

Tyrosine Kinase Inhibitors and Phosphatases: Overcoming the BCR-ABL T315I Mutation in CML with a Synergistic Combination of Ponatinib and Forskolin

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Introduction

The Philadelphia (Ph⁺) chromosome is pathognomonic for the myeloproliferative disease, chronic myeloid leukemia (CML). It is the result of a translocation between the long arms of chromosome 9 and 22, which functionally give rise to the constitutively active protein tyrosine kinase, BCR-ABL [1]. Currently, the initial treatment of CML revolves around targeting the kinase activity of BCR-ABL, by utilizing a first generation tyrosine kinase inhibitor (TKI) imatinib [2]. Since the clinical employment of imatinib, multiple second generation TKIs have been developed. Second generation TKIs, such as dasatinib, were developed to target BCR-ABL with more potent capabilities to overcome disease resistance to first line TKI treatment [3]. Although survival and remission rates of CML disease have significantly increased since the advent of TKI therapies, many patients go on to develop resistance or intolerance to treatment. Usually, TKI treatment is associated with very limited to mild side effects. However, there are severe symptoms that develop, such as neutropenia, thrombocytopenia, anemia, and cardiovascular complications that may be life threatening, ultimately leading to the patient being taken off TKI therapy [4].

A significant portion of patients will have CML disease that develops resistance towards TKI treatment. Resistance can be classified into two categories: primary and secondary. With the former entailing the TKI failing to achieve a desired response, and the latter being associated with relapse from initial treatment that displayed some level of response early on in the course of treatment [5]. Although various mechanisms lead to TKI resistance, one of the most clinically significant is the result of a T315I mutation within the BCR-ABL kinase domain that directly impacts the binding of all first and second generation TKIs, by removal of a key hydrogen bond necessary for TKI binding [6]. The T315I mutation has been associated with a poor clinical outcome, and poses a major hurdle for treating CML due to the lack of TKIs capable of binding BCR-ABL^{T315I} [7]. Ursan et al. in 2015, conducted an extensive meta-analysis of 12 clinical studies which involved a total of 1,698 patients diagnosed with CML. Results demonstrated the T315I mutation to be one of the most commonly reported mutations in patients displaying TKI resistance. Concluding this study, it was estimated within the meta-analysis that 13% of patients with imatinib resistance contained the T315I mutation [8]. These findings coincided with prior studies conducted by Jabbour et al. and Nicolini et al., which also demonstrated the T315I mutation to appear in 5% to 15% of CML patients who developed resistance to TKI

therapy [9,10]. Being that the T315I mutation requires a complex strategy to treat, these studies highlight the frequent obstacle clinicians encounter when treating patients afflicted with CML that harbors the T315I mutation [11].

Ponatinib, a third generation TKI is a potent inhibitor of BCR-ABL^{T315I}

Currently there are only five TKIs approved by the United States Food and Drug Administration (FDA) for treating CML. These TKIs include: Imatinib (Gleevec, Novartis Pharma), dasatinib (Sprycel, Bristol-Myers Squibb Pharma), nilotinib (Tasigna, Novartis Pharma), bosutinib (Bosulif, Pfizer Inc.), and ponatinib (Iclusig, ARIAD Pharmaceuticals) [4]. Less than two decades ago the first TKI, imatinib, was developed to selectively target BCR-ABL, opening an avenue for targeted therapies to treat cancer with limited side effects, compared to conventional chemotherapies [3]. Second generation TKIs, dasatinib and nilotinib, were developed to be more potent against CML that became insensitive to imatinib treatment. Although the second generation TKIs proved efficacious in treating several imatinib resistant types of CML, point mutations within the BCR-ABL protein began to clinically appear which displayed resistance to both second generation TKIs [5]. Bosutinib, a late second generation TKI, is only US FDA approved to treat CML that has failed all TKI therapies. Bosutinib displays increased potency in overcoming most drug resistant CML, with the exception of CML possessing the T315I mutation [12]. Ponatinib, a TKI used in the treatment of drug resistant CML, is currently the only third generation TKI developed to overcome T315I positive CML [13]. Prior TKIs relied on a hydrogen bond interaction within the kinase domain of the BCR-ABL protein, but with the T315I mutation, this hydrogen bond is lacking. Ultimately leading to the inability of the TKI to inactivate the kinase activity of the BCR-ABL protein. Ponatinib is a designer drug that was developed using BCR-ABL crystal structure guidance, resulting in a TKI capable of overcoming the T315I mutation via van der Waals interactions [14]. For this reason, in 2012 the US FDA granted ponatinib accelerated approval for the treatment of CML patients who failed, or were intolerant to, initial TKI treatment regimens. Although ponatinib proved efficacious in treating aggressive and drug resistant CML, in October of 2013 the US FDA recommended the suspension of all marketing and sales of ponatinib. This was in response to the increased incidences of patients experiencing vascular complications such as thrombosis and narrowing of blood vessels while taking ponatinib. The US FDA later reinstated ponatinib, under conditions of more stringent monitoring and evaluation, since few treatment options were available for CML patients with a T315I mutation. However, ponatinib carries a boxed warning of arterial thrombotic events [15].

Molecular applications of forskolin in cell-signaling

The adenylate cyclase activator, forskolin, has been shown to modulate platelet signaling via suppression of platelet derived growth factor (PDGF), or alteration of intracellular cyclic AMP levels [16,17]. Such properties of forskolin have been useful in multiple studies involving mechanisms associated with platelet formation and aggregation [18-20]. Taking this into account, it would prove worthwhile to investigate whether the synergistic combination of ponatinib and forskolin has any implications on reducing common adverse cardiovascular events seen in patients treated with ponatinib alone. In addition to the possible reduction in thrombotic events, forskolin has also been shown to hinder CML proliferation through activation of Protein Phosphatase 2A (PP2A) [21].

PP2A regulates BCR-ABL tyrosine kinase activity

BCR-ABL activity has been shown to modulate the activity of PP2A. The manipulation of PP2A activity coincides with the stages of CML disease progression, with dramatic decreases in PP2A activity observed during the CML blast phase [21]. The mechanism of PP2A inhibition has been correlated to the tumor suppressor inhibitor, SET. The expression and activity of SET is enhanced by BCR-ABL; therefore favoring CML progression through the SET mediated inhibition of PP2A. This SET/PP2A relationship is altered when CML cells are treated with PP2A activators, such as forskolin [22,23].

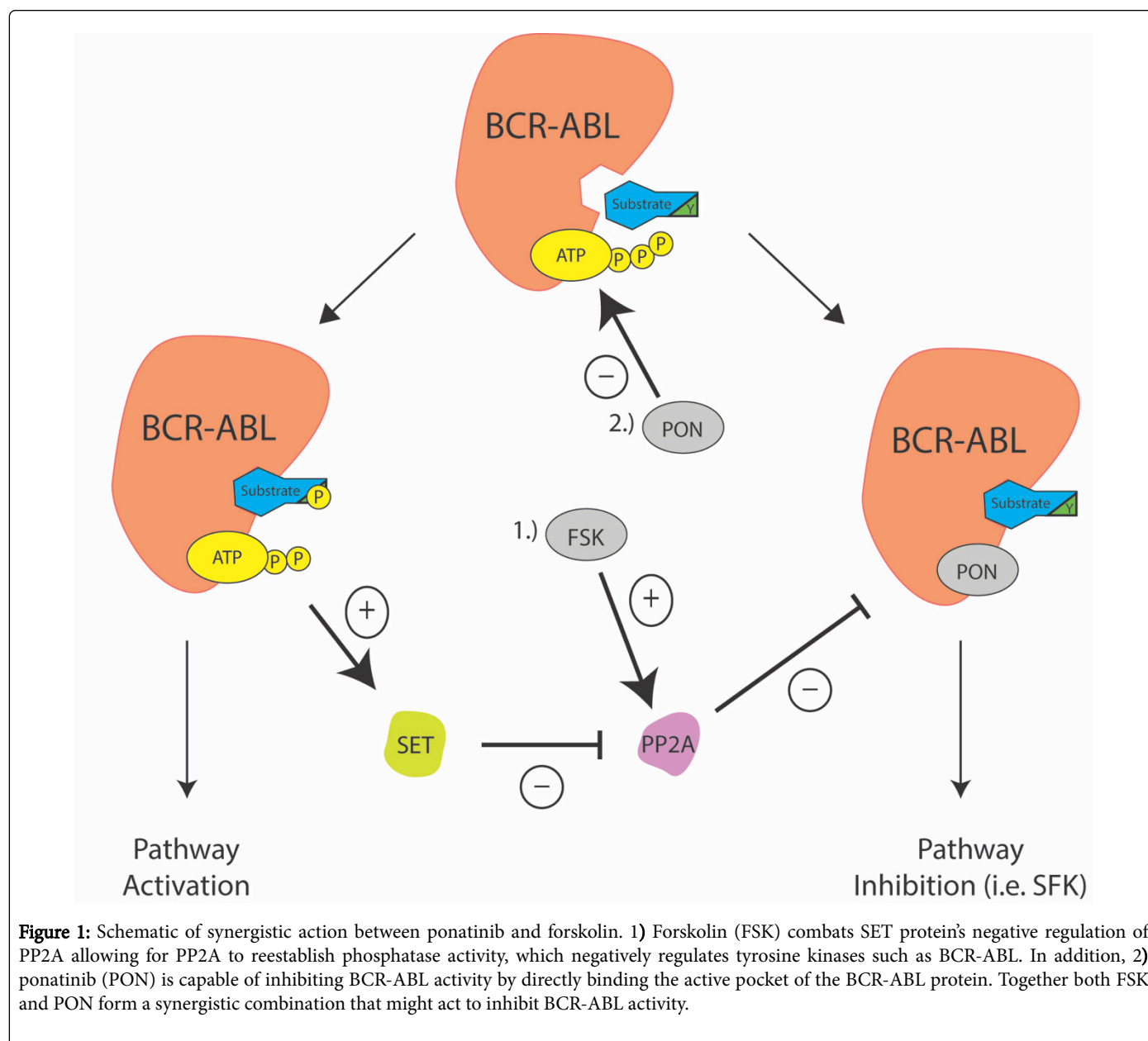


Figure 1: Schematic of synergistic action between ponatinib and forskolin. 1) Forskolin (FSK) combats SET protein's negative regulation of PP2A allowing for PP2A to reestablish phosphatase activity, which negatively regulates tyrosine kinases such as BCR-ABL. In addition, 2) ponatinib (PON) is capable of inhibiting BCR-ABL activity by directly binding the active pocket of the BCR-ABL protein. Together both FSK and PON form a synergistic combination that might act to inhibit BCR-ABL activity.

Sensitivity of imatinib-resistant T315I BCR-ABL CML to a synergistic combination of ponatinib and forskolin

When investigated, ponatinib and forskolin (PF) demonstrated a synergistic effect in a highly imatinib-resistant T315I BCR-ABL CML cell line. Used in combination with 20 μ M of forskolin, ponatinib IC50 concentrations were attained at 0.5 nM [24]. Achieving such potency towards T315I positive CML may warrant *in vivo* and clinical investigation into whether such a combination possesses clinical value over standard ponatinib therapy. While the synergistic mechanism of action remains to be elucidated, PF dual treatment is able to induce cell cycle arrest within the G1 phase. In addition, apoptosis associated protein, BAD, showed increased activity with PF treatment. Furthermore, SRC family tyrosine kinases and Signal Transducer and Activator of Transcription 3, STAT3, activity was negatively impacted with PF treatment [24]. One could speculate the synergistic mechanism observed in CML cell lines treated with PF treatment is two-fold (Figure 1). First, there is a direct inhibition of BCR-ABL because ponatinib would compete for the ATP binding site within the enzymes' catalytic domain. Second, forskolin might reactivate PP2A, which would then act to inhibit BCR-ABL directly [23]. In summary, the model for the proposed synergistic mechanism suggests that PP2A and ponatinib inhibit BCR-ABL activity thus reducing the amount of ponatinib required to achieve therapeutic effectiveness [24]. Current guidelines in managing CML cases that contain the T315I mutation recommend using the lowest effective dose of ponatinib. This is due to the uncertainty of adverse events being associated with increased doses of ponatinib [4]. Ponatinib remains to be the only TKI available that is US FDA approved for treating T315I positive CML. It is imperative that drug combinations are identified that allow for its usage at lower concentrations. The study described herein reveals such a combination, allowing for ponatinib to be used at effective concentrations that are several times less than its recommended dose [24]. Forskolin is a natural herb that is used globally for treating various ailments including, cardiovascular conditions, skin disorders, hypertension, asthma, aiding fat metabolism, and treating other disorders [25]. The safety profile of forskolin is very extensive, with limited adverse events reported in humans [26,27]. Ponatinib has been utilized in combating extremely drug resistant CML in the last three years, and its usage is limited by its reported toxicity profile. An international phase 3 clinical trial demonstrated promising preliminary data of ponatinib's efficacy over imatinib in treating newly diagnosed CML. However, the phase 3 trial was terminated due to increased adverse events reported in patients receiving ponatinib therapy [28]. To date, ponatinib remains to be the only US FDA approved TKI capable of overcoming the T315I BCR-ABL mutation. As discussed earlier, the T315I mutation remains to be a frequent clinical problem encountered after patients fail first-line TKI therapy. In conclusion, more studies are needed for identifying safer analogues of ponatinib, or discovering strategic drug combinations that allow for ponatinib to be effectively utilized at lower and safer concentrations.

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