

PCI-45292, a Novel Btk Inhibitor with Optimized Pharmaceutical Properties, Demonstrates Potent Activities in Rodent Models of Arthritis

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ABSTRACT

Purpose: Btk is an essential element of BCR signaling in B cells and FcγR signaling in monocytes/macrophages, and is considered an ideal molecular target for the treatment of autoimmune diseases such as rheumatoid arthritis. PCI-45292, was derived from the chemical scaffold of PCI-32765^{1,2,3}, a first-in-man Btk inhibitor currently in Phase I/II clinical trials in patients with NHL/CLL⁴. Both PCI-32765 and PCI-45292 are irreversible inhibitors of Btk, forming a covalent bond with the sulfhydryl group of Cys-481 at the ATP-binding site. The two compounds were evaluated in vitro for their effects on human lymphocytes, monocytes, and basophils, and in vivo for therapeutic effects in multiple models of arthritis.

Methods and Results: In Btk biochemical assays, PCI-32765 and PCI-45292 have an IC₅₀ of 0.7 nM and 1.4 nM, respectively. PCI-45292 was a more selective inhibitor of Btk than either PCI-32765 or dasatinib, with no significant inhibition of VEGFR2, EGFR, JAK1, 2 or 3, or Abl. Both PCI-32765 and PCI-45292 were potent inhibitors of human B cell activation following BCR stimulation by anti-IgM with an EC₅₀ of 2 nM while failing to inhibit T cell activation at concentrations up to 2,000 nM. Both compounds also inhibited anti-IgE mediated upregulation of CD63 in human whole blood basophils with an EC₅₀ of 20-100 nM. In addition, PCI-45292 inhibited cytokine release from human monocytes at 20-100 nM but did not inhibit IgG-mediated phagocytosis at concentrations up to 10,000 nM. In vivo, both Btk inhibitors dose-dependently inhibited inflammatory synovitis, pannus formation, synovial fluid cytokines, cartilage damage, and bone erosion in both preventive and established murine collagen-induced arthritis (CIA) models. PCI-32765 and PCI-45292 inhibited overt manifestations of arthritis in mice with ED₅₀ values of 2.23 and 0.61-0.75 mg/kg/day, respectively. In a murine collagen-antibody-induced arthritis model (CAIA model), PCI-45292 completely suppressed the development of arthritis at doses 0.8 mg/kg/day. In glutathione binding assays, the rate of glutathione conjugation was 20 fold lower for PCI-45292 than for PCI-32765. In human liver microsomes, the half-life of PCI-32765 was 2.5 min compared to 19.2 min for PCI-45292. Pharmacokinetic studies in rats showed that the bioavailability of PCI-32765 and PCI-45292 was 22.8% and 24.7%, respectively. Hepatic extraction ratio, a measurement of first-pass metabolism, was 0.690 and 0.289 for PCI-32765 and PCI-45292, respectively. Based on pharmacokinetic/efficacy relationships in mice and inter-species scaling of clearance, the daily efficacious dosage of PCI-45292 was estimated to be <10 mg/patient/day.

Conclusions: PCI-45292 is a potent irreversible inhibitor of Btk that suppresses B cell activation following BCR stimulation and inhibits monocytes/macrophage release of pro-inflammatory cytokines and chemokines following FcγR activation. PCI-45292 was shown to have increased selectivity for Btk inhibition over other tyrosine kinases, a reduced potential for off-target protein binding, and improved metabolic stability. Also, PCI-45292 had a more potent effect in ameliorating inflammation and tissue damage in CIA and CAIA models when compared to PCI-32765. PCI-45292 is currently undergoing preclinical development for autoimmune applications.

INTRODUCTION

Bruton tyrosine kinase Btk is required for B-cell receptor (BCR) signaling

- Mutations in Btk cause the human genetic disease X-linked agammaglobulinemia (XLA). In XLA patients, inactivation of Btk blocks signals from the BCR that are required for normal B cell maturation.
- A wide range of studies have shown that antigen binding to the BCR leads to Btk phosphorylation and activation. Btk in turn phosphorylates PLCγ, and is required for sustained Ca²⁺ signaling and activation of the downstream effectors NFκB and ERK.
- The effect of XLA mutations is primarily limited to B cells. Thus Btk is an attractive kinase target for selectively inhibiting the BCR pathway and subsequent autoantibody production.

Btk is important in immune-complex mediated FcγR and FcεR pathways.

- Immune complex (IC) mediated FcγR and FcεR activation of pro-inflammatory cytokine/chemokine release contribute to infiltration of neutrophils, macrophages, and mast cells; pannus, cartilage erosion and bone resorption.

BCR signaling and IC mediated activation of FcγR and FcεR pathways are important for pathogenesis of autoimmune diseases, such as rheumatoid arthritis.

- PCI-32765 is a selective Btk inhibitor with potent clinical activity in B cell malignancies⁴, served as a chemical scaffold for lead optimization.
- Preclinical development candidates were evaluated for off-target tyrosine kinase inhibition, glutathione binding, human liver microsomal metabolism, and in vivo pharmacokinetic/pharmacodynamic characteristics.
- Optimized compound PCI-45292 was shown to have improved activities in the mouse CIA, CAIA and Arthus models.

RESULTS

Figure 1. PCI-45292 is a selective and covalent inhibitor of Bruton's Tyrosine Kinase (Btk)

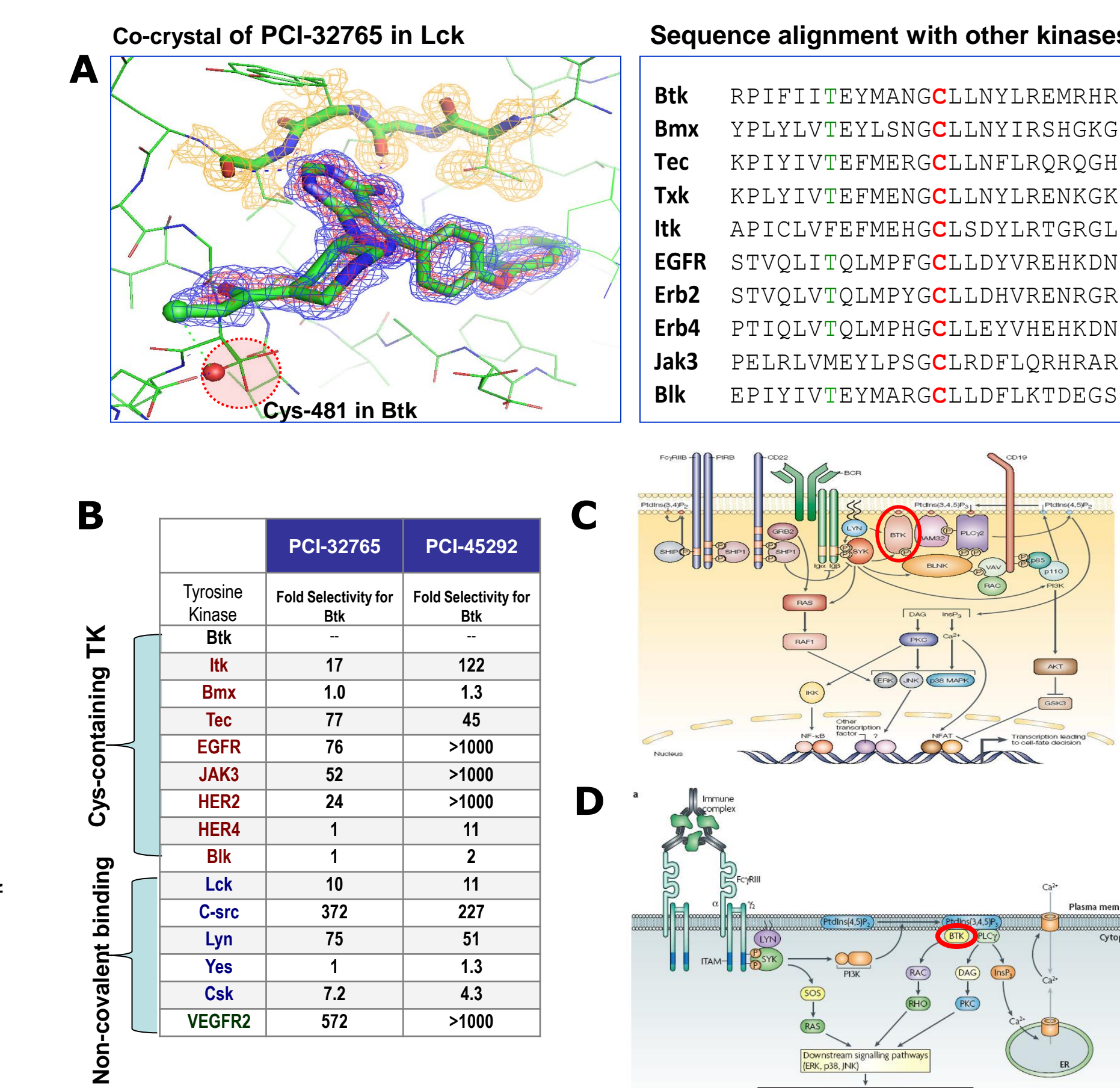
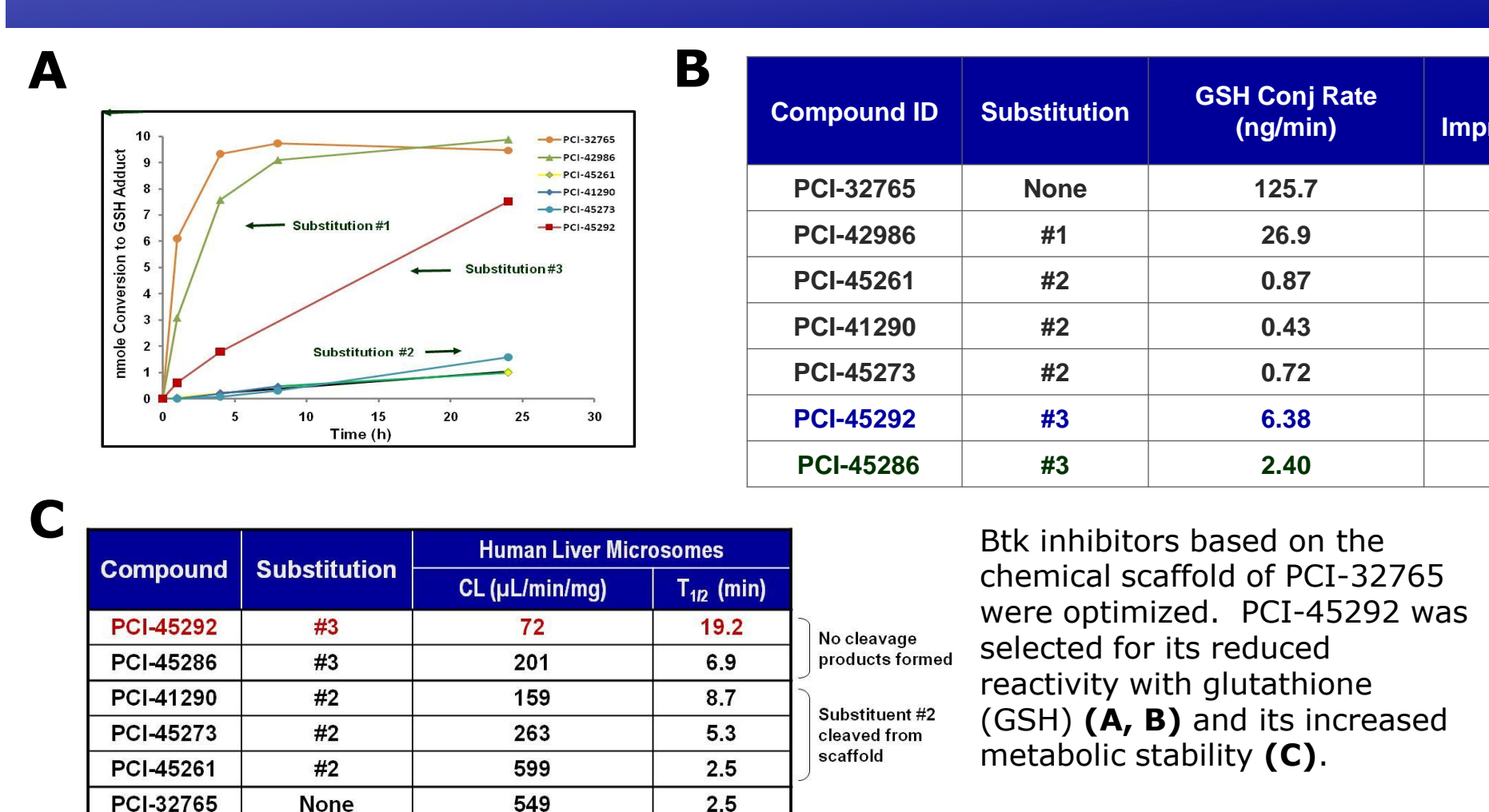


Figure 2. PCI-45292 inhibits BCR driven human primary B cell function and FcγR and FcεR pathways in human primary monocytes, macrophages and mast cells, respectively.

Figure 3. PCI-45292 is optimized for a reduced potential of off-target protein binding and increased metabolic stability

Cell	Activation	Assay Readout	IC ₅₀ μM
Human primary B	BCR	CD69	0.01
Human primary B	BCR	proliferation	0.01
Human PBMC	BCR	PhosphoFlow pBtk (Y223), pPLCγ, pErk1/2	0.03-0.1
Human PBMC	BCR	TNFα, IL-6 release	0.05
THP-1	FcγR	TNFα, IL-6, MCP-1 release	0.01-0.05
Human Monocytes	FcγR	TNFα, IL-6, MCP-1 release	0.01-0.05
Human Macrophages	FcγR	TNFα, IL-6, MCP-1 release	0.01-0.2
Human Mast Cells	FcεR	TNFα, IL-8 release	0.003
Human Basophils (WB)	FcεR	CD63 upregulation	0.02-0.1

Figure 4. PCI-45292 dose-dependently ameliorates clinical arthritis in treatment CIA models



RESULTS

Figure 5. PCI-45292 dose-dependently ameliorates clinical arthritis in treatment CIA models

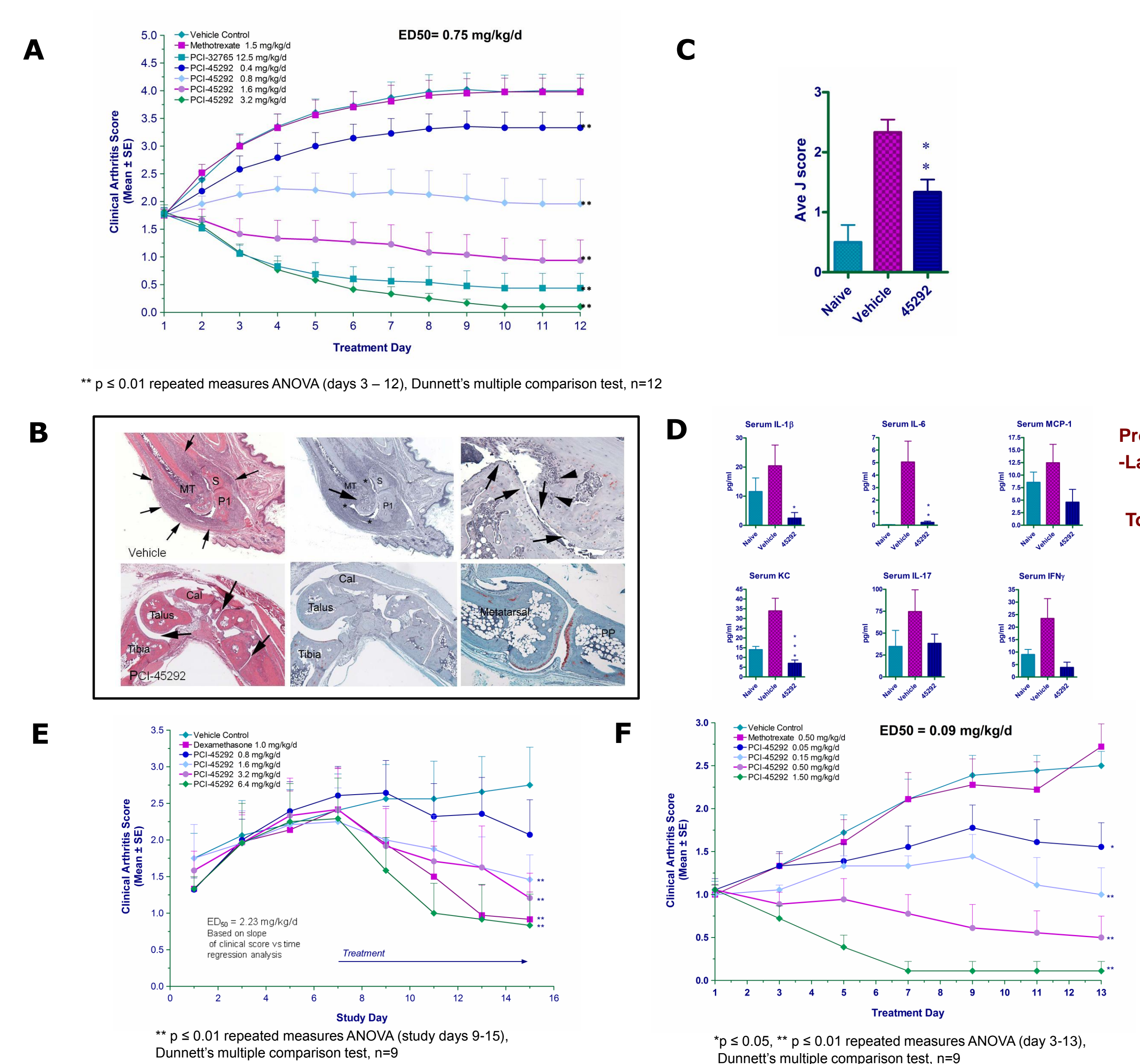


Figure 6. Dose-dependent Btk active-site occupancy in splenocytes from mice administered with PCI-45292

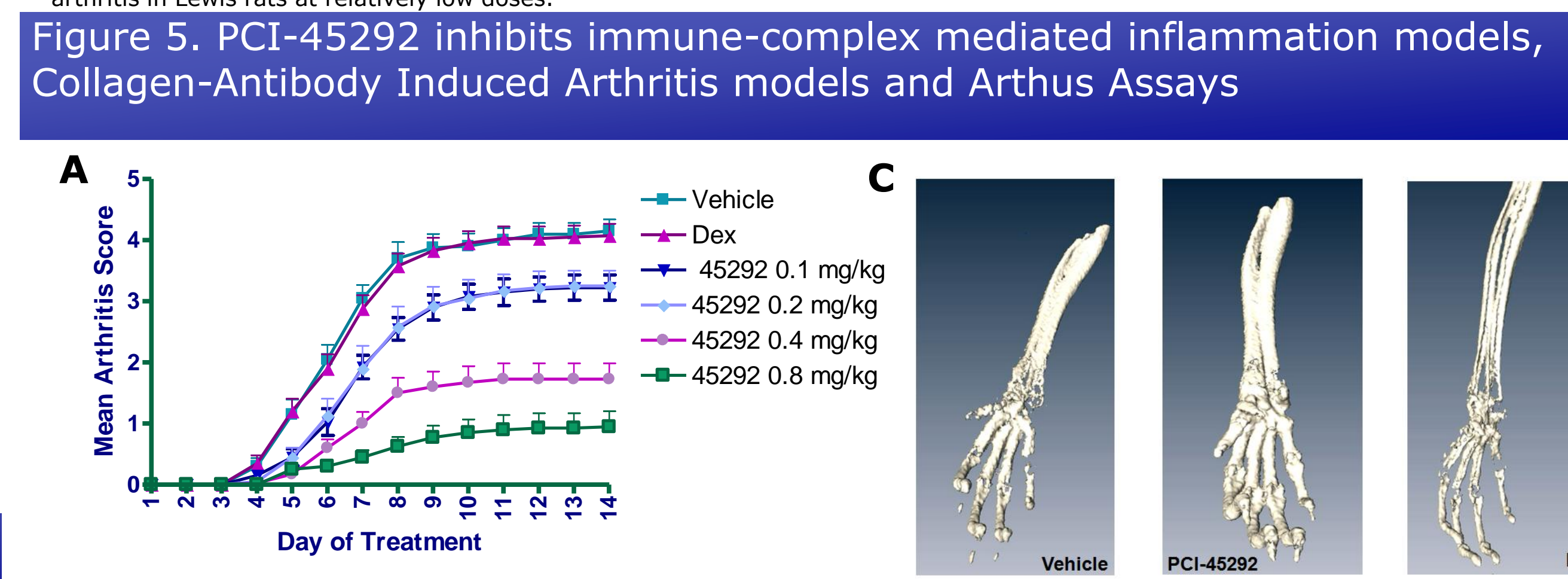
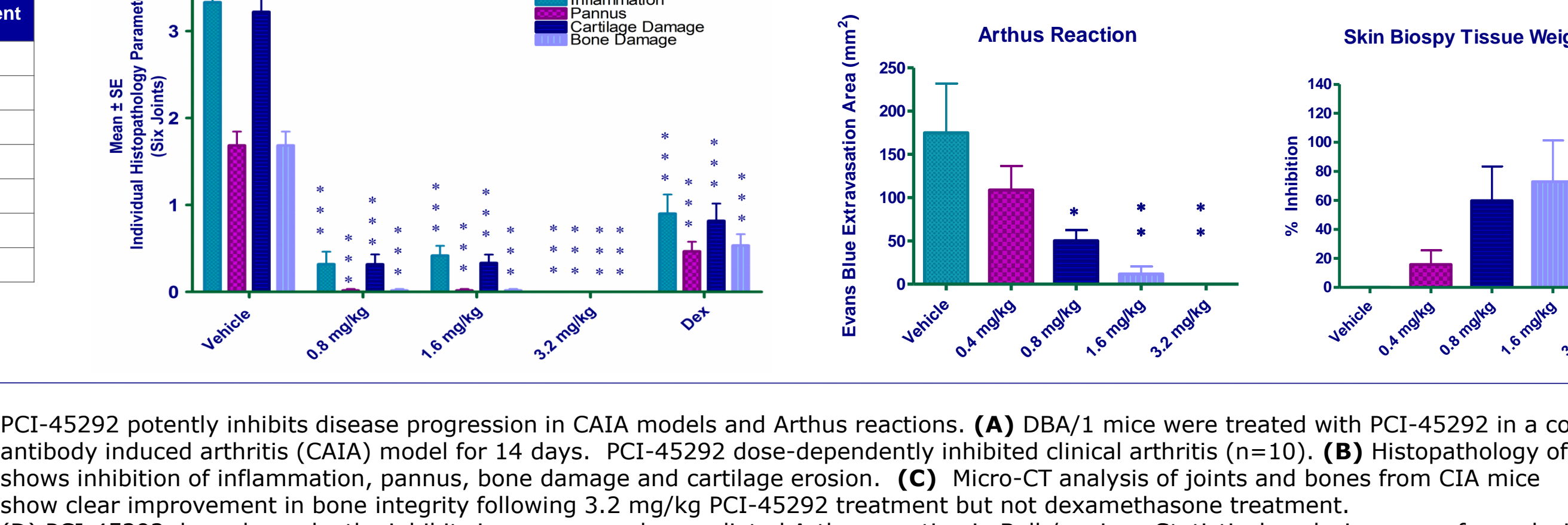
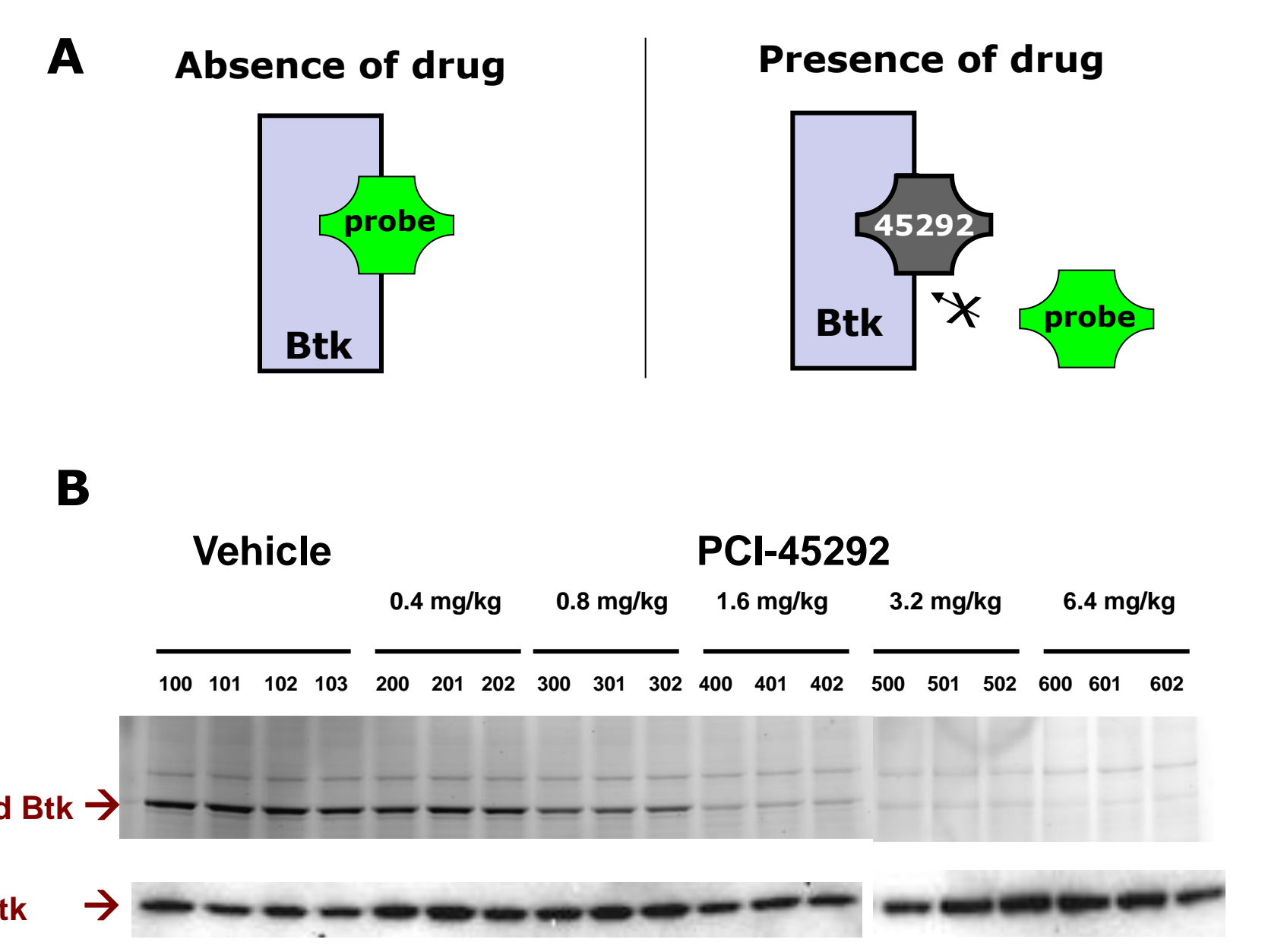


Figure 7. Reduced hepatic extraction ratio in rats and projected median oral efficacious dose (ED50) of PCI-45292 in humans



CONCLUSIONS

Figure 8. Postulated mechanism of action of PCI-45292 in collagen-induced arthritis model

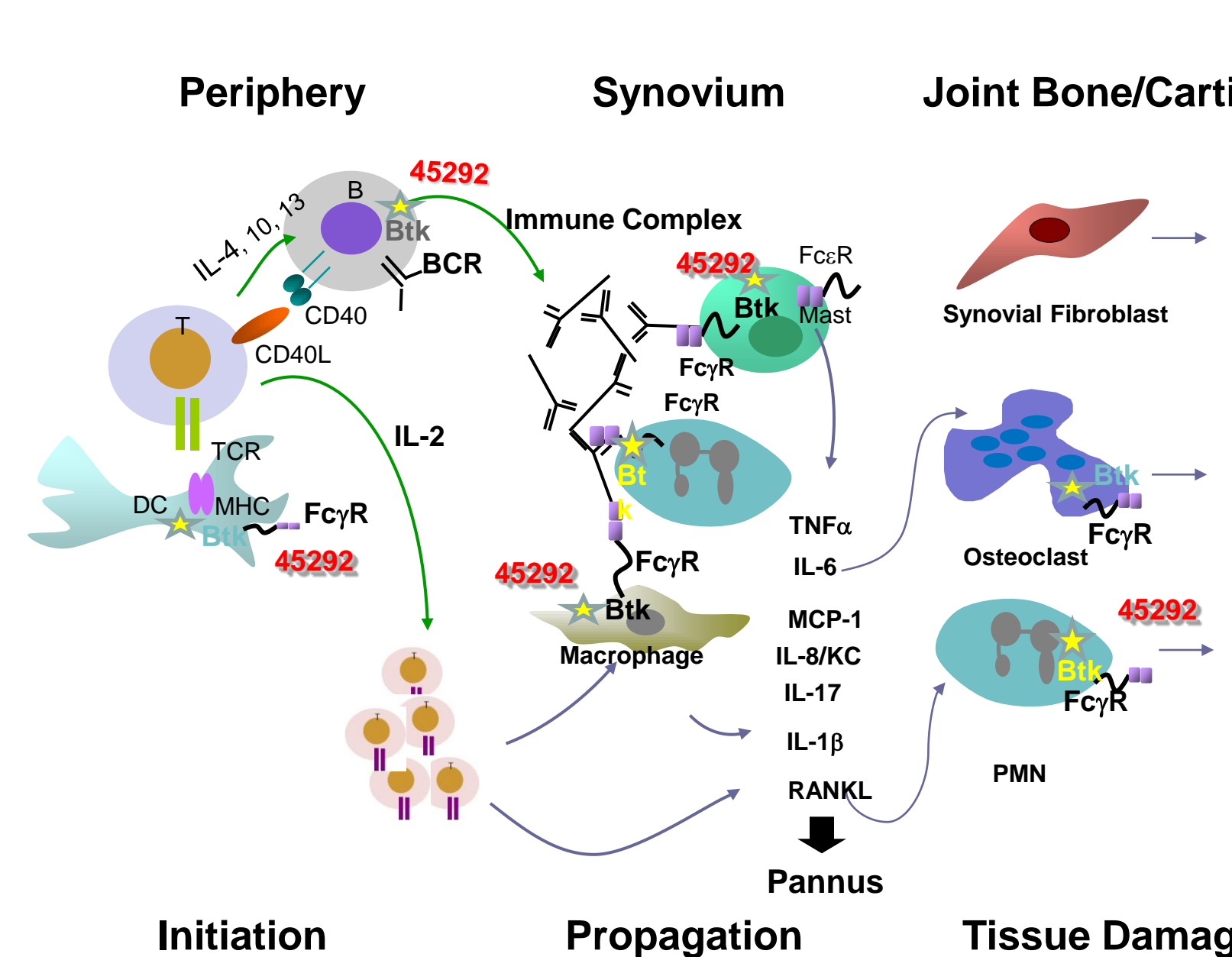


* Modified from Wong et al., (6)

- PCI-45292 is a potent and selective small-molecule inhibitor of Btk. PCI-45292 inhibits downstream events mediated by BCR and FcγR/FcεR stimulations in B lymphocytes and monocyte/macrophage/mast cells. Human primary B lymphocyte activation, proliferation and cytokine secretion were compromised. Monocyte/macrophage and mast cell cytokine/chemokine (TNFα, IL-6, MCP-1) release was inhibited following FcγR/FcεR activation. These cells all contribute to the pathogenesis of rheumatoid arthritis (RA).

- PCI-45292 potently and dose-dependently inhibits clinical arthritis in CIA studies in mice and rats with ED₅₀ values of 0.61 to 0.75 mg/kg/d and 0.09 mg/kg/d, respectively. Of note, PCI-45292 treatments suppressed myeloid cell infiltration into joints, and protected cartilage and bone integrity.
- PCI-45292 potently inhibits Arthus reactions and disease progression in CAIA models. These responses do not rely on suppression of B cell functions.
- PCI-45292 is optimized for selectivity, reduced potential for off-target protein-binding, reduced first-pass clearance, and potent activity in preclinical models of arthritis following oral administration.
- ED₅₀ values for the active-site Btk occupancy assay in mice (0.63 mg/kg) and the activity in the murine CIA model (0.61 to 0.75 mg/kg) were similar.
- Btk inhibition is a promising approach for the treatment of autoimmune diseases such as RA. PCI-45292 is currently in preclinical development.

Figure 8. Postulated mechanism of action of PCI-45292 in collagen-induced arthritis model



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Disclosure: All Pharmacyclics affiliated authors are employees and shareholders of Pharmacyclics.

- Significant clinical activity expected at daily doses ≤ 10 mg per patient per day