Preclinical Development

In Vivo Activity of Combined PI3K/mTOR and MEK Inhibition in a Kras<sup>G12D</sup>;Pten Deletion Mouse Model of Ovarian Cancer

Kathryn M. Kinross<sup>1,2,3</sup>, Daniel V. Brown<sup>1,2,3</sup>, Margarete Kleinschmidt<sup>1,2,3</sup>, Susan Jackson<sup>2,3</sup>, James Christensen<sup>8</sup>, Carleen Cullinane<sup>2,3,7</sup>, Rodney J. Hicks<sup>2,3,4,6</sup>, Ricky W. Johnstone<sup>2,7</sup>, and Grant A. McArthur<sup>1,2,3,5,6,7</sup>

Abstract
The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is commonly dysregulated in human cancer, making it an attractive target for novel anticancer therapeutics. We have used a mouse model of ovarian cancer generated by Kras<sup>G12D</sup> expression promoting cell survival and that the therapeutic effect of PF-04691502 would be enhanced by combinatorial inhibition of MEK using PD-0325901.

Introduction
The phosphatidylinositol 3-kinase (PI3K) pathway is an essential regulator of cellular proliferation, survival, and growth acting through disparate downstream effectors including the AKT and mTOR pathways. Genomic changes leading to activation of this pathway, such as mutation or loss of PTEN, activating mutations or amplification of PI3K or activating mutations in upstream receptor tyrosine kinases such as epidermal growth factor receptor and ERBB2 are common in human cancers, making this pathway an attractive target for novel anticancer therapeutics (1). Multiple small-molecule inhibitors have been targeted to different kinases throughout the pathway, including AKT inhibitors (MK-2206), mTOR inhibitors (everolimus, temsirolimus, AZD8055, OSI-027), PI3K inhibitors (GDC-0941, PX866, BKM120), and dual PI3K/mTOR inhibitors [BEZ235: reviewed in (2, 3)]. Recently, PF-04691502, a novel dual PI3K/mTOR inhibitor, has been described (4) and has been entered in phase I clinical trials for the treatment of solid cancers. PF-04691502 is orally bioavailable and is a highly potent ATP-competitive kinase inhibitor of class 1 PI3Ks and mTOR. As yet, minimal characterization of the preclinical predictors of the efficacy of this drug has been reported.

Despite evidence of improving outcomes, ovarian cancer remains one of the leading causes of gynecological cancer deaths because of its propensity to present with an advanced clinical stage (5). These tumors commonly exhibit activation of the PI3K/Akt pathway via PIK3CA mutation (6), loss of PTEN, PTEN mutation (7), or increased expression of mir-214 targeting PTEN (8). Current therapeutic strategies for ovarian cancer involve
Materials and Methods

Transgenic mice

We obtained LSL-KrasG12D heterozygous mice (B6.129-Kras<sup>instTy</sup>) from the Mouse Models of Human Cancer Consortium [National Cancer Institute (NCI)] and Pten<sup>del</sup> mice (c:129S4-Pten<sup>tm1Hwu/J</sup>) from Jackson Laboratories. All LSL-KrasG12D<sup>+/−</sup>;Pten<sup>del</sup> mice were maintained on a mixed (Black6; BalbC; C3H/HeJ) background. All BalbCnu/nu mice were obtained from the Animal Research Centre or WEHI. All in vivo experiments were carried out with adherence to the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with approval from the Peter MacCallum Cancer Centre Animal Experimentation Ethics Committee.

Adenoviral-Cre recombination in ovary

Ovarian surface epithelium was infected with replication-deficient recombinant adenovirus expressing Cre recombinase (15), a kind gift from Walter Thomas’ Laboratory, Baker Institute, Australia, by methods previously described (13, 16, 17). Briefly, ovulation was synchronized by i.p. injection of 5U of pregnant mare serum gonadotropin (Intervet), followed by i.p. injection of 5U human chorionic gonadotropin (Intervet) 48 hours later. 1.5 days later, mice were anesthetized with an i.p. injection of ketamine and xylazine (86 and 17 mg/kg, respectively, in saline) and a dorsal excision was made to expose the ovary. A 32-gauge needle and Hamilton syringe was used to inject 2.0 × 10<sup>9</sup> optical particle units of Adenoviral-Cre (AdCre) in 5 µL (precipitate prepared in 1:1 Dulbecco’s Modified Eagle’s Medium and PBS/virus mix; supplemented with 0.2 mol/L CaCl<sub>2</sub>) into the ovarian bursa. The ovary was returned to the body and the incision sealed with sutures and staples. Mice were monitored for ovarian tumor growth by small animal ultrasound (Vevo 770, Visual Sonics) and palpation.

Orthotopic transplant of ovarian tumors

Tumors were harvested from KrasG12D,Pten<sup>del</sup> mice and dissected into approximately 1 mm diameter pieces and stored in 10% DMSO:90% fetal calf serum at −80°C. Tumor pieces were thawed and washed in 3 changes of cold PBS and dissected further under a dissecting microscope to equal sizes approximately 0.1 mm in diameter. Female BalbCnu/nu mice were anesthetized with ketamine/xylazine mix and the left ovary was exposed as described above. A small incision was made in the ovarian bursa and a tumor piece placed between the bursal lining and the ovary using fine forceps. The ovary was returned to the body and the incision sealed with sutures and staples. Mice were monitored for ovarian tumor growth by ultrasound and palpation.

In vivo drug studies

2-amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-pyridin-3-yl)-4-methylpyrido[2,3-d]pyrimidin-7(8H)-one (PF-04691502; ref. 4) was prepared in 0.5% methylcellulose. Mice were dosed daily at either 7.5 or 10 mg/kg in a volume of 2.5 µL/g of mouse body weight by oral gavage. N-(2R)-2,3-dihydroxypropoxy-3,4-difluoro-2-[4-fluoro-4iodophenyl]aminobenzamide (PD-0325901; ref. 18) was prepared in 0.5% hydroxypropyl-methylcellulose (Biochemika fluka), 0.2% Tween 80 (Sigma), and mice were dosed daily at either 1.5 or 10 mg/kg in a volume of 2.5 µL/g of mouse body weight by oral gavage. For chemical structures, see Supplementary Figure S1.

In vivo imaging

The volume of the left ovarian mass was monitored by ultrasound imaging at the beginning of therapy and again at various intervals to monitor change in tumor burden (Vevo 770, Visualsonics; fixed with a 707b or 704 scanner). FDG-PET imaging was conducted as previously described (19) using a dedicated small-animal PET scanner (Mosaic, Philips).

Histology and immunohistochemistry

Tumors were fixed in 10% neutral-buffered formalin and paraffin embedded. Sections were stained with hematoxylin and eosin for morphological examination. Immunohistochemistry was conducted following antigen retrieval by boiling slides in antigen target retrieval solution pH 9 (DAKO) in a pressure cooker for 5 minutes. Antibodies used were phosphorylated (p)-AKT (S473, CS787), p-ERK (CS2211) and p-ERK (CS4370) from Cell
Signaling Technology and bromodeoxyuridine (BrdU; 347580; BD Biosciences). For BrdU analysis, mice were injected with 100 mg/kg BrdU (Sigma) i.p. 1 hour before sacrifice. BrdU quantitation was determined using the MetaMorph computer program (Universal Imaging), where the percentage of BrdU positive cells was counted relative to nuclei over 5 microscopic fields at ×40 magnification. TUNEL staining was conducted using the ApopTag Peroxidase In Situ Apoptosis Detection Kit following the manufacturers instructions (Chemicon International). All images were taken on an Olympus BX51 microscope.

Western blot analysis

Tumors were snap frozen in liquid nitrogen and lysed in RIPA buffer (1 mmol/L EDTA; 1% NP40; 0.5% sodium deoxycholate; 0.1% SDS; 50 mmol/L sodium fluoride; 1 mmol/L sodium pyrophosphate in PBS) supplemented with phosphatase inhibitor cocktail (Roche) for Western blotting. Antibodies used were p-AKT (Ser473, CS9271), total AKT (CS9272), p-ERK (p44/42 MAPK, Thr202/Tyr204; CS9101), p-RPS6 (CS2215), and total S6 (CS2217), each from Cell Signaling Technology, and total ERK (SC-94, Santa Cruz), Mcl-1 (600-401-394, Rockland Immunochemicals Inc.), Bim (AAP-330, ENZO Life Sciences), caspase 3 (611048, BD Biosciences), and pan-actin (clone C4, MAB1501, Millipore).

Results

Dual PI3K/mTOR inhibition with PF-04691502 inhibits PI3K signaling and glucose metabolism in a KrasG12D;Ptendel mouse model of ovarian cancer

To evaluate the effects of PF-04691502 in vivo, we used the KrasG12D;Ptendel mouse model of ovarian cancer (13) characterized by constitutive activation of KRAS and deletion of PTEN specifically in the ovarian surface epithelium. Female mice were superovulated and exposed to AdCre at 6 to 10 weeks old in the left ovarian bursa. Mice were monitored twice weekly by palpation and weight gain. When mice appeared to display bloating or rapid weight gain and small-animal ultrasound confirmed presence of tumors, mice were recruited to therapy groups. The median time to recruitment was 17 weeks post-AdCre exposure (range 9.6–41.9 weeks). Mice were randomized into 4 groups receiving drug (PF-04691502; 10 mg/kg daily orally) or vehicle for 2 or 7 days. To directly assess if PF-04691502 could inhibit the PI3K pathway in the tumors that develop in these mice, we conducted biomarker analysis on whole tumors following sacrifice (Fig. 1A). These tumors exhibit high basal levels of both p-AKT (Ser473) and p-RPS6, which were each reduced following treatment with PF-04691502 for 2 days or 7 days. However, the reduction in p-AKT was not as robust in mice treated for 7 days compared with 2 days. In a subset of these mice, we used FDG-PET to noninvasively detect a metabolic response to PF-04691502 therapy (Fig. 1B and C). We observed a reduction in FDG-uptake, indicating decreased glucose metabolism that correlates with the observed decrease in PI3K pathway activation. These findings suggest that FDG-PET imaging may be used as a biomarker of target inhibition in response to PF-04691502 therapy.

PF-04691502 inhibits tumor growth in an orthotopic transplant model of KrasG12D;Ptendel ovarian cancer

Because of the long latency, abdominal location and complication of peritoneal spreading and ascites often making identification of these tumors and quantification by ultrasound difficult, we did not find evaluation of primary tumors in this mouse cancer model to be readily amenable to larger preclinical studies. To enable higher
throughput studies, we isolated tumors arising from transgenic mice and used them as a source for primary, orthotopic transplantation into multiple recipients. BalbCnu/nu recipients were used because of the mixed background of the original tumor-bearing transgenic mice. These tumors were stored as tumor pieces in a frozen tumor bank and before transplant were thawed, dissected into small pieces approximately 0.1 mm diameter, and surgically inserted under the bursa of the left ovary. Two independent tumors, B84 and B87, were typically used in experiments. Recipients developed tumors greater than 50 mm³ by 2 weeks following transplant with a take rate of approximately 75%. These tumors were each resistant to cisplatin (4 mg/kg weekly) and docetaxel (15 mg/kg weekly) upon initial therapy (Supplementary Fig. S2).

To confirm that we could use ultrasound imaging to accurately monitor growth of these transplanted tumors, we initially correlated the volumes obtained using ultrasound imaging with the \textit{ex vivo} tumor weight following dissection of the tumor and found a high correlation using these 2 measurements (Supplementary Fig. S3).

To assess the efficacy of PF-04691502, we transplanted tumors and began therapy on day 13 posttransplantation when tumors reached greater than 50 mm³. The average tumor size was 172 ± 24 mm³ (SEM), with a range of 54.3 to 327.7 mm³. Mice were randomized to receive either drug or vehicle daily by oral gavage for 7 days and ultrasound and FDG-PET scans were conducted on days 0, 2, and 7 of drug treatment. PF-04691502 inhibited tumor growth rate significantly at 2 days (1.85 ± 0.18-fold tumor growth in vehicle-treated vs. 1.07 ± 0.14-fold tumor growth in drug-treated mice; Student’s \( t \) test \( P < 0.01 \)) and 7 days (4.31 ± 0.63-fold tumor growth in vehicle-treated vs. 2.14 ± 0.65-fold tumor growth in drug-treated mice; Student’s \( t \) test \( P < 0.05 \)). Tumor weights measured postmortem on day 7 posttherapy also showed a significant lower tumor mass in treated mice (0.78 ± 0.13 g in vehicle-treated vs. 0.38 ± 0.06 g in drug-treated mice; Student’s \( t \) test \( P < 0.05 \); Fig. 2C). FDG-PET imaging revealed that PF-04691502 reduced glucose metabolism dramatically at 48 hours, and this reduction was maintained for the 7-day treatment duration (Fig. 2D and E).

To confirm that the PI3K/mTOR pathway was successfully inhibited with PF-04691502, Western blots and immunohistochemistry were carried out on tumor samples harvested at 7 days posttreatment (Fig. 3A and B). Similar to results seen in transgenic mice treated with PF-04691502 (Fig. 1A), mice bearing transplanted \( \text{Kras}^{G12D}; \text{Ptendel} \) ovarian tumors also showed inhibition of p-AKT.

![Figure 2. Single agent PF-04691502 reduces tumor growth rate and FDG uptake \textit{in vivo}. Mice were transplanted orthotopically with \( \text{Kras}^{G12D};\text{Ptendel} \) ovarian tumors and following tumor establishment was treated with vehicle (\( n = 5 \)) or PF-04691502 (\( n = 8 \)) at 10 mg/kg daily for 7 days. A, representative serial 3-dimensional reconstructions and volumetric quantitation of ovarian tumor regions from ultrasound imaging. B, tumor volume change determined by ultrasound imaging expressed relative to that on day 0. C, Mice were all sacrificed on day 7 and tumor weights determined. D, serial FDG-PET maximum-intensity-projection (top) and transaxial (bottom) images from a representative mouse treated with either vehicle or drug. Arrows indicate tumor region. E, change in FDG uptake in response to drug. FDG uptake ratio refers to the maximum pixel intensity in the tumor region of interest on each day expressed relative to day 0. Data is represented as mean ± SEM.](mct.aacrjournals.org)
We next examined incorporation of BrdU in these tumors as a marker of proliferation (Fig. 3C). Surprisingly, we observed only a marginal decrease in BrdU-positive cells in PF-04691502–treated tumors (Fig. 3C), suggesting that despite initial stasis in tumor growth at 2 days post-treatment (indicated by ultrasound volumetric analysis, Fig. 2A), in response to PF-04691502, following 7 days continuous treatment, these effects had been overcome. Given the presence of the activated KRAS in this model, we hypothesized these cells may be able to survive and reinstate proliferation through increased activation of the RAS/RAF/MEK/ERK (RAS/MAPK) signaling cascade downstream of \( \text{Kras}^{G12D} \). We assessed the levels of p-ERK as a readout of RAS/MAPK signaling (Fig. 3D and E). p-ERK expression was variable in this model in both vehicle-treated and PF-04691502–treated tumors, which is consistent with the findings of others that in some contexts p-ERK can be an unreliable marker of activated KRAS in tumors (20–22). To determine if these tumors exploit this pathway to limit the efficacy of PF-04691502 therapy, we next evaluated susceptibility to combined inhibition of the RAS/MAPK and PI3K pathways using the MEK inhibitor, PD-0325901, in combination with PF-04691502.

**Combined inhibition of PI3K/mTOR (PF-04691502) and MEK (PD-0325901) leads to tumor regression in vivo**

We transplanted \( \text{Kras}^{G12D};\text{Ptenu}^{ld} \) ovarian tumors orthotopically and following establishment of tumors began treatment with vehicle, PF-04691502 (7.5 mg/kg, daily), PD-0325901 (10 mg/kg, daily), or combined PF-04691502 and PD-0325901 (7.5 mg/kg and 10 mg/kg, respectively). These doses were determined to be the combined maximum–tolerated dose causing less than 20% total body weight loss. Ultrasound was used to monitor tumor growth over 7 days of therapy (Fig. 4A and B). PF-04691502 alone led to tumor growth inhibition of 55% ± 10% at the lower dose of 7.5 mg/kg. Comparison to previous experiments shows greater tumor growth inhibition with 10 mg/kg daily dosing of PF-04691502 (Fig. 2B) compared with 7.5 mg/kg, indicating that the effects of PF-04691502 are dose dependent. Interestingly, PD-0325901 alone led to tumor regression (>20% volume reduction) in 6/10 mice (mean volume 36.3% ± 20.5 smaller on day 7 compared with day 0 tumor volume). Combining PF-04691502 and PD-0325901 led to a greater effect than either agent alone, with striking tumor regression (>50% volume reduction) in all 9 mice (mean volume 80.7% ± 5.0 smaller on day 7 compared with day 0 tumor volume). Images of representative tumors taken from each group following 7 days therapy show these differences in tumor volume (Supplementary Fig. S4).
tolerability. This schedule was well tolerated, causing no weight loss over the treatment period (data not shown). Tumors were allowed to reach a relatively large volume (average 281.6 ± 19.4 mm³) before commencing therapy to better enable the evaluation of tumor regression in the combination group. Tumor growth was monitored with ultrasound volumetric analyses (Supplementary Fig. S4), and the time to reach the predefined endpoint of clinical deterioration necessitating sacrifice or sacrifice at a tumor volume of 1.3 cm³ was determined (Fig. 4C). The low-dose PF-04691502 did not significantly improve survival (median survival of 7 and 8 days in vehicle and PF-04691502 groups, respectively), whereas therapy with low-dose PD-0325901 led to a modest survival advantage (median survival of 13 days in PD-0325901 group; t test comparison to vehicle; P < 0.001). Combined therapy with PF-04691502 and PD-0325901 led to a substantial long-term survival advantage, with median survival extended to 37 days (P < 0.0001, Fig. 4C).

Biomarker studies were conducted on tumor bearing mice treated with vehicle, PF-04691502, PD-0325901, and the drug combination (Fig. 5; Supplementary Fig. S5). As expected, treatment with PF-04691502 alone resulted in decreased p-AKT and p-RPS6, whereas PD-0325901 decreased p-ERK and partially decreased p-AKT. Interestingly, PF-04691502 alone led to a mild reduction in expression of the antiapoptotic protein Mcl-1 and PD-0325901 alone caused an increase in expression of the BH3-only protein, Bim. Treatment of the tumor-bearing mice with a combination of PF-04691502 and PD-0325901 resulted in a total loss of detectable p-AKT, p-RPS6, and p-ERK, and increased expression of Bim and decreased Mcl-1. The combination of PF-04691502 and PD-0325901 caused an increase in cleavage and accumulation of caspase-3 (Fig. 5A) and increased TUNEL staining (Fig. 5B), indicating that this treatment regimen caused increased tumor apoptosis.

Discussion

Here we present an in vivo efficacy study of the PI3K/mTOR inhibitor, PF-04691502 in a genetically engineered mouse model of ovarian cancer driven by Kras<sup>G12D</sup>-activating mutation and Pten deletion. To enhance preclinical studies using this model, we transplanted primary tumors from these mice orthotopically into the ovarian bursa and used in vivo imaging to noninvasively monitor tumor growth and metabolic activity over time and in response to treatment. Several advantages of this transplant model include that the tumors are transplanted as pieces rather than single cells and therefore include the cancer-associated stromal tissue; the tumors are never used in cell culture and thus, do not undergo any genomic changes associated with the adhering to plastic; and they are transplanted orthotopically, thus being exposed to appropriate microenvironmental factors. We showed that PF-04691502 had limited long-term single agent activity in this model, and hypothesized that this was because of sustained RAS/MAPK signaling in treated tumors. Accordingly, combination therapy using PF-04691502 and an inhibitor of MEK (PD-0325901) resulted in robust and prolonged tumor regression.
PF-04691502 is a novel, orally bioavailable and highly potent dual PI3K and mTOR inhibitor (4). Early preclinical and clinical development of PI3K pathway inhibitors has been focused on the identification of clinical settings in which single agent PI3K pathway inhibitors may be effective. For example, the single agent use of the mTORC1 inhibitor, temsirolimus, has proved efficacious for mantle cell lymphoma therapy (26) and is approved for the treatment of renal cell carcinoma (27). Recently, the PI3K inhibitor, GDC-0941, has been shown to inhibit tumor growth in a subset of breast cancer lines with PIK3CA mutations, HER2 amplification, or alterations in 2 PI3K pathway components (28). Preclinically, dual inhibition of PI3K and mTOR has shown antitumor efficacy in the context of Pik3ca(H1047R)-driven lung (29) and breast (30, 31) tumors and HER2-amplified breast cancers (31). However, doubt remains as to the ability of PI3K pathway inhibitors to enable long-term tumor growth inhibition across a range of tumor types without the development of resistant tumors. In this study, KrasG12D;Ptendel ovarian tumors display high activity of the PI3K pathway and were hypothesized to be susceptible to single agent PF-04691502 therapy. Indeed, PF-04691502 led to robust PI3K pathway inhibition and tumor growth delay. However, these tumors are able to overcome their dependency on this pathway, to survive and continue proliferating despite ongoing exposure to the drug. The swift adaptation following PF-04691502 therapy in the KrasG12D;Ptendel ovarian tumor model highlights the extensive cross-talk between parallel cancer-signaling pathways and consequently, the requirement for multi-pathway inhibition for the long-term prevention of proliferation or induction of cell death. Activation of the RAS/MAPK signaling pathway is a known mechanism by which tumors can become resistant to PI3K pathway inhibition, with activating mutations in Kras conferring resistance to PI3K pathway inhibition in vitro (32, 33). As such, combined inhibition of PI3K and RAS/MAPK pathways has had success preclinically. In vivo studies in LSL-KrasG12D lung cancer mice (29), breast cancer MCF7 xenografts (34), PTEN-deleted/Nxk3.1-deleted androgen-independent prostate cancer–bearing mice (35), and TPO-KrasG12D;Ptendel thyroid cancer mice (36) have each shown improved efficacy with combined PI3K and MEK pathway inhibition.

MEK inhibitors have shown promise as anticancer agents (37, 38), and one such agent, PD-0325901, showed target inhibition and some clinical activity in a phase I clinical trial (24). However, a phase II clinical trial in advanced non–small cell lung cancer was negative for its primary endpoint, with no patients achieving partial or complete responses (23, 24). These studies highlight the possibility that MEK inhibitors, as with PI3K pathway inhibitors, may need to be developed with alternate scheduling, appropriate patient selection criteria, or be combined with other rationally determined inhibitors to be clinically successful. Consistent with a dual dependency of the KrasG12D;Ptendel ovarian tumors on both the PI3K/mTOR and the RAS/MAPK pathway, PD-0325901 also had limited efficacy as a single agent. While MEK inhibition did result in greater effects compared with PF-04691502 alone, including tumor regression in some mice, these effects were short lived in the context of continuous long-term dosing. Combined inhibition of both signaling...
pathways led to improved efficacy because of the enhanced induction of apoptosis, which can be in part attributed to the combined activation of pro-apoptotic BH3-only protein Bim as a result of MEK inhibition and repression of Mcl-1 as a result of PI3K/mTOR inhibition (35, 39). Here, the ability of high Bim and low Mcl-1 expression to cooperatively initiate apoptosis in response to PI3K pathway and MEK inhibition is consistent with similar observations in human leukemias (40) and human lung cancer cell lines (41).

In addition to exploring the effects of combining 2 targeted therapies, it is advantageous to exploit preclinical models to understand the effect of standard chemotherapies in combination with targeted therapeutics, given the established role of standard chemotherapy in ovarian cancer. Each of our KrasG12D;Pten−/− ovarian cancer transplanted clones showed intrinsic resistance to current ovarian cancer platinum- and taxane-based chemotherapeutics, cisplatin, and docetaxel, respectively. When we combined PF-04691502 (5 mg/kg, daily) with cisplatin or docetaxel, we only observed very minimal tumor growth inhibition (Supplementary Fig. S2). Attempts at dose escalation to increase efficacy were limited by toxicity of the combined drugs. Notably, we observed greater tumor growth inhibition with high dose PF-04691502 as a single agent (10 mg/kg, daily) compared with the low doses achievable (5 mg/kg, daily) when used in combination with either cytotoxic agent. This may be an important observation going forward in clinical development, where PF-04691502 may be suitable for patients subsequent to failing platinum and taxane therapy, but would only be suitable in combination with those drugs if efficacious PF-04691502 drug concentrations can be achieved without adverse toxic effects. However, more extensive analysis of in vivo models with various levels of chemotherapy resistance and various dosing schedules would need to be carried out before we understand how widely applicable this finding is.

The use of molecular imaging such as FDG-PET to noninvasively monitor drug responses is aiding the development and testing of novel anticancer compounds (42). It is well described that the PI3K/AKT pathway is a major driver of glucose metabolism in cancer cells (43), therefore FDG-PET is likely to be an appropriate method to identify response to inhibitors of this pathway. Here, we show that high basal FDG-PET signal observed in the KrasG12D;Pten−/− ovarian tumors was greatly reduced in response to PF-04691502, corresponding with inhibition of p-AKT and p-RPS6 protein expression in ex vivo biomarkers analyses following 1 week of therapy. However, there was discordance between the dramatic inhibition of glucose uptake compared with the lack of effect on BrdU incorporation and the minimal long-term effects of single agent PF-04691502. This is most likely explained by FDG-PET metabolic activity corresponding only to glucose uptake, and it does not measure a cell's ability to proliferate or generate energy by alternate metabolic pathways such as via glutaminolysis (44). This has also been observed recently with mTOR inhibitors, where inhibition of PI3K/AKT pathway signaling and glucose metabolism was independent of tumor proliferation (45), and highlights the need to develop other PET tracers that correlate with drug target inhibition, tumor viability, and tumor proliferation. Despite the limitations of FDG-PET response to reflect the antitumor efficacy of PF-04691502, importantly, it does reflect successful target pathway inhibition and may therefore be a valuable biomarker of kinase inhibition during clinical development of this drug.

In conclusion, we have found PF-04691502 to be a potent inhibitor of the PI3K pathway in an in vivo mouse model of ovarian cancer driven by KrasG12D and Pten deletion, and this pathway inhibition may be imaged via FDG-PET. While there may be clinical scenarios in which single agent activity of PF-04691502 is efficacious, in this model driven by mutant Kras, at least, the therapeutic benefit of PF-04691502 alone is limited. Importantly, the efficacy of PF-04691502 can be enhanced by combined inhibition of MEK using PD-0325901.

Disclosure of Potential Conflicts of Interest

J. Christensen: employee and shareholder, Pfizer.

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References


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