Evaluation of Selective γ-Secretase Inhibitor PF-03084014 for Its Antitumor Efficacy and Gastrointestinal Safety to Guide Optimal Clinical Trial Design

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Abstract

Aberrant regulation of Notch signaling has been implicated in tumorigenesis. Proteolytic release of the Notch intracellular domain (NICD) by γ-secretase plays a key role in Notch-dependent nuclear signaling. γ-Secretase is an attractive pharmaceutical target for therapeutic intervention in cancer. We describe the potent antitumor effects of PF-03084014, a small molecule that is a reversible, noncompetitive, and selective γ-secretase inhibitor. The ability of PF-03084014 to inhibit γ-secretase activity was shown by the reduction of endogenous NICD levels and by the downregulation of Notch target genes Hes-1 and cMyc in the T-cell acute lymphoblastic leukemia (T-ALL) cell line HPB-ALL. PF-03084014 caused cell growth inhibition of several T-ALL cell lines via cell cycle arrest and induction of apoptosis. PF-03084014 treatment also resulted in robust NICD reduction in HBP-ALL xenograft models. Broad antitumor efficacy at well-tolerated dose levels was observed in six Notch-dependent models. Additional mechanism-of-action studies showed inhibition of tumor cell proliferation and induction of apoptosis in HPB-ALL tumors, suggesting that the antitumor activity of PF-03084014 may be mediated by its direct effects on tumor cell growth or survival. Further studies on PF-03084014–induced gastrointestinal toxicity identified an intermittent dosing schedule that displayed reduced body weight loss and sustained antitumor efficacy. We also showed that glucocorticoids abrogated PF-03084014–induced gastrointestinal toxicity and delayed administration of glucocorticoids did not compromise its protection effect. Collectively, the results show that inhibition of Notch signaling by PF-03084014 while minimizing gastrointestinal toxicity presents a promising approach for development of therapies for Notch receptor–dependent cancers. This compound is being investigated for the treatment of T-ALL and advanced solid tumors in phase I clinical trials.

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Introduction

The oncogenic potential of aberrant Notch signaling in human cancers was discovered in T-cell acute lymphoblastic leukemia (T-ALL), an aggressive neoplasm of immature T cells. The Notch1 gene in humans was first identified from the t(7;9) translocations found in a small subset of T-ALL (1). Approximately 10% of T-ALL cases harbor deletions of the extracellular domain of Notch1, which generate constitutively active Notch1 proteins (2). A much broader involvement of Notch in this cancer type was revealed when activating Notch1 mutations were found to be present in ~50% of T-ALL patients, making Notch1 the most commonly activated oncogene in this disease (3). The identified Notch1 receptor mutations cluster in the heterodimerization domain (HD) or the COOH-terminal PEST region. Heterodimerization domain mutations enhance ligand-independent generation of Notch intracellular domain (NICD) by γ-secretase cleavage, whereas PEST mutations increase the half-life of NICD. Both the heterodimerization domain and PEST domain mutations enhance ligand-independent generation of Notch intracellular domain (NICD) by γ-secretase cleavage, whereas PEST mutations increase the half-life of NICD. Both the heterodimerization domain and PEST domain mutations require γ-secretase cleavage, whereas PEST mutations increase the half-life of NICD. Both the heterodimerization domain and PEST domain mutations are associated with a broad panel of solid tumors. At least three Notch members (Notch 1, 3, and 4) are found to be involved in solid tumors. For example, activated Notch1 was identified in...
breast cancer (4, 5), multiple myeloma (6), pancreatic cancer (7), and melanoma (8). Interestingly, in ~40% of breast cancers, aberrant activation of Notch1 signaling is correlated with loss of Numb activity, a negative regulator of the Notch pathway (5). Furthermore, elevation of both Notch1 and its ligand JAG1 is linked to poor overall survival in human breast cancer (9).

The discovery of activating Notch1 mutations in T-ALL has made the Notch pathway an attractive target for the development of small molecule drugs, with γ-secretase inhibitors (GSI) having the most immediate therapeutic potential (10). Inhibition of Notch signaling by GSIs leads to cell or tumor growth inhibition that is potentially mediated by induction of apoptosis, cell cycle arrest, and reduction of cell proliferation (3, 8, 11, 12). More recently, Rao et al. showed that inhibition of Notch pathway activity signature results in the upregulation of cyclin-dependent kinase inhibitors CDKN2D (p19 INK4d) and CDKN1b (p27 Kip1) and the subsequent derepression of Rb. These results further support the role of Notch in cell cycle regulation (13).

Despite the potential of GSIs in cancer therapies, clinical success has been hindered by the development of grade 3/4 gastrointestinal toxicity due to Notch inhibition. GSI treatment causes significant accumulation of goblet cells in the small intestine (14–17) that results in severe diarrhea in patients in clinical trials. Therefore, it is important to mitigate this mechanism-based toxicity for future clinical development of GSIs. The most promising approach to manage gastrointestinal toxicity is glucocorticoid combination therapy recently discovered by Real et al. (18). Glucocorticoids ameliorate the gastrointestinal toxicity induced by GSIs (19). These results may provide opportunities to improve the safety of GSIs in clinical application.

The present study describes the identification and characterization of PF-03084014, a potent small molecule that is a selective reversible, noncompetitive GSI. PF-03084014 potently inhibits Notch signaling by reducing NICD and Notch target genes Hes-1 and cMyc in both cells and tumors. As a result of NICD modulation, robust antitumor efficacy was achieved in six Notch-driven human xenograft models at well-tolerated doses in vivo. PF-03084014 may exert its antitumor activities through its direct effects on tumor cell cycle and apoptosis mechanisms. Furthermore, we identified an intermittent dosing schedule showing reduced gastrointestinal toxicity as well as sustained antitumor efficacy. We also confirmed enteroprotection by glucocorticoid combination. PF-3084014 is currently in clinical development for the treatment of T-ALL patients and advanced solid tumors.

Materials and Methods

**Compound**

PF-03084014 [(S)-2-((S)-5,7-difluoro-1,2,3,4-tetrahydro-3-ylamino)-N-(1-(2-methyl-1-(neopentylami-
manufacturer’s instructions. cDNA was generated using the iScript Select cDNA Synthesis Kit (Bio-Rad) and analyzed by quantitative real-time PCR using TaqMan Gene Expression Assays (Applied Biosystems) and the iCycler-iQ muticolor real-time PCR detection system (Bio-Rad). Relative expression levels were normalized against 18S rRNA expression that was used as a reference control.

Quantification of cell cycle and apoptosis by flow cytometry
DNA content analysis was done using Cycle Test Plus DNA Reagent Kit (BD Biosciences), and apoptosis was assessed using Vybrant Apoptosis Assay Kit (Molecular Probes) according to the manufacturer’s instructions. Labeled cells were quantified using FACS Calibur flow cytometer (BD Biosciences).

Animals
Athymic female mice (nu/nu, 6–8 weeks) were used for all in vivo studies. Mice were obtained from Charles River Laboratories and housed in specific pathogen-free conditions, according to the guidelines of the Association for the Assessment and Accreditation for Laboratory Animal Care, International. Animals were provided sterile rodent chow and water ad libitum. All in vivo studies were conducted in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and with the Pfizer Animal Care and Use Committee guidelines.

Subcutaneous xenograft models in athymic mice
Cells for implantation into athymic mice were harvested and pelleted by centrifugation at 450 × g for 5 to 10 minutes. The cell pellets were washed once and resuspended in sterile serum-free medium. Tumor cells were supplemented with 50% Matrigel (BD Biosciences) to facilitate tumor take and growth of selected tumor cells as xenografts. Cells (5–10 × 10⁶ in 200 μL) were implanted s.c. into the hind flank region of the mouse and allowed to grow untreated to a size of 300 to 800 mm³. Mice were administered PF-03084014 as a single oral dose [for acute pharmacokinetics/pharmacodynamics (PK/PD) studies] at the designated dose levels. At the indicated times following PF-03084014 administration, individual mice were humanely euthanized, blood samples were isolated from the cardiac left ventricle using a syringe and subsequently transferred to tubes primed with heparin sulfate, and tumors were resected. Plasma samples were analyzed for PF-03084014 concentration using liquid chromatography/mass spectrometry analysis. Resected tumors were snap frozen and homogenized in cold 1× cell lysis buffer (Cell Signaling Technologies). Proteins were extracted from the tumor lysate by centrifuging at 13,000 rpm for 10 minutes at 4°C. Protein concentrations were determined using a BCA assay (Fierce). The level of NICD in each tumor sample was determined using Western blot analysis described above.

Combination of PF-03084014 and glucocorticoids and gastrointestinal histologic analysis
Mice received oral dosing of vehicle or PF-03084014 at 150 mg/kg, twice daily for 14 days. Dexamethasone was administered with i.p. injection at 15 mg/kg for 14 days, 7 days plus 7 days break or 7 days break plus 7 days. Duodenum samples were collected at day 14 and analyzed by H&E, Ki67, or Alcian blue staining. Stained sections were analyzed using an Olympus microscope.

Immunohistochemistry
At least three animals were included in each treatment group for immunohistochemistry analysis. Tumor specimens were fixed in 10% neutral buffered formalin for 24 hours, followed by standard tissue processing and embedding. Sections were cut at 4 μm and dried overnight at 37°C onto microscope slides. The tissue sections were stained with primary antibodies, Notch1 (Epitomics), caspase-3 (Cell Signaling), and Ki67 (NeoMarkers) in a Bond fully automated immunohistochemistry stainer (Leica Microsystems). These antibodies were detected using the Bond polymer refine detection system (Leica Microsystems). All of the sections were counterstained using hematoxylin, dehydrated, cleared, and mounted before examination using an Olympus microscope.

Results

**PF-03084014 is a potent and selective inhibitor of γ-secretase**
PF-03084014 (Supplementary Fig. S1) was originally identified as a potent inhibitor of γ-secretase. The IC₅₀ of PF-03084014 for γ-secretase enzyme inhibition in cell-free assay for Aβ production using detergent solubilized membranes derived from HeLa cells was determined.
to be 6.2 nmol/L. When tested for inhibition of Notch receptor cleavage in cellular assays using HPB-ALL cells that harbor mutations in both the heterodimerization and PEST domains in Notch1, the cell IC<sub>50</sub> was determined to be 13.3 nmol/L.

PF-03084014 was evaluated across a number of other proteases, receptors, ion channels, and kinases. Overall, PF-03084014 failed to show significant activity (<1 μmol/L) against a broad panel of enzymes and receptors. Activity (IC<sub>50</sub>) in a broad panel of common receptors was >1 μmol/L in all assays. The IC<sub>50</sub> was determined for all receptors where inhibition was greater than 50% at the screening concentration of 10 μmol/L. Testing at other aspartic proteases (pepsin A, BACE 1, and cathepsin D) or serine proteases (chymotrypsin A, trypsin) showed negligible inhibition at 20 μmol/L. PF-03084014 also exhibited <25% inhibition at 10 μmol/L in a panel of 10 kinase enzymes, including ABL, CK1d, GSK3β, IKK2, IKK1, LCK, MK2, P38, PKA, and PKCζ. Thus, PF-03084014 is very selective for γ-secretase compared with other proteases, receptors, ion channels, and kinases tested in the study (Supplementary Table S1).

**PF-03084014 downregulates Notch target genes in HPB-ALL cells**

To investigate the effect of PF-03084014 on Notch target genes, HPB-ALL cells were treated with PF-03084014 for 3 days. In these cells, PF-03084014 caused a significant decrease in NICD levels in a dose-dependent manner (Fig. 1A). Expression of two Notch genes, Hes-1 and cMyc, was greatly downregulated in the same cell population treated with PF-03084014 (Fig. 1B), confirming the inhibition of the Notch receptor–dependent cell signaling by this compound.

**PF-03084014 leads to cell growth inhibition through induction of cell cycle arrest and apoptosis**

To examine the effect of cell growth, a subset of human T-ALL cell lines was treated with PF-03084014 for 7 days. Four sensitive cell lines (HPB-ALL, DND-41, TALL-1, and Sup-T1) responded to PF-03084014 treatment with various IC<sub>50</sub> values (Supplementary Table S2). In contrast, Jurkat cells that lack PTEN expression (21) and express a mutated form of FBW7 (22) were resistant to this compound.

We further investigated the cause of cell growth inhibition by PF-03084014. Compound-treated HPB-ALL cells were analyzed for cell cycle population based on their DNA content. PF-03084014 caused a marked arrest in cell cycle progression after a 7-day treatment, as characterized by the loss of cells in S-G2-M phases and an accumulation of cells in G0-G1. Induction of cell cycle arrest by PF-03084014 was dose dependent, with greatest effect obtained at 1 and 10 μmol/L (Fig. 2A). A slight increase in sub-G<sub>1</sub> population (from 15% to 23%) was also noted, suggesting DNA fragmentation as a result of apoptosis. In accordance with cell cycle block, we observed robust regulation of total Rb protein and cyclin dependent kinase inhibitor p27 Kip1 by PF-03084014 (Fig. 2B). PF-03084014 caused a significant increase in caspase-3 activities in HPB-ALL and TALL-1 cells (Fig. 2C) as well as an induction of cleaved PARP and cleaved caspase-3 (Fig. 2D) after a 7-day treatment.

Our results suggest that growth inhibition of T-ALL cells by PF-03084014 is in part due to its effect on induction of cell cycle block and apoptosis.

**The effect of PF-03084014 on NICD, cell cycle arrest, and apoptosis show different durations of effect in vitro**

The unusual aspect of Notch-dependent signaling mediated by γ-secretase is that the key signaling step is mediated by the proteolytic cleavage of the Notch receptor leading to the release of NICD which then translocates to the nucleus and activates gene expression. Given this mechanism, we were interested in examining how this impacts the duration of effect on NICD levels, cell cycle arrest, and cell apoptosis as a result of γ-secretase inhibition by PF-03084014. We monitored the effect of continuous exposure to PF-03084014 as well as the reversibility on...
compound removal. We investigated regulation of cell cycle, apoptosis, and the appropriate protein markers in HPB-ALL cells after 3 or 7 days of continuous compound treatment. The same end points were further examined on days 1, 3, and 7 after the compound was removed from the cells. Induction of cell cycle block was not evident until day 7 after compound treatment (Fig. 3A). Consistent with cell cycle arrest, modulation of the two
cell cycle proteins was much greater on day 7 although a slight reduction in Rb and a moderate increase in p27 Kip1 were observed on day 3 (Fig. 3B). Similarly, full induction of cleaved PARP and apoptosis was obtained after day 7 of compound treatment. On compound removal from the cells, NICD levels returned to normal within 1 day. However, the effect on cell cycle block and apoptosis lasted for a longer time in the absence of compound (>3 days for cell cycle block and >7 days for apoptosis, respectively). Taken together, the data suggest that continuous treatment with PF-03084014 for ≥7 days is required for its maximal effect and that intermittent dosing of the compound after 7 days may not compromise its efficacy significantly.

PF-03084014 causes a significant reduction of NICD levels and dose-dependent tumor growth inhibition in HPB-ALL tumors

To evaluate the response of NICD levels to PF-03084014, xenograft HPB-ALL tumors were used due to the robust and consistent level of NICD observed in tumor xenografts when using this model. Tumor samples were harvested at several time points following oral administration in single dose at 50 mg/kg. NICD levels in tumors were quantitated by Western blot analysis. In this study, >50% reduction of NICD was observed as early as 4 hours and a greater effect (70–80%) was obtained around 24 hours (Fig. 4A). In subsequent single-dose studies with higher doses (200 mg/kg) and longer time points (up to 72 hours), we achieved maximal NICD inhibition for ~80% and the duration covering 40% to 80% NICD inhibition lasted up to 48 hours (data not shown). Similar NICD and Aβ modulation was also obtained with an additional T-ALL model Sup-T1 (Supplementary Fig. S2).

To assess antitumor efficacy in HPB-ALL model, repeat dose studies were done at two dose levels (75 and 150 mg/kg, twice daily). Tumor volume was measured utilizing electronic Vernier calipers. PF-03084014 showed robust antitumor activity in this model on 14-day twice daily dosing (Fig. 4B). Tumor growth inhibition was dose dependent, with maximal tumor growth inhibition of ~92% obtained at high dose levels (150 mg/kg). PF-03084014 was also evaluated for its effect on NICD/Notch1, tumor mitotic index (Ki67), and apoptosis (activated caspase-3) using immunohistochemical methods. PF-03084014 caused a significant reduction in perinuclear staining revealed by Notch1 antibody staining, indicating a decrease in NICD by the compound. Reduction of NICD and target genes (Hes-1 and cMyc) was confirmed in the same tumor samples using Western blot analysis and real time PCR respectively (Supplementary Fig. S3).

A marked increase in activated caspase-3 levels was also observed in the tumor samples treated with PF-03084014, whereas mitogenesia was significantly inhibited as indicated by a reduction of Ki67 staining (Fig. 4C and Supplementary Fig. S4).

The antitumor efficacy of PF-03084014 was also tested on a variety of Notch-dependent tumors. This compound was efficacious in these tumors with various degrees of tumor growth inhibition (Supplementary Table S4).

Collectively, these results suggest that robust antitumor efficacy of PF-03084014 is mediated through its modulation of Notch signaling, which may subsequently lead to induction of apoptosis and reduction of proliferation in tumor cells. The broad efficacy of this compound in other Notch-dependent tumors suggests its potential...
as a novel agent for treatment of human cancers with aberrant Notch signaling.

**Intermittent dosing (7 days on/7 days off) of PF-03084014 reduces its gastrointestinal toxicity and is efficacious in tumor growth inhibition**

It is well known that Notch inhibition causes severe gastrointestinal toxicity in mice. In tumor growth inhibition studies where mice received repetitive twice daily dosing for more than a week, PF-03084014 was well tolerated at dose levels below 100 mg/kg as no significant weight loss, morbidity, or mortality was observed. When the dose was increased to 150 mg/kg, however, mice had diarrhea and showed weight loss (10–15%) approximately 10 days after compound administration. The body weight of treated animals usually returned to normal if dosing holidays were given, suggesting that the toxicity of PF-03084014 was reversible. To explore dosing schedules with reduced toxicity and sustained efficacy, we dosed mice bearing HPB-ALL tumors at 150 mg/kg twice daily with either continuous or two intermittent dosing schedules, 3 days on/4 days off and 7 days on/7 days off. Mice on both intermittent dosing schedules displayed less body weight loss when compared with the group receiving continuous dosing of the compound (Fig. 5A). The results suggest that the toxicity of PF-03084014 was reversible and could be mitigated by dosing breaks. Interestingly, the tumor efficacy of mice on a 7-days-on/7-days-off schedule was largely sustained whereas much less tumor efficacy was observed with a

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**Figure 4.** Reduction of NICD and tumor growth inhibition by PF-03084014 in HPB-ALL xenograft model. Athymic mice bearing HPB-ALL tumors were orally given PF-03084014 at the indicated dose or vehicle alone over the designated treatment schedule. A, plasma and tumor samples were harvested at designated time points post single dose at 50 mg/kg. Tumor lysates were subjected to immunoblot analysis for NICD. Data were presented as % NICD inhibition (inh.) of treatment versus vehicle (veh.) groups. Plasma samples were analyzed for PF-03084014 concentrations using liquid chromatography/mass spectrometry analysis. B, PF-03084014 was administered twice daily (bid) for indicated time. Tumor volume was measured using Vernier calipers on the indicated days with the median tumor volume ± SE indicated for groups of 10 to 15 mice. C, tumors from mice receiving 150 mg/kg PF-03084014 twice daily for 7 days were resected and fixed in 10% neutral buffered formalin. Fixed tumors were cut into 4-mm sections, and immunostained for NICD (red arrows), cleaved caspase-3 (red arrows), or Ki67. Photomicrographs were taken at 20× (NICD), 10× (caspase-3), and 40× (Ki67) magnifications.
3-days-on/4-days-off schedule (Fig. 5B). Sustained antitumor efficacy with a 7-days-on/7-days-off schedule was consistent with in vitro findings showing that 7 days' continuous exposure to the compound was required to achieve significant effect on cell cycle arrest and apoptosis (Fig. 3). Taken together, intermittent dosing schedules that maintain continuous dosing for ≥7 days followed by dosing breaks may allow achieving optimal tumor efficacy with minimal toxicity.

Dexamethasone coadministration abrogates gastrointestinal toxicity induced by PF-03084014

GSI-induced gastrointestinal toxicity is believed to be caused by goblet cell hyperplasia in the gut, as a result of altered differentiation of progenitor cells in this tissue on Notch inhibition (14, 15). It was recently reported that the combination of glucocorticoids with GSIs reduces the gastrointestinal toxicity associated with GSI treatment in mice (18). To test if dexamethasone can abrogate PF-03084014–induced goblet cell hyperplasia, we analyzed duodenum samples from mice treated with PF-03084014 (150 mg/kg, orally, twice daily) only, or PF-03084014 plus dexamethasone (15 mg/kg, i.p., daily or qd). Histologic analysis revealed that on day 14, PF-03084014 alone caused a marked increase in goblet cells in the intestine (Fig. 6A). This was accompanied with inhibition of cell proliferation in the same tissues (represented by reduced Ki67 immunostaining). In contrast, mice treated with both PF-03084014 and dexamethasone displayed a normal amount of goblet cells seen in control animals and restored cell proliferation, showing the protective effect of dexamethasone on PF-03084014–induced gastrointestinal toxicity (Fig. 6A).

To understand if simultaneous dexamethasone administration with PF-03084018 was required to obtain full protection, mice receiving continuous PF-03084014 were divided into two groups. Dexamethasone was given to one group simultaneously for the first 7 days then discontinued for the last 7 days (Dex 7 d on/7 d off). Dexamethasone administration for the other group was the opposite, with the mice in this group receiving no dexamethasone for the first half of the study followed by 7 days dosing of dexamethasone (Dex 7 d off/7 d on). Interestingly, the mice receiving dexamethasone for the first 7 days but not the last week displayed reduced levels of goblet cells compared with the mice on continuous PF-03084014, whereas the number of goblet cells in mice that were given dexamethasone in the second week of the study was completely normal (Fig. 6B). These results indicated that dexamethasone protection was temporary and reversible. They also suggested that delayed administration of dexamethasone did not compromise its gastrointestinal protection effect.

Collectively, the results suggest that glucocorticoids may protect against GSI-induced gut toxicity by antagonizing the effects of Notch inhibition in the intestinal epithelium. Thus, combination therapies with GSIs plus glucocorticoids may provide new opportunities to improve the safety of anti-Notch therapies in human cancers.

Discussion

In the present report, we show that PF-03084014 has broad antitumor activity against Notch receptor–driven tumors via its potent inhibition of γ-secretase. This novel orally available small molecule, PF-03084014, was identified as a selective reversible, noncompetitive inhibitor of γ-secretase. PF-03084014 potently modulated the Notch receptor signaling pathway and inhibited the growth of a panel of T-ALL cells potentially through the induction of cell cycle arrest and apoptosis. This compound also displayed significant antitumor activity in a broad spectrum of Notch-driven xenograft models.

Previous studies have shown that activated Notch signaling causes aberrant cell cycle progression of T-ALL cells and GSI treatments lead to cell cycle arrest and exit (3, 12, 13, 23, 24). We confirm and extend these findings by showing that PF-03084014 completely inhibited Notch activity (NICD levels) and expression of Notch target...
Figure 6. Dexamethasone (Dex) reduces GSI-induced goblet cell hyperplasia in mice. A, histologic analysis of small intestine from mice continuously treated with vehicle (14 d), PF-03084014 (14 d; 150 mg/kg, orally), Dex (14 d; 15 mg/kg, i.p.), or Dex (14 d) plus PF-03084014 (14 d). B, histologic analysis of small intestine from mice continuously treated with vehicle (14 d), PF-03084014 (14 d; 150 mg/kg, orally), Dex (14 d; 15 mg/kg, i.p.), or Dex (14 d) plus PF-03084014 (14 d). Red arrows indicate examples of goblet cell hyperplasia.
genes Hes-1 and cMyc after 7 days of treatment. Notch inhibition caused a dose-dependent cell cycle arrest (Fig. 2) and upregulation of cyclin-dependent kinase inhibitor p27 Kip1, a direct target of Hes-1 (25). In addition, PF-03084014 treatment caused a complete reduction of total Rb, a similar effect previously reported for another GSI, MRK-003 (13).

In an attempt to understand if continuous exposure to PF-03084014 was required for its effect on cell cycle block and apoptosis, we evaluated the time course for PF-03084014 treatment. We found that although reduction of NICD by PF-03084014 was complete on day 3 of treatment, its effects on cell cycle block or apoptosis were not evident until day 7. This delayed response on cell cycle regulation has been previously reported for other GSIs as well (12, 13, 24). In addition to confirmation of this delayed response, we observed lasting effects on the cell cycle and apoptosis after compound removal (>3 days). These effects were reversible as the cells returned to normal by day 7 in the absence of the compound. In agreement with these in vitro observations, we showed that in xenograft models, continuous dosing for up to 7 days was essential for desirable tumor inhibition and that discontinuation of compound for the next 7 days did not significantly comprise the efficacy (Fig. 5). These findings suggest that continuous exposure to PF-03084014 is not required for tumor efficacy. Recently, sustained efficacy using intermittent dosing regimens was also achieved for RO4929097 and GSI/MRK-003 in their preclinical studies (26, 27).

The development of severe gastrointestinal toxicity due to Notch inhibition in this tissue by GSIs or other means has been well documented (14–17). Future success in the clinical development of GSIs relies on the mitigation of their gastrointestinal toxicity without compromising their efficacy. Along this line, we explored approaches with aims to reduce the toxicity and improve treatment outcomes. Because Notch inhibition only shifts the balance of cell types from nutrient-absorbing cells to mucus-secreting cells and does not affect the parental stem cell population (28), GSI-induced gastrointestinal toxicity may be temporary and reversible as intestinal tissues are self-renewal with high cell turnover. In such case, intermittent dosing regimens may confer reduced gastrointestinal toxicity in animal models. Indeed, we found that mice on both intermittent dosing regimens showed reduced toxicity reflected by the improvement of the body weight compared with those on continuous dosing schedule. In accordance with body weight changes, total compound exposure at termination was reduced significantly in the group receiving continuous PF-03084014 treatment, whereas reduction of compound exposure was less in mice on either intermittent dosing schedule (data not shown). Therefore, intermittent dosing regimens can mitigate the risk of GSI-induced gastrointestinal toxicity by allowing the gastrointestinal tract to recover from enteroendocrine architectural changes caused by continuous exposure to GSIs. Similar efficacious intermittent dosing regimens that minimize gastrointestinal toxicity have been reported by others in preclinical models (27). Whether these regimens can provide better treatment outcome in the clinical development of this class of compounds is currently under investigation (26).

The enteroprotective effects of glucocorticoids against GSI-induced gastrointestinal toxicity may offer another opportunity for the use of GSIs in the treatment of human cancers (18). However, chronic glucocorticoid treatment is associated with significant toxicities including osteopenia, hypertension, and muscle atrophy. In addition, the combination of GSIs and glucocorticoids has deleterious effect on the immune system (18). Therefore, it is important to determine the optimal safe dosage and schedule for dexamethasone cotreatment. It is evident that continuous exposure to dexamethasone was not required for its protective effect because full protection was obtained when dexamethasone was administered after PF-03084014 (Fig. 6B). These results suggest that intermittent regimens may provide possible ways to scale down the overall dosage of glucocorticoids and hence to reduce undesired toxic effects of chronic glucocorticoid treatment. Given the potential for gastrointestinal protection effect of glucocorticoids, more extensive dose-range-finding studies for optimal dexamethasone doses are being conducted in preclinical models. This approach will be further evaluated in ongoing clinical trials for PF-03084014.

In summary, our studies illustrate the biological and pharmacologic effects of PF-03084014–mediated γ-secretase inhibition on Notch receptor–dependent cell signaling, cell growth, and cell apoptosis in T-ALL models. The broad antitumor efficacy of this compound on a variety of Notch-dependent tumors supports its potential as a novel agent for treatment of human cancers with aberrant Notch signaling. Identification of efficacious intermittent dosing regimen or glucocorticoid combination therapy that mitigates GSI-induced gastrointestinal toxicity provides guidance for optimizing clinical designs. These approaches could increase the safety margin for this class of agents, and hence potentially achieve a better clinical outcome in patients. PF-03084014 is currently being investigated for the treatment of T-ALL and advanced solid tumors in phase I clinical trials.

Disclosure of Potential Conflicts of Interest

Authors are employees and stockholders of Pfizer, Inc.

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References


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