



Executive Summary

Bruton's Tyrosine Kinase (Btk) Inhibitor Programs for Oncology and Autoimmune Diseases

Stage of development:	PCI-32765 – Phase I clinical study (B cell lymphoma and chronic lymphocytic leukemia), <i>enrolling</i> New series for compounds for autoimmune diseases – Preclinical development
Description of compounds:	Small-molecule, orally bioavailable inhibitors of Bruton's Tyrosine Kinase (Btk), a kinase required for B-cell receptor (BCR) signaling.
Indications:	<u>Oncology</u> : non-Hodgkin's lymphomas, chronic lymphocytic leukemia, and Ewing's sarcoma. <u>Autoimmune diseases</u> : rheumatoid arthritis, systemic lupus erythematosus, and Sjögrens syndrome.
Status:	<p>An IND (no. 102688) for PCI-32765 was opened with the FDA Division of Drug Oncology Products on October 3, 2008. An ascending multidose Phase 1 clinical trial in patients with recurrent B-cell lymphoma is currently enrolling. At initial dose levels of 1.25 and 2.5 mg/kg/day clinical responses were achieved in 5 of 16 patients. Full occupancy of the Btk active site by PCI-32765 has been achieved in the peripheral blood mononuclear cells of patients at a dose of 2.5 mg/kg/day. A robust kilogram-scale GMP synthesis has been developed.</p> <p>Preclinical development of two new Btk inhibitors, PCI-45292 and PCI-45308, is underway. Selection of a clinical development candidate is projected for Q2 '10.</p>
Enabling technologies:	<p>Initial research compounds were co-crystallized with the tyrosine kinase Lck as a surrogate for Btk and structural data guided lead identification. Covalent interaction between our inhibitors and a specific amino acid at the Btk active site results in durable pharmacodynamic activity and increased selectivity.</p> <p>Pharmacyclics has developed multiple pharmacodynamic assays to monitor inhibition of B cell lymphocytes and basophils in peripheral blood including a proprietary assay that can be used to monitor Btk active-site occupancy by our inhibitors.</p>

I. Background

Pharmacyclics is developing multiple orally bioavailable inhibitors of Bruton's Tyrosine Kinase (Btk), a non-receptor tyrosine kinase, for hematologic malignancies (Non-Hodgkin's lymphomas and chronic lymphocytic leukemia) and autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus and other B-cell and mast cell mediated diseases). Btk is an essential component of the B-cell receptor and mast cell signaling pathway. Specific mutations in the gene encoding Btk that result in the loss of

functional Btk have been described in humans. Loss of functional Btk results in X-linked agammaglobulinemia (XLA), a disorder characterized by a lack of mature B cells that express surface immunoglobulin (Ig), a lack of antibody-producing plasma cells, and reduced serum Ig. This provides human genetic evidence that Btk plays an important role in B cell maturation, and suggests that a selective Btk inhibitor will not affect the function of cell types outside of the immune system. Btk also functions in mast cells, suggesting that inhibition of Btk may suppress the release of chemical mediators, including histamine and serine proteases.

II. Btk inhibitor Programs

A. Btk inhibitor for oncology – PCI-32765

In vitro profile

PCI-32765 is an inhibitor that covalently binds to Cysteine-481 in the Btk kinase domain (as described in our earlier publication *Chem Med Chem* **2007**, 2, 58–61). This interaction with Btk makes PCI-32765 uniquely selective over closely related kinases lacking this cysteine. PCI-32765 is highly potent, inhibiting purified Btk with an IC_{50} of 0.46 nM. In *ex vivo* assays using whole blood, PCI-32765 inhibits human BCR activation ($IC_{50} \approx 200$ nM), while not affecting T cell activation. PCI-32765 also inhibits key phosphorylation events downstream of the BCR at similar concentrations. In cellular assays, a 1-hour pulse of PCI-32765 is sufficient to inhibit B cell activation for ~18 hours.

B cell receptor signaling is also implicated in the survival of malignant B cells. We have demonstrated that our inhibitors are cytotoxic to a subset of lymphoma cell lines ($GI_{50} < 1$ μ M). PCI-32765 (2 mg/kg) also inhibits BCR-driven tumor growth in a transgenic *myc*/BCR mouse tumor model (presented at AACR 2009, see **Figure 1**). This model relies on the cooperation between *Myc* overexpression and BCR signaling in B cells to produce a lymphoma phenotype that resembles Burkitt's Lymphoma. These cells, which also express green fluorescence protein (GFP), populate the lymph nodes of mice when delivery by intravenous injection. In a study using this model to evaluate PCI-32765, mice received once daily intraperitoneal injections of PCI-32765 for 8 consecutive days beginning at first appearance of lymph node swelling. Treatment with PCI-32765 completely inhibited tumor growth (lack of green cells) and significantly reduced lymph node size.

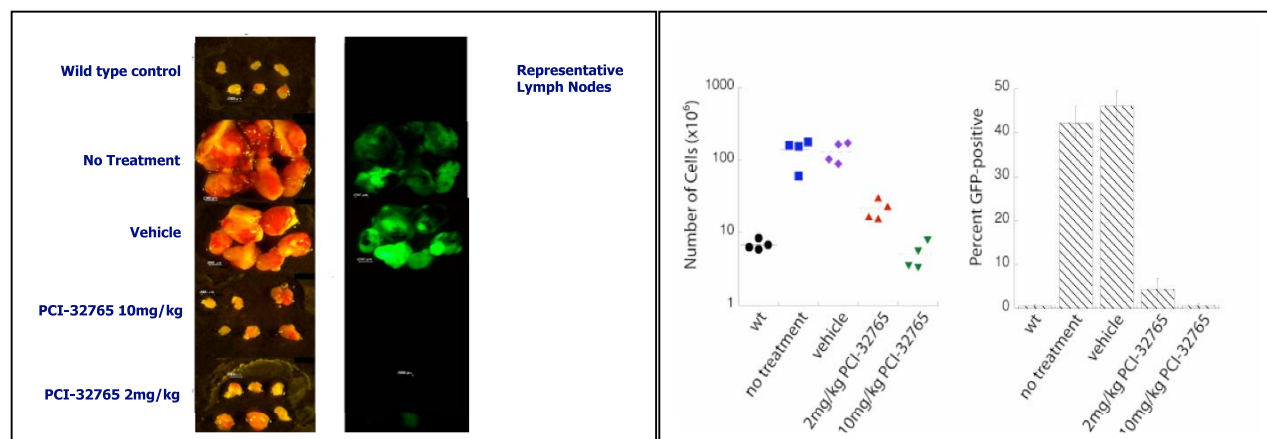


Figure 1: PCI-32765 in a transgenic *myc*/BCR murine model



In dogs with naturally occurring lymphoma, a partial response (RECIST criteria) was observed for 2/4 dogs treated with PCI-32765 via capsules at a dosage of 2.5, 5.0, or 7.5 mg/kg/day. Sustained inhibition of Btk in peripheral blood mononuclear cells and lymph nodes was confirmed. At dose levels of ≤ 7.5 mg/kg/day, no significant toxicities (greater than Grade I) were observed.

Preclinical DMPK

The oral bioavailability of PCI-32765, when administered as a suspension formulation to rats was approximately 20% and approximately 10% when administered as a capsule formulation to dogs. The terminal half-life of PCI-32765 following oral dose administration was 1.0 to 4.7 hours in rats and 3.3 to 6.4 hours in dogs. Following administration to rats by IV injection, 94% of radiolabeled PCI-32765 was eliminated in the feces and 6% was eliminated in the urine.

IND-Enabling Safety Studies

Safety Pharmacology Assessment

IC₅₀ for hERG channel inhibition was 1.0 μ M, a concentration 128 to 1280 times the unbound C_{max} values projected for the range of dose levels in the Phase 1 clinical trial. No neuro-behavioral (CNS) or pulmonary effects were observed in rats. No prolongation of QT_c interval was observed in radio-telemetered dogs.

Toxicology

PCI-32765 was negative in a bacterial mutagenicity assay and was negative in *in vivo* and *in vitro* tests for clastogenicity. One-month GLP toxicology studies in rats and dogs have been completed. The NOAEL in rats was set at 40 mg/kg/day. The NOAEL in dogs was set at 24 mg/kg/day. Based on PK data from these safety studies, preclinical efficacy studies, and predicted PK in human, >10-fold safety margins were determined for at a clinical dose of 5 mg/kg/day.

B. Btk inhibitors for Autoimmune Diseases

Using the same chemical scaffold as PCI-32765, work was initiated on a new series of Btk inhibitors with the goal of increasing therapeutic index and target selectivity. Two optimized inhibitors, PCI-45292 and PCI-45308 are now in preclinical development. In a series of enzyme selectivity, efficacy, pharmacokinetic, and safety assays these molecules were shown to retain potent inhibition of Btk while exhibiting a better selectivity profile. Preclinical development candidate PCI-45292 has a greater than 5000-fold selectivity for Btk over the tyrosine kinases EGFR and JAK-3. We have demonstrated that orally dosed PCI-45292 is significantly more efficacious in a murine collagen-induced arthritis model (ED₅₀ < 0.75 mg/kg/d, **Figure 2**) than methotrexate. Efficacious doses below 10 mg per patient per day are predicted for PCI-45292 in humans based on interspecies scaling.

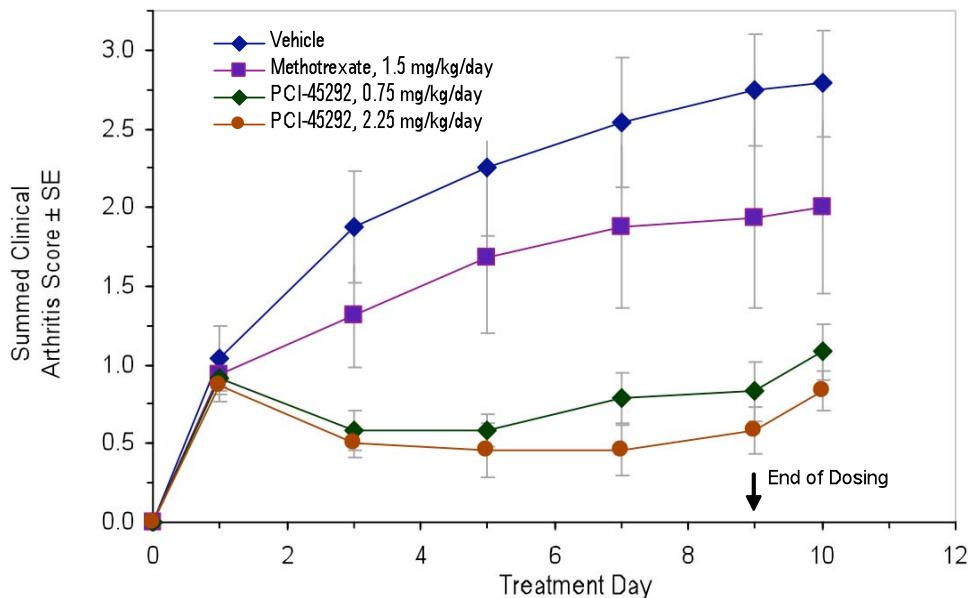


Figure 2. PCI-45292 efficacy in a murine collagen-induce arthritis model

Autoimmune disease models using PCI-32765 as a proof of concept molecule

In vivo, once daily oral dosing of PCI-32765 inhibited collagen-induced arthritis in the mouse ($ED_{50} = 4.6$ mg/kg/day). In a scheduling study (Figure 3), PCI-32765 rapidly reversed clinical signs of fully established collagen-induced arthritis. Upon cessation of dosing with PCI-32765 disease inhibition was sustained for an additional 3 days. In the mouse MRL/lpr lupus model, PCI-32765 also significantly reduced disease progression, inhibiting proteinuria (Figure 4) and anti-dsDNA antibody levels.

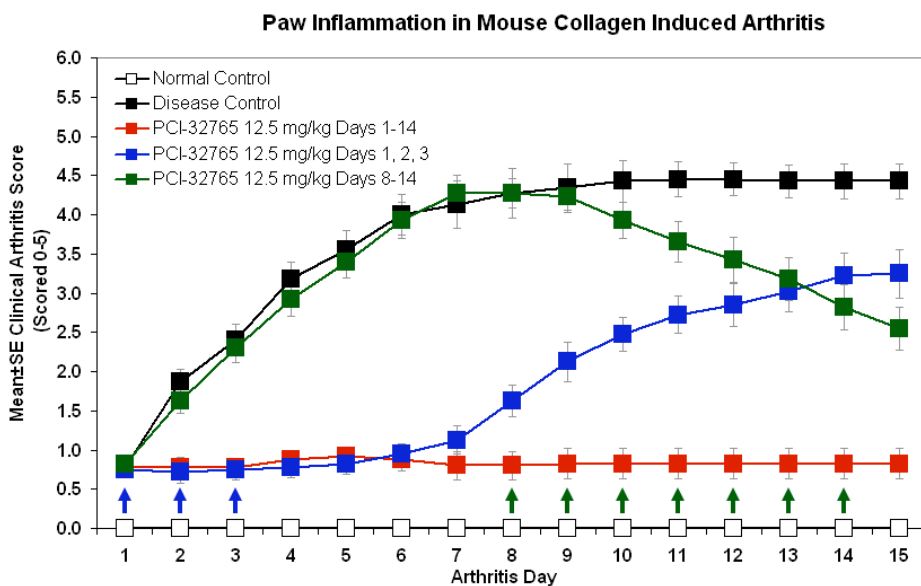


Figure 3. Scheduling study of PCI-32765 in mouse collagen induced arthritis (CIA) model.

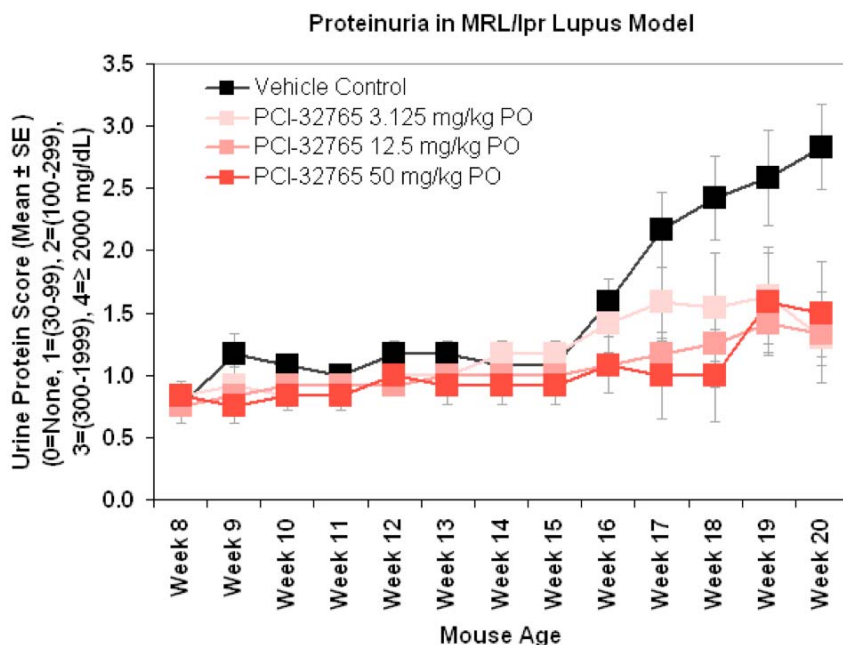


Figure 4. PCI-32765 efficacy in mouse MRL/lpr lupus model

PCI-32765 also prevents mast cell degranulation *in vitro* and inhibits the passive cutaneous anaphylaxis reaction *in vivo*. Dual inhibition of mast cell and B cell activation may explain the significant efficacy of Btk inhibitors in animal models.

C. Clinical Development

Pharmacodynamic Assay

Pharmacyclics has developed a fluorescent probe that covalently binds to the Btk active site. The extent to which the Btk active site is occupied by PCI-32765 or other inhibitors can be measured with this probe. We have confirmed that responses in autoimmune models correlate with the degree to which the Btk active site is occupied by our inhibitors. In addition, we have adapted the probe assay so that it can be used to monitor Btk active-site occupancy by PCI-32765 in human peripheral blood mononuclear cells (PBMCs).

Trials

Phase I in B-Cell Lymphoma (04753), enrolling

A Phase I trial in surface immunoglobulin positive B cell lymphoma is enrolling at multiple clinical sites. The objective of this study is to determine the safety and tolerability of daily oral dosing and to evaluate effects on pharmacodynamic assays and tumor response.

In the first 2 dose cohorts, 16 heavily pretreated and progressing lymphoma patients with a variety of B-cell malignancies were evaluated. In the first dose cohort, 7 patients were treated with PCI-32765 resulting in 2 partial responses (e.g. a 50% decrease in sum of the product of the diameters of up to 6 largest dominant masses; no increase in size of other nodes per the Revised Response Criteria for Malignant Lymphoma [Bruce D. Cheson *J Clin Oncol* **25**:579-586]) one in mantle cell lymphoma, one in



follicular lymphoma and one patient with stable disease for approximately 5 cycles. In the second dose cohort, 9 patients were treated resulting in 3 partial responses (one patient with mantle cell lymphoma and two patients with chronic lymphocytic leukemia (CLL/SLL)) and 2 patients with stable disease for approximately 2 cycles. The overall response rate (ORR), considering only partial and complete responses, was 31% for the first two dose cohorts.

Additionally, as of December 5, 2009, an interim evaluation has been made on 3 of the 6 patients in the third dose cohort. Each of these 3 patients suffered from CLL/SLL. All three have been evaluated as partial responders. At this point the Partial Response Rate in CLL/SLL patients is 5 out of 6.

Preliminary findings from dose cohorts 1, 2, and 3 (1.25, 2.5, and 5.0 mg/kg/day, respectively) indicate good patient tolerability. Full Btk active site occupancy in PBMCs was achieved at a dose of 2.5 mg/kg/day. The half-life of PCI-32765 was determined to be approximately 6 hours.

D. Business Development & Intellectual Property

Partnering

Pharmacyclics seeks strategic pharma/biotech partner(s) to co-develop and commercialize PCI-32765 for oncology and the clinical development candidate for autoimmune diseases.

Patents

A variety of non-provisional PCT applications have been filed for the lead Btk inhibitor, PCI-32765, and the series of compounds for autoimmune diseases and for the Btk fluorescent probe (PD marker). Patents also include cysteine targeted discovery platform and therapeutic use claims and method of inhibitor development. For clinical development molecule, PCI-32765, we expect patent protection till at least December 2026 (without including pharmaceutical extensions) in the United States (issued patent) and other international Territories.