Effective Targeting of Triple-Negative Breast Cancer Cells by PF-4942847, a Novel Oral Inhibitor of Hsp 90

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Abstract

**Purpose:** Triple-negative breast cancer (TNBC) patients have poor prognoses and survival outcomes such that the development of new targeted therapies is in strong demand. Mechanisms associated with high proliferation and aggressive tumor progression, such as PI3K/PTEN aberration, epidermal growth factor receptor (EGFR) overexpression, and cell-cycle upregulation, play important roles in TNBC. The molecular chaperone Hsp90 is required for the conformational maturation and stability of a variety of proteins in multiple pathways, such as EGFR, AKT, Raf, cdk4, etc. Therefore, an Hsp90 inhibitor may show therapeutic benefit in TNBC by targeting multiple pathways.

**Experimental Design:** The novel oral Hsp90 inhibitor PF-4942847 was characterized in multiple in vitro and in vivo assays to determine its antitumor activity in TNBC cell lines. In addition, the correlation of AKT degradation and Hsp70 induction in host peripheral blood lymphocytes (PBL) and xenograft tumors was determined.

**Results:** PF-4942847 induces degradation of multiple client proteins, cell-cycle block, apoptosis, and inhibits cell proliferation in TNBC lines, subsequently leading to tumor growth inhibition in mouse xenograft models. The correlation of AKT degradation and Hsp70 induction between PBLs and xenograft tumors reveals a differential modulation of Hsp90 activity between host and tumor tissues, and suggests that AKT degradation in PBLs may serve as a pharmacodynamic biomarker in future clinical development.

**Conclusions:** The novel oral Hsp90 inhibitor, PF-4942847, is a candidate for clinical development in TNBC by collaboratively targeting multiple signaling pathways. In addition, AKT degradation in PBLs may serve as a biomarker in clinical development.

**Introduction**

Heat shock protein 90 kDa (Hsp90) is a molecular chaperone that regulates the conformation, stability and activity of numerous key signaling proteins, including protein kinases (e.g., C-Raf, AKT, ErbB family, Cdk4), steroid receptors [e.g., androgen receptor and estrogen receptor (ER)], and transcription factors (e.g., HIF1α). These Hsp90 client proteins are involved in multiple pathways of cell transformation and tumor progression; therefore, targeting Hsp90 offers an opportunity to inhibit multiple pathways in cancer (1, 2). Several natural products, such as geldanamycin and radicicol, bind to Hsp90 at the NH₂-terminal ATP pocket and inhibit the ATPase activity of Hsp90 subsequently leading to client protein degradation through ubiquitin ligase machinery (3). The geldanamycin derivative 17-allylamino-17-demethoxy-geldanamycin (17-AAG), 17-DMAG, and IPI-504 have been developed as potential therapeutics in a variety of clinical trials (4–6). However, 17-AAG is poorly soluble and has low oral bioavailability, metabolism issues and hepatotoxicity (7, 8). Because of the potential toxicity of geldanamycin derivatives, specific small molecular weight Hsp90 inhibitors may be more effective clinical agents. Several small molecular weight Hsp90 inhibitors, including SNX-5422, CNF2024, STA 9090, and AIYL 922, are currently in clinical trials in various tumor types; it is too early to differentiate these inhibitors from the geldanamycin derivatives in the clinical setting (9–12).

Breast cancer is a heterogeneous disease for which there are a variety of biological features, natural history, and treatment options. Gene expression profiling has allowed us to classify breast cancers into 5 subtypes based on distinctive gene expression signatures (13). These 5 subtypes are luminal A, luminal B, HER2 positive, basal-like, and normal-like breast cancers. Basal-like tumors are characterized by the expression of genes that are specific for basal epithelial cells and involved in cellular proliferation, suppression of apoptosis, cell migration, and cell invasion (13–15). Basal-like breast cancers (BLBC) are...
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Translational Relevance

There is a strong need to develop new targeted therapies for triple-negative breast cancer (TNBC) patients. The work presented here shows that a novel oral Hsp90 inhibitor, PF-4942847, exhibits in vitro and in vivo efficacy in multiple TNBC cell lines; these results further suggest that PF-4942847 may be of clinical benefit in TNBC patients. The correlation of AKT degradation between mouse PBLs and xenograft tumors was determined in response to drug treatment and indicates that AKT degradation in patient PBLs may have the potential to serve as a biomarker to predict drug modulation in tumors.

Materials and Methods

Hsp90 inhibitor

PF-4942847 was synthesized as previously described (26). PF-4942847 was dissolved in DMSO for in vitro cellular assays and formulated in 40% PEG-400/60% saline (v/v) for animal studies.

Cell line

All cell lines were purchased from the American Type Culture Collection (ATCC) and cultured according to ATCC instructions. The gene mutation status of cell lines was obtained from the Sanger COSMIC database: http://www.sanger.ac.uk/.

Immunoblotting

Cells and xenograft tumors were lysed in lysis buffer (50 mmol/L Tris-HCl, 0.5% NP-40, 0.5% Triton X-100, 150 mmol/L NaCl, 1 mmol/L Na3VO4, 1 mmol/L NaF, and protease inhibitor cocktail). Protein (50 μg) was resolved by SDS-PAGE and Western blots were done with various antibodies (Actin from Sigma, all others from Cell Signaling) to detect proteins of interest.

Luminex and MesoScale assays

Cells (10,000/well) were seeded in a 96-well microtiter plate and cultured overnight. PF-4942847 was added to each well at a top concentration of 10,000 nmol/L with a 3-fold serial dilution ending at 0.0169 nmol/L. After 24 hours, cells lysates were prepared and analyzed following the manufacturer’s instructions. The Luminex 100 system (Upstate) was used for Hsp70 protein measurement, and MesoScale Discovery technology was used for Hsp70 protein measurement.

Cell-cycle profiling

TNBC cells were seeded and cultured overnight prior to compound treatment. Cells were harvested, fixed, stained with propidium iodide (PI), and analyzed by flow cytometry. A total of 10,000 events were analyzed for each sample, and the experiments were repeated at least 3 times.

Cell proliferation and caspase 3/7 assay

For cell proliferation assays, TNBC cells were seeded at 3,000 cells/well in a 96-well plate; PF-4942847 was added to each well as described above and incubated for 72 hours, followed by addition of 250 μg/mL of Resazurin (Sigma). After incubation for an additional 6 hours at 37°C, plates were analyzed by a fluorescence reader. For the caspase 3/7 assay, cells were plated and compound was added as described; the cells were assessed by the Caspase 3/7 Assay (Promega) following the manufacturer’s instructions.

often stained negative by immunohistochemistry for ER, progesterone receptor, and HER2; this BLBC subpopulation is thus called triple-negative breast cancers (TNBC). Although BLBC and TNBC share numerous clinical and pathologic features, they are not identical (16, 17). In the majority of cases, however, these 2 categories share similar clinical features, prognoses, and treatment options. For convenience, the term "TNBCs" will be used in this study to collectively describe BLBC and TNBC cell lines and patient populations. Clinical studies have shown that TNBCs are more aggressive and patients have poorer prognoses than the other breast cancer subtypes (18, 19). Therefore, the development of targeted therapies for TNBCs is clearly needed to help this patient population. More than 60% of TNBCs express epidermal growth factor receptor (EGFR) and this could serve as a prognostic marker for TNBC outcomes (20, 21). Activation of the PI3K pathway either by frequent RUNT alteration, elevated PIK3CA expression or oncogenic mutation has been identified in TNBCs and is also associated with a poor prognosis (22–24). Hsp90 regulates multiple signal transduction pathways by maintaining stability and activity of client proteins such as EGFR, AKT, Raf, and Cdk4; therefore, targeting Hsp90 function may provide an opportunity to inhibit tumor progression of TNBCs through EGFR, PI3K, and other proteins (25).

A series of 2-amino-5,7-dihydro-pyrrolo[3,4-d]pyrimidine-6-carboxylic acid amide compounds were previously described as potent and specific Hsp90 inhibitors; the detailed structure activity relationship (SAR) has also been reported (26). One of the compounds from this series, PF-4942847, had a low Ki value (6 nmol/L) and exhibited selectivity in the Invitrogen Kinase Panel against 35 other measured kinases (<10% inhibition at 1 μmol/L; ref. 26). PF-4942847 was characterized in this study to show in vitro and in vivo antitumor activity in a variety of human TNBC cell lines. PF-4942847 induces AKT protein degradation, cell-cycle block, apoptosis, and inhibits cell proliferation in TNBC lines, subsequently leading to tumor growth inhibition (TGI) of MDA-MB-231 and Mx-1 in mouse xenograft models. The correlation of AKT degradation and Hsp70 induction between peripheral blood lymphocytes (PBL) and xenograft tumor was also determined and reveals a differential modulation of Hsp90 activity between host and tumor tissues in response to drug treatment suggesting that AKT degradation in PBLs can potentially be used to guide clinical studies for TNBC.
Animal studies

Six to 8-week-old nu/nu athymic female mice and severe combined immunodeficient mice (SCID) female mice were obtained from Jackson Labs; the mice were maintained in pressurized ventilated cages at the Pfizer La Jolla animal facility. All studies were done in compliance with Institutional Animal Care and Use Committees guidelines. Tumors were established by injecting 2 × 10⁶ cells suspended 1:1 (v/v) with reconstituted basement membrane (Matrigel, BD Biosciences). For PK/PD studies, mice with established tumors of approximately 400 mm³ were randomized and treated with PF-4942847 QD for 2 days to establish steady state of drug exposure. For TGI studies, mice with established tumors of approximately 150 mm³ were randomized and treated with vehicle, PF-4942847, or 17-DMAG. Tumor dimensions were measured with vernier calipers, and tumor volumes were calculated using this formula: \( V = \pi/6 \times (\text{larger diameter}) \times (\text{smaller diameter})^2 \). TGI% was calculated as 100 × (1 – ΔT/ΔC). For the survival study, dosing was stopped as indicated in the graph for all animals and measurements were taken 3 times per week; animals were sacrificed and noted “dead” when the tumor size reached 1,500 mm³. For the tumor regression study, mice with established tumors of approximately 300 mm³ were randomized and treated with vehicle or PF-4942847 3 times per week.

Determination of PF-4942847 concentration in plasma

Plasma concentrations of PF-4942847 were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) following protein precipitation of plasma samples as previously described (27).

Statistical analysis

Prism 5.0 Software (Graphpad Software) was used for statistical analysis and graph generation. Unless otherwise stated, error bars indicate SD, and \( P \) values of less than 0.05 are denoted by an asterisk (*), \( P \) values of less than 0.005 are denoted by 2 asterisks (**), and \( P \) values of less than 0.0005 are denoted by 3 asterisks (***)

Results

PF-4942847 induces degradation of multiple Hsp90 client proteins in human TNBC cells

We first examined the cellular activity of PF-4942847 (Supplementary Fig. S1) in a number of human TNBC cell lines. PF-4942847 was added to MDA-MB-231 cells for multiple treatment time points, and total protein and phosphorylation levels of Akt, EGFR, cMet, B-Raf, C-Raf, ERK, and Hsp70 were determined by Western blot. The effect of PF-4942847 on protein degradation and reduction in phosphorylation of Hsp90 clients was observed beginning at 6 hours after compound addition and was more definite after the 18 hour time point (Fig. 1A). We also observed a preferential loss of p-AKT versus total AKT at 6 hour and 18 hour (Fig. 1A) suggesting that there may be other key clients degraded by PF-4942847 that account for this activity. This is most likely due to PF-4942847 interference of Hsp90 maintenance of the stability and functional conformation of client proteins. Similar results were observed with HCC-70 and MX-1 cells treated with PF-4942847 (Fig. 1B). In addition, PF-4942847 induced B-Raf protein degradation selectively in the cells harboring a mutant B-Raf allele (MDA-MB-231 and MX-1) over those with 2 wild-type copies (HCC-70). This is consistent with previous studies that B-Raf mutant protein, but not wild-type B-Raf, is degraded by Hsp90 inhibitors (28–30). In addition, the degradation of RTKs such as EGFR and cMet by PF-4972847 leads to the suppression of ERK phosphorylation in TNBCs, while Hsp70 induction in treated cells is due to the feedback mechanism in the HSP machinery (31, 32). Next, a panel of TNBC cell lines was used to investigate Akt protein degradation and Hsp70 protein induction by plate-based assays to quantitatively determine the Akt degradation IC₅₀ and Hsp70 induction EC₅₀ values. As shown in Figure 1C and D and summarized in Table 1, PF-4942847 exhibits IC₅₀ values ranging from 20 to 100 nmol/L in the Akt degradation assay and EC₅₀ values ranging from 20 to 40 nmol/L in the Hsp70 induction assay in a variety of TNBC cell lines. 17-DMAG was included in the Akt and Hsp70 assays and results indicate that PF-4942847 and 17-DMAG have comparable effects on the inhibition of Hsp90 activity (Table 1).

PF-4942847 blocks cell-cycle progression and induces apoptosis resulting in inhibition of cellular proliferation in TNBCs

PF-4942847 has the ability to induce protein degradation in the signaling pathways of RTKs (including cMet, EGFR, Ras-Raf-ERK, and AKT); as such, it was very likely that PF-4942847 would block cell-cycle progression and induce cellular apoptosis via targeting of multiple important signaling pathways in TNBCs. Therefore, we next investigated cell-cycle block and apoptosis activation by the treatment of PF-4942847 in TNBCs. Results indicate that PF-4942847 induces cell-cycle block at the G2/M transition in both MDA-MB-231 and MX-1 cells (Fig. 2A and B, Supplementary Fig. S2). Moreover, high concentrations of PF-4942847 also increase the subG₁ cell population indicating an increase in apoptotic cells (Fig. 2A and B, Supplementary Fig. S2). PF-4942847 also induced cellular apoptosis in MDA-MB-231 and MX-1 cells as determined by the induction of caspase 3/7 activity (Fig. 2C). We next measured the inhibitory activity of PF-4942847 on cellular proliferation. Results presented in Figure 2D and summarized in Table 1 show that PF-4942847 effectively inhibits cell proliferation in TNBCs with IC₅₀ values ranging from 20 to 60 nmol/L. The inhibitory potency of PF-4942847 and 17-DMAG is comparable. PF-4942847 was also evaluated in cell lines representative of other breast cancer subtypes; these subtypes showed increased subG₁ population and caspase 3/7 activity (Supplementary Table S1).
results show that PF-4942847 inhibited cellular proliferation in a number of breast cancer cell lines with different backgrounds (Supplementary Table S1). This further suggests that the inhibition of multiple pathways by disruption of Hsp90 activity is effective in inducing antiproliferative activity in a variety of breast cancers. These results clearly indicate that PF-4942847 is a potent Hsp90 inhibitor that induces protein degradation of multiple key signaling pathways, blocks cell-cycle progression, and induces apoptosis; these combined activities subsequently inhibit cellular proliferation in TNBCs.

**PF-4942847 induces Hsp90 client protein degradation and Hsp70 elevation in MDA-MB-231 xenograft tumors**

We next determined the *in vivo* activity of PF-4942847 in mouse xenograft studies. A MDA-MB-231 xenograft model was used to conduct the *in vivo* experiments and
investigate the relationship between plasma drug exposure and modulation of Hsp90 activity by PF-4942847. To reach the steady state of drug exposure, PF-4942847 was orally administered QD for 2 days to MDA-MB-231 tumor-bearing mice, and free unbound PF-4942847 was detected in mouse plasma in a dose- and time-dependent manner after the second dose (Fig. 3A). The free unbound concentrations of PF-4942847 in mouse plasma at 7 hours post-dose were 103 and 152 nmol/L at 30 and 50 mg/kg, respectively, both of which are above the in vitro AKT degradation IC₅₀ value (20 nmol/L, Table 1). Thus, it was determined that PF-4942847 was likely to inhibit Hsp90 activity in xenograft tumors. At the 30 mg/kg dose, MDA-MB-231 tumors were dissected at

| Table 1. Summary of IC₅₀ values for AKT degradation, Hsp70 induction, and cell proliferation inhibition by PF-4942847 and 17-DMAG treatment in a panel of TNBCs |
|-----------------|-----------------|-----------------|-----------------|
| AKT degradation | 17-DMAG         | Hsp70 induction | 17-DMAG         |
| IC₅₀ (nmol/L)   | IC₅₀ (nmol/L)   | EC₅₀ (nmol/L)   | IC₅₀ (nmol/L)   |
| PF-4942847      | 17-DMAG         | PF-4942847      | 17-DMAG         |
| Mean        | SD      | Mean        | SD      | Mean        | SD      | Mean        | SD      |
| MDA-MB-231    | 19.4     | 1.18       | 24.8     | 1.2        | 24.1     | 3.3        | 3.5      | 1.4        | 259     | 6.5       | 8.6      | 1.2       |
| HCC-70        | 61.4     | 2.13       | 22.8     | 6.5        | 23.4     | 4.9        | 7.7      | 1.9        | 579     | 18.8      | 4.9      | 1.8       |
| MX-1          | 93.7     | 15.6       | 87.4     | 20.7       | 17.2     | 1.6        | 2.7      | 0.2        | 24.7    | 2.4       | 5.2      | 0.2       |
| Hs-5786T      | 28.5     | 3.6        | 9.5      | 3.2        | 23.6     | 3.4        | 3.4      | 1.4        | 18.1    | 8.3       | 7.5      | 0.2       |
| BT-549        | 74.5     | 15.4       | 22.7     | 10.6       | 21.4     | 4.2        | 4.6      | 1.3        | 19.1    | 6.9       | 5.5      | 0.3       |
| MDA-MB-468    | 86.9     | 19.5       | 89.1     | 20.3       | 37.8     | 6.2        | 12.3     | 2.9        | 37.0    | 4.1       | 13.5     | 3.6       |
| HCC-38        | 51.7     | 7.3        | 35.8     | 6.3        | 35.6     | 8.1        | 13.8     | 1.5        | 23.7    | 2.4       | 11.1     | 0.3       |

Figure 2. PF-4942847 blocks cell-cycle progression and induces apoptosis resulting in inhibition of proliferation of TNBC cell lines. MDA-MB-231 (A) or MX-1 (B) cells were treated with compounds for 24, 48, or 72 hours, fixed and stained with PI, and analyzed by flow cytometry. The percentages of the cell population in each cell-cycle phase were as indicated. C, cells were treated with compound for 24 or 48 hours, then analyzed by caspase 3/7 assay to determine caspase activity. D, cells were cultured and treated with compound for 72 hours before utilizing the resazurin to determine inhibition of cell proliferation.
various times to determine time-dependent modulation of Hsp90 activity (Fig. 3B). In addition, PF-4942847 was administered orally QD for 2 days to MDA-MB-231 tumor-bearing mice at 10, 30, and 50 mg/kg; plasma concentration of PF-4942847 was determined and plotted at select time points. B, MDA-MB-231 tumor-bearing mice were treated QD for 2 days with PF-4942847 at 30 mg/kg, and tumors were harvested at 4, 7, and 24 hours after the second dose to determine time-dependent protein changes. Or, mice were treated QD for 2 days with PF-4942847 at 10, 30, and 50 mg/kg, and tumors were harvested at 4 hours after the second dose to determine dose-dependent protein changes. Tumor lysates were subject to Western blot analysis for various Hsp90 client proteins as indicated.

PF-4942847 triggers differential responses of AKT degradation and Hsp70 induction between xenograft tumors and mouse PBLs

To further aid future clinical development studies, we investigated the relationship of AKT degradation and Hsp70 induction in response to PF-4942847 between xenograft tumors and mouse PBLs to determine whether it was possible to correlate Hsp90 activity changes between tumors and surrogate tissues. Both MDA-MB-231 tumors and mouse PBLs from the same animal were collected to measure AKT (by Luminex) and Hsp70 levels (by MesoScale Discovery technology) to determine Hsp90 activity changes induced by PF-4942847 between tumors and normal host tissue. Both Luminex and MesoScale methods had been previously optimized to ensure that appropriate amounts of tumor and PBL samples were analyzed and that all data points were within the linear range. Therefore, the Luminex and MesoScale readout of units per μg of protein tested can be quantitatively compared from sample to sample.
sample. At 4 hours, PF-4942847 induced similar degrees of AKT degradation in both tumors and PBLs (Fig. 4A). Similarly, at the 30 mg/kg dose, we observed comparable AKT degradation in tumors and PBLs (Fig. 4B). Although AKT degradation might be slightly higher in tumors than PBLs, the differences were not significant with the exception of the 72-hour time point as shown in Figure 4B. Interestingly, we observed a significant difference in Hsp70 induction fold change between tumors and PBLs (Fig. 4C and D). At 4 hours, Hsp70 induction is dose dependent and significantly higher fold changes were observed in mouse PBLs than tumors at the 50, 30, and 10 mg/kg doses while the differences between tumors and PBLs were less pronounced at the 3 and 1 mg/kg doses as indicated in Figure 4C. At the 30 mg/kg dose, we observed significant differential fold changes of Hsp70 induction in tumors and PBLs up to the 120-hour time point (Fig. 4D). We further analyzed the data by comparing the AKT and Hsp70 level per µg of protein (Supplementary Fig. S4); the analysis revealed that PF-4942847 induced dose- and time-dependent AKT degradation and Hsp70 induction in both tumors and PBLs. However, while the total amount of AKT per µg of protein is similar between tumors and PBLs, the total amount of Hsp70 per µg of protein is much less in PBLs than in tumors. Of note, recovery of AKT and Hsp70 in both the tumor samples and PBLs was delayed even when the drug concentration was below the lower limit of detection after 24 hours, and the recovery time seemed to be longer in tumors than in PBLs in response to PF-4942847 treatment. Detailed percent inhibition of AKT and fold increase of Hsp70 in tumors and PBLs are summarized in Supplementary Table S2. Taken together, the data show that PF-4942847 inhibits Hsp90 activity in both MDA-MB-231 tumors and host PBLs, and furthermore exhibits differential modulation of AKT and Hsp70 between tumors and PBLs. In addition, these results reveal that the relative percent change of AKT in MDA-MB-231 tumors and mouse PBLs is similar while the relative fold increase of Hsp70 in tumors is much less than those in PBLs.

**PF-4942847 inhibits in vivo tumor growth of human TNBC cell lines MDA-MB-231 and MX-1 in mice**

PF-4942847 has shown in vitro and in vivo inhibition of Hsp90 activity in a variety of TNBCs and in xenograft MDA-MB-231 tumors. We next determined whether PF-4942847 was able to induce in vivo TGI in mouse xenograft models. MDA-MB-231 and MX-1 were implanted subcutaneously in nude or SCID-bg mice, respectively, and treated orally once a day with vehicle or PF-4942847 to conduct the TGI experiments. PF-4942847 was well tolerated at 25 mg/kg in nude mice and at 20 mg/kg in SCID-bg mice with minimal body weight loss (≤5%). PF-4942847 induces 91% and 80% TGI, in MDA-MB-231 and MX-1 xenograft models, respectively (Fig. 5A and B). Furthermore, the effective concentration (EC_{50}) to induce AKT degradation and TGI of MDA-MB-231 was calculated using a PK/PD modeling approach as previously described and those results indicated that AKT degradation (EC_{50} ~19 nmol/L) was well correlated to TGI (EC_{50} ~12 nmol/L) in MDA-MB-231 (27). Although 17-DMAG exhibits comparable cellular
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Potency as PF-4942847 in TNBC lines, 17-DMAG at the maximal tolerated dose (10 mg/kg, BID, 5 days/wk) only showed 56% TGI in the MDA-MB-231 model. This is consistent with previous findings where 17-AAG and 17-DMAG have shown only moderate in vivo TGI activity in breast cancer models (33, 34). Starting from day 29, MDA-MB-231 tumor-bearing mice were maintained without further dosing, and tumor volume continued to be measured and animal survival was assessed (Fig. 6A). Mice treated with PF-4942847 at 25 mg/kg had a longer period compared with the vehicle-treated animals ($P < 0.005$). Two mice in the 25 mg/kg dose group remained tumor free at day 120 as shown in Figure 6A. In the MX-1 model, PF-4942847 treatment at 20 mg/kg also increased survival of tumor-bearing mice ($P < 0.05$); however, there were no tumor-free animals at the end of the study, and MX-1 tumors progressed very quickly in the low-dose groups such that there was not a significant survival benefit over the vehicle control group ($P > 0.05$; Fig. 6B). This suggests that PF-4942847 is critical in stopping tumor progression but not sufficient to induce tumor cell death in the MX-1 tumor model. On the basis of our results where AKT degradation and Hsp70 induction were sustained beyond PF-4942847 plasma clearance, we also tested an alternative dosing schedule with PF-4942847. MDA-MB-231 tumor-bearing mice were treated with PF-4942847 orally 3 times a week (Monday, Wednesday, and Friday) at 75 mg/kg without significant bodyweight loss; we observed 78% tumor regression in this study. At the end of study day 69, 4 mice were tumor free and the tumor measurements of the remaining mice all had a decreasing trend (Fig. 6C). Therefore, results indicate that PF-4942847 is a potent Hsp90 oral inhibitor that inhibits tumor growth in TNBC models.

Discussion

Preclinical studies have shown the notable sensitivity of HER2-overexpressing breast tumors to Hsp90 inhibitors (9, 10). 17-AAG (Tanespimycin) is being developed in the clinic for HER2-positive breast cancer and shows a moderate clinical response in combination with trastuzumab in patients (35). However, aside from a study with the IV Hsp90 inhibitor PI-1171 (25), it has not been widely reported that an Hsp90 inhibitor is effective in inhibition of TNBC. Therefore, we wanted to investigate the potential development opportunities for the oral Hsp90 inhibitor PF-4942847 in the treatment of TNBC. Our results show that PF-4942847 inhibited cellular proliferation in a panel of TNBC lines, eliminated tumor growth in MDA-MB-231 and MX-1 xenograft models, and induced tumor regression of a MDA-MB-231 xenograft model in mice. In this study, PF-4942847-induced modulation of AKT and Hsp70 between tumors and host PBLs was also quantitatively determined and data suggest that AKT degradation in PBLs may be a better biomarker than Hsp70 induction to predict tumor pharmacodynamic changes in response to PF-4942847 treatment. Although a number of Hsp90 inhibitors have been investigated in preclinical and clinical stages, there is not yet an approved drug targeting Hsp90. PF-4942847 is an orally bioavailable Hsp90

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PF-4942847 induces tumor regression in the MDA-MB-231 xenograft model. MDA-MB-231 (A) or MX-1 (B) tumor-bearing mice were treated with various dose concentrations of PF-4942847. When the average tumor size in the vehicle group was above 1,500 mm³, mice were taken down from the study, and PF-4942847 treatment was stopped for all other groups to conduct the survival study. The last dosing days are as indicated in each graph. One-way ANOVA analysis was done to compare each group to the vehicle group. P values less than 0.05 are indicated by 1 asterisk (*) and P values less than 0.005 are indicated by 2 asterisks in the graph (**). MDA-MB-231 (C) cells were implanted into nude mice and allowed to reach approximately 300 mm³. Once tumors reached sufficient size, mice were dosed with PF-4942847 3 times per week at 75 mg/kg PO to examine whether PF-4942847 would induce tumor regression.

Figure 6. PF-4942847 induces tumor regression in the MDA-MB-231 xenograft model. MDA-MB-231 (A) or MX-1 (B) tumor-bearing mice were treated with various dose concentrations of PF-4942847. When the average tumor size in the vehicle group was above 1,500 mm³, mice were taken down from the study, and PF-4942847 treatment was stopped for all other groups to conduct the survival study. The last dosing days are as indicated in each graph. One-way ANOVA analysis was done to compare each group to the vehicle group. P values less than 0.05 are indicated by 1 asterisk (*) and P values less than 0.005 are indicated by 2 asterisks in the graph (**). MDA-MB-231 (C) cells were implanted into nude mice and allowed to reach approximately 300 mm³. Once tumors reached sufficient size, mice were dosed with PF-4942847 3 times per week at 75 mg/kg PO to examine whether PF-4942847 would induce tumor regression.

In MDA-MB-231 tumors, even when the drug had been cleared from mouse plasma, the recovery of AKT and Hsp70 was not immediate but relatively slow to reach basal levels. Therefore, we investigated whether an alternative dosing schedule with higher dose concentration of PF-4942847 would have better antitumor activity by stronger inhibiting of Hsp90 activity in tumors and allowing recovery of Hsp90 clients in the normal tissues. Our results indicated that QOD dosing of PF-4942847 in MDA-MB-231 model (Fig. 6C) induced tumor regression and was superior to QD dosing, even though a 91% TGI was achieved in the latter. Traditionally, oral daily dosing has been preferred in the clinic, however, our current data
suggest that alternative dosing schedules should be explored in the clinic to avoid systemic toxicity triggered by daily dosing of Hsp90 inhibitors while maintaining a low level of client proteins in tumors.

In conclusion, we have characterized a novel oral Hsp90 specific inhibitor, PF-4942847, and have shown that PF-4942847 is an effective antitumor agent to induce in vitro and in vivo antitumor activity in a panel of TNBC cells. Our data suggest that PF-4942847 is a potential candidate for clinical development to address the unmet need for therapy targeting TNBC. Moreover, the biomarker studies to determine AKT and Hsp70 changes in tumors and PBLs provide preliminary evidence that AKT degradation in PBLs is a feasible biomarker approach to evaluate target modulation in tumors.

References


Retraction: Effective Targeting of Triple-Negative Breast Cancer Cells by PF-4942847, a Novel Oral Inhibitor of Hsp 90

The article entitled, “Effective Targeting of Triple-Negative Breast Cancer Cells by PF-4942847, a Novel Oral Inhibitor of Hsp 90,” which was published in the August 15, 2011, issue of Clinical Cancer Research (1), is being retracted at the request of Pfizer, the study sponsor.

In early 2016, the CCR editorial office became aware of potential image manipulation in the article through a record posted on pubpeer.com. The CCR editorial office contacted the corresponding author about the matter and received a response from a Pfizer representative, who informed us that the company assembled a review team to investigate the matter further. A Pfizer representative recently contacted the CCR editorial office with the results of the investigation: Most or all of the images in question appear to be duplications, and most of the authentic, original images that should have been used in place of the duplicated images cannot be located. As a result, Pfizer is requesting retraction of the article and has obtained consent from all six authors.

The matter was reviewed by members of the AACR Publications staff and the CCR editors, who agree that the figure manipulation present in the article merits retraction. Five authors have consented to this retraction notice; the CCR editorial office did not receive a response from Dr. Pramod P. Mehta.

Reference

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