Antiarthritic Effect of a Novel Bruton’s Tyrosine Kinase (BTK) Inhibitor in Rat Collagen-Induced Arthritis and Mechanism-Based Pharmacokinetic/Pharmacodynamic Modeling: Relationships between Inhibition of BTK Phosphorylation and Efficacy

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ABSTRACT

Bruton’s tyrosine kinase (BTK) plays a critical role in the development, differentiation, and proliferation of B-lineage cells, making it an attractive target for the treatment of rheumatoid arthritis. The objective of this study was to evaluate the antiarthritic effect of GDC-0834 [R-N-(3-(6-(4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenylamino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide], a potent and selective BTK inhibitor, and characterize the relationship between inhibition of BTK phosphorylation (pBTK) and efficacy. GDC-0834 inhibited BTK with an in vitro IC50 of 5.9 and 6.4 nM in biochemical and cellular assays, respectively, and in vivo IC50 of 1.1 and 5.6 μM in mouse and rat, respectively. Administration of GDC-0834 (30–100 mg/kg) in a rat collagen-induced arthritis (CIA) model resulted in a dose-dependent decrease of ankle swelling and reduction of morphologic pathology. An integrated disease progression pharmacokinetic/pharmacodynamic model where efficacy is driven by pBTK inhibition was fit to ankle-diameter time-course data. This model incorporated a transit model to characterize nondrug-related decreases in ankle swelling occurring at later stages of disease progression in CIA rats. The time course of ankle swelling in vehicle animals was described well by the base model. Simultaneous fitting of data from vehicle- and GDC-0834-treated groups showed that overall 73% inhibition of pBTK was needed to decrease the rate constant describing the ankle swelling increase (kinc) by half. These findings suggest a high degree of pBTK inhibition is required for maximal activity of the pathway on inflammatory arthritis in rats.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints, the tissues that surround joints, and other organs in the body. Approximately 1% of the general population is afflicted by RA, in which the occurrence is two to three times more prevalent in women than men (Smolen and Steiner, 2003). The disease can begin at any age, but the onset is most frequent in woman older than 65 years (Scott et al., 2010). RA is a systemic inflammatory disease that principally affects diarthrodial joints. The major characteristic of the disease is a symmetric polyarthritis involving the small joints of the hands and feet, although other joints are often involved. It has been estimated that 55 to 70% of patients with RA have progressive disease, resulting in joint destruction and disability. RA is also associated with a reduced life expectancy predominantly...
caused by cardiovascular disease and connective tissue diseases (Van Doornum et al., 2006). Although many aspects of the etiology and pathogenesis of RA remain unknown, it is thought that an immune response is initiated and/or perpetuated by one or more antigens present in the synovial compartment. Many cell populations, including B cells, monocytes/macrophages, T cells, endothelial cells, and fibroblasts, participate in the ongoing inflammatory process (Scott et al., 2010), suggesting the presence of multiple cellular targets for immunotherapy of RA. B cell-directed therapy, such as rituximab, a chimeric monoclonal antibody that depletes B cells by binding to the CD20 cell-surface antigen (Mease, 2008), has highlighted the pivotal role of B cells in the pathogenesis of RA and other inflammatory diseases (Eisenberg and Albert, 2006; Townsend et al., 2010).

Bruton’s tyrosine kinase (BTK) is a member of the Tec family of nonreceptor tyrosine kinases, which is expressed in all cells of hematopoietic lineage except plasma cells, natural killer cells, and T lymphocytes (Satterthwaite and Witte, 2000; Brunner et al., 2005). BTK is activated by phosphatidylinositol 3-kinase-dependent plasma membrane recruitment and phosphorylation on tyrosine 551 by the Src-family kinase Lyn. Once activated, BTK induces phospholipase C γ2- and Ca2+-dependent signaling leading to the activation of nuclear factor-κB- and nuclear factor of activated T cell-dependent pathways (Niirro and Clark, 2002). In B cells, BTK is important for B cell antigen receptor-, CD40-, and Toll-like receptor 4-mediated activation and proliferation (Khan et al., 1995). Furthermore, BTK plays a role in B cell antigen processing and presentation (Sharma et al., 2009). It is noteworthy that BTK is also essential in Fcγ receptor-mediated inflammatory cytokine production [tumor necrosis factor α, interleukin (IL)-1β, and IL-6] in monocytes/macrophages and therefore can contribute to immune complex-induced disease (Di Paolo et al., 2011).

Animal models of arthritis are used to study pathogenesis of disease and test potential new therapies for clinical use. The three most commonly used models for the testing of potential RA therapeutic agents are adjuvant-induced arthritis in rats and collagen-induced arthritis (CIA) in rats and mice (Bendele, 2001; Hegen et al., 2008). The rat CIA model was first described by Trentham et al. (1977) and, together with mouse CIA, is the most widely accepted animal model for human RA. As in human RA, female rats are more susceptible to arthritis in this model. The onset of arthritis is rapid, typically developing 10 to 13 days after immunization with homologous or heterologous type II collagen, peaking at approximately days 15 to 20 and then gradually declining. The resulting polyarthritis is characterized by marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption, periosteal proliferation, and moderate to marked synovitis and periarticular inflammation (Bendele, 2001). Collagen-specific T and B cells both are required for disease induction, and CIA can be transferred with serum from diseased animals into recipient strains. Rat CIA differs from human RA in that it is self-limiting and not characterized by exacerbations and remissions. In addition, the inflammatory cell infiltrate in rat CIA consists predominantly of polymorphonuclear cells, whereas a high proportion of mononuclear cells are seen in human RA (Hegen et al., 2008).

GDC-0834 [R-N-3-(6-(4-(1,4-dimethyl-3-oxo-1,2-phenyllumino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylnyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide] is a highly selective, reversible, ATP-competitive, small-molecule inhibitor of BTK and has been developed as a potential therapeutic for RA. We show that GDC-0834 inhibits BTK phosphorylation (pBTK) in vitro and in vivo in rodents and suppresses arthritis in the rat CIA model in a dose-dependent manner. In addition, GDC-0834 significantly inhibited joint inflammation, cartilage damage, and bone resorption. Finally, we developed a disease progression pharmacokinetic (PK)/pharmacodynamic (PD) model to provide an understanding of the relationship between target modulation (inhibition of pBTK) and efficacy (reduction of ankle swelling) in this animal model.

Materials and Methods

Materials and Animals. GDC-0834 (Fig. 1) was provided by Genentech (South San Francisco, CA) and CGI Pharmaceuticals (Branford, CT). Bovine type II collagen was purchased from Elastin Products (Owensville, MO). Female Lewis rats weighing 130 to 162 g (mean approximately 148 g) were obtained from Charles River Laboratories, Inc. (Wilmington, MA), and BALB/c mice were obtained from Harlan (Indianapolis, IN). Rats were acclimated for 8 days before being immunized with type II collagen. During the acclimation and study periods, animals were housed in a laboratory environment with temperatures ranging between 67 and 76°F and relative humidity ranging between 30 and 70%. Automatic timers provided 12 h of light and 12 h of dark. Animals were allowed access ad libitum to Harlan Teklad Rodent Chow and fresh municipal tap water. Animal care including room, cage, and equipment sanitation conformed to the guidelines cited in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the applicable standard operating procedures of the University of Colorado and CGI Pharmaceuticals vivaria.

Measurement of BTK Enzyme Inhibition by GDC-0834. Btk activity was quantified by determining phosphorylation of an exogenous peptide product in a Lanthascreen assay (Lebakken et al., 2009). In the validation of the assay conditions, both enzyme concentration and time dependence experiments were performed ensuring linearity at 60 min before assessing compound potency (data not shown). The K_{app} values for ATP and peptide were also assessed before selecting the final reaction conditions. In a final reaction volume of 25 μl, Btk [human, full-length, C-terminal V5–6× His, expressed in Sf9 cells; 0.075 ng/25 μl reaction] was incubated with 50 mM HEPES, pH 7.5, 10 mM MgCl2, 2 mM MnCl2, 2 mM dithiothreitol, 0.2 mM NaVO4, 0.01% casein, 0.01% Triton X-100 (Bio-Rad Laboratories, Hercules, CA), 2.5% glycerol, and 0.4 μM fluorescein poly-Glu/Ala/Tyr (Invitrogen, Carlsbad, CA). The reaction was ini-
tiated by the addition of ATP to 25 μM ($K_m$ of ATP). After incubation for 60 min at room temperature, the reaction was stopped by the addition of a final concentration of 2 mM Tb-PY20 detection antibody (Invitrogen) in 60 mM EDTA for 30 min at room temperature. Detection was determined on a PerkinElmer Envision (PerkinElmer Life and Analytical Sciences, Waltham, MA) with 340-nm excitation and emission at 495 and 520 nm. The response versus BTK inhibitor concentration data were fitted with GraphPad Prism version 5.00 (GraphPad Software Inc., San Diego, CA).

**Measurement of BTK Cellular Inhibition by GDC-0834.** The rat splenocytes were obtained from male Sprague-Dawley rats (Charles River Laboratories, Inc) by homogenization of rat spleena. Rat spleens were manually disrupted and homogenized with a tissue homogenizer in 10 ml of RPMI media. The homogenate was passed through a cell strainer into a 50-ml Falcon tube followed by red blood cell lysis. A total of 1 × 10⁷ cells/ml were added to serum-free RPMI media for 1 to 2 h. Five hundred microliters of cell suspension was incubated with GDC-0834 for 1 h at 37°C, followed by stimulation with 3 μg/ml Oligomycin (final concentration) for 3 min. Cells were briefly centrifuged and resuspended in 1× Cell Signaling lysis buffer (Cell Signaling Technology, Danvers, MA), supplemented with a protease inhibitor cocktail (Roche, Indianapolis, IN) and phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s directions and lysed on ice for 15 min. Cells were centrifuged at 10,000 rpm for 10 min. The supernatants were added to Tris-glycine SDS sample buffer with 5% β-mercaptoethanol, heated to 74°C for 10 min, and loaded on Novex (San Diego, CA) NuPAGE 4 to 12% Bis-Tris SDS-polyacrylamide gel electrophoresis gels. Western blots were performed according to standard procedures (Burnette, 1981), blotted on nitrocellulose, and detected using an Odyssey Western blot detection system (LI-COR Biosciences, Lincoln, NE). Primary antibodies were purchased for pBTK-Tyr223 (BTK autophosphorylation on tyrosine 223) (Cell Signaling Technology) and total BTK (BD Biosciences, Franklin Lakes, NJ). The response versus BTK inhibitor concentration data were fitted with GraphPad Prism version 5.00.

**pBTK Inhibition by GDC-0834 in BALB/c Mice.** To investigate the in vivo potency of GDC-0834 on inhibiting pBTK-Tyr223 in blood in mice, BALB/c mice were dosed orally with GDC-0834 at 25, 50, 100, and 150 mg/kg, and the terminal blood samples were collected from mice at 2, 4, or 6 h postdose. Three animals per each time point were sacrificed for the blood sampling. GDC-0834 plasma levels were quantitated using LC/MS/MS as described below. Levels of BTK-pTyr223 (pBTK) and total BTK were determined in blood by Western blot. A rabbit polyclonal pBTK from Cell Signaling Technology and a mouse monoclonal total BTK from BD Biosciences Transduction Laboratories (Bedford, MA) were used. The bands were quantified with LI-COR Odyssey imager and software. The percentage of inhibition of pBTK inhibition relative to the GDC-0834 blood concentrations. No hysteresis was evident in plots of pBTK inhibition versus GDC-0834 concentration.

**Induction of Arthritis and Treatment with GDC-0834 in Rats.** Arthritis was induced in female Lewis rats at Boulder BioPATH (Boulder, CO). In brief, animals (10 rats per group) were anesthetized with isoflurane and injected with 300 μl of Freund’s incomplete adjuvant (Difco, Detroit, MI) containing 2 mg/ml bovine type II collagen at the base of the tail and two sites on the back on days 0 and 6. Oral dosing of GDC-0834 (vehicle, 1, 3, 10, 30, and 100 mg/kg b.i.d. [twice daily]) at 12-h intervals, vehicle, 10, 30, 100 mg/kg once daily (QD) at 24-h intervals, and 100 mg/kg every other day (Q2D) at 48 intervals] was initiated on day 0 of the study and continued through day 16. The caliper measurements of ankles were taken every day starting from day 9 to day 17, and the area under the ankle diameter-time curves were calculated based on the trapezoidal rule (Gibaldi and Perrier, 1982). After final body weight measurement on day 17, animals were anesthetized for terminal serum collection and then euthanized for tissue collection. Ankle diameters were also measured in the normal rats treated with the vehicle ($n = 6$).

**Morphologic Pathology of Ankles in Rat CIA Model.** Preserved and decalcified (5% formic acid) ankle joints were cut in half longitudinally, processed through graded alcohols and a clearing agent, infiltrated and embedded in paraffin, sectioned, and stained with toluidine blue. The tissues were examined microscopically. Collagen arthritic ankles were given scores of 0 to 5 for inflammation, pannus formation, cartilage damage, and bone resorption according to the following criteria: For ankle inflammation, 0 = normal; 1 = minimal infiltration of inflammatory cells in synovium/periarticular tissue; 2 = mild infiltration; 3 = moderate infiltration with moderate edema; 4 = marked infiltration with marked edema; and 5 = severe infiltration with severe edema. For ankle pannus, 0 = normal; 1 = minimal infiltration of pannus and subchondral bone; 2 = mild infiltration (<1/4 of tibia or tarsals at marginal zones); 3 = moderate infiltration (1/4 to 1/3 of tibia or small tarsals affected at marginal zones); 4 = marked infiltration (1/2–3/4 of tibia or tarsals affected at marginal zones); and 5 = severe infiltration (>3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture).

For cartilage damage (emphasis on small tarsals), 0 = normal; 1 = minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption; 2 = mild = mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption; 3 = moderate = moderate loss of toluidine blue staining with multifocal moderate (depth to middle zone) chondrocyte loss and/or collagen disruption; 4 = marked = marked loss of toluidine blue staining with multifocal marked (depth to deep zone) chondrocyte loss and/or collagen disruption, one or more small tarsals have full thickness loss of cartilage; and 5 = severe = severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption.

For bone resorption, 0 = normal; 1 = minimal = small areas of resorption, not readily apparent on low magnification, rare osteoclasts; 2 = mild = more numerous areas of resorption, not readily apparent on low magnification, osteoclasts more numerous, <1/4 of tibia or tarsals at marginal zones resorbed; 3 = moderate = obvious resorption of medullary trabecular and cortical bone without full thickness defects in cortex, loss of some medullary trabeculae, lesion apparent on low magnification, osteoclasts more numerous, 1/4 to 1/3 of tibia = tarsals affected at marginal zones; 4 = marked = full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, 1/2–3/4 of tibia or tarsals affected at marginal zones; and 5 = severe = full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts, >3/4 of tibia or tarsals affected at marginal zones, severe distortion of overall architecture.

**Inhibition of pBTK in CIA Rats Treated with GDC-0834.** The percentage of inhibition of BTK-Tyr223 autophosphorylation in blood was determined at 8 h after the morning dose on day 7 in satellite groups of rats that were treated with various doses (3, 10, 30, and 100 mg/kg b.i.d. and 30 and 100 mg/kg QD) of GDC-0834. Levels of pBTK and total BTK were determined in the rat blood by Western blotting (with the mouse blood as the positive control). A rabbit polyclonal pBTK from Cell Signaling Technology and a mouse monoclonal total BTK from BD Transduction Laboratories were used. The bands were quantified with LI-COR Odyssey imager and software. The percentage of inhibition of pBTK-Tyr223 normalized to
the total BTK was calculated relative to the level determined from the vehicle-treated animals as described previously. GDC-0834 plasma levels were quantified using LC/MS/MS (as described below).

PK/PD Modeling of In Vivo pBTK Inhibition in Rodents. Because there was no evidence of hysteresis observed in preliminary plots of pBTK inhibition and GDC-0834 plasma concentrations in mice, a sigmoidal direct response model (eq. 1) was used to fit the GDC-0834 plasma concentration versus pBTK inhibition data from the BALB/c mice and the CIA rats using GraphPad Prism version 5.00.

\[ \text{pBTK}_{\text{inh\%}} = \frac{C_m}{IC_{50} + C} \]  

where pBTK_{inh\%} is the percentage of inhibition of pBTK in the mouse and rat blood, C is the plasma concentration of GDC-0834, IC_{50} is the drug concentration that produces 50% pBTK inhibition, and n is the Hill slope representing the curve steepness.

Pharmacokinetics of GDC-0834 in the Rat CIA model. Blood samples were collected on day 16 from all groups (n = 10) in the rat CIA study. Blood samples were collected from the first subgroup of five animals at 2 and 8 h (and 24 when dosed as QD or Q2D) postdose. For the second subgroup of five rats, blood was collected at 4 and 12 h postdose. GDC-0834 plasma concentrations were quantitated using LC/MS/MS (Thermo Finnigan TSQ; Thermo Fisher Scientific, Waltham, MA) for PK analysis. The assay limit of quantitation was 5 ng/ml. The PK parameters, including C_{max} (highest observed plasma concentration), t_{max} (time at which C_{max} occurred), AUC_{0–24} (area under the plasma concentration curve from time 0 to 24 h), and t_{1/2, p, o} (terminal half-life after an oral dose) for each group were determined by noncompartmental methods using the extravascular input model (model 200, WinNonlin, version 5.2.1; Pharsight, Mountain View, CA). For modeling and simulation purposes, a one-compartment model with oral absorption was fit to the GDC-0834 concentration-time data using SAAM II version 1.2.1 (University of Washington, Seattle, WA) to obtain the compartmental PK parameters, including absorption rate constant (k_in), elimination rate constant (k_out), and oral volume of distribution (V/F).

Disease Progression PK/PD Modeling of Relationship between pBTK Inhibition and Efficacy. Based on the pathophysiology of the rat CIA model, an integrated disease progression model (Fig. 2) was used to fit the time course of ankle diameter change (mean data) in the vehicle- and/or GDC-0834-treated groups using SAAM II version 1.2.1. The Runge-Kutta integrator using a standard forward integrating order 5-4 method (SAAM II user guide) was applied, and the convergence criterion was 0.0001. Initial attempts at using population PK/PD analysis and a two-stage approach for this study were not successful, so a model using mean ankle diameter data was used. An indirect response model was used to describe the impact of pBTK inhibition on ankle diameter changes (eqs. 2 and 3). Equation 2 constitutes a positive feedback (autocatalysis or exponential growth), which is not an unreasonable concept in inflammation involving cell proliferation. A transit model was used to describe the nondrug-related decrease in ankle swelling occurring at the later stages of disease progression in the arthritic ankles of CIA rats (eqs. 4–7).

\[ \frac{dAD}{dt} = (k_{in} - E)(AD - AD_0) - k_{out}(AD - AD_0) \]  

\[ E = k_0\text{pBTK}_{\text{inh\%}} \]  

\[ \text{pBTK}_{\text{inh\%}} = \frac{C_m}{IC_{50} + C} \]  

where AD is the ankle diameter, AD_0 is a heuristic parameter required to describe the exponential increase of ankle diameter that is obtained from fitting, k_in is the ankle swelling increase rate constant, k_out is the rate constant describing nondrug-related reduction in ankle swelling (Fig. 2, top), pBTK_{inh\%} is the percentage of inhibition of pBTK in blood, IC_{50} is the percentage of inhibition of pBTK that produces 50% of the maximum inhibition of k_in, and n is the Hill slope.

The pBTK_{inh\%} was simulated based on eq. 1 where the GDC-0834 concentration (C) was simulated based on the PK parameters from the one-compartment model with oral absorption as described previously. The transit model of k_out is described by Fig. 2, top, and eqs. 4 to 7, where k is the transit rate constant from k_in to k_out and R is the input rate of k_out into the transit model. The described transit model allows k_out to increase with time. The initial time of the pharmacokinetics of GDC-0834 is 0 h; 216 h (day 9) was used as the initial time for the disease progression (eqs. 2–7) in the fitting as the ankle diameter was first measured on the day 9 when it started to increase in the rat CIA model. The initial conditions on day 9 for eqs. 2 to 7 are k_1 = k_2 = k_3 = k_4 = 0 and AD = 0.26 inch.

\[ \frac{dk_1}{dt} = R - k \times k_1 \]  

\[ \frac{dk_2}{dt} = k \times k_1 - k \times k_2 \]  

\[ \frac{dk_3}{dt} = k \times k_2 - k \times k_3 \]  

\[ \frac{dk_4}{dt} = k \times k_3 - k \times k_4 \]  

\[ \frac{dk_{out}}{dt} = k \times k_4 - k \times k_{out} \]

Results

BTK Enzyme and Cellular Inhibition by GDC-0834. GDC-0834 suppressed BTK kinase activity with an IC_{50} value of 5.9 ± 1.1 nM with Hill slope value of −0.84 ± 0.070 (mean ± S.E.) (Fig. 3A). In the rat splenocytes, GDC-0834 suppressed pBTK activity with an IC_{50} value of 6.4 ± 1.6 nM with Hill slope value of −0.76 ± 0.19 (mean ± S.E.) (Fig. 3B).

pBTK Inhibition by GDC-0834 in Rodents. The treatment of BALB/c mice with GDC-0834 resulted in dose-dependent inhibition of pBTK-Tyr223. Animals dosed with 150 or 100 mg/kg GDC-0834 for 2 h showed complete inhibition of pBTK-Tyr223 levels in blood, with a mean inhibition of 97 and 96%, respectively. Individual animal plasma GDC-0834 concentrations from different time points and percentage of inhibition of pBTK-Tyr223 levels in blood were plotted to assess a PK/PD relationship (eq. 1). Because there was no evidence of a hysteresis, a direct response model was used to fit the relationship between GDC-0834 concentration and pBTK inhibition. The fit resulted in an estimated IC_{50} value.
of 1.1 ± 0.16 µM with m (Hill slope) = 1.1 ± 0.22 (mean ± S.E.) (Fig. 4).

In the rat CIA study, GDC-0834 inhibited pBTK-Tyr223 in rat blood in a dose-dependent manner (Fig. 5, A and B). Like with the mouse data, a direct response model (eq. 1) was used to fit inhibition of pBTK-Tyr223 levels in blood and GDC-0834 plasma levels. The IC<sub>50</sub> estimate of pBTK-Tyr223 inhibition in rats was determined to be 5.6 ± 1.6 nM with m of 0.51 ± 0.087 (mean ± S.E.) (Fig. 5C).

Antiarthritis Effect of GDC-0834 in the Rat CIA Model. Significant inhibition of ankle diameter between day 9 (onset of increase in ankle diameter) and day 17 was observed in rats treated with GDC-0834 at 100 mg/kg (b.i.d., QD, or Q2D) and 30 mg/kg (b.i.d., QD). Treatment with GDC-0834 resulted in a significant reduction of the area under the ankle diameter-time curve in rats at the following doses: 100 mg/kg b.i.d. (88%), 30 mg/kg b.i.d. (47%), 100 mg/kg QD (81%), 30 mg/kg QD (41%), and 100 mg/kg Q2D (49%). Non efficacious doses for the area under the ankle diameter-time curve parameter ranged from 1 mg/kg (b.i.d.) and 3 mg/kg (b.i.d.) to 10 mg/kg (b.i.d., QD) (Fig. 6). In the normal rats treated with vehicle, the ankle diameters did not change and were 0.26 inch from days 9 to 17 (S.D. = 0.0010 inch on each day). Ankles from normal rats were significantly different (P < 0.05) compared with the CIA rats treated with the vehicle from days 10 to 17.

Morphologic Pathology Findings of Ankles. Histopathology was performed on all groups with the exception of animals dosed with 3 and 1 mg/kg b.i.d.. Representative photomicrographs are shown in Fig. 7. Compared with the normal rats treated with vehicle (Fig. 7A), the vehicle-treated disease control rats had marked to severe synovitis and periarticular inflammation in at least one, and usually both, ankle joints with minimal to moderate pannus and bone resorption and minimal to marked cartilage damage (Fig. 7B). In contrast, ankles of arthritic rats treated b.i.d. with 100 mg/kg GDC-0834 had mild synovitis and periarticular inflammation, minimal cartilage damage, and minimal pannus and bone destruction (Fig. 7C). All mean ankle histopathology parameters were significantly reduced toward normal in rats treated b.i.d. (78% reduction), QD (78% reduction), or Q2D (38% reduction) with 100 mg/kg GDC-0834 and b.i.d. (38% reduction) or QD (42% reduction) with 30 mg/kg GDC-0834 (Table 1 and Fig. 8).

Pharmacokinetics of GDC-0834 in CIA Rats. The PK parameters produced by the noncompartmental analysis of GDC-0834 plasma data in CIA rats are listed in Table 2. In general, the AUC estimates were reasonably dose-proportional, and the terminal half-lives were similar in all groups. For the modeling and simulation purposes, a one-compartment model with oral absorption was fit to the same GDC-0834 concentration-time data. The following pharmacokinetic parameter estimates were obtained: absorption rate constant (k<sub>a</sub>) = 0.21 h<sup>-1</sup> [coefficient of variation (CV) = 3.5%]; elimination rate constant (k<sub>e</sub>) = 1.7 h<sup>-1</sup> (CV = 47%); and oral volume of distribution (V/F) = 0.12 L/kg (CV = 50%). A plot of the measured versus model predicted concentrations is presented in Fig. 9 with R<sup>2</sup> = 0.92.

Disease Progression PK/PD Model for Vehicle-Treated Group. The base disease progression model described in Fig. 2 was applied to the ankle diameter data from the vehicle-treated rat CIA group. As seen in Fig. 10, the model was able...
to describe the mean ankle diameter data from a vehicle group (i.e., b.i.d. vehicle group shown in Fig. 6) reasonably well, providing estimates of $k_{in} = 0.029 \text{ h}^{-1}$, $r = 0.00086 \text{ h}^{2}$, and $k = 0.029 \text{ h}^{-1}$ (Table 3). The parameters associated with the vehicle group were estimated with good confidence with all CV values being $\leq 15\%$ (Table 3). The value of AD$_{0.5}$, 0.25 inch ($CV = 1.6\%$), was obtained by fitting with this vehicle-treated data and was used in the disease progression PK/PD modeling for the GDC-0834-treated groups.

**Disease Progression PK/PD Model for GDC-0834-Treated Groups.** The ankle diameter data for the vehicle and GDC-0834-treated rat CIA groups (11 groups) were simultaneously fitted to the full disease progression model shown in Fig. 2. In general, the model described the data well and resulted in the following pharmacodynamic parameter estimates: $k_{in} = 0.022 \text{ h}^{-1}$, $r = 0.0010 \text{ h}^{-2}$, $k = 0.032 \text{ h}^{-1}$, IpBTK$_{50} = 73\%$, and $n = 24$ (Table 3 and Fig. 11). The CVs of each parameter were $<5\%$ except that of $n$ ($CV = 39\%$). Figure 12 shows a plot describing the change in $k_{out}$ with time. The increase in $k_{out}$ over time in the model nicely captures the nondrug-related decrease in ankle swelling in the vehicle and treatment groups that occur after day 15 based on the fitting of the data shown in Fig. 6. A plot of the effect ($E$) based on eq. 3 versus %pBTK inhibition is shown in Fig. 13. The estimate of the %pBTK inhibition needed to decrease the $k_{in}$ by half was 73\%.

**Discussion**

The discovery of a highly selective inhibitor for BTK has provided convincing evidence that BTK is an attractive target for the treatment of RA and B cell-related diseases (Pan et al., 2007, 2008; Honigberg et al., 2010; Di Paolo et al., 2011). However, no drug has been approved for this target to date. GDC-0834 is a selective, potent, and reversible BTK inhibitor with a biochemical IC$_{50}$ of 5.9 nM (Fig. 3A) and cellular IC$_{50}$ of 6.4 nM (Fig. 3B). Functionally, this antagonist prevented murine and human B cell proliferation in response to B cell antigen receptor or CD40 stimulation (data not shown). In the in vivo studies, the pBTK-Tyr223 inhibition by GDC-0834 was demonstrated in mice and rats (Figs. 4 and 5). After normalized to the free fraction of GDC-0834 in mouse (0.7%) and rat (1%) plasma, the free IC$_{50}$ values from BALB/c mice (7.7 nM) and CIA rats (5.6 nM) were comparable with the determined biochemical and cellular IC$_{50}$ estimates. This is not surprising considering the high homology BTK has in rodents and humans. No hysteresis was observed in the time-course data from the mouse (Fig. 4), suggesting that the inhibition of pBTK is a rapid event. These data serve as justification for why we used a sigmoidal direct effect model in the rat when describing the relationship between GDC-0834 plasma concentrations and pBTK inhibition (Fig. 5C).
strongly suggesting that GDC-0834 would be efficacious in therapeutic CIA models (although not specifically tested) because CGI1746 demonstrated comparable efficacy in both the therapeutic and prophylactic CIA models. Administration of GDC-0834 resulted in a dose-dependent reduction of severity of the arthritic condition characteristic of CIA rats. At a dose of 100 mg/kg b.i.d. GDC-0834, synovitis and periarticular inflammation were mild, whereas cartilage damage, pannus, and bone destruction were minimal in the ankles of arthritic rats (Fig. 7C). These results were comparable with findings in rats from the active control group treated with methotrexate (data not shown). The described morphologic improvement of arthritic ankles by GDC-0834 was associated with dose-dependent decreases in ankle diameters with time (Fig. 6). Because the lesions in the rat CIA model exhibit common aspects to those seen in human RA, in that there is more extensive pannus associated cartilage destruction (Bendele, 2001), the antiarthritis effect of GDC-0834 may translate into clinical relevance.

Mechanism-based PK/PD modeling is a useful tool that integrates dynamic changes of a drug in the body with the time course of its pharmacological effects to better understand the mechanisms of drug action and disease progression (Mager et al., 2003, 2009; Yamazaki et al., 2008). Although the application of mechanism-based PK/PD modeling in preclinical models of arthritis is relatively new, previous work has provided a better understanding of factors contributing to disease progression in the rat CIA model (Earp et al., 2008a,b, 2009). In the current integrated disease progression PK/PD model (Fig. 2), we have incorporated an indirect response model (Fig. 2, bottom) where efficacy is driven by pBTK inhibition and a transit model (Fig. 2, top) used to describe the nondrug-related decrease in ankle swelling occurring at the later stages of disease progression in the arthritic ankles of CIA rats. To our knowledge, the use of transit compartments to model disease progression in CIA rats has not been described previously. The nondrug-related decrease in ankle swelling observed in CIA rats is associated with the time course of the relative expression of the proinflammatory cytokines, such as tumor necrosis factor α, IL-1β, and IL-6, and anti-inflammatory cytokines such as transforming growth factor β, during the course of arthritis (Marinova-Mutafchieva et al., 2006; Earp et al., 2008b). The natural disease progression in the control vehicle-treated group was well described by the model with the transit compartments (Fig. 10). The fitted parameter estimates (Table 3) show that the increase in ankle swelling (controlled by $k_{in}$) was greater than $k_{out}$ (Fig. 12) at the early and middle stages of the rat CIA study. At approximately day 15 (360 h), $k_{in}$ is surpassed by $k_{out}$, which is consistent with the observed changes in the ankle diameter (Fig. 6). The value of incorporating the transit model in the integrated PK/PD model is that the antiarthritis effect of GDC-0834 on ankle swelling can be differentiated from the nondrug-related decrease in ankle swelling that is part of the natural disease progression in CIA rats.

We have previously quantified the relationship between mitogen-activated protein kinase kinase pathway modulation (phospho-mitogen-activated protein kinase kinase 1 inhibition) and tumor growth inhibition through the use of an integrated PK/PD model (Wong et al., 2009). In this article, we have used a similar method to characterize the relation-
ship between pBTK inhibition and antiarthritis effect ($E$) (Fig. 13). The current analysis using GDC-0834 as a tool BTK inhibitor suggests that an approximate threshold of $\approx 60\%$ pBTK inhibition is required for any activity on ankle diameter (i.e., $k_{in}$). Approximately $73\%$ inhibition of pBTK is required for half-maximal activity, and a higher degree of pBTK inhibition is required for maximal activity of the pathway on reduction of ankle diameter in CIA rats. A “PD marker-response curve” showing the relationship between pBTK inhibition and $E$ was sigmoid in nature with a Hill coefficient ($n$) of $\approx 24$ (Fig. 13), which suggested a steep PD marker-response curve. This suggests that onset of efficacy is rapid beyond a certain threshold of target modulation.

In addition to the debate on the suitability of the inflammatory arthritis animal models, there are several assumptions and caveats associated with preclinical PK/PD modeling itself. Whether our improved understanding of target modulation requirements for efficacy derived from the studies shown here will translate to that in humans is still a question. Nevertheless, the development of a disease progression PK/PD model for arthritis disease provides a quan-

**TABLE 1**
The individual histopathologic (inflammation, pannus, cartilage damage, and bone resorption) scores and the sum of four scores of the ankles with the different treatments in the rat CIA model. Values are expressed as mean ± S.E.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inflammation Score</th>
<th>Pannus Score</th>
<th>Cartilage Damage Score</th>
<th>Bone Resorption Score</th>
<th>Sum of Four Histopathologic Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle b.i.d.</td>
<td>4.65 ± 0.22</td>
<td>2.55 ± 0.18</td>
<td>3.30 ± 0.24</td>
<td>2.55 ± 0.18</td>
<td>13.05 ± 0.81</td>
</tr>
<tr>
<td>100 mg/kg b.i.d.</td>
<td>1.50 ± 0.29</td>
<td>0.45 ± 0.15</td>
<td>0.60 ± 0.27</td>
<td>0.35 ± 0.15</td>
<td>2.90 ± 0.83</td>
</tr>
<tr>
<td>30 mg/kg b.i.d.</td>
<td>3.30 ± 0.36</td>
<td>1.40 ± 0.20</td>
<td>2.00 ± 0.31</td>
<td>1.35 ± 0.21</td>
<td>8.05 ± 1.07</td>
</tr>
<tr>
<td>10 mg/kg b.i.d.</td>
<td>4.40 ± 0.24</td>
<td>2.25 ± 0.22</td>
<td>3.15 ± 0.28</td>
<td>2.25 ± 0.22</td>
<td>12.05 ± 0.94</td>
</tr>
<tr>
<td>Vehicle QD</td>
<td>4.60 ± 0.28</td>
<td>2.35 ± 0.21</td>
<td>3.25 ± 0.27</td>
<td>3.25 ± 0.27</td>
<td>12.55 ± 0.94</td>
</tr>
<tr>
<td>100 mg/kg QD</td>
<td>1.75 ± 0.22</td>
<td>0.35 ± 0.11</td>
<td>0.35 ± 0.13</td>
<td>0.25 ± 0.10</td>
<td>2.70 ± 0.52</td>
</tr>
<tr>
<td>30 mg/kg QD</td>
<td>3.00 ± 0.44</td>
<td>1.25 ± 0.25</td>
<td>1.75 ± 0.35</td>
<td>1.25 ± 0.25</td>
<td>7.25 ± 1.26</td>
</tr>
<tr>
<td>10 mg/kg QD</td>
<td>4.15 ± 0.36</td>
<td>2.15 ± 0.26</td>
<td>3.00 ± 0.26</td>
<td>2.15 ± 0.26</td>
<td>11.45 ± 1.23</td>
</tr>
<tr>
<td>100 mg/kg Q2D</td>
<td>3.35 ± 0.30</td>
<td>1.25 ± 0.19</td>
<td>1.90 ± 0.30</td>
<td>1.25 ± 0.19</td>
<td>7.75 ± 0.85</td>
</tr>
</tbody>
</table>
A quantitative tool by which to evaluate a drug’s antiarthritis efficacy and provide a better understanding of the degree of pathway suppression required for this efficacy. Finally, this type of mathematical model makes it possible to make clinical predictions and influence clinical trial design through the integration of predicted or actual human PK and PD biomarker data.

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**Authorship Contributions**

*Participated in research design:* Liu, Di Paolo, Barbosa, Rong, and Wong.

*Conducted experiments:* Di Paolo, Barbosa, and Rong.

*Performed data analysis:* Liu, Di Paolo and Wong.

*Wrote or contributed to the writing of the manuscript:* Liu, Di Paolo, Reif, and Wong.

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