REVIEW

Resistant mutations in CML and Ph+ALL – role of ponatinib

Geoffrey D Miller
Benjamin J Bruno
Carol S Lim

Department of Pharmaceutics and
Pharmaceutical Chemistry, College
of Pharmacy, University of Utah,
Salt Lake City, Utah, USA

Abstract: In 2012, ponatinib (Iclusig®), an orally available pan-BCR-ABL tyrosine kinase inhibitor (TKI) developed by ARIAD Pharmaceuticals, Inc., was approved by the US Food and Drug Administration for use in resistant or intolerant chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL). Ponatinib is the only approved TKI capable of inhibiting BCR-ABL with the gatekeeper T315I kinase domain mutation, known to be the cause for 20% of resistant or relapsed CML cases. In 2013, ponatinib sales were temporarily suspended due to serious side effects seen in nearly 12% of the patient population. These side effects are thought to stem from the potent nature and pan-activity of this TKI. ARIAD Pharmaceuticals, Inc. has since been permitted to resume sales and marketing of ponatinib to a limited patient population with an expanded black box warning. In the following review, the use of ponatinib in CML and Ph+ALL will be discussed. Mechanisms of resistance in CML are discussed, which provide insight and background into the need for this third generation TKI, followed by the molecular design and pharmacology of ponatinib, which lead to its success as a therapeutic. Finally, the efficacy, safety, and tolerability of ponatinib will be highlighted, including summaries of the important clinical trials involving ponatinib as well as its current place in therapy.

Keywords: BCR-ABL, T315I, Ph+ALL, PACE trial, EPIC trial, ARIAD, compound mutations

Management issues in CML and Ph+ALL

Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome, which results from a chromosomal translocation between chromosomes 9 and 22. This genetic aberration in turn forms the fusion oncoprotein tyrosine kinase BCR-ABL, whose kinase activity is responsible for the constitutive activation of growth, survival, and proliferation signaling pathways seen in CML cells.[1–3] The end result is an expansion of functionally normal cells of the myeloid lineage causing a leukemia phenotype. Inhibition of the BCR-ABL kinase domain results in leukemic cell death and often successful management of the disease.[4]

The Ph chromosome (and thus, BCR-ABL) is also present in some cases of acute lymphoblastic leukemia referred to as Ph chromosome-positive acute lymphoblastic leukemia (Ph+ALL), which is a subset of leukemia characterized by an expansion of immature cells from the lymphocyte lineage. Unlike CML, only 20%–30% of adult cases and 3%–5% of childhood cases contain the Ph chromosome and subsequent BCR-ABL protein.[5] Specifically, presence of the Ph chromosome in ALL correlates with a

Correspondence: Carol S Lim
University of Utah, 30 South 2000 East,
Room 2916, Salt Lake City, UT 84112,
USA
Tel +1 801 587 9711
Email carol.lim@pharm.utah.edu

less favorable prognosis.[6] Whereas in CML the leukemic cells are normally dependent on BCR-ABL signaling for growth and survival, solely inhibiting the BCR-ABL tyrosine kinase in Ph+ALL is not sufficient to inhibit leukemic cell growth.[7] Thus, more broad, intense therapies are needed to eradicate Ph+ALL.

Current treatments of CML and Ph+ALL

In 2001, imatinib mesylate (Gleevec®), a small molecule targeting the adenosine triphosphate (ATP)-binding region of the BCR-ABL tyrosine kinase domain, was US FDA approved for first-line treatment of CML.[8–10] While the introduction of this drug aided in improving 10-year survival rates to 80% to 90%,[11] not all patients responded to imatinib therapy.[9] In many of these cases, resistance was due to the presence or acquisition of mutations in the kinase domain that prevented imatinib binding and activity. Thus, second and third generation tyrosine kinase inhibitors (TKIs) were designed and developed to overcome this resistance or intolerance. Dasatinib (Sprycel®), a dual-Src family kinase inhibitor, and nilotinib (Tasigna®), a more potent BCR-ABL

inhibitor, were approved for CML therapy in patients who were intolerant or nonresponsive to imatinib in 2006 and 2007, respectively. Each of these inhibitors was designed to inhibit a broader range of kinases, thereby exhibiting greater activity against a number of kinase domain mutations that could not be inhibited by imatinib.[4]

Currently, newly diagnosed CML patients in the chronic phase have the option of beginning treatment on imatinib, dasatinib, or nilotinib. A majority of patients will initially respond to one of these treatments. However, in nearly one-third of patients, first-line TKI treatment will eventually become ineffective, forcing patients to start on another TKI.[12] Most patients will convert to either nilotinib or dasatinib. However, for some patients, especially those possessing the T315I kinase domain mutation, ponatinib (Iclusig®) will be the ideal TKI. Additionally, omacetaxine, a protein synthesis inhibitor, has been approved recently for relapsed CML patients. While this drug shows a marginal success rate, significant side effects (including, for example, myelosuppression, bleeding, and hyperglycemia) are seen in 99% of patients. Such side effects are likely due to the nonspecificity of the molecule making it undesirable for many CML patients, and thus will not be

Table 1 Select approved drugs for CML therapy, kinase specificity, and notable adverse effects and toxicity.

Drug name	Type of inhibitor	Binds which kinase conformation?	Kinase specificity	Adverse reactions/toxicity
Imatinib	TKI	Inactive	Abl, PDGFR, KIT	Rash, decreased LVEF, CHF, arterial thrombotic events, noninfectious pneumonitis/diffuse alveolar damage/pulmonary fibrosis, mucositis/stomatitis, diarrhea/colitis, neutropenia, thrombocytopenia, transaminase elevations, hyperlipasemia, hypophosphatemia
Dasatinib	TKI	Active	Abl, Src family (SRC, YES, FYN, LYN), KIT, PDGFR, BMX, Eph family, VEGFR2, TIE2	Rash, decreased LVEF, CHF, QT prolongation, arterial thrombotic events, noninfectious pneumonitis/diffuse alveolar damage/pulmonary fibrosis, pulmonary arterial hypertension, diarrhea/colitis, neutropenia, thrombocytopenia, transaminase elevations, hypophosphatemia
Nilotinib	TKI	Inactive	Abl, PDGFR, KIT	Rash, decreased LVEF, CHF, QT prolongation, arterial thrombotic events, diarrhea/colitis, hyperglycemia, neutropenia, thrombocytopenia, transaminase elevations, hyperlipasemia, hypophosphatemia
Ponatinib	TKI	Inactive	Abl, FLT3, FGFR, VEGFR, KIT, PDGFR, RET, EPH, Src family	Rash, decreased LVEF, CHF, QT prolongation, arterial thrombotic events, mucositis/stomatitis, diarrhea/colitis, hyperglycemia, neutropenia, thrombocytopenia, transaminase elevations, hyperbilirubinemia, hyperlipasemia, hypophosphatemia
Omacetaxine	Protein synthesis inhibitor	(Not applicable)	(Not applicable)	Toxic: 99% of patients in chronic CML had adverse effects, 18% requiring drug withdrawal (w/d); 100% in accelerated CML had adverse effects, 37% requiring drug w/d; 3% death from myelosuppression; 2% death from cerebral hemorrhage; embryo-fetal death in animals. Severe thrombocytopenia (>80% patients), hyperglycemia

Note: Data from.[13,23,45,60,81,88–90]
Abbreviations: Abl, abelson kinase; BMX, member of Bruton's tyrosine kinase family; CHF, congestive heart failure; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; EGF, epidermal growth factor; Eph, ephrin family receptor tyrosine kinases; EPH, ephrin receptor tyrosine kinase; EPIC, Evaluation of Ponatinib versus Imatinib in Chronic Myeloid Leukemia; FGFR, fibroblast growth factor receptor; FLT, FMS-like tyrosine kinase; FYN, fyn kinase; KIT, kinase receptor; LVEF, left ventricular ejection fraction; LYN, Lck/yes novel tyrosine kinase; PACE, Ponatinib Ph+ALL and CML Evaluation; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection kinase; Src, sarcoma family of kinases; SRC, sarcoma protein kinase; TIE, tyrosine kinase with immunoglobulin-like and EGF-like domains; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor; w/d, withdrawal; YES, yes kinase.

further discussed.[13] Table 1 shows some of the approved therapies for CML, including their mode of action, usage indications, and common side effects.

While the treatment of Ph⁺ALL includes the use of the aforementioned TKIs, the overall treatment strategy differs from CML. Current frontline treatment of childhood Ph⁺ALL includes chemotherapy with a rigorous induction phase, often using cyclophosphamide, vincristine, adriamycin, and dexamethasone (known as hyper-CVAD) in combination with a TKI such as imatinib or dasatinib.[14] Even if remission is achieved, the only possibility of a cure is hematopoietic stem cell transplantation. Most patients who do not undergo hematopoietic stem cell transplantation eventually relapse.[14] Depending on the molecular status of BCR-ABL transcripts during follow-up, patients may be administered a TKI as maintenance therapy.

In the following sections, the mechanism of resistance to TKIs will be discussed, as well as the role of ponatinib in these scenarios. Additionally, the pharmacology and mode of action of ponatinib will be discussed to describe how and why ponatinib is often effective against both native and mutant BCR-ABL. Finally, the efficacy and safety of ponatinib will be discussed, including a summary of various clinical trials; and the current place and future use of ponatinib in therapy will be described and speculated. The majority of this review will focus on the potential of ponatinib in the treatment of CML. Although CML and Ph⁺ALL involve different treatment strategies, overlapping issues arise in TKI-resistant CML and BCR-ABL inhibition in Ph⁺ALL.

Mechanisms of mutational resistance in CML and Ph⁺ALL

It is well documented how the introduction of imatinib (and other TKIs) has profoundly changed the therapeutic management of CML and Ph⁺ALL.[15–17] Despite these successes, it is estimated that one-third of CML patients on first-line imatinib therapy will require other therapy due to imatinib intolerance or resistance.[12] BCR-ABL independent resistance represents another mechanism of TKI resistance, which will not be discussed in this review. For excellent reviews on this topic, two other papers provide excellent topical summaries.[18,19]

Resistance to TKIs can be classified into two types: primary resistance and secondary resistance. Primary resistance refers to the lack of initial response, presumably due to the presence of baseline mutations in the kinase domain. On the other hand, secondary resistance refers to the loss of an established response, often due to the prolonged exposure to the TKI.[20] Whether it is primary or secondary, the most common and well understood mechanism of resistance against all TKIs is the appearance or development of point mutations in the BCR-ABL kinase domain.[19] In patients with primary resistance, many leukemic cells harbor mutations from the onset of disease. Conversely, a clonal model of resistance is often used to describe secondary resistance from a molecular standpoint. Here, nonresistant cells are first eliminated by TKI therapy giving surviving resistant clones a selective advantage for future outgrowth. The observation that sequential treatment with TKIs often leads to enhanced TKI resistance supports this theory.[21,22] In a subset of patients who were treated sequentially with imatinib and dasatinib but did not reach disease remission, new BCR-ABL mutations were observed after dasatinib treatment, which had not been recognized after imatinib treatment. These double mutants, as described in the "Emergence of compound mutations" section, can confer resistance to multiple drugs and enhance BCR-ABL oncogenicity.[21]

Importantly, the kinase domain mutations that are responsible for resistance occur at strategic locations, which prevent TKI activity. When these residues are mutated, they inhibit TKI binding but still allow ATP binding and kinase activity.[23] Imatinib, dasatinib, and nilotinib each bind the BCR-ABL kinase in a characteristic manner (Table 1). Due to these binding specifics, resistance profiles specific to each TKI will exist. In some cases, mutations will occur directly in the kinase active site, sterically hindering TKI binding. In other cases, mutations will occur at residues that affect the overall three-dimensional structure of the kinase. Because imatinib and nilotinib bind BCR-ABL in its inactive conformation (Table 1), mutations may occur in resistant patients that destabilize or prevent adoption of the inactive conformation of the kinase. Failure to adopt this conformation prevents the binding and activity of imatinib and nilotinib.[18,21] Most importantly, clinically relevant mutations in the BCR-ABL kinase domain all occur on residues such that the integrity of the tyrosine kinase itself is not eliminated. Specifically, more than 55 residues showing varying levels of resistance to imatinib have been identified.[18] It should be noted that while many of the studied kinase domain mutations are observed in CML, mutations in BCR-ABL also appear in Ph⁺ALL with similar effects.

Emergence of compound mutations

In a 2013 study, 1,700 patient samples with BCR-ABL kinase domain mutations were analyzed using direct DNA sequencing. In this set of samples, 11.4% showed two or more mutations in the kinase domain.[24] Double mutants in the BCR-ABL

kinase domain can exist in two forms: compound or polyclonal. Compound mutations, which account for 70.2% of double mutations in the BCR-ABL kinase domain, refer to multiple mutations in the same BCR-ABL molecule.[24] The other 29.8% of double mutations are represented as polyclonal mutants, where mutations occur in multiple BCR-ABL molecules within a single cell. As previously mentioned sequential treatments with TKIs can lead to the emergence of multiple mutations in a single patient. Not surprisingly, the composition of the compound mutations noted in this study reflected the TKI treatment history, where one or more mutational components were associated with a typical clinical or in vitro resistance profile to the specific TKIs.[24] Altogether, 30 different compound mutations were observed, with the T315I mutation being the most frequently observed mutation among all compound mutants. It has been suggested that the best way to prevent the onset of compound mutations is to initially treat with a drug that can "collectively suppress" all single point mutations. A molecule such as ponatinib, which was shown to inhibit all clinically important kinase domain mutations including T315I during preclinical evaluation, has the potential to achieve this feat. It is unlikely, however, that ponatinib will be used for first-line therapy at its current dosing regimen.[21,25]

Pharmacology of ponatinib

Although dasatinib and nilotinib have shown efficacy against many of the clinically relevant mutations for which imatinib is ineffective, neither has shown the ability to inhibit BCR-ABL with the T315I mutation (BCR-ABL[T315I]).[26] Thus, the need arose for a new TKI that not only inhibits BCR-ABL[T315I] but also inhibits native BCR-ABL and other commonly seen BCR-ABL mutations. In order to design a molecule that could meet these criteria, it is important to understand the structural features of the BCR-ABL kinase domain containing the T315I mutation.

Effects of the T315I mutation

Although the T315I mutation does not disturb the overall structure of the BCR-ABL protein, it affects the topology of the ATP binding region.[27] Current TKIs are unable to inhibit this mutant form of the enzyme for two main reasons. The T315I mutant form of BCR-ABL lacks a threonine residue, which provides a hydroxyl group critical for hydrogen bonding with first and second generation TKIs. This hydrogen bond has been described to drive both the potency and specificity of these ABL inhibitors.[27,28] The hydrogen bond disappears when the threonine is replaced with an isoleucine (Ile) residue. Additionally, residue 315 is located directly in front of the hydrophobic pocket, which is why it is referred

to as the "gatekeeper" residue. When mutated to Ile, the bulkier Ile side chain protrudes further into the enzyme active site. This initiates a steric clash subsequently blocking the entrance of a TKI into the hydrophobic pocket while still allowing access to ATP. For these reasons, first and second generation TKIs fail to inhibit BCR-ABL[T315I].[25,27,29]

Design of ponatinib

Overcoming both losses of an active-site hydrogen bond and the steric hindrance caused by the T315I mutation were the main focus during the design process of ponatinib.[30] While still binding to the kinase active site of BCR-ABL, the overall design of the ponatinib molecule was still based on the ATP-mimetic template provided by the previous TKIs imatinib, nilotinib, and dasatinib. The structure of ponatinib is shown in Figure 1. The five critical regions of the molecule and the structure–activity relationships of each region are described in the following sections.

Hinge region

The hinge region (Figure 1A) was optimized to improve binding affinity, lipophilicity, and overall oral bioavailability. The final product resulted in a 6-5 fused ring system with an imidazole[1,2]pyridazine chemical moiety that occupies the adenine-binding pocket of the kinase domain.[30] Chemically, the imidazole moiety forms hydrogen bonds with the hinge of the enzyme pocket (Figure 2) while the pyridazine ring interacts hydrophobically with F382 of the DFG region and Y253 in the P-loop of BCR-ABL (Figure 2). Finally, the lack of atomic substitutions and substituents in this fused ring system has led to optimal lipophilicity and oral bioavailability.[29]

Ethynyl linker

Ponatinib differs from previous BCR-ABL TKIs due to the presence of a triple bond ethynyl linker (Figure 1B), which connects the hinge region and A ring. Most importantly, this ethynyl linker allows ponatinib to span the bulky isoleucine side chain present in BCR-ABL[T315I] (Figure 2). Structurally, this Ile side chain protrudes into the active site in front of the hydrophobic pocket. Previous TKIs could not span this side chain and thus could not dock into the hydrophobic pocket. Structure–activity studies revealed that the presence of the triple bond allows a ten-fold increase in potency when compared to similar molecular designs containing single or double bonded systems.[29] Vinyl and acetylene linkages were also attempted during the design process. While vinyl and acetylene linkages also showed promise, the final ethynyl linkage showed superior chemical and pharmacological stability.
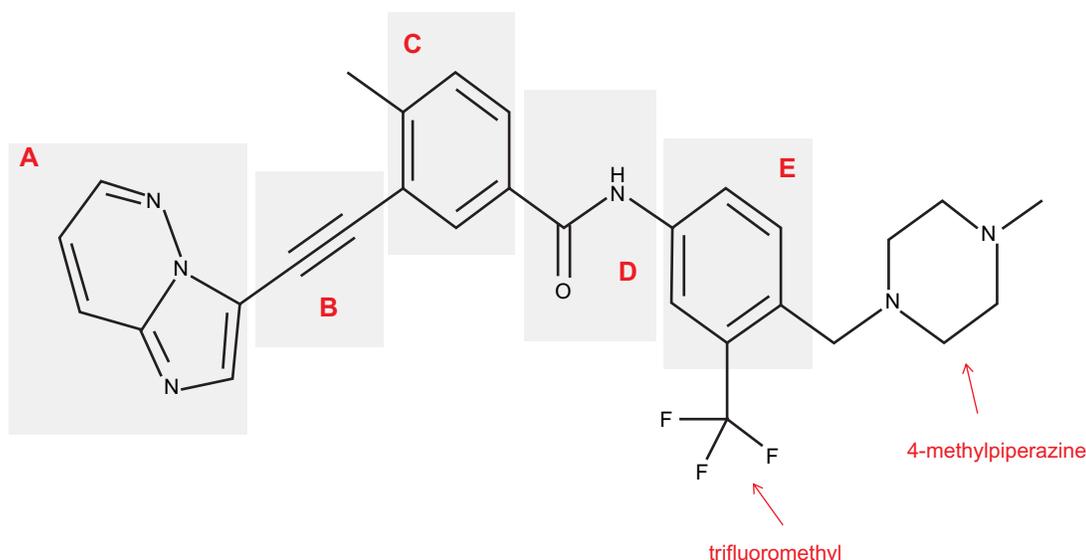
Figure 1 Chemical structure of ponatinib.
Notes: Chemical structure of ponatinib with labels for the (**A**) hinge region, (**B**) ethynyl linker, (**C**) A ring, (**D**) linker 2, and (**E**) B ring. The trifluoromethyl and 4-methylpiperazine groups, which are added during the optimization process for maximal BCR-ABL inhibition, are also shown.

A ring

The A ring of ponatinib (Figure 1C) binds in the hydrophobic pocket of the BCR-ABL enzyme located just behind the gatekeeper residue. Existing as a methylphenyl moiety, the A ring appeared very sensitive to any substitutions or chemical modifications and thus, was not changed.[29,30].

Linker 2

Linker 2 (Figure 1D) chemically exists as an amide linkage with the purpose of connecting the A and B rings, spatially aligning the

Figure 2 Hydrogen bonding pattern between ponatinib and BCR-ABL[T315I].
Notes: Pymol figure showing ponatinib (pink) binding in the ATP-binding region of BCR-ABL[T315I]. Hydrogen bonds involving the residues E286, M318, I360, and D381 of BCR-ABL[T315I] are represented by yellow dashed lines. Oxygen atoms are shown in red, nitrogen atoms in blue, sulfur in gold, and fluorine in cyan. Protein Data Bank, ID 3IK3.
Abbreviations: ASP, aspartate; ATP, adenosine triphosphate; BCR-ABL[T315I], BCR-ABL with the T315I mutation; GLU, glutamate; ILE, isoleucine; MET, methionine.

molecule with the structure of the BCR-ABL.[29] Hydrogen bonds are formed between the linker carbonyl group and residue D381, and between the NH group and residue E286 (Figure 2).[28]

B ring

The B ring (Figure 1E) consists of a trifluoromethyl ring with a 4-methylpiperazine substituent. This B ring binds into the aspartate-phenylalanine-glycine (DFG)-out pocket of the enzyme active site. The addition of these particular substituents has allowed for optimization of maximal BCR-ABL[T315I] inhibition due to the formation of critical hydrogen bonds between the piperazine group and the enzyme backbone, notably at residue I360 (Figure 2).[28] Specifically, methylpiperazine increases potency and molecular recognition, while piperazine itself is a solubilizing group, improves permeability, and decreases lipophilicity and protein binding.

Taken together, the optimization process of the five regions listed above led to the final design of ponatinib. Although the overall structure remains similar in many ways to other approved TKIs, the ability to potently inhibit native BCR-ABL, BCR-ABL[T315I], and many other mutant forms of BCR-ABL made ponatinib a highly versatile drug with great potential moving into clinical evaluation.
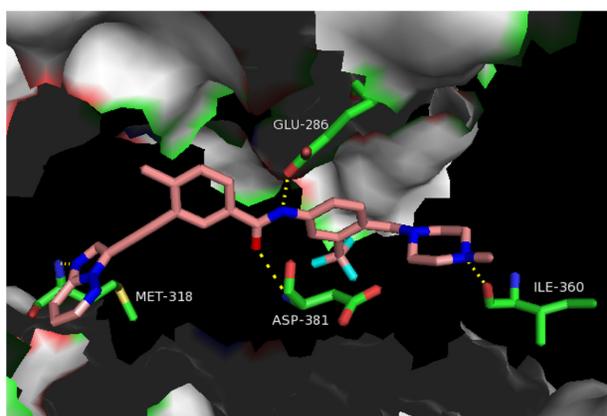
Efficacy, safety, and tolerability of ponatinib
Preclinical testing of ponatinib

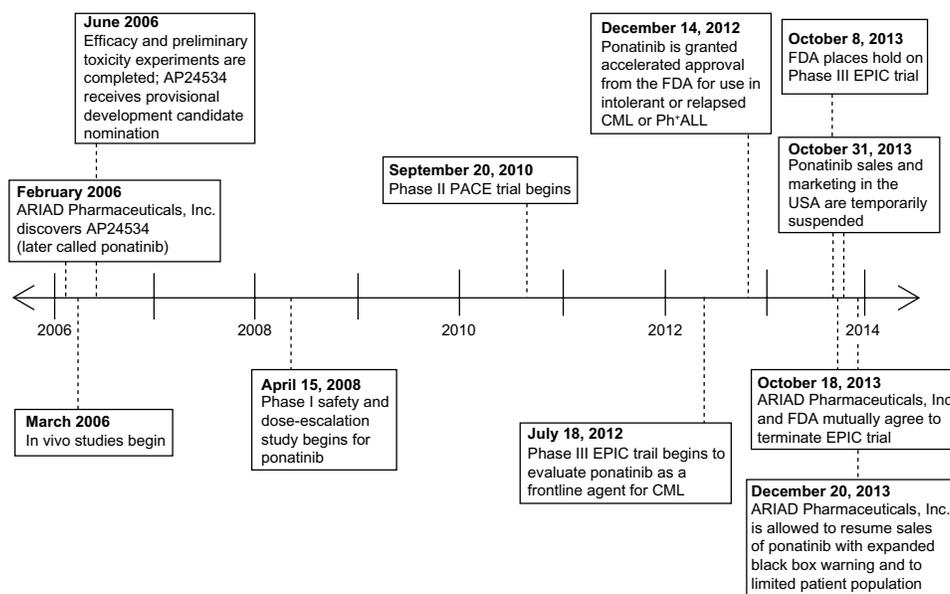The design and preclinical testing of ponatinib began in 2006 as the need for a CML therapy capable of inhibiting

June 2006
Efficacy and preliminary toxicity experiments are completed; AP24534 receives provisional development candidate nomination

February 2006
ARIAD Pharmaceuticals, Inc. discovers AP24534 (later called ponatinib)

September 20, 2010
Phase II PACE trial begins

December 14, 2012
Ponatinib is granted accelerated approval from the FDA for use in intolerant or relapsed CML or Ph⁺ALL

October 8, 2013
FDA places hold on Phase III EPIC trial

October 31, 2013
Ponatinib sales and marketing in the USA are temporarily suspended

March 2006
In vivo studies begin

April 15, 2008
Phase I safety and dose-escalation study begins for ponatinib

July 18, 2012
Phase III EPIC trail begins to evaluate ponatinib as a frontline agent for CML

October 18, 2013
ARIAD Pharmaceuticals, Inc. and FDA mutually agree to terminate EPIC trial

December 20, 2013
ARIAD Pharmaceuticals, Inc. is allowed to resume sales of ponatinib with expanded black box warning and to limited patient population

2006 2008 2010 2012 2014

Figure 3 Timeline of ponatinib events from discovery to present day.
Notes: This figure highlights the dates of the discovery of ponatinib (formerly AP24534), including preliminary studies, clinical trials, and events involving the approval, suspension, and resumption of sales. Data from.[36,39,75–80]
Abbreviations: CML, chronic myeloid leukemia; EPIC, Evaluation of Ponatinib versus Imatinib in Chronic Myeloid Leukemia; FDA, US Food and Drug Administration; PACE, Ponatinib Ph⁺ALL and CML Evaluation; Ph⁺ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia.

many resistant forms of BCR-ABL arose (Figure 3). Initial preclinical testing involved demonstrating that ponatinib could both decrease autophosphorylation of the BCR-ABL kinase as well as inhibit the proliferation of cells dependent on BCR-ABL. Namely, these in vitro experiments utilized K562 (BCR-ABL positive human cells) and Ba/F3 (pro-B mouse cells), which stably transduced to express BCR-ABL with a wild-type or mutant tyrosine kinase domain. From these studies, it was found that ponatinib inhibits both wild-type and mutant BCR-ABL kinases with IC_{50} (50% inhibitory concentration of the drug) values between 0.37–2.0 nM.[25] Further, cell proliferation assays demonstrated inhibition with IC_{50} values ranging from 0.3–0.5 nM for non-mutated and up to 36 nM for mutant BCR-ABL. It was also found that a dose of 40 nM was able to completely suppress the emergence of BCR-ABL resistant mutants in vitro, although efficacy may be limited particularly in examples with the E255V point mutation (IC_{50} of 36 nM). Finally, ponatinib also inhibited the activity of members of the vascular endothelial growth factor (VEGF), fibroblast growth factor receptor (FGFR), and platelet-derived growth factor receptor (PDGFR) families at similar doses, but it showed IC_{50} values more than 1,000-fold higher for inhibition of insulin receptor and members of the cyclin kinase and aurora kinase families.[25]

Based on this information, researchers proceeded to a xenograft mouse model (Figure 3) as is standard for in vivo CML studies. Cells harboring native BCR-ABL and

BCR-ABL^T315I were subcutaneously injected into the right flank in female mice, and these mice were given ponatinib at doses of 10, 30, and 50 mg/kg. The 10 mg/kg and 30 mg/kg doses were able to suppress tumor growth, while the 50 mg/kg dose was able to cause 96% reduction in the mean tumor volume.[25] Pharmacokinetic studies of the 30 mg/kg dose revealed mean plasma levels of 782, 561, and 8 nM at 2, 6, and 24 hours posttreatment, respectively. No pharmacokinetic data for the 50 mg/kg dose was reported. This demonstrated that plasma concentrations of ponatinib effective against mutated forms of BCR-ABL could be sustained for 24 hours using the 30 mg/kg dose.

Moving ponatinib into clinical trials
The first Phase I trial involving ponatinib enrolled CML and Ph⁺ALL patients who were resistant or had relapsed while they were on the standard of care therapy. The goal of the study was to determine the maximum tolerated or recommended daily dose of ponatinib, while also investigating the safety, anti-leukemic activity, and pharmacokinetic/pharmacodynamic profile of the drug.[31] Eighty-one patients were enrolled, 65 of whom had Ph⁺ disease. Daily doses of 2–60 mg were tested. No dose-limiting toxic effects were observed up to the 30 mg/day dose, while the 60 mg/day dose led to toxicity in six out of 19 patients. Adverse effects included elevation of pancreatic enzymes, clinical pancreatitis, fatigue, and increases in liver enzymes aspartate transaminase (AST) and alanine transaminase (ALT)

(Table 1). Pharmacokinetic studies demonstrated a linear relationship between dose and peak blood concentration. For the 30 mg/day dose, the half-life was 22 hours; and the trough blood concentration was above 40 nM, which is the minimal concentration thought to be required to prevent the emergence of BCR-ABL mutants resistant to ponatinib. The anti-leukemic activity of the drug was also promising; 42/43 (98%) of patients with chronic phase (CP)-CML (Table 2) had a complete hematologic response (see Table 3 for clinical terms); 31/43 (72%) had a major cytogenetic response (MCyR), 27/43 (63%) had a complete cytogenetic response (CCyR), and 19 patients had a major molecular response (MMR). Further, all 12 patients with CP-CML and the T315I mutation had a complete hematologic response, and 8/12 (67%) had a MMR. Despite the fact that the a dose of 30 mg/day showed excellent results in the Phase I trial, researchers proceeded to Phase II study using the 45 mg/day dose to ensure coverage of any and all BCR-ABL TKI resistant clones.[31–33]

The Phase II Ponatinib Ph⁺ALL and CML Evaluation (PACE) trial enrolled 449 patients: 267 with CP-CML, 83 with accelerated phase (AP)-CML, and 94 with blast crisis (BC)-CML or Ph⁺ALL (Table 2).[32] Patients were eligible for the study if they were resistant or had unacceptable side effects to dasatinib or nilotinib, or harbored the T315I mutation. The trial showed that 37% of patients had received two TKIs, and 55% had received three or more. Of those who had received dasatinib or nilotinib, 88% were resistant. At 12 months after the initiation of ponatinib therapy, 56% of patients with CP-CML had a MCyR, 46% had CCyR, and 34% had MMR (Table 3). Interestingly, higher response rates were reached for those with the T315I mutation, but the presence of this mutation was determined not to be a significant prognostic factor.[34] Six patients had an unsustained MCyR, but no change in mutation status was seen in those with unsustained responses. In patients with AP-CML and BC-CML (Table 2) or Ph⁺ALL,

rates of major hematologic response at 6 months were 39% and 31%, respectively. Importantly, no single mutation conferring resistance was seen.

Responses were observed for each of 15 mutations present at baseline for CP-CML patients, and 26 unique mutations in total.[35] Response rates were similar for those with and without mutant BCR-ABL, with MCyR occurring in 56% of all patients, 49% of patients with native BCR-ABL, 64% for those with one mutation, 62% for those with two or more mutations, and 74% for those with the T315I mutant.[35] Though no single point mutation has shown resistance against ponatinib, compound mutations developed in 21 patients, mainly those in BC-CML or Ph⁺ALL. While ponatinib should still inhibit a number of compound mutants based on their in vitro cell proliferation IC_{50} values, a select few clinically reported compound mutants, including G250E/T315I, E255K/T315I, and E255V/T315I (IC_{50} values of 49 nM, 106 nM, and 425 nM, respectively[22]), have shown increased resistance. Because these compound mutants appear only infrequently, and based on these results from the clinical trials described above, ponatinib was granted accelerated FDA approval in December 2012 for use in resistant or intolerant CML or Ph⁺ALL or in patients harboring BCR-ABL^T315I (Figure 3).[36]

Safety profile of ponatinib

Minimal safety concerns were initially reported. Sixty-seven percent of patients had dose interruptions, with 55% having at least one dose reduction. Serious nonhematologic adverse drug events (ADEs) included pancreatitis, abdominal pain, and myocardial infarction (MI). Serious hematologic ADEs included thrombocytopenia, anemia, neutropenia, and pancytopenia. Cardiovascular, cerebrovascular, and peripheral vascular events were observed in 7.1%, 3.6%, and 4.9% of the population, respectively, although it was speculated

Table 2 World Health Organization (WHO) definitions and descriptions of CML stages, and commonly seen symptoms

Chronic phase	Accelerated phase	Blast crisis
≤10% blast cells in blood and bone marrow	10%–19% blast cells in blood and bone marrow	≥20% blast cells in blood and bone marrow
• WBCs still capable of fighting infection	• High basophil count (≥20% of WBC differential) • Persistent thrombocytopenia unrelated to therapy • Persistent thrombocytosis unresponsive to therapy • Increasing WBC and spleen size unresponsive to therapy • Cytogenic evidence of clonal evolution	• Large clusters of blasts in bone marrow • Extramedullary blast proliferation
Mild symptoms, if any	Fever, poor appetite, weight loss, fatigue	Fever, loss of appetite, weight loss, fatigue, bleeding, infections common, stomach pain, bone pain

Note: Data from.[82–86]
Abbreviations: WBCs, white blood cells; CML, chronic myeloid leukemia.

Table 3 Clinical terminology describing patient response in CML

Clinical terminology	Abbreviation	Description
Hematologic response	HR	Decrease in the number of WBCs from the time of diagnosis
Complete hematologic response	CHR	WBC count returns to normal level and no immature cells seen in the blood
Cytogenetic response	CyR	Any reduction in Ph chromosome levels from baseline established at diagnosis
Major cytogenetic response	MCyR	0%–35% of cells are Ph$^+$
Complete cytogenetic response	CCyR	0% Ph$^+$ cells measured by cytogenetic testing
Molecular response	MR	Any reduction in BCR-ABL transcript levels as measured by PCR
Major molecular response	MMR	≥3 log reduction in BCR-ABL transcript levels
Complete molecular response	CMR	Undetectable BCR-ABL transcript levels

Note: Data from Kantarjian et al.[87]
Abbreviations: WBC, white blood cell; CML, chronic myeloid leukemia; Ph, Philadelphia; PCR, polymerase chain reaction.

these were minimally related to the treatment. Serious arterial thrombotic events were seen in 8.9% of patients on ponatinib, predominantly in patients with either documented ischemic conditions or with one or more risk factors at baseline.

An analysis of the cardiovascular risk profile of ponatinib was performed with the results of the PACE trial.[37] There were 14 cases of MI, five of coronary artery disease, and two of angina (21 in total). At the start of the study, 10/21 had active cardiovascular disease, and five had valvular or pericardial disease. Further, 81% of these patients had at least two cardiovascular risk factors, while 95% had at least one. At the time of the drug's approval, the label noted that 8% of patients had cardiovascular, cerebrovascular, and peripheral vascular thrombosis including fatal MIs and strokes.[38] However, at a 2.7-year follow-up of the Phase I study, 48% of patients reported adverse vascular events. At a follow-up of the Phase II study (median time of 1.3 years), 24% of patients had an MI, stroke, limb ischemia, or stenosis of the vessels of the heart, extremities or brain requiring revascularization.[38] It is unlikely that these events can be fully explained by underlying cardiovascular risk, as numbers this high were not seen in late phase trials of other TKIs. Further, adverse events occurred as soon as two weeks into therapy and even in patients with no cardiovascular risk factors.[38]

As the increased cardiovascular risks associated with ponatinib were uncovered, ponatinib was removed from the market in October 2013.[39] Additionally, the Evaluation of Ponatinib versus Imatinib in Chronic Myeloid Leukemia (EPIC) trial, a Phase III trial investigating the use of ponatinib versus imatinib for first-line use, was halted following this decision. The FDA and ARIAD Pharmaceuticals, Inc. "mutually agreed the trial should be terminated" due to the now understood increased cardiovascular risks associated with ponatinib.[40] Ponatinib has since been put back on the market for a narrowed patient population: treatment of adults with T315I-positive CML (any phase) and Ph$^+$ALL, and treatment of adult patients for whom no other TKI therapy is indicated.[38] A timeline highlighting important events in the discovery and approval process of ponatinib is provided in Figure 3.

One question that still remains concerning TKI therapy is whether or not the therapy can ever be discontinued without resulting in disease recurrence. The Evaluation of the Persistence of the Complete Molecular Remission After Stopping Imatinib Chronic Myeloid Leukemia (STIM) was undertaken to determine if those with the deepest level of disease suppression could successfully be taken off of imatinib therapy without disease recurrence.[41] Briefly, patients on imatinib for at least 3 years and in complete molecular response (CMR) (deepest response) for at least 2 years were eligible for the study. They were taken off imatinib therapy and monitored for a median follow-up time of 17 months. Fifty-four of 100 patients had a molecular relapse, and 46 were relapse-free at a median follow up time of 14 months. The majority of relapses (35/54) occurred in the first 3 months, and only one patient relapsed after 7 months. Males, those with a good prognosis (based on the Sokal scale for CML prognosis,[42]) and those with imatinib therapy duration of more than 50 months were more likely to remain in CMR. Encouragingly, all of the patients who relapsed remained sensitive to imatinib therapy, and the median time to CMR return was 3 months. Imatinib therapy infrequently leads to sustained CMR for the 2 years required for acceptance into this study. The cumulative incidence of CMR on imatinib therapy was 0.5% at 1 year and 8.3% at 5 years in newly diagnosed CP-CML patients.[43] Studies with the second-generation TKI nilotinib showed that patients on nilotinib were more likely to be in CMR at 24 months than those on imatinib (22.1% versus 8.7%).[44] The PACE trial results indicated that 15% of patients who entered treatment with CP-CML and 23% of patients with the T315I mutation were in CMR at 12 months.[32] Therefore, patients on ponatinib

may be more likely to successfully discontinue treatment than those taking imatinib.

Conclusion and place in therapy for ponatinib

Ponatinib is currently FDA-approved for T315I-positive CML (in any phase) and T315I-positive Ph⁺ALL, or any phase of CML or Ph⁺ALL for which no other TKIs are indicated.[45] As it has only been available on the market since December 2012, more time is required to sufficiently analyze the effect that ponatinib will have on long-term CML and Ph⁺ALL therapy. It is speculated that more potent inhibition of BCR-ABL earlier in therapy with drugs like ponatinib may lead to decreased development of resistance.[12,32] Regardless of the time of therapy, detection and quantitative monitoring of patients on ponatinib for potentially resistant (poly)mutant subclones could be performed using newer methods including ligase-dependent polymerase chain reaction (PCR)[46] or long-range next generation sequencing.[47] The toxicity profile of ponatinib, however, precludes its use alone as a frontline agent.[48] To circumvent this unwanted toxicity, one possibility may include using ponatinib in combination with other therapeutic agents to allow for dose-lowering of ponatinib. Our lab has taken a multidomain targeting approach of concurrently inhibiting both the tyrosine kinase domain (with ponatinib) and the N-terminal coiled-coil domain of BCR-ABL with a rationally designed peptidomimetic.[49] Using this therapeutic strategy, we have shown the ability to lower the dose of ponatinib necessary for BCR-ABL inhibition,[50] which would likely result in a safer toxicity profile.[33] This coiled-coil dimerization inhibitor has in fact shown activity by itself against the BCR-ABL[T315I],[50] as well as the BCR-ABL E255V/T315I compound mutant (Woessner et al., unpublished data, 2014), which is not targetable by any current TKI, and could be used as an alternative to ponatinib. Other strategies include concurrently targeting BCR-ABL along with proteins and signaling pathways important to leukemia progression including MUC-1 (Mucin-1, cell surface associated),[51] Alox5 (arachidonate 5-lipoxygenase),[51] STAT3 (signal transducer and activator of transcription 3),[52] and Hedgehog,[53] to name a few. It is also expected that combination targeting with ponatinib may result in reducing the emergence of resistance in cells, which could lead to greater long-term success.[19,54] Lastly, it has also been reported that dosing of potent drugs such as ponatinib with a high risk of ADEs should be lowered to the minimum effective dose rather than the maximum tolerable dose, which in this case turned out to be toxic in clinical trials.[33]

While ponatinib is the only approved TKI that targets BCR-ABL[T315I], other drugs in development capable of targeting this resistant form of BCR-ABL in either CML or Ph⁺ALL include: rebastinib (formerly DCC-2036), an ABL switch control inhibitor;[55,56] HG-7-85-01, a multi-targeted kinase inhibitor;[57] and AT9283, a multi-targeted kinase inhibitor.[58,59] For more comprehensive lists and discussions of ATP-competitive, allosteric, and other inhibitors of BCR-ABL, other reviews focus specifically on these topics.[4,60] Additionally, drugs are being developed to target CML stem cells by attacking other signaling pathways.[18,61] One example includes SL-401, a therapeutic targeting the IL-3 receptor that is overexpressed on CML stem cells.[62,63]

Due to the broad-spectrum activity of ponatinib for tyrosine and other kinase families (Table 1),[64–68] the drug is also being examined for use in other cancers and malignancies. As of March 2014, ponatinib was either being used in or currently suspended from six clinical trials for cancers and malignancies other than CML or Ph⁺ALL via inhibition of FGFR (fibroblast growth factor receptor), EGFR (epidermal growth factor receptor), ALK (anaplastic lymphoma kinase), and RET (rearranged during transfection).[69–74] Ultimately, ponatinib may prove to be useful in combination with other agents that robustly target BCR-ABL (multidomain targeting),[50] or other pathways involved in cancer progression besides BCR-ABL (for example, in combination with STAT3 inhibitors[52]) for a synthetically lethal approach for full eradication of CML or Ph⁺ALL.

Disclosure
The authors have no conflicts of interest in this work.

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