GDC-0941, a Novel Class I Selective PI3K Inhibitor, Enhances the Efficacy of Docetaxel in Human Breast Cancer Models by Increasing Cell Death *In Vitro* and *In Vivo*

Jeffrey J. Wallin, Jane Guan, Wei Wei Prior, Leslie B. Lee, Leanne Berry, Lisa D. Belmont, Hartmut Koeppen, Marcia Belvin, Lori S. Friedman, and Deepak Sampath

Abstract

**Purpose:** Docetaxel is a front-line standard-of-care chemotherapeutic drug for the treatment of breast cancer. Phosphoinositide 3-kinases (PI3K) are lipid kinases that regulate breast tumor cell growth, migration, and survival. The current study was intended to determine whether GDC-0941, an orally bioavailable class I selective PI3K inhibitor, enhances the antitumor activity of docetaxel in human breast cancer models *in vitro* and *in vivo*.

**Experimental Design:** A panel of 25 breast tumor cell lines representing HER2+, luminal, and basal subtypes were treated with GDC-0941, docetaxel, or the combination of both drugs and assayed for cellular viability, modulation of PI3K pathway markers, and apoptosis induction. Drug combination effects on cellular viability were also assessed in nontransformed MCF10A human mammary epithelial cells. Human xenografts of breast cancer cell lines and patient-derived tumors were used to assess efficacy of GDC-0941 and docetaxel *in vivo*.

**Results:** Combination of GDC-0941 and docetaxel decreased the cellular viability of breast tumor cell lines *in vitro* but to variable degrees of drug synergy. Compared with nontransformed MCF10A cells, the addition of both drugs resulted in stronger synergistic effects in a subset of tumor cell lines that were not predicted by breast cancer subtype. In xenograft models, GDC-0941 enhanced the antitumor activity of docetaxel with maximum combination efficacy observed within 1 hour of administering both drugs. GDC-0941 increased the rate of apoptosis in cells arrested in mitosis upon cotreatment with docetaxel.

**Conclusion:** GDC-0941 augments the efficacy of docetaxel by increasing drug-induced apoptosis in breast cancer models. *Clin Cancer Res; 18*(14); 3901–11. ©2012 AACR.

Introduction

Breast cancer is the leading cause of nonsmoking cancer-related death in women and continues to be a major health concern globally (1). Although a number of genetic and environmental factors contribute to the development of mammary epithelial neoplasia and malignancy, tumorigenesis is initially controlled by estrogen-mediated induction of growth factors, transcription factors, cell-cycle regulators, and antiapoptotic factors (2, 3). In addition, genetic alterations such as HER2 amplification can transform mammary epithelial cells in preclinical models and is clinically validated in a subset of patients with breast cancer who overexpress HER2 and respond to biologic therapeutics such as trastuzumab (4, 5).

Taxanes, such as paclitaxel and docetaxel, inhibit microtubule function by altering their dynamic equilibrium and are used as standard-of-care treatment of breast cancer in combination with chemotherapeutics and targeted agents (6, 7). However, combination therapy with drugs that target additional growth factor signaling pathways may increase the therapeutic index and thereby provide greater clinical benefit (8–12). The phosphoinositide 3-kinase (PI3K) pathway plays an essential role in regulating tumor cell growth, migration, and survival upon growth factor receptor or integrin activation (13). Activating and transforming mutations in the PIK3CA gene of the p110α subunit are commonly found in HER2+ and estrogen receptor (ER)+ breast tumors (14–17). In addition, genetic deletion or loss-of-function mutations within the tumor suppressor PTEN, a phosphatase with opposing function to PI3K, also results in aberrant activation of PI3K pathway signaling (18). As such, PI3K may be an ideal target for combination therapy in breast cancer.
Translational Relevance

Docetaxel is commonly used as a front-line treatment option for breast cancer but patients ultimately relapse and succumb to disease progression. Therefore, combination therapies that enhance the antitumor activity of docetaxel may provide greater clinical benefit and outcomes. Given that phosphoinositide 3-kinase (PI3K) is frequently activated in breast cancer and induces chemoresistance, it is an attractive target for combination therapy with standard-of-care drugs such as docetaxel.

We report that the combination of a class I selective PI3K inhibitor, GDC-0941, enhances antitumor activity of docetaxel in human breast cancer models in vitro and in vivo by rapid induction of proapoptotic mechanisms. Our data provide a preclinical rationale for evaluating GDC-0941 in combination with docetaxel for breast cancer treatment.

PI3K inhibitors such as wortmannin and LY2942004 have been shown to increase efficacy of paclitaxel and docetaxel in preclinical tumor models (19–21). However, their lack of selectivity confounds the ability to specifically define the contribution of PI3K inhibition to the antitumor effects observed when combined with taxanes (22). GDC-0941 is an orally bioavailable inhibitor of class I PI3K that is 100-fold more potent against class I than against class II, III, and IV family members and is in clinical development for solid tumor indications including breast cancer (23–26). Initial evaluation of the combination effects of GDC-0941 and docetaxel showed increased antitumor activity in the HER2+ BT474M1 breast cancer model in vitro and in vivo (27). We aimed to expand upon these results by evaluating a broader panel of human tumor cell lines representing the major breast cancer molecular subtypes (HER2+, luminal, and basal) in response to the combination of GDC-0941 and docetaxel. An additional goal was to ascertain the mechanism of action of the drug combination in sensitive breast tumor models.

Materials and Methods

Materials

GDC-0941 was synthesized at Genentech, Inc., as described before (23), and docetaxel was purchased from ChemShuttle. Caspase inhibitor, Z-VAD-FMK, was purchased from Promega. Primary antibodies used were pAkt, Akt, pPRAS40, PRAS40, pS6, S6, pGSK3β, p70S6K, pFOXO1, PTEN, cyclin D1, phospho-Histone H3, cleaved caspase-3, cleaved PARP (Cell Signaling Technology), anti-HER2 clone 4D5 (Genentech), and β-actin (Sigma).

Cell culture

Human breast tumor cell lines were obtained from the American Type Culture Collection (ATCC) and maintained in RPMI or Dulbecco’s Modified Eagle’s Media (DMEM) supplemented with 10% FBS, 100 units/mL penicillin, 2 mmol/L L-glutamine, and 100 mg/mL streptomycin at 37°C under 5% CO2. MCF10A mammary epithelial cells were also obtained from ATCC and cultured in DMEM: F12 (50:50) medium supplemented with 20 ng/mL human EGF and 10 μg/mL insulin. Human breast cancer MCF7-neo/HER2 cells were generated at Genentech by ectopically overexpressing HER2 in MCF7 cells. HER2 expression was confirmed by fluorescence-activated cell-sorting (FACS) analysis using anti-HER2 primary antibodies (clone 4D5). The level of HER2 expression in the MCF7-neo/HER2 cell line is comparable with breast cell lines with known HER2 amplification such as SK-BR-3 (Supplementary Fig. S1). However, on the basis of gene expression profiling [including estrogen and progesterone receptor (PR) positivity], MCF7-neo/HER2 cells are phenotypically luminal.

Cell viability assay

All drug treatments were tested in quadruplicate during a 4-day incubation period, and the relative number of viable cells was estimated using CellTiter-Glo (Promega). Total luminescence was measured on a Wallac Multilabel Reader (Perkin-Elmer). Cells were treated simultaneously with docetaxel (dose range = 0.0003–0.020 μmol/L) or GDC-0941 (dose range = 0.083–5 μmol/L) in an 8 × 10 matrix of concentrations chosen to encompass clinically relevant doses (24). The concentration of drug resulting in 50% maximal effective concentration (EC50) was determined using Prism software (GraphPad). Combination synergy of GDC-0941 and docetaxel was determined by Bliss independence analyses (28).

A Bliss expectation for a combined response (C) was calculated by the equation:

\[
C = (A + B) - (A \times B)
\]

where A and B are the fractional growth inhibitions of drug A and B at a given dose. The difference between the Bliss expectation and the observed growth inhibition of the combination of drugs A and B at the same dose is the “Delta.Bliss.” Delta. Bliss scores were summed across the dose matrix to generate a Bliss sum. Bliss sum = 0 indicates that the combination treatment is additive (as expected for independent pathway effects); Bliss sum > 0 indicates activity greater than additive (synergy); and Bliss sum < 0 indicates the combination is less than additive (antagonism).

Statistical analysis comparing the Bliss sums for each cell line was conducted by the Student t test.

Western blotting

Cells were treated at EC50 concentrations of GDC-0941, docetaxel, or both for 4 or 24 hours and lysed in 1 × Cell Extraction Buffer (BioSource) supplemented with protease inhibitors and Phosphatase Inhibitor Cocktails 1 and 2 (Sigma). Protein concentrations were determined using the Pierce BCA Protein Assay Kit. For immunoblotts, equal amounts of protein were separated by electrophoresis through NuPAGE Bis-Tris 10% gradient gels (Invitrogen), transferred onto polyvinylidene difluoride membranes.
using the Criterion system, and probed with monospecific primary antibodies. Specific antigen–antibody interactions were detected with IRDye 680 or IRDye 800 infrared secondary antibodies using a LI-COR imaging system.

FACS analysis
For cell-cycle analysis, cells were treated with EC50 concentrations of GDC-0941 and/or docetaxel for 24 hours, fixed in 100% ice-cold ethanol, and incubated in propidium iodide (PI) solution for 30 minutes and analyzed with a FACSscan flow cytometer (Becton Dickinson). For cell death analyses, cells were incubated with GDC-0941, docetaxel, or both drugs for 48 hours, stained with Annexin V-FITC (BD Biosciences) and PI solution (Sigma) according to the manufacturer’s instructions and analyzed with a FACScan flow cytometer (Becton Dickinson).

Time lapse microscopy imaging
Cells were seeded onto glass-bottom 24-well plates (Greiner Bio-one) and, 24 hours later, incubated with drug-containing media. Cells were treated with EC50 concentrations of GDC-0941 and/or docetaxel, multiple fields per condition were selected, and fluorescent and phase-contrast images were recorded with a 10× objective every 15 minutes for 72 hours on an AxioObserver inverted microscope (Carl Zeiss), equipped with an environmental chamber (Okolab), MS2000 XY stage (Applied Scientific Instruments), and a CoolSNAP CCD camera (Hewlett Packard). Mitotic events and cell death were scored as previously described (29). Cells were also cotreated with caspase inhibitor Z-VAD-FMK at a final concentration of 2 μmol/L. Statistical analysis was conducted by the Student t test.

Cell synchronization
Cells were synchronized with a double thymidine block and released into drug-containing media. Adherent cells were washed twice with cold PBS and lysed in 1× Cell Extraction Buffer (BioSource) supplemented with protease inhibitors (Roche), 1 mmol/L phenylmethylsulfonylfluoride, and Phosphatase Inhibitor Cocktails 1 and 2 (Sigma). Protein concentration was determined using the Pierce BCA Protein Assay Kit.

In vivo xenograft models
Female nu/nu mice were inoculated subcutaneously with MCF7-neo/HER2 or MX-1 breast cancer cells. When tumors reached a mean volume of 200 to 250 mm3, animals were size-matched and distributed into groups consisting of 10 animals per group. Docetaxel formulated in 3% EtOH, 97% saline was administered intravenously once weekly. GDC-0941, formulated in MCT (0.5% methylcellulose, 0.2% Tween-80) was dosed orally and daily. MAXF1162 is an HER2+/ErbB3/PR+ patient-derived breast cancer tumor xenograft model established at Oncotest, Inc., by directly implanting tumors subcutaneously from patient to NMRI nu/nu mice. Tumor volume was calculated as follows: tumor size (mm3) = (longer measurement × shorter measurement2) × 0.5. Tumor sizes were recorded twice weekly over the course of a study. Following data analysis, P values were determined using the Dunnett t test.

For pharmacodynamic studies, tumor samples (n = 4) were immediately frozen or fixed in 10% neutral-buffered formalin. Tumors were dissociated in cell extraction buffer, and lysates were analyzed by Western blotting as described above. Immunohistochemistry was conducted using 5-μm paraffin sections of formalin-fixed tissue on a Ventana Benchmark XT instrument (VMSI) by deparaffinization, treatment with antigen retrieval buffer (VMSI), and incubation with anti-cleaved caspase-3 primary antibody (Cell Signaling Technology) at 37°C. Bound antibody was detected using DABMap technology (VMSI), and sections were counterstained with hematoxylin.

Results

Combination effects of GDC-0941 and docetaxel on human breast cancer cell viability in vitro
We previously observed that the combination of GDC-0941 and docetaxel increased antitumor activity in the HER2+ BT474M1 breast cancer model (27). Therefore, our aim was to expand upon these preliminary results by evaluating the combination effects of GDC-0941 and docetaxel in a panel of 25 human breast cancer cell lines representing the major breast cancer molecular subtypes (HER2+, luminal, and basal) and were either P13Kα wild-type, P13Kα-mutant (E545K or H1047R), or PTEN-null (30).

Breast tumor lines were treated with docetaxel and GDC-0941 in an 8 × 10 dose matrix, and the combination effects on cellular viability were evaluated using the Bliss independence model as described in Materials and Methods. In comparison to single-agent treatments the combination of GDC-0941 and docetaxel reduced tumor cell viability by 80% or greater in the breast cancer cell lines tested in vitro (Supplementary Table S1). A Bliss sum of 0 was determined in the MDA-MB-453 cell line indicating an additive combination effect whereas Bliss sums >0 were calculated in the other tumor cell lines indicating a synergistic effect (Supplementary Table S1). However, the degree of synergy varied across the tumor lines tested as a range of Bliss sums from 1 to 451 were noted (Supplementary Table S1). For example, in the Hs578T1.2 line, a Bliss sum of 73 indicates synergy between both drugs whereas stronger synergetic effects were observed in the MCF7-neo/HER2 and MX-1 models in which Bliss sums equaled 250 and 375, respectively, and illustrated as representative “heat maps” in Fig. 1A. A drug combination effect (Bliss sum = 61) was also observed in the spontaneously immortalized MCF10A human mammary epithelial cell line (Fig. 1A). As a result of the latter, we focused our analysis on those breast tumor cell lines in which a stronger combination effect was observed relative to the nontransformed MCF10A cell line (i.e., Bliss sums > 61). A subset of breast tumor lines (11 of 25) met the latter criteria (Supplementary Table S1). Within this subset, there was no significant difference in drug
synergy across subtypes, PI3Kα mutational or PTEN expression status (Fig. 1B and C).

**Modulation of PI3K pathway markers upon treatment with GDC-0941 and docetaxel in breast cancer cell lines**

To further analyze the combination effects of GDC-0941 and docetaxel, we focused our attention on those tumor lines in which strong synergy was observed relative to the MCF10A line by evaluating their effects on modulation of the PI3K pathway biomarkers. As representative models, we used Hs578T1.2 (PI3Kα wild-type), MCF7-neo/HER2 (PI3Kα-mutant), and MX-1 (PTEN-null). In all 3 models, GDC-0941 inhibited Akt phosphorylation and downstream targets of Akt signaling such as pPRAS40 and pS6 within 4 hours of treatment and this effect was sustained for 24 hours (Fig. 2). The phosphorylation of Akt substrates FOXO1 and GSK3β as well as phosphorylation of the downstream target of Akt-TORC1 signaling, p70S6K, were also inhibited through 24 hours after GDC-0941 treatment (Fig. 2). Treatment with docetaxel alone for 24 hours did not alter the PI3K pathway markers evaluated, but an increase in phospho-Histone H3 was detected, confirming that the drug was pharmacologically active (Fig. 2).

**GDC-0941 decreases the time of docetaxel-induced mitotic arrest prior to apoptosis**

Given that docetaxel induces cell death in sensitive tumor cell lines (31), we determined whether the increase in tumor growth inhibition observed in combination with GDC-0941 was due to increased apoptosis. Within
48 hours of combination treatment with GDC-0941 and docetaxel, there was an increase in the percentage of apoptotic cells compared with cells treated with either drug alone (Table 1). The fold increases in the percentage of apoptotic cells after combination treatment were similar for Hs578T1.2, MCF7-neo/HER2, and MX-1 breast tumor cells (Table 1).

To investigate the mechanism of apoptosis that was induced by combination of GDC-0941 and docetaxel, we conducted time lapse microscopy in the HCC-1954 (Bliss sum = 212) and MCF7-neo/HER2 tumor lines to determine the timing and extent of cell-cycle arrest and appearance of apoptotic markers following drug treatment. We detected apoptotic cells (based on fragmented morphology) with GDC-0941 treatment in HCC-1954 breast tumor cells (Supplementary Fig. S2). However, the majority of cells treated with docetaxel or the combination of GDC-0941 and docetaxel (82% and 100%, respectively) became fragmented after a prolonged mitotic arrest (Fig. 3A; Supplementary Fig. S2). Interestingly, the time in mitotic arrest before fragmentation was significantly reduced when docetaxel was combined with GDC-0941 (P < 0.001). In addition, similar results were obtained in the MCF7-neo/HER2 cell line, albeit to a lesser extent, thereby confirming acceleration of cell death induced by the combination of GDC-0941 and docetaxel (Fig. 3A). The proapoptotic effect of combination drug treatment was caspase-dependent, as cotreatment with a caspase inhibitor, Z-VAD-FMK, reduced the number of fragmented cells and increased the duration of mitotic arrest (Fig. 3A).

Modulation of apoptosis pathway markers by the drug combinations during mitosis was assessed in HCC-1954 cells 12 hours after synchronization release that allowed the cells to progress through S-phase before any treatment. Cells were synchronized by double thymidine block, and after release from synchronization, the cells advanced through the cell cycle and reached mitosis within approximately 24 hours, based on increased levels of phospho-Histone H3 and cyclin B1 (Supplementary Fig. S3). Consistent with our observations made with time-lapsed microscopy, we detected increased cleaved PARP to a greater extent in cells treated with the combination of GDC-0941 and docetaxel 24 hours after synchronization release (Fig. 3B). An increase in pBcl-xLSer62 after docetaxel treatment was also observed, but Mcl-1 protein levels were not affected by either drug treatment (Fig. 3B). The levels of pAktSer473 and

### Table 1. Quantification of apoptotic cells after combination drug treatment

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Hs578T1.2</th>
<th>MCF7-neo/HER2</th>
<th>MX-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Cell death</td>
<td>Fold increase</td>
<td>% Cell death</td>
</tr>
<tr>
<td>GDC-0941</td>
<td>6.6 (±3.0)</td>
<td>1.4</td>
<td>5.8 (±2.2)</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>6.7 (±2.3)</td>
<td>1.4</td>
<td>8.2 (±3.8)</td>
</tr>
<tr>
<td>GDC-0941 + docetaxel</td>
<td>12.2 (±4.1)</td>
<td>2.5</td>
<td>11.4 (±2.6)</td>
</tr>
</tbody>
</table>

NOTE: Fold increase in percentage of apoptotic cells based on Annexin V positivity relative to vehicle (dimethyl sulfoxide) control after treatment for 48 hours with GDC-0941, docetaxel, or the combination of both drugs in Hs578T1.2, MCF7-neo/HER2, and MX-1 tumor cell lines.
Combination of GDC-0941 and docetaxel leads to enhanced antitumor efficacy and apoptosis in vivo

To confirm our in vitro observations that the combination of GDC-0941 with docetaxel led to increased tumor growth inhibition, we evaluated tumor xenograft models that were either PI3Kα-mutant (MCF7-neo/HER2), PTEN-null (MX-1) or PI3Kα wild-type (MAXF1162). Treatment of animals bearing MCF7-neo/HER2 breast cancer xenografts with 7.5 mg/kg docetaxel or 150 mg/kg GDC-0941 led to tumor growth delay and tumor stasis, respectively (Fig. 4A). In contrast, the combination of GDC-0941 and docetaxel resulted in tumor regressions during the treatment period leading to enhanced antitumor responses (Fig. 4A). Single-agent and combination treatments were at maximum tolerated doses based on minimal changes in animal body weights (Fig. 4B). Similar to the MCF7-neo/HER2 xenograft model, we observed greater than additive effects when GDC-0941 was administered in combination with docetaxel in the MX-1 xenograft model resulting in increased tumor regressions during the treatment period (Fig. 4C).

Because the Hs578T1.2 tumor cell line is nontumorigenic in vivo, we evaluated the MAXF1162 patient-derived breast tumor xenograft model, which is HER2+/ER+/PR−, PI3Kα wild-type, and PTEN-positive (Oncotest Inc; personal communication). Treatment of MAXF1162 primary breast tumor xenografts with 15 mg/kg docetaxel in vivo as a single agent resulted in tumor growth delay (Fig. 4D). However, the combination of 100 mg/kg GDC-0941 and docetaxel resulted in tumor stasis during the treatment period that was sustained after dosing ended (Fig. 4D). GDC-0941 and docetaxel were administered at maximum tolerated doses, and no additional change in animal body weights were noted when both drugs were combined (data not shown).

To confirm that the mechanism of action was primarily due to induction of apoptosis, we evaluated the pharmacodynamic activity of both drugs as single agents and in combination. Treatment with 150 mg/kg GDC-0941 alone resulted in decreased levels of pAkt, pPRAS40, and pS6 within 1 hour of administration showing that the drug was pharmacologically active (Fig. 4E). Treatment with 7.5 mg/kg docetaxel had no effect on PI3K pathway suppression, but increased phospho-Histone H3 levels within 24 hours, total time after synchronization release.

**Dosing schedule of GDC-0941 in combination with docetaxel in vivo**

Given that PI3K activity has been described to be necessary for progression through G1, S, and G2 phases...
of the cell cycle (32, 33), we determined whether the combination effects of GDC-0941 and docetaxel were dependent on the order of drug treatment. Increased apoptosis was detected when docetaxel was dosed 1 to 4 hours before GDC-0941 compared with docetaxel or GDC-0941 alone (Fig. 5A). Similarly, when docetaxel was dosed 4 hours before GDC-0941, an increase in the sub-G1 cell population occurred indicative of increased cell death (Supplementary Fig. S4). However, increased apoptosis compared with docetaxel alone was not observed when GDC-0941 was dosed 4 hours before docetaxel (Fig. 5A).

We next investigated whether the drug combination efficacy observed in vivo was dependent on the order of administration. Maximum combination efficacy was observed in the MCF7-neo/HER2 model when GDC-0941 was administered 1 hour before or after docetaxel (Fig. 5B). Interestingly, maximum combination efficacy was also observed when GDC-0941 was dosed 4 hours after docetaxel administration but was abolished when GDC-0941 was dosed 4 hours before or 24 hours after docetaxel administration (Fig. 5C and D). We have previously reported that GDC-0941 has a half-life of approximately 2.5 hours in immunocompromised mice (34).
addition, docetaxel reaches peak plasma concentrations within 1 hour of dosing, which is within the window of time that maximum GDC-0941 drug concentrations are achieved (Supplementary Fig. S5A and S5B). Therefore, given the short half-lives of both docetaxel and GDC-0941 in vivo, maximum combination efficacy in the MCF7-neo/HER2 xenograft model is achieved within 1 hour of dosing both drugs.

Discussion

Docetaxel is frequently used as front-line standard-of-care therapy for breast cancer and has proven to be effective
but patients often become resistant to treatment or are initially refractory (7). As a result, there remains an unmet medical need to identify novel therapeutic agents to provide greater clinical benefit. The PI3K pathway is frequently activated in breast cancer as a result of growth factor receptor activation, HER2 amplification, gain-of-function mutations in PI3K, or loss of PTEN (13–18). In addition, activation of the PI3K pathway has been shown to confer resistance to targeted agents, conventional chemotherapeutics, including taxanes, and radiation therapy (8, 35–38). Thus, targeting PI3K in combination with docetaxel may provide greater therapeutic benefit in the treatment of breast cancer. In support of the latter hypothesis, inhibition of PI3K with nonselective PI3K inhibitors such as LY294002 or wortmannin in combination with paclitaxel enhanced antitumor activity and increased paclitaxel-induced apoptosis in preclinical ovarian cancer models (19, 20). In addition, treatment of oral squamous carcinoma cells with wortmannin or LY294002 enhanced docetaxel-induced apoptosis by regulating the expression and posttranslational modification of both proapoptotic (Bax) and antiapoptotic (Bcl-2, cleaved inhibitor of apoptotic protein (cIAP)-1, and X-linked inhibitor of apoptotic protein [XIAP]) factors, respectively (21). However, given that wortmannin and LY294002 are known to have off-target activities, the specific role of PI3K inhibition in taxane potentiation is not well-defined.

The discovery of potent and selective inhibitors confers an opportunity to pharmacologically dissect the role of PI3K itself in combination with chemotherapeutics. In comparison to single-agent treatments, the combination of a selective class I PI3K inhibitor, GDC-0941, and docetaxel decreased cellular viability in a panel of breast tumor cell lines in vitro but to variable degrees of drug synergy. In addition, diminished cellular viability was also observed in the spontaneously immortalized nontransformed MCF10A human mammary epithelial cells when treated with the combination of both drugs. The latter may be due to a dependence on PI3K, given the cellular growth requirement for insulin and EGF in vitro (39). When benchmarked to the MCF10A cell line, stronger synergy was observed in a subset of tumor cell lines (11 of 25) that was not predicted by breast cancer subtype, PTEN expression, or PI3K mutation (E545K or H1047R) status. The variability in drug synergy suggests that differential biologic responses to treatment may be a reflection of the genetic heterogeneity in each model. In addition, the sensitivity of PI3K wild-type and PTEN-positive breast tumor lines to the drug combinations may be due to other mechanisms of PI3K pathway activation such as those driven by Akt1 mutations, Ras activation, or Erb downmodulation (40–43). The heterogeneity in drug synergy suggests that identification of additional biomarkers of PI3K pathway activation may improve the predictability of responses to GDC-0941 in combination with docetaxel.

The robust drug combination effects observed in a subset of breast tumor lines in vitro translated in vivo in human xenograft models. For example, we observed increased tumor regressions when GDC-0941 was combined with docetaxel in the MCF7-neo/HER2 and MX-1 xenograft model. A combination effect was also observed in a patient-derived breast cancer model (MAXF1162) that was HER2+/ER+/PR+ but PI3K-wild-type and PTEN-positive. The MCF7-neo/HER2 and MAXF1162 xenograft models are resistant to trastuzumab (ref. 25 and Oncotest Inc; personal communication), which suggests that combination therapy of GDC-0941 with docetaxel may be effective in treating tumors that are refractory to anti-HER2 therapy.

Potentiation of docetaxel by GDC-0941 is primarily due to increased apoptosis as a consequence of inefficient escape from mitotic arrest when both drugs are combined. This result in acceleration of the time to mitotic catastrophe and a lowering of the cell death threshold, which may occur when the PI3K survival pathway is inhibited upon treatment with antimitotic drugs as cells attempt to escape mitotic arrest (44). Specific members of the Bcl-2 family that regulate intrinsic apoptosis may govern the cell death mechanisms that are activated by the combination of docetaxel and GDC-0941 during mitosis. For example, phosphorylation of Bcl-xL, which we observed in HCC-1954 breast tumor cells, has been reported to inhibit its prosurvival function after treatment with microtubule-targeted agents including paclitaxel (45, 46). Consistent with the role of Akt in phosphorylating Bad and promoting cell survival (47, 48), we observed decreased levels of pAkt and pBad after GDC-0941 treatment. Therefore, concomitant activation of the proapoptotic BH3-only protein Bad by inhibition of Akt and inactivation of Bcl-xL by docetaxel may rapidly increase cell death during mitosis after combination treatment with both drugs. Interestingly, similar results were obtained with ABT-263 (navitoclax), a Bad mimetic that antagonizes Bcl-xL, in which combination with paclitaxel or docetaxel accelerated apoptosis in epithelial cells during drug-induced mitotic arrest (49, 50).

Our findings with GDC-0941 confirm that selectively inhibiting class I PI3K is sufficient to augment the anti-tumor effects of docetaxel in breast cancer models in vitro and in vivo. The primary mechanism of action for the combination effect is shortening the duration in mitosis resulting in increased cell death. Our preclinical data provide a rationale to evaluate GDC-0941 in combination with docetaxel for the treatment of breast cancer.

Disclosure of Potential Conflicts of Interest
J. Guan and L.S. Friedman have ownership interest (including patents) in Genentech, a member of the Roche group. The other authors disclosed no potential conflicts of interest.

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Study supervision: D. Sampath

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References


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