Expert Opinion

PCI-32765: a novel Bruton's tyrosine kinase inhibitor for the treatment of lymphoid malignancies

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Introduction: There has been a significant paradigm shift in the manner in which lymphoid malignancies are treated and managed. Treatment has been moving away from conventional chemotherapy and towards targeted therapy. The success of new classes of agents such as monoclonal antibodies, proteasome inhibitors and immunomodulatory derivatives has sparked further searches for novel pathways to inhibit. The Bruton's tyrosine kinase (Btk) pathway is a downstream mediator of the B-cell receptor (BCR) pathway, which is crucial in B-cell production and maintenance, and a potential therapeutic target.

Areas covered: This review will summarize the current knowledge of the Btk pathway and its role in lymphoid malignancies. It will also discuss the present data about PCI-32765 in both the preclinical and clinical setting.

Expert opinion: PCI-32765 is an oral irreversible Btk inhibitor with high potency and both preclinical and clinical activity in chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL). Phase I studies have demonstrated that it is well tolerated and has an excellent safety profile. Further studies are ongoing as a single agent and in combination with other targeted and conventional therapies. PCI-32765 is a very promising targeted therapy, and the data from these trials will ultimately decide its future role and success.

Keywords: Bruton's tyrosine kinase, leukemia, lymphoma

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1. Introduction

The treatment of lymphoid malignancies, and cancer as a whole, has markedly changed over the past decade. The use of monoclonal antibodies (mAbs) and biologic targeted therapy has revolutionized the manner in which cancer is treated, particularly the hematologic malignancies. For example, the addition of rituximab (Rituxan®, Genentech, Inc., South San Francisco, CA, USA) to standard chemotherapy has increased both response rates and overall survival in non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) [1,2]. There has been a paradigm shift in the treatment of cancer from non-specific antiproliferation chemotherapy to therapy targeting specific molecular pathways known to be active in cancer proliferation and anti-apoptosis.

One pathway which has gained significant attention for the treatment of lymphoid malignancies is the B-cell antigen receptor (BCR) pathway. The chronic activation of this pathway plays a significant role in proliferation and survival in B-cell NHL [3,4]. On activation by antigen, signaling is mediated through CD79A and CD79B and creates a cascade of downstream signals to nuclear factor-kappaB (NF-κB), phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK). BCR stimulation also leads to phosphorylation of tyrosine kinases

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such as the splenic tyrosine kinase (Syk), protein kinase Cβ (PKCβ) and Bruton's tyrosine kinase (Btk) (**Figure 1**). Recent trials have been initiated with success with inhibitors of these pathways, such as the use of the Syk inhibitor fostamatinib (FosD, AstraZeneca, London, UK) [5] and the selective PI3Kδ inhibitor CAL-101 (Calistoga Pharmaceuticals, Seattle, WA, USA) [6].

The Btk pathway is also an interesting target for novel therapeutics in the treatment of lymphoid malignancies. PCI-32765 (Pharmacyclics, Sunnyvale, CA) is a novel selective and irreversible Btk inhibitor available in oral formulation. This review will describe the mechanisms of the Btk pathway, summarize the preclinical and clinical data of PCI-32765 and discuss the possible clinical uses and potential benefits of this new therapy.

2. The Btk pathway

Btk is a member of the Tec family of kinases and is a cytoplasmic protein predominantly expressed in hematopoietic cells [7,8]. Btk is predominantly expressed on B lymphocytes, marrow-derived stem cells, lymphocyte progenitors and developing myeloid cells, with little expression on resting mature cells prior to activation [9]. In an early experiment by de Weers *et al.*, *btk* expression was investigated in numerous cell lines including human leukemias. Expression was noted in mature B-cell lines, lymphoma, myeloid cell line and multiple myeloid leukemia cell lines [10]. Btk is not at all expressed in plasma cells or T lymphocytes [10,11].

Loss of function of the *Btk* gene inhibits B-lymphocytes production due to a maturation inhibition between the pro- and pre-B cell stages. This inhibition causes an inability to make all classes of immunoglobulins [12,13]. The implication of Btk in human disease was first discovered by Vetrie *et al.*, who isolated the defective gene using cDNA from a yeast artificial chromosome (YAC) clone [14]. The phenotypic disease is called X-linked agammaglobulinemia (XLA), and patients had a failure of B-cell development and therefore markedly decreased production of all classes of immunoglobulins [15]. These patients are particularly susceptible to bacterial infections, have increased susceptibility to viral and parasitic infections. With the advent of intravenous immunoglobulin preparations (IVIG), most of these patients reach adulthood and although have more frequent work absences and hospitalization, lead productive lives [16]. Further work has also been done in parallel looking at the Btk activity in mice. Initial studies demonstrated that mutations in the Btk region, particularly one at position 219 resulting in an amino acid change from argenine to cysteine resulted in immunodeficient XID mice, primarily from arrested B-cell development. It was also noted that these XID B cells did not respond to B-cell activation signals [17]. Mice deficient in the mouse-Btk gene also have X-linked immunodeficiency [18]. It is also interesting to note that mice with a genetic disruption of the p85α of PI3K displays a virtually identical phenotype to the XID mice, suggesting that there is link between this subunit and the Btk pathway [19].

Btk is generally thought to be cytosolic, although it has to be localized to the plasma membrane for phosphorylation and activation [20]. Although there is some cellular data demonstrating Btk in the nucleus of cells in small quantities, it is felt that the signaling process begins in the plasma membrane [21]. After BCR activation, PI3K is activated which generates phosphatidylinositol-3,4,5-triphosphate (PIP_3), and after sufficient accumulation of PIP_3 at the inner surface, Btk is recruited to the plasma membrane. Experiments initially performed by Rawlings *et al.* [22] demonstrated that Btk then undergoes phosphorylation at site Y551 by the Src family kinases Blk, Lyn and Fyn [23,24]. This then leads to autophosphorylation at Y223. Btk then phosphorylates phospholipase Cγ2 (PLCγ2), which leads to activation of downstream effectors protein kinase Cβ (PKCβ), and ultimately the transcription factor NF-κB [8,25]. Although the most prominent role of NF-κB is to inhibit apoptosis, it also induces transcription of the Btk gene [26]. Stimulation of this pathway has been shown to aid in the proliferation and prolonged survival in lymphoid malignancies [27].

Although there is plethora of evidence demonstrating the vital nature of Btk for B-cell proliferation and differentiation, there are also data which demonstrate that Btk plays a role in apoptosis. It has been identified as the first dual function regulator of apoptosis [28]. In further studies, Btk mediates apoptosis, although this is dependent on membrane localization and kinase activity and involves p38 MAPK [29].

3. Preclinical data

PCI-32765 is a selective and irreversible Btk inhibitor. PCI-32765 inhibits BCR signaling by covalently bonding to a cysteine residue (Cys-481) in the active site of Btk. It inhibits BCR signaling but has no impact on T-cell signaling. PCI-32765 also blocks mast cell and basophil degranulation [30].

Davis *et al.* found that in the activated B-cell-like (ABC) subtype of diffuse large B-cell lymphomas (DLBCL) driven by activated BCR, Btk was an essential kinase for survival. Two short hairpin RNAs that targeted Btk were found to be toxic for wild-type CARD11 ABC DLBCL subtype. However, no toxicity was demonstrated in those cell lines possessing mutated CARD11. In survival assays, Btk kinase activity was required for rescue of cell lines possessing Btk knockdown. The group also demonstrated that PCI-32765 selectively induced apoptosis in DLBCL cell lines with chronically active BCR signaling via Btk [3].

In vitro studies have attempted to elucidate the effects of PCI-32765 on CLL cell viability. In co-cultures with nurse-like cells, CLL viability was significantly decreased. Further studies showed that Btk inhibition reduced secretion of chemokines CCL3 and CLL4, as well as homing chemokines CXCL12 and CXCL13 [31]. Furthermore, in NHL *ex vivo* tumor testing, 1 of 7 DLBCL and 6 of 15 follicular lymphomas (FL) responded. In a follow-up experiment, the authors found a microRNA (miRNA) signature that could predict

the sensitivity of FL tumors, although it was not known if this was direct inhibition of Btk or its downstream effectors [32].

Honigberg *et al.* [33] conducted studies that examined the *in vivo* use of PCI-32765 utilizing mouse models. The focus was on autoimmune diseases where Btk or B-cell function plays a role. Collagen-induced arthritic mice were assigned to treatment groups when their disease had progressed. The mice were treated with PCI-32765 daily at varying doses. After treatment, the clinical arthritis scores were significantly improved. A significant reduction in the amount of anticollagen autoantibodies was noted. Modestly reduced total IgG levels were also seen. A companion study evaluated a lupus model (MRL-Fas (Ipr)) treated with PCI-32765. At the conclusion of treatment, the mice had reduced proteinuria and blood urea nitrogen. Anti-dsDNA levels were also reduced. A second study evaluated PCI-32765 in multiple murine models of immune complex disease, including collagen-induced arthritis, collagen antibody-induced arthritis, reversed passive anaphylactic reaction and passive cutaneous anaphylaxis. PCI-32765 was able to reduce inflammation in the anaphylaxis models, and also prevented clinical arthritis in the arthritis models. *In vitro* analysis also noted inhibition of TNF-α, IL-1β and IL-6 [34].

Honigberg's group also looked at the efficacy of PCI-32765 in spontaneous canine B-cell lymphomas. Canine NHL shares many characteristics with human NHL, such as response to CHOP-based chemotherapy and diagnostic classification [35]. Both treatment naïve and relapsed dogs were included. At the time of their publication, eight dogs had been treated with three partial responses and three instances of stable disease.

The results from these earlier studies coupled with increasing understanding of the BCR signaling lead Herman *et al.* to examine the role of PCI-32765 in CLL. The impact of PCI-32765 on apoptosis, proliferation and microenvironment stimulation in CLL cells was assessed. *ex vivo* studies using CLL cells from 10 patients utilizing increasing concentrations of PCI-32765 on a log scale induced cytotoxicity in a dose-dependent manner and PCI-32765 increased apoptosis through the caspase pathway. Treatment did not increase the rate of cell death among T cells, although it did lead to a decrease in production of T-cell cytokines such as IL-6, IL-10 and TNF-α. Additionally, PCI-32765 altered signaling in the MAPK, PI3K and NF-κB signaling pathways [36].

Herman *et al.* also examined PCI-32765 on CLL cells proliferation in their microenvironment [37]. Cytokines such as TNF-α, IL-6 and IL-4 have been shown to be active in the microenvironment of CLL patients, reducing spontaneous apoptosis [38,39]. Treatment with PCI-32765 reduced the protective effect of these cytokines. PCI-32765 also inhibited proliferation of CLL cells even in setting of CpG oligonucleotides which served as a stimulating agent. PCI-32765 was also evaluated for the ability to maintain cytotoxicity in the presence of stromal cells, which have been shown to portend a survival benefit in CLL cells *in vivo* and provide means to drug resistance [40,41]. No reduction in cell death was seen in cells co-treated with stromal cell lines and PCI-32765.

4. Clinical development

4.1 Phase I studies

There have been two Phase I studies evaluating PCI-32765 in lymphoid malignancies. One study evaluated two cohorts of patients with CLL, one with *de novo* disease in patients over 65 years of age and the other with relapsed/refractory (R/R) disease [42,43]; Two dose levels of oral PCI-32765 (420 and 840 mg) were examined and the drug was administered daily for 28-day cycles until disease progression. An interim analysis was presented after 39 patients were evaluable. The treatment was well tolerated with grade 3 or greater toxicities seen in 26% of patients, although grade > 3 cytopenias was limited to < 5% of patients. Two serious adverse events were noted: viral adenitis (grade 3) and a viral infection (grade 2), whereas two adverse events led to discontinuation of PCI-32765 (small bowel obstruction and exacerbation of chronic obstructive pulmonary disease). In the R/R group, 87% had a poor risk cytogenetic or molecular features. The nodal response rate was 89%, and although there initially was an increase in absolute lymphocyte count, the majority had an eventual decrease. At a median of 4 months time, 39% had a partial response (PR) and 5% had a complete response (CR); 4 of the 12 patients with del(17p) had a response. At the time of presentation, 78 patients had been enrolled.

A second study looked at PCI-32765 in relapsed aggressive NHL [44]. The dosing in this study was weight-based, at 6 dose levels 1.25 mg/kg/day and further levels of 2.5, 5, 8.3, 12.5 and 17.5 mg/kg/day; dose increases were based on a safety evaluation and that the cohort achieved > 90% occupancy of Btk. Of the 29 patients, 12 had FL, 7 CLL, 4 DLBCL, 4 mantle cell lymphoma and 2 marginal zone lymphoma. Of the first 22 patients enrolled in the first three dose levels, treatment was found to be well tolerated, with the majority of toxicities grade < 2. Pharmacodynamics noted > 95% enzyme occupancy of Btk, and T-cell responses were not changed, nor was there a depletion of peripheral blood lymphocytes. The overall response rate in this diverse group was 42%, with 1 of the 19 patients achieving CR. The responses were 5 of 7 CLL, 2 of 4 mantle cell lymphomas and 1 of 12 FL patients. In a follow-up analysis [45], 47 patients were evaluable at greater than 6 months and the overall response rate was 43% with three CR.

These two studies used a novel fluorescent probe to evaluate the binding of PCI-32765 to Btk, which showed 95% enzyme occupancy 4 h postdose, and also measured basophil degranulation as a surrogate of Btk inhibition; this was completely inhibited up to 24 h.

5. Current studies

5.1 Single agent PCI-32765

The two aforementioned Phase I studies are still ongoing (NCT01105247; NCT00849654). A further single agent study looks at PCI-32765 in patients with R/R mantle cell lymphoma (MCL). It is a Phase II study with the primary end point as

response, and secondary end point as safety and pharmacokinetics. The planned enrollment is 100 patients (NCT01236391). Studies in refractory DLBCL (NCT01325701) are also ongoing.

5.2 Combination regimens with PCI-32765

The results of the Phase I trials have spawned further Phase I/II trials using PCI-32765 in combination with other agents. Based on the number of responses in CLL, many of these trials look at this patient population. One of these trials is a Phase Ib/II study using PCI-32765 in combination with ofatumumab (Arzerra®, GlaxoSmithKline, Research Triangle Park, NC, USA) in R/R CLL. The goal enrollment of this study is 27 patients (NCT01217749). Another CLL study evaluates PCI-32765 at a dose of 420 mg/day in combination with either fludarabine (Fludara®, Genzyme Corp., Cambridge, MA, USA)/cyclophosphamide/rituximab (FCR) or bendamustine (Treanda®, Cephalon, Frazer, PA, USA)/rituximab (BR), with the primary end point of prolonged hematologic toxicity (NCT01292135). The goal enrollment is 60 patients who have R/R disease.

6. Expert opinion

The landscape of cancer therapy has vastly changed over recent years. The advent of novel agents in combination with conventional chemotherapy has become the standard treatment for B-cell malignancies such as NHL and CLL. Although the initial targeted therapies were mAbs such as rituximab [1,2], the fully humanized anti-CD20 mAb ofatumumab [46] and the anti-30 antibody-drug conjugate brentuximab vedotin (Adcetris®, Seattle Genetics, Bothell, WA, USA) [47], other biologic agents such as the proteasome inhibitor bortezomib (Velcade®, Millennium, Cambridge, MA, USA) have also been found to be effective in the treatment of MCL [48]. These drugs are clear examples that novel, targeted therapeutics can be used in conjunction with conventional chemotherapy and provide significant improvements in complete responses and overall survival.

Recently, the BCR signaling pathway has been examined as a potential target for drug therapy. There are multiple signals and substrates that have potential for blockage of downstream effectors and thereby halting the proliferation of B cells. Although this review focuses on the Btk pathway, other pathways that have been examined for potential inhibition are the Syk pathway, the PI3K pathway and the PKCβ pathway. Inhibitors have been synthesized for each of these pathways, and there has been moderate efficacy with each of these agents. Interestingly, these agents seem more active in CLL than the more aggressive lymphomas, although the sample size is currently small.

The Btk pathway provides an intriguing target for treatment of B-cell malignancies. Btk is clearly integral in the development, maturation and proliferation of B cells and its absence leads to the human disease of XLA. Because it activates multiple downstream effectors such as MEK, PI3K, PLCγ2 as well as NF-κB, it is an attractive kinase to inhibit and prevent proliferation. Its promiscuity of localization between plasma membrane, cytosolic and cell nucleus also make it important in B-cell growth regulation, thereby increasing its potential as a target. The dual function role in apoptosis, although not completely understood may lead to further benefit via Btk inhibition as it may play a role not only in proliferation but also in targeted cell death.

PCI-32765 is a potent, selective, irreversible and orally available Btk inhibitor, and has been shown to be effective in a variety of lymphoid malignancies in both the preclinical and clinical settings. The initial *in vitro* studies demonstrates a covalent bonding with Btk, and can induce apoptosis in cell lines with chronic active BCR signaling. *ex vivo* studies of lymphocytes from patients with CLL demonstrated a dose-dependent cytotoxicity through the caspase pathway, but did not have an effect on T cells. Two Phase I trials have been reported with interim results. The CLL trial demonstrated a response rate of 44%, but more exciting was the 33% response rate in patients carrying del(17p), who have been classically hard to treat given poor responses and survival with current therapies. The NHL trial showed a response rate of 43%, and using a novel technique to evaluate binding exhibited > 95% occupancy of Btk enzyme sites at 4 h.

There are many appealing features of PCI-32765. Beyond its moderate anti-lymphoid activity in a heavily pretreated population, it appears to be very well tolerated, with grade 3 or greater toxicities noted in 26% of patients. In the CLL study, there was an initial increase in absolute lymphocyte count, although the majority of these patients later had a reduction. It is notable that a similar effect was noted in the single agent clinical trial using CAL-101, selective PI3Kδ inhibitor, in patients with CLL [49], but not in a further CLL study using CAL-101 in combination with rituximab and bendamustine [50]. This lymphocyte count flare may be a class effect for agents that target effectors of the BCR pathway, however, it appears that it may be nullified by combination chemotherapy regimens.

The most promising area for future trials of PCI-32765 is in the treatment of B-cell malignancies. In the two Phase I studies available, the disease which seems to have the most activity is in CLL, however, there seems to be responses in the other low-grade lymphomas as well. With the small, preliminary data currently available, there have been no responses noted in the four patients with DLBCL. The *in vitro* studies, though, show Btk as essential in these cell lines and therefore DLBCL warrants further evaluation in this disease; one such study is currently enrolling patients (NCT01325701). Data previously mentioned from Davis *et al.* and described by Rui *et al.* demonstrate that the Activated B Cell-like (ABC) DLBCL are dependent on BCR signaling, and further evaluation in the ABC DLBCL is warranted [51,3]. The data in mantle cell are also notable, especially since this disease is often refractory to conventional chemotherapy and has short durations of remission. It is highly unlikely that there will be future studies in multiple myeloma or T-cell lymphomas since these cells do not show Btk expression.

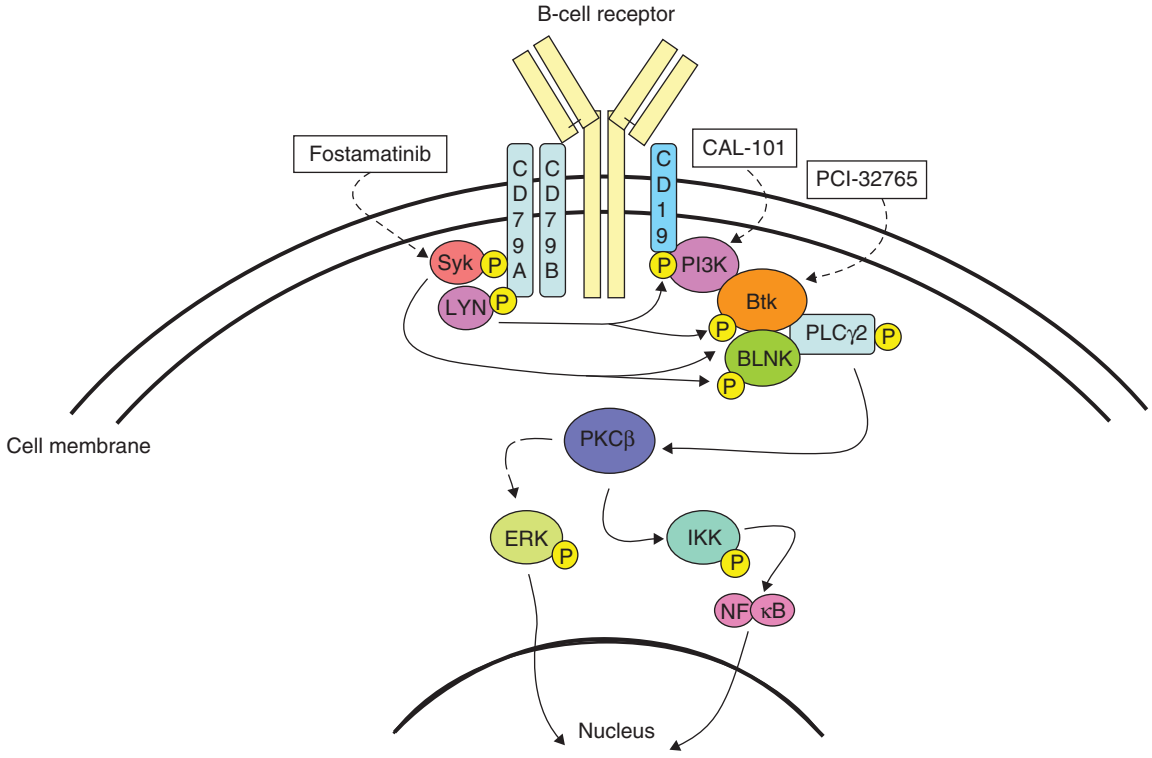

Figure 1. The B-cell receptor pathway is activated by antigen causing protein tyrosine kinase (PTK), Lyn, to be stimulated by CD79A and CD79B proteins. An additional PTK, splenic tyrosine kinase (Syk), is recruited by phosphorylation of tyrosine residues within CD79 proteins. Activation of Lyn and Syk in turn cause a cascade of events leading to phosphorylation of phosphatidylinositol 3-kinase (PI3K), which generates phosphatidylinositol-3,4,5-triphosphate and ultimately leads to activation of Bruton's tyrosine kinase (Btk). Activation of Btk causes phosphorylation of phospholipase Cγ2 (PLCγ2), which thereby activates downstream effectors protein kinase Cβ (PKCβ). This creates a cascade of downstream signals to nuclear factor-kappaB (NF-κB) leading to an upregulation of anti-apoptotic genes. PTKs turned on by antigen stimulation lead to activation of the extracellular signal-regulated kinase (ERK) pathway. Activation of the ERK pathway also blocks apoptosis. The novel agents fostamatinib, CAL-101 and PCI-32765 inhibit Syk, PI3K and Btk, respectively.

The use of PCI-32765 will likely not be limited to malignancy. The preclinical data demonstrated significant inhibition of B-cell activation without affecting T-cell receptor signaling. PCI-32765 also was effective in disease suppression in mouse models with lupus (MRL-Fas(lpr)) and in mice with collagen-induced arthritis and collagen antibody-induced arthritis. Clinical trials will likely be initiated to evaluate the effect of PCI-32765 in patients with rheumatoid arthritis or other B-cell-mediated autoimmune disease such as systemic lupus erythematosis. Further studies may also begin to assess the effects of PCI-32765 in other autoimmune B-cell disorders, such as autoimmune hemolytic anemia or multiple sclerosis, based on its prominent effect on B cells.

It is unlikely that PCI-32765 will be used as a single agent but rather in combination with other chemoimmunotherapies. Unfortunately, there have not been studies evaluating the potential synergistic effects of PCI-32765 with other agents, but this should be examined. There are current studies in CLL evaluating PCI-32765 with ofatumumab as well as a CLL study with PCI-32765 with FCR and BR. The combination with BR is an attractive option because the BR regimen is highly active in both CLL and low-grade NHL. Synergy with this regimen could result in high remission rates. Combination regimens in DLBCL, such as with cyclophosphamide, adriamycin, vincristine and prednisone (CHOP) and rituximab will likely be assessed. Similarly, a study will likely be initiated evaluating PCI-32765 with bortezomib and rituximab in mantle cell lymphoma. Lastly, a novel concept would be to combine PCI-32765 with one of the other inhibitors of the BCR pathway, such as fostamatinib, the Syk inhibitor or CAL-101, the selective PI3Kδ inhibitor to compound the inhibitor effects of the BCR pathway.

In summary, PCI-32765 is an exciting novel drug that has an oral formulation and irreversibly inhibits Btk, a critical kinase involved in the BCR pathway. Single agent studies have demonstrated moderate responses in B-cell malignancies, particularly CLL. PCI-32765 holds significant promise in the treatment of lymphoid

malignancies. In the initial Phase I studies it has a very acceptable safety profile, and ongoing and future trials will evaluate its synergy with other chemotherapeutic or immunologic agents.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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