lipase (18%), and fatigue (18%). Among the patients with CP-CML, 98% achieved and maintained complete hematologic response (CHR), although more than half entered the study in CHR, 72% MCyR, 65% complete cytogenetic response (CCyR), 51% major molecular response (MMR), and 23% MR4 (4-log reduction of BCR-ABL transcripts). Kaplan–Meier estimates suggest that 83% of the MCyR and 55% of MMR will maintain their response at 2 years. Among the 12 patients with CP-CML with T315I mutation, 100% CHR, 92% MCyR, 83% CCyR, and 67% MMR were achieved. Two-year estimates of maintaining MCyR (82%) and MMR (50%) in patients with T315I mutation were similar to that of the remaining CP-CML population. Among the patients with advanced phase disease, 40% achieved major hematologic response (MHR) that lasted 3.6 (0.02–14.7) months, 32% MCyR, and 18% CCyR (25, 26).

In the PACE phase II clinical trial of 45 mg of daily oral ponatinib for 449 heavily pretreated patients, 98% received

at least 2 and 53% received at least 3 prior TKIs, with majority (88%) of patients being resistant to the second-generation TKIs. After median follow-up for the entire CML-CP cohort of 15.3 (0.1–25) months, 63% remained on the study, whereas 12% discontinued for progression/lack of efficacy and 13% for AE; MCyR was 56% (70% for T315I and 51% for other CP-CML), CCyR 46%, MMR 34%, MR4.5 15%. Similarly to the results of the phase I trial, 91% CP-CML in MCyR retained that response at 1 year (27).

As expected, patients with advanced forms of Ph⁺ leukemia fared less well, although responses were seen among all the subgroups. MHR was achieved in 57% of AP-CML, 29% of myeloid BP-CML, 40% lymphoid CB-CM, and 41% of Ph⁺ ALL. MCyR was achieved in 39% of AP-CML, 19% of myeloid BP-CML, 40% lymphoid CB-CM, and 47% of Ph⁺ ALL. MMR was achieved in 24% of AP-CML, 15% of myeloid BP-CML, 30% lymphoid CB-CM, and 38% of Ph⁺ ALL. The median duration of the MHR was 12 months in AP-CML and 5 months in BP-CML/Ph⁺ ALL. At a median follow-up of 16 months, half the patients with AP-CML (53%) remained on the study, whereas only a minority of BP-CML/Ph⁺ ALL continued. The main reason for the discontinuation was disease progression. Multivariate analysis of the trial showed that the presence of T315I mutation did not affect the response to ponatinib; however, fewer prior therapies, younger age, and higher dose were associated with better response rates (28).

Discontinuation rates in the PACE trial due to the treatment-emergent AEs were 13% for CP-CML, 11% for AP-CML, 17% for myeloid BC-CML, 10% for lymphoid BC-CML, and 6% Ph⁺ ALL. Therapy-related bone marrow suppression was common, particularly in patients with advanced phase disease, although many of them had abnormal counts at baseline. Cardiovascular, cerebrovascular, and peripheral vascular thrombosis, including fatal myocardial infarction were reported, 11% of any grade, 8% serious AE (SAE), although most patients who experienced SAE had at least one cardiovascular risk factor. Treatment-related hypertension, defined as systolic blood pressure \geq 140 mmHg or diastolic blood pressure \geq 90 mmHg on at least one occasion, occurred in 67% of the patients, with 2% considered to be SAE. Clinical pancreatitis occurred in 6%, 5% being at least grade 3 AE; 6% required treatment interruption or discontinuation. Other common AEs included rash (38%, 4% grade 3/4), abdominal pain (38%, 9% grade 3/4, 4% SAE), headache (35%, 2% grade 3/4), dry skin (35%, 2% grade 3/4), constipation (34%, 2% grade 3/4), fatigue (27%, 2% grade 3/4), pyrexia (27%, 2% grade 3/4), nausea (26%, 1% grade 3/4), arthralgia (25%, 2% grade 3/4), lipase elevation (19%, 12%, grade 3/4, 2% SAE), and amylase elevation (7%, 2% grade 3/4). Patients should be monitored for the development of hepatotoxicity (acute liver failure and death occurred in 3 patients), hemorrhage (5% SAE, 24% bleeding usually in a setting of thrombocytopenia), fluid retention (3% SAE, 23%–16% peripheral edema, 7% pleural effusions, 3% pericardial effusions), cardiac arrhythmia, and tumor lysis syndrome (27).

Integration of Ponatinib into the CML Therapy Paradigm

Following FDA indications, ponatinib should be used in patients who failed or were unable to tolerate first and second-generation TKIs, and because it is the only active clinically available TKI for T315I mutation, it should be used in that setting as well. In the context of the current National Comprehensive Cancer Network and European LeukemiaNet recommendations for the optimal management of CML, at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (Chicago, IL), we use ponatinib as following:

Ponatinib is considered in the following instances:

1. Patients on imatinib, more than 10% BCR-ABL transcript by quantitative real-time PCR (qRT-PCR; international scale) or less than partial cytogenetic response (PCyR) at 3 months, intolerant to second-generation TKIs
2. Patients on second-generation TKIs who remain more than 10% BCR-ABL transcript by qRT-PCR (IS) or less than PCyR at 3 months
3. Patients on imatinib fail to achieve a CCyR at 12 months, intolerant to second-generation TKIs
4. Patients on second-generation TKIs fail to achieve a CCyR at 12 months
5. Patients on imatinib fail to achieve an MMR at 18 months, intolerant to second-generation TKIs
6. Patients on second-generation TKIs fail to achieve an MMR at 18 months
7. Patients with T315 mutation
8. Patients with V299L mutation intolerant to nilotinib
9. Patients with T315A or F317L/V/I/C mutations intolerant to nilotinib and bosutinib
10. Patients with F359V/C/I or Y253H or E255K/V mutations intolerant to dasatinib and bosutinib
11. Patient with clinical disease progression with or without other mutations

Further Directions/Questions

The goals of the current approach to the CML management have increasingly been to achieve more profound responses earlier in a course of therapy, leading to usage of the second-generation TKIs in the first-line setting. This tactic is based on the following observations. First, it seems that resistance and loss of response occur early in a course of the disease. Second, achievement of rapid and deep molecular responses correlates with improved outcomes. For example, one-log reduction of BCR-ABL transcript after 3 months of imatinib therapy is associated with the improved progression-free survival (PFS) and OS (29, 30). Early molecular responses, with time, appear increasingly more stable, enough so that questions of TKIs discontinuation in a setting of deep molecular remission and achievement of actual cure are being studied. Third, comparing imatinib with dasatinib, nilotinib, and bosutinib in DASISION, ERNEST, and BELA phase III trials, second-generation TKIs

were associated with lower rates of CML progression. Fourth, disease progression occurs in patients with all Sokal score risk groups (including the low risk), making it impossible to determine at the time of the diagnosis, which patients would benefit for more intensive first-line therapy. Fifth, sequential TKI therapy may predispose to the development of multiresistant compound mutations in a specific patient.

Acknowledging the caveats of historical comparisons, ponatinib shows efficacy superior to that of second-generation TKIs in the refractory setting (Tables 1 and 2). Considering the importance of achieving less than 10% BCR-ABL transcript after 3 months of therapy, 59% of the patients reached that landmark in the PACE trial. Given this remarkable activity, the question of whether ponatinib would be more effective than other TKIs as an up-front

therapy by preventing CML progression and occurrence of BCR-ABL mutations are being addressed (NCT01570868, NCT01650805). It remains to be seen whether the toxicity profile of ponatinib would allow for its widespread use in untreated patients with CML. Another important issue is whether ponatinib will improve the outcomes of patients with Ph⁺ ALL, given its robust activity against both native BCR-ABL kinase and T315I, a mutation that is particularly prevalent in this population after the exposure to prior TKIs. Early results of a phase II clinical trial combining Hyper-CVAD and ponatinib in untreated Ph⁺ ALL showed 100% complete response after the first cycle, 100% CCyR after 2 cycles, and 55% CMR at median of 10 weeks after starting the therapy (ref. 31; NCT01424982). The effect of ponatinib on leukemic stem cells and its potential for curing the patients with CML undoubtedly will be explored.

Table 2. Summary of the pharmacologic, efficacy, and toxicity data on the FDA-approved CML therapy

	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib	Omacetaxine
Main targets	Abl1, Kit, PDGFR, DDR1, NQO2	SRC, Abl1, PDGFR, Kit, EPHA2	Abl1, Kit, PDGFR, DDR1, NQO2, VEGF	Abl1, SRC	Pan BCR-ABL, SRC, VEGF	Protein translation inhibitor
Potency relative to imatinib	1	325-fold	30-fold	20-fold	>400-fold	N/A
Target Abl1 conformation	Inactive	Active and inactive	Inactive	Active	Inactive	N/A
Activity against T315I	None	None	None	None	Highly active	Active ^e
Dose	400 mg daily	100 mg daily	300 mg b.i.d. ^{a, b} or 400 mg b.i.d	500 mg daily	45 mg daily	1.25 mg/m ² s.c. ^c b.i.d × 14 days
Metabolism	CYP34A	CYP34A	CYP34A	CYP34A	CYP34A	Not CYP34A
Activity against CML stem cells	None	None	None	None	Unknown	Unknown
Main Toxicities	Pancytopenia, periorbital and peripheral edema, rash, nausea, diarrhea, transaminitis, cramps, skin discoloration	Pancytopenia, pleural effusions, headache, QT prolongation, rash, diarrhea, bleeding,	Transaminitis, clinical and laboratory pancreatitis, pancytopenia, hyperglycemia, QT prolongation, diarrhea, rash, electrolyte abnormalities	Diarrhea, pancytopenia, transaminitis, edema, rash	Arterial thrombosis, HTN, clinical and laboratory pancreatitis, transaminitis, pancytopenia, rash	Pancytopenia, infections, fatigue, injection site erythema, diarrhea
Price ^d	6,395/month	8,582/month	9,735/month	8,181/month	9,580/month	23,380/14 days

Abbreviation: HTN, hypertension.

^aTwice per day.

^b300 mg b.i.d for first-line therapy; 400 mg b.i.d second line.

^cSubcutaneously.

^d<https://passport.amerisourcebergen.com/irj/portal>, vendor for Robert H. Lurie Comprehensive Cancer Center, Northwestern Memorial Hospital (accessed May 7 2013).

^eOmacetaxine mepesuccinate, a first-in-class cephalotaxine that acts as a reversible protein translation inhibitor with clinical efficacy in patients with CML independent of BCR-ABL, also showed activity against T315I mutation. In a phase II clinical study in heavily pretreated patients (100% prior imatinib failure, 74% failure of ≥2 prior TKIs), subcutaneously administered omacetaxine lead to 84% CHR, 23% MCyR, 16% CCyR with median PFS of 7.7 months.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: O. Frankfurt

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): O. Frankfurt

Writing, review, and/or revision of the manuscript: O. Frankfurt, J.D. Licht

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): O. Frankfurt

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