Functional Characterization of an Isoform-Selective Inhibitor of PI3K-p110β as a Potential Anticancer Agent

Jing Ni, Qingsong Liu, Shaozhen Xie, Coby Carlson, Thanh Von, Kurt Vogel, Steve Riddle, Cyril Benes, Michael Eck, Thomas Roberts, Nathanael Gray, and Jean Zhao

ABSTRACT

Genetic approaches have shown that the p110β isoform of class Ia phosphatidylinositol-3-kinase (PI3K) is essential for the growth of PTEN-null tumors. Thus, it is desirable to develop p110β-specific inhibitors for cancer therapy. Using a panel of PI3K isoform-specific cellular assays, we screened a collection of compounds possessing activities against kinases in the PI3K superfamily and identified a potent and selective p110β inhibitor: KIN-193. We show that KIN-193 is efficacious specifically in blocking AKT signaling and tumor growth that are dependent on p110β activation or PTEN loss. Broad profiling across a panel of 422 human tumor cell lines shows that the PTEN mutation status of cancer cells strongly correlates with their response to KIN-193. Together, our data provide the first pharmacologic evidence that PTEN-deficient tumors are dependent on p110β in animals and suggest that KIN-193 can be pursued as a drug to treat tumors that are dependent on p110β while sparing other PI3K isoforms.

SIGNIFICANCE: We report the first functional characterization of a p110β-selective inhibitor, KIN-193, that is efficacious as an antitumor agent in mice. We show that this class of inhibitor holds great promise as a pharmacologic agent that could be used to address the potential therapeutic benefit of treating p110β-dependent PTEN-deficient human tumors. Cancer Discov; 2(5); 425–33. © 2012 AACR.

INTRODUCTION

The class Ia phosphatidylinositol-3-kinase (PI3K) pathway is arguably the most important signaling pathway in cells because of its roles in the control of cell growth, survival, and death (1, 2). The PI3K pathway is activated at the cell membrane by an important lipid signaling molecule known as phosphatidylinositol 3,4,5-trisphosphate (PIP3). Under normal conditions, the level of PIP3 is tightly regulated by the activities of 2 enzymes, PI3K (a lipid kinase) and PTEN (a lipid phosphatase), which act as “on/off” switches in opposition to each other. In response to the extracellular signals mediated by receptor tyrosine kinases (RTK), G protein-coupled receptors (GPCR), or GTPases, class Ia PI3Ks are recruited to the cell membrane...
and subsequently phosphorylate phosphatidylinositol-4, 5-bisphosphate (PIP₂) to produce PIP₃. This in turn activates the Ser/Thr kinase AKT and other downstream effectors to regulate multiple cellular functions, including proliferation, survival, and migration. Class Ia PI3Ks are heterodimeric lipid kinases consisting of a p110 catalytic subunit (p110α, p110β, or p110γ) and a p85 regulatory subunit. Although the expression of p110β is largely restricted to the immune system, p110α and p110β are commonly expressed in all tissues. The tumor suppressor PTEN catalyzes the dephosphorylation of PIP₃ back to PIP₂ and thereby antagonizing PI3K activity.

Aberrant activation of the class I PI3K signaling pathway is a common event in many types of cancer. Frequently observed mechanisms of PI3K pathway hyperactivation include gain-of-function mutations in p110α, loss-of-function mutations or deletions in PTEN, and activation of RTKs (2). No activating mutations have been found in p110β so far with the exception of gene amplification in breast and ovarian cancers (3, 4). Interestingly, however, we have recently found that genetic ablation of p110β, but not p110α, is sufficient to inhibit tumor formation driven by Pten loss in the anterior prostate in a mouse prostate tumor model (5). Other recent studies have shown that certain PTEN-deficient human cancer cell lines are sensitive to inactivation of p110β rather than p110α (6, 7).

To investigate whether the dependence on p110β can be recapitulated with pharmacologic inhibitors of p110β kinase activity, several groups have been developing p110β-specific inhibitors. However, only a few selective p110β inhibitors have been reported. Perhaps the best described p110β-specific inhibitor to date is TGX-221, which has been used in defining p110β as an important new target for antithrombotic agents (8), but none of these compounds has been reported for tumor studies in vivo. We sought to identify alternative compounds that are potent and selective p110β inhibitors with properties suitable for use in tumor studies in vivo. Here we show that KIN-193 is a potent and selective p110β inhibitor when evaluated in a battery of biochemical and cellular assays. In addition, we show that this compound can inhibit the growth of tumors driven by p110β or PTEN loss in vivo. Together, our study has identified and characterized KIN-193 as a potential antitumor agent that can be used to treat tumors that are dependent on p110β while sparing other PI3K isoforms.

**RESULTS**

To screen for new selective PI3Kβ inhibitors, we generated a set of isogenic human mammary epithelial cell (HMEC) lines that stably express myristoylated (Myr)-tagged PI3K class Ia p110 isoforms (p110α, p110β, or p110γ), respectively, designated as HMEC-CA-p110α, HMEC-CA-p110β, and HMEC-CA-p110γ. In these cell lines, endogenous PI3K signaling is inactive under serum-free condition, whereas the ectopically expressed Myr-p110 isoforms are membrane-targeted and constitutively active as a result of N-terminal myristoylation (9, 10), thus driving the phosphorylation of AKT, a downstream target of PI3K (Supplementary Fig. S1, first lane vs. second lane). Notably, activation of p110α can also be achieved by N-terminal addition (11). We validated the specificity of this system by monitoring the ability of well-characterized p110 isoform-specific inhibitors, for example, PIK-75 for p110α (12), TGX-221 for p110β (8), IC87114 for p110β (13), and a pan-inhibitor GDC-0941 (14), to inhibit phosphorylation of AKT at both Thr308 and Ser473 in a dose-dependent manner (Supplementary Fig. S1).

The high degree of sequence similarity among p110 catalytic isoforms of PI3K makes it extremely challenging to develop isoform-specific PI3K inhibitors de novo (15); therefore we assembled a collection of 19 compounds possessing activity against PI3Ks (Supplementary Table 1) for our study. To facilitate systematic analyses of these compounds, we used the BacMam gene delivery technology to express GFP-AKT in these isogenic HMEC cells, which enables a time-resolved fluorescence resonance energy transfer (TR-FRET)–based assay termed LanthaScreen (16). The phosphorylation status of AKT at both Thr308 and Ser473 was measured by the binding of terbium-labeled phosphate-specific antibodies that undergo FRET with the green fluorescent protein–labeled AKT (Supplementary Fig. S2A). The most promising candidate to emerge from this profiling was KIN-193 (Fig. 1A and Supplementary Fig. S2B), a compound recently described as a p110β-selective inhibitor (17). Interestingly, KIN-193 is a close structural analog of TGX-221, a p110β isoform-specific inhibitor that has been used in defining p110β as an important new target for antithrombotic agent (8) (Fig. 1B). KIN-193 has comparable selectivity and potency against p110β compared with TGX-221 as measured by AKT phosphorylation in HMECs through Western blot analysis (Supplementary Fig. S3).

We next determined the target spectrum of KIN-193 against the PI3K superfamily as well as the kinome. An in vitro kinase assay showed that KIN-193 is highly potent in the inhibition of p110β’s kinase activity (IC₅₀ of 0.69 mmol/L) and has 200-, 20-, and 70-fold selectivity over p110α, p110γ, and p110γ isozymes, respectively (Fig. 1C). KIN-193 also exhibited selectivity of approximately 80-fold over PI3K-C2 and DNA-PK and more than 1,000-fold over other PI3K-related kinases (Fig. 1C). An inhibitor-kinase interaction profiling of KIN-193 against a panel of 433 kinases using the KinomeScan approach (DiscoverX) showed that KIN-193 is highly selective in its interaction with PI3Ks (Fig. 1D and Supplementary Table 2). Together, these data suggest that KIN-193 is a selective kinase inhibitor that targets the p110β isoform of PI3K.

Recent studies have shown that certain PTEN-deficient tumors are critically dependent on p110β’s activity (5–7). To determine whether KIN-193 selectively targets PTEN-deficient tumors, we tested the effect of KIN-193 on cell proliferation on a large panel of 422 cancer cell lines using high-throughput tumor cell line profiling (18). As shown in Figure 2A, 35% of cell lines with PTEN mutations (20 of 57) and 16% of cell lines with wild-type PTEN (58 of 365) were sensitive to KIN-193 with a threshold of EC₅₀ less than 5 μmol/L. The statistical analysis suggested that cell lines harboring mutations in PTEN exhibited significantly higher sensitivity to KIN-193 (P = 0.0014, 2-tailed Fisher exact test; Fig. 2A and Supplementary Table 3). We further evaluated the effect of KIN-193 along with other pan- or isoform-selective PI3K inhibitors on p110β signaling on a number of PTEN-null cancer cell lines,
including HCC70, MDA-MB-468, BT549, and PC3 cell lines (Supplementary Fig. S4). Our results show that both KIN-193 and GDC-0941 significantly inhibited AKT phosphorylation, whereas PIK-75 and IC87114 had much less effect (Fig. 2B). Taken together, these data suggest that KIN-193 strongly impairs PI3K signaling in PTEN-deficient cancer cells.

To facilitate in vivo efficacy studies of KIN-193, we performed pharmacokinetic analyses of KIN-193 and found that intraperitoneal (i.p.) delivery to be the optimal route to achieve robust in vivo exposure (Supplementary Fig. S5A). To determine the pharmacodynamics of KIN-193 in tumors in vivo, we engineered rat fibroblast (Rat1) cells to express both p53DD, a dominant negative mutant of p53 (10), and a constitutively activated myr-p110β (Rat1-CA-p110β) to enable these cells to form xenograft tumors in mice. For comparison, we also generated an isogenic Rat1 cell line expressing p53DD and myr-p110α (Rat1-CA-p110α), which is also tumorigenic in vivo. We introduced Rat1-CA-p110α and Rat1-CA-p110β cells subcutaneously into the contralateral flanks of athymic mice such that tumors driven by activated p110α or p110β would be exposed to identical conditions and that concern about animal-to-animal variability could be eliminated. When tumors reached a volume of approximately 500 mm³, the tumor-bearing mice received a single i.p. injection of KIN-193 (10 mg/kg). The plasma concentration of KIN-193 was highest
Figure 2. Effects of KIN-193 on PTEN-deficient cancer cells. A, effects of KIN-193 on the proliferation of a panel of 422 cancer cell lines. IC_{50} values of KIN-193 are ordered from low to high. Pink bars indicate cell lines known to be mutant for PTEN. The subset of cell lines with IC_{50} values <5 μmol/L were defined as KIN-193–sensitive cell lines. Thirty-five percent of cell lines with PTEN mutations (20 of 57) versus 16% of cell lines with wild-type PTEN (58 of 365) were sensitive to KIN-193 (P = 0.0014, Fisher exact test). B, effects of KIN-193, GDC-0941, PIK-75, and IC87114 on AKT phosphorylation in PTEN-deficient cell lines as indicated. Representative Western blots are shown. Bar graphs represent mean SD of Western blot quantitations of AKT^{T308} (n = 3). *P < 0.05, **P < 0.01 (one-way analysis of variance followed by Dunnett’s multiple comparison test). DMSO, dimethyl sulfoxide.
at 1 hour postinjection and declined to undetectable levels by 4 hours (Supplementary Fig. S5B). Concentrations of KIN-193 in both the CA-p110α- and CA-p110β-driven tumors paralleled the plasma concentrations (Fig. 3A). Analyses of tumor lysates harvested at various time points after KIN-193 injection revealed that the phosphorylation of AKT was significantly reduced at 1 hour after KIN-193 injection in Rat1-CA-p110β tumors but remained unchanged in Rat1-CA-p110α tumors (Fig. 3A). These in vivo pharmacokinetic and pharmacodynamic properties suggest that KIN-193 holds promise as an effective in vivo p110β-specific inhibitor.

To evaluate the efficacy of KIN-193 in blocking tumor growth in vivo, we generated additional cohorts of mice bearing tumors driven by Rat1 cell expressing CA-p110α or CA-p110β. When tumors size reached approximately 500 mm³, these mice were grouped and administered with vehicle control or KIN-193 by i.p. injection (20 mg/kg) once or twice daily. Although administration of KIN-193 significantly impaired Rat1-CA-p110β-driven tumor growth in a dose-dependent manner, KIN-193 had little effect on the growth of Rat1-CA-p110α-derived xenograft tumors (Fig. 3B), demonstrating the specific antitumor effect of KIN-193 on p110β-driven tumors in vivo. Remarkably, all mice receiving KIN-193, either 1 or 2 doses daily, maintained their body weight throughout the entire treatment course of 13 days (Fig. 3C), indicating that KIN-193 is well tolerated in mice.

We next assessed the antitumor activity of KIN-193 on the growth of PTEN-deficient tumors in vivo using cohorts of mice bearing PTEN-deficient tumor xenografts (HCC70 and PC3) and PTEN wild-type tumor xenografts (HCC1954). KIN-193 significantly inhibited tumor growth of both HCC70 and PC3 tumors but failed to block the growth of HCC1954 tumors (Fig. 4A-C). However, HCC1954, a wild-type PTEN cancer cell line harboring an activated mutant p110α, responded to treatment with the pan-PI3K inhibitor, GDC-0941 (Fig. 4C). Concurrently, immunohistochemistry analyses of tumor specimens isolated from tumor-bearing mice at 4 days after treatment revealed that KIN-193 dramatically reduced levels of both AKT phosphorylation and Ki67 signal in xenograft tumors of both PTEN-deficient cancer cell lines, HCC70 and PC3 (Fig. 4D, 4E). In contrast, a pan-PI3K inhibitor, GDC-0941, but not KIN-193, blocked AKT phosphorylation and cell
proliferation in HCC1954 tumor xenografts (Fig. 4F). We conclude that KIN-193, a p110β-selective inhibitor, can specifically suppress both the PI3K pathway activation and oncogenic transformation induced by PTEN deficiency.

**DISCUSSION**

Accumulating evidence has suggested that distinct PI3K isoforms are specifically involved in a variety of different disease conditions, including cancer, metabolic disorders, immunity, and cardiovascular dysfunction (18–21). Previous reports have shown that p110β is important in thrombosis and that a selective p110β small molecular inhibitor, TGX-221, prevents platelet aggregation in an extracorporeal circulation model (8). Recently our group and others have provided compelling evidence that p110β is involved in PTEN loss–induced tumorigenesis (5–7). Additional aspects of p110 isoform dependency of PTEN-deficient cancer cell lines were presented at the 2012 Cold Spring Harbor conference on PTEN Pathways and Targets (22). However, no p110β-specific inhibitors have been described in tumor studies in vivo. Here we show for the first time that a p110β-selective inhibitor, KIN-193, can block both the signaling and tumor growth driven by PTEN loss, providing the first pharmacologic evidence for tumor dependence on p110β kinase activity and suggesting that PTEN-null tumors would be an appropriate genetic background to deploy these inhibitors. Notably, IC50 values for KIN-193 differ with the system of study, for example, it is approximately 1 nmol/L in vitro and 100 to 500 nmol/L in cell culture (Figs. 1A and 1C). It can reach as high as 1 μmol/L in vivo. Although enzymatic assays are useful, they are poor predictors of whether bona fide cellular selectivity will be achieved. We show that in cell culture, we can achieve selectivity of p110β over p110α and p110δ (Fig. 1A). In mice we have only shown that KIN-193 inhibits the PI3K signaling and tumor growth driven by activated p110β but not p110α. However, because the concentrations in vivo are in the range that other PI3K family members (e.g., p110δ and DNA-PK) may be inhibited, we cannot exclude that they contribute to the antiproliferative effects.

The waterfall profiling of cancer cell lines for sensitivity to KIN-193 is particularly interesting for two notions. First, although there is a significant correlation between PTEN mutation and sensitivity to KIN-193, not all PTEN-null cell lines are impacted by treatment with KIN-193. This is perhaps not surprising. Our prior finding of the importance of
p110β in PTEN-loss driven tumorigenesis was based on a defined genetic mouse model, whereas human cancer lines are far more complex in their genetic makeup. Because loss of PTEN simply removes the “brakes” on the PI3K pathway, the dependence of PTEN-null tumors on p110β may be altered by coexisting mutations of the tumor. Thus, if PTEN-null tumor cells also harbor a p110α gain-of-function mutation or an upstream mutation that primarily drives p110α activation, then the tumor might depend on p110α, not p110β. It is also possible that the presence of other oncogenic mutations downstream of PI3K (e.g., AKT) or in PI3K-independent pathways may render PTEN-null tumors less reliant on p110β. Recent studies showed that p110β signals downstream of certain GPCRs or integrins (5, 23). It has also been proposed that p110β is responsible for the basal lipid kinase activity that can be enhanced in the absence of PTEN to drive transformation (5, 12, 24). Therefore, only those PTEN-null tumors in which the PI3K pathway is activated by certain GPCRs or integrins that drive p110β activation or perhaps through the background PI3K activity contributed by p110β are expected to remain dependent on p110β. The second feature of the profiling is perhaps more interesting. There are a number of the cell lines that respond to KIN-193 that are not PTEN-null by mutation. Although some of these lines may have lost PTEN expression by other means (e.g., epigenetic alterations), it is possible that there are PTEN-independent mechanisms that activate p110β in tumors.

To date, the array of PI3K inhibitors that are in preclinical and clinical development consists largely of pan inhibitors, and patients with PTEN-deficient tumors are potential candidates for such PI3K-targeted therapy. However, isoform-specific molecules are emerging in the clinic. The promising early clinical results of the p110β-selective inhibitor CAL-101 in treating lymphoid malignancies suggest that isoform-selective inhibitors may have efficacy and safety advantages over pan-PI3K inhibitors (25). This study identifies KIN-193 as a selective and efficacious p110β inhibitor and shows its potent anticancer activity in PTEN-deficient tumor models, providing a starting point from which to develop orally bioavailable compounds that could ultimately be used to assess the potential therapeutic benefit of treating p110β-dependent tumors.

**METHODS**

**Cell Culture**

Cancer cell lines (HCC1954, HCC70, BT549, BT474, and PC3) were obtained from the American Type Culture Collection. The MDA-MB-468 cell line was from MD Anderson Cancer Center. These cells were frozen after receiving and freshly thawed cells were used at early passage, and no authentication was done by the authors. These cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 100 µg/mL penicillin-streptomycin. HMEC derivative cell lines were cultured as previously described (10). Rat1 cells and its derived cell lines were maintained in DMEM supplemented with 10% FBS and 100 µg/mL penicillin–streptomycin. Rat1 stable cell lines were generated by serial infection with retroviral supernatants carrying p53DD and Myr-HA-p110α or Myr-HA-p110β in the presence of 8 µg/mL polybrene. The cells were then selected with blastidin S 5 µg/mL and puromycin 2 µg/mL.

**Compounds and Reagents**

PIK-75 and TGX-221 were from Chemdea. IC87114 was from Selleck Chemicals. GDC-0941 and KIN compounds were purchased from MedChemExpress. Anti-PTEN, anti-p110α, anti-p110β, anti-phospho-AKT (Thr308), anti-phospho-AKT (Ser473), and anti-AKT were all from Cell Signaling Technology. Anti-p85 antibody was from Upstate Biotechnology. Anti-vinculin and anti-α-tubulin antibodies were from Sigma. Anti-Ki67 antibody was from Vector Labs.

**LanthaScreen Cellular Assay**

Experiments were performed according to the manufacturer’s instructions (Inventrogen) and a previous report (16). Briefly, a 10% BacMam GFP-AKT virus (10% v/v) solution was incubated with HMECs overnight with 0.5× Enhancer Solution. The next day, transfected cells were harvested and seeded in a white 384-well plate (Corning #3570) at a density of 5,000 cells/well in 36 µL of Opti-MEM reduced serum medium. The next day, cells were treated with serial dilutions of compounds (4 µL of 10× solution in 1% dimethyl sulfoxide) for 1.5 hours. After compound treatment, the assay medium was removed and cells were lysed through the addition of LanthaScreen lysis buffer in 20 µL volume supplemented with protease and phosphatase inhibitor cocktails and either Tb-anti-pAKT or Tb-anti-pAKT antibody (2 nmol/L final concentration) for 2 hours at room temperature. The TR-FRET signal (emission ratio 520 nm/495 nm) was read on an EnVision fluorescence plate reader from PerkinElmer. Compounds were tested in duplicate and the data presented are from at least two independent experiments. Curve fitting analysis and IC50 value determination were performed using GraphPad Prism 4 (GraphPad Software).

**Ambit In Vitro KinomeScan Kinase Selectivity Profile**

KIN-193 was profiled at a concentration of 10 µmol/L against a diverse panel of 433 kinases by Ambit Biosciences. Scores for primary screen hits are reported as percent of the dimethyl sulfoxide control (% control). For kinases in which no score is shown, no measurable binding was detected. The lower the score, the lower the Kd is likely to be such that scores of zero represent strong hits. Scores are related to the probability of a hit but are not strictly an affinity measurement. At a screening concentration of 10 µmol/L, a score of less than 10% implies that the false-positive probability is less than 20% and the Kd is most likely less than 1 µmol/L. A score between 1% and 10% implies that the false-positive probability is less than 10%, although it is difficult to assign a quantitative affinity from a single-point primary screen. A score of less than 1% implies that the false-positive probability is less than 5% and the Kd is most likely less than 1 µmol/L.

**High-Throughput Cell Viability Assay**

Cell viability was determined as previously described (18). Briefly, cells were seeded in medium containing 5% FBS at a density ensuring cell growth throughout drug treatment (approximately 15% for most cell lines). Drug treatment was started 24 hours postseeding and continued for 72 hours. Cells were fixed and stained using Syto60 (Invitrogen), a red fluorescent DNA stain. The relative cell number was calculated by taking the ratio of the relative fluorescence intensity from drug-treated wells over untreated wells after background subtraction (cell-free wells). Nine doses of KIN-193 were used in twofold dilution steps ranging from 5.12 µmol/L to 0.02 µmol/L. IC50 corresponding to 50% cell number compared with control (untreated) wells, determined using a fixed top and bottom sigmoidal fitting algorithm implemented in PipelinePilot (Accelrys).

**Western Blot Analysis**

Tumor samples or cells were lysed in ice-cold NP40 buffer (1% NP40, 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate...
in 1X phosphate-buffered saline) containing protease inhibitor cocktail (Roche) and phosphatase inhibitor (Thermo) and lysates were clarified by centrifugation. For experiments with compound treatments, the 80% confluent cells in 6-well plates were treated with the indicated compound for 1 hour, then the cells were lysed in SDS loading/lysis buffer (4% sodium dodecyl sulfate, 20% glycerol, 120 mmol/L Tris pH 6.8, and pyronin Y). Equal amounts of lysates were resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes. After incubation with blocking buffer (50% LICOR blocking buffer in phosphate-buffered saline) for 1 hour, the membranes were incubated with primary antibody overnight at 4°C and then incubated with fluorescently labeled secondary antibodies (Rockland Immunochemicals) for 1 hour at room temperature. The protein signal was detected using an Odyssey scanner (LI-COR).

**In Vivo Pharmacokinetics and Pharmacodynamic Assays**

Approximately 6- to 8-week-old female nude mice (Taconic) were injected subcutaneously with Rat1-Myr-HA-p110 (Rat1-CAp110α) cells (1 × 106 cells in 40% matrigel) (BD Biosciences) in one flank (Site 1) and Rat1-Myr-HA-p110B (Rat1-CAp110B) cells (0.5 × 106 cells in 10% matrigel) in the contralateral flank (Site 2). When tumors grew to approximately 500 mm3, mice were dosed once by i.p. injection with KIN-193 formulated in 7.5% NMP (Sigma), 40% PEG400 (Sigma), 52.5% dH2O at 0.1 mL/10 g body weight, and 10 mg/kg. Tumors were collected at 0, 1, 4, 8, and 24 hours after compound administration and blood samples were obtained by direct heart puncture. Serum was separated and stored at −80°C. The drug concentrations in serum and tumor samples were assessed by liquid chromatography–tandem mass spectroscopy analysis by the DMPK group. The phosphorylation status of AKT in tumor samples was assessed by Western blot analysis and quantified using ImageJ software with statistical calculations performed using Prism software.

**Xenograft Tumor Growth**

Each flank of athymic nude mice was injected subcutaneously with 1 × 106 cells resuspended in 30% matrigel. KIN-193 was given by i.p. injection either once or twice a day at 20 mg/kg. GD0941 (0.1% methylone, 0.1% Tween 80) was given by gavage at 150 mg/kg once per day. Rat1-CA-p110α, Rat1-CA-p110β, HCC70, and HCC1954 xenograft tumor growth was assessed in female nude mice. PC3 xenograft tumor growth was assessed in male nude mice. Tumor-bearing animals were treated with KIN-193, GD0941, or vehicle control as described previously. Tumor volumes were calculated using the formula (length × width2)/2. All the animal experiments were done in accordance with National Institutes of Health animal use guidelines and protocols approved by the Dana-Farber Cancer Institute Animal Care and Use Committee.

**Immunohistochemical Staining**

Decarboxinized tissue sections were heated in 10 mmol sodium citrate buffer for 20 minutes using a microwave oven. Primary antibodies were incubated on slides overnight at 4°C. Sections were then incubated with biotinylated secondary antibody (Vector Labs) and ABC solution (Vector Labs). Tissues were stained by diaminobenzidine (Vector Labs) followed by Meyer’s hematoxylin counterstaining (BioGenex Laboratories).

**Disclosure of Potential Conflicts of Interest**

M. Eck, T. Roberts, and J. Zhao are consultants for Novartis Pharmaceuticals, Inc. M. Eck is a consultant/advisory board member for Novartis Institutes for Biomedical Research. No potential conflicts of interest were disclosed by the other authors.

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