GDC-0980 is a novel class I PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway

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Disclosure of Potential Conflicts of Interest

Abstract = 250 words, Text < 5000 words, Figures = 6, References = 29
ABSTRACT

Alterations of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway occur broadly in cancer via multiple mechanisms including mutation of the PIK3CA gene, loss or mutation of phosphatase and tensin homolog (PTEN), and deregulation of mammalian target of rapamycin (mTOR) complexes. The dysregulation of this pathway has been implicated in tumor initiation, cell growth and survival, invasion and angiogenesis, thus, PI3K and mTOR are promising therapeutic targets for cancer. We discovered GDC-0980, a selective, potent, orally bioavailable inhibitor of Class I PI3 Kinase and mTOR kinase (TORC1/2) with excellent pharmacokinetic and pharmaceutical properties. GDC-0980 potently inhibits signal transduction downstream of both PI3K and mTOR, as measured by pharmacodynamic (PD) biomarkers, thereby acting upon two key pathway nodes to produce the strongest attainable inhibition of signaling in the pathway. Correspondingly, GDC-0980 was potent across a broad panel of cancer cell lines, with the greatest potency in breast, prostate, and lung cancers and less activity in melanoma and pancreatic cancers, consistent with KRAS and BRAF acting as resistance markers. Treatment of cancer cell lines with GDC-0980 resulted in G1 cell cycle arrest, and in contrast to mTOR inhibitors, GDC-0980 induced apoptosis in certain cancer cell lines, including those with direct pathway activation via PI3K and PTEN. Low doses of GDC-0980 potently inhibited tumor growth in xenograft models including those with activated PI3K, loss of LKB1 or PTEN, and elicited an exposure-related decrease in PD biomarkers. These preclinical data demonstrate that GDC-0980 is a potent and
effective dual PI3K/mTOR inhibitor with promise for the clinic.
Introduction

Upregulation of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway is a common feature in most cancers (reviewed in (1)). Genetic deviations in the pathway have been detected in many human cancers (2) and act primarily to stimulate cell proliferation, migration and survival. Activation of the pathway occurs following activating point mutations or amplifications of the \( PIK3CA \) gene encoding the p110\( \alpha \) PI3K isoform (3, 4). Genetic deletion or loss of function mutations within the tumor suppressor PTEN, a phosphatase with opposing function to PI3K, also increases PI3K pathway signaling (5). These aberrations lead to increased downstream signaling through kinases such as Akt and mTOR and increased activity of the PI3K pathway has been proposed as a hallmark of resistance to cancer treatment (6-10).

Oncogenes such as PI3K, AKT, epidermal growth factor receptor (EGFR) and human epithelial growth factor receptor 2 (HER2) stimulate proliferation, growth and survival by activating mTOR kinase (reviewed in (11)). mTOR kinase activity has been attributed to two protein complexes, mTORC1 and mTORC2 (12). Rapamycin specifically disrupts the mTORC1 complex and rapalogs have activity in renal cancers though patients all progress eventually, which may be due, in part, to feedback to IRS-1 and activation of AKT (reviewed in (13)). Therefore, inhibiting both mTOR complexes with an mTOR kinase inhibitor is predicted to counter feedback by blocking the PDK2 function of mTORC2. Additionally, inhibiting PI3K in the same molecule creates a blockade higher in the pathway where IRS1 signals to PI3K.
Therapeutic targeting of the PI3K pathway with small molecule inhibitors may have clinical benefit, either as single agents in PI3K-addicted cancers or used more broadly in combination with other conventional or targeted therapies. Several inhibitors targeting the PI3K pathway have now entered clinical trials (14-16). Here we describe preclinical data for the novel PI3K/mTOR inhibitor GDC-0980. We show that GDC-0980 potently inhibits pathway signaling and viability in the majority of solid tumor cancer cell lines investigated. Furthermore we show that GDC-0980 is efficacious in the majority of xenograft models investigated. The pharmacokinetic properties of GDC-0980 allow for intermittent in vivo dosing without sacrificing anti-tumor efficacy.

**Materials and Methods**

**Cell culture.** Cell lines were obtained from the American Type Culture Collection (ATCC, VA) or from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DMSZ, Germany). Cell lines were tested and authenticated using gene expression and single nucleotide polymorphism genotyping arrays, as previously described (17, 18). Lines were cultured in DMEM or RPMI supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C under 5% CO2. MCF7-neo/HER2 is an in vivo selected tumor cell line developed at Genentech and derived from the parental MCF7 human breast cancer cell line.

**Materials.** GDC-0941 and GDC-0980 were generated at Genentech, Inc. (South San Francisco, CA). mTOR1/2 inhibitor is from patent WO 2008/023159
A1. Antibodies used include phospho-Akt\textsuperscript{Thr308}, phospho-AK\textsuperscript{Ser473}, AKT, phospho-PRAS40\textsuperscript{Thr246}, phospho-S6\textsuperscript{Ser235/236}, phospho-S6\textsuperscript{Ser240/242}, S6, phospho-ERK\textsuperscript{Thr202/Tyr204}, ERK, cleaved PARP and cyclin D1 obtained from Cell Signaling (Danvers, MA) and a βActin antibody was obtained from Sigma (St. Louis, MO).

Mutation detection. Screening for mutation was done by sequencing of exons and adjacent intronic junctions (Polymorphic DNA Technologies, Inc., CA). Primers for PCR and sequencing were designed using the primer3 program (Integrated DNA Technologies, Inc. CA). Nested PCR products were treated with ExoSAP from USB (Santa Clara, CA). PCR products were sequenced using BigDye Terminator Mix using an ABI3730xl (Applied Biosystems, Foster City, CA). Trace files were analyzed using the software program Agent (Paracel, Pasadena, CA), Sequencher (Genecodes, Ann Arbor, MI) and Mutation Surveyor 3.0 (SoftGenetics, State College, PA).

Cell viability assays. 384-well plates were seeded with 2000 cells/well in a volume of 54 μl per well followed by incubation at 37°C under 5% CO\textsubscript{2} overnight (~16 hours). Compounds were diluted in DMSO to generate the desired stock concentrations then added in a volume of 6 μL per well. All treatments were tested in quadruplicate. After 4 days incubation, relative numbers of viable cells were estimated using CellTiter-Glo (Promega, Madison, WI) and total luminescence was measured on a Wallac Multilabel Reader (PerkinElmer, Foster City, CA). The concentration of drug resulting in 50% inhibition of cell viability (IC\textsubscript{50}) or 50% maximal effective concentration (EC\textsubscript{50}) was
determined using Prism software (GraphPad, La Jolla, CA). For cell lines that failed to achieve an IC50 the highest concentration tested (20 μM) is listed.

**Protein assays.** 10 cm² dishes were seeded with two million cells in a volume of 10 mL followed by incubation at 37°C under 5% CO2 overnight (~16 hours). Cells were treated with the indicated concentration of GDC-0941, GDC-0980 or mTOR1/2 inhibitor for the time indicated. Following treatment, cells were washed with cold PBS and lysed in 1X Cell Extraction Buffer from Biosource (Carlsbad, CA) supplemented with protease inhibitors (Roche, Germany), 1mM PMSF, and Phosphatase Inhibitor Cocktails 1 and 2 from Sigma (St. Louis, MO). Protein concentration was determined using the Pierce BCA Protein Assay Kit (Rockford, IL). For immunoblots, equal protein amounts were separated by electrophoresis through NuPage Bis-Tris 10% gradient gels (Invitrogen, Carlsbad, CA); proteins were transferred onto PVDF membranes using the Criterion system and protocol from Bio-Rad (Hercules, CA).

Levels of phospho-AktSer473 and total Akt were assessed in xenograft tumors using biomarker kits from Meso Scale Discovery (Gaithersburg, MD).

**Xenograft studies.** In vivo efficacy of compounds was evaluated in the following cancer cell line tumor xenograft models: breast cancer cell lines MX-1, MCF7, MDA-MB-231, Cal-51, MCF7-neo/HER2, KPL4, the Fo5 HER2+ mouse transplant model, and the MAXF1162 primary human transplant model (Oncotest; Freiburg, Germany); NSCLC lines - NCI-H1299, NCI-H2122, A549 LXFL529 (Oncotest; Freiburg, Germany); pancreatic cancer cell lines - KP4 and MiaPaCa-2; colon cancer cell lines - HCT-116, Colo205, LoVo and DLD-1;
prostate cancer lines - LuCap35V and PC3; melanoma line A375. Cells or tumor fragments were implanted subcutaneously into the flank of each mouse. Prior to cell inoculation with MCF7 or MCF7-neo/HER2 cells, 17 b-estradiol pellets (0.36 mg/pellet, 60-day release, No. SE-121; Innovative Research of America (Sarasota, FL)) were implanted into the dorsal shoulder. After implantation of cells into mice, tumors were monitored until they reached mean tumor volumes of 180 to 350 mm$^3$ and were distributed into groups of no less than 8 animals/group ensuring each group had equivalent mean tumor volumes prior to initiating dosing. Female nude (nu/nu, athymic nude or NMRI nude) mice that were 6-8 weeks old and weighed 20-30 g were obtained from Charles River Laboratories (Hollister, CA), Harlan Laboratories (Somerville, NJ) or Taconic (Hudson, NY). GDC-0980 was formulated at various concentrations in 0.5% methylcellulose with 0.2% Tween-80 (MCT) to achieve the indicated dosages and was administered daily, every 4 days, or weekly with 100 μL via oral gavage. Docetaxel (Sanofi Aventis; Bridgewater, NJ) was dosed at 5 or 10 mg/kg, intraperitoneally, every week. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc., Newton, MA) using the formula ($L \times W \times \frac{W}{2}$). Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that %TGI = 100 x 1 – $rac{(AUC_{treatment/day} - AUC_{vehicle/day})}{AUC_{vehicle/day}}$. Curve fitting was applied to Log$_2$ transformed individual tumor volume data using a linear mixed-effects model using the R package nlme, version 3.1-97 in R v2.12.0.
Tumor sizes and body weights were recorded twice weekly over the course of the study. Mice with tumor volumes $\geq 2000 \text{ mm}^3$ or with losses in body weight $\geq 20\%$ from their body weight at the start of treatment were euthanized per IACUC guidelines.

For pharmacodynamic marker analysis, PC3 or MCF7-neo/HER2 xenograft tumors were excised from animals and immediately snap frozen in liquid nitrogen. Frozen tumors were weighed and processed using a pestle (Scienceware; Pequannock, NJ) in Lysis Buffer (see above). Levels of phospho-Akt$^{\text{Ser473}}$ and total Akt were assessed in xenograft tumors using biomarker kits from Meso Scale Discovery (Gaithersburg, MD).

**Statistics.** Significant differences (p values) comparing lines with and without evaluated genetic abnormalities was determined by two-tailed Mann-Whitney test calculated using the JMP statistical software, version 5.1.2 (JMP Software, Cary, NC).

**Results**

**GDC-0980 reduces viability in cancer cell lines by cell cycle inhibition and induction of apoptosis**

GDC-0980 is a potent small-molecule inhibitor of class I PI3K isoforms and mTOR kinase and displays excellent selectivity against a large panel of other kinases, including closely related family members DNA-PK, VPS34, c2alpha and c2beta (Figure 1 and (19)). We assessed a panel of breast, non-small-cell lung, colon, pancreatic, melanoma, and prostate cancer cell lines in cell viability.
experiments to profile the activity of GDC-0980 in vitro (Figure 1). GDC-0980 was most effective in prostate (IC\textsubscript{50} <200 nM 50\%), <500 nM 100\%), breast (IC\textsubscript{50} <200 nM 37\%, <500 nM 78\%) and non-small-cell lung (NSCLC) lines (IC\textsubscript{50} <200 nM 29\%, <500 nM 88\%), and was less effective in pancreatic (IC\textsubscript{50} <200 nM 13\%, <500 nM 67\%) and melanoma cell lines (IC\textsubscript{50} <200 nM 0\%, <500 nM 33\%). Taken together, GDC-0980 had broad cellular activity with potency below 500 nM in 124 out of the 167 cell lines tested.

GDC-0941 is a potent inhibitor of class I PI3K isoforms, but not mTOR and is also currently being evaluated in clinical trials (14, 20). Overall, GDC-0980 was more effective than GDC-0941 in cell viability experiments (Figure 1). The increased potency of GDC-0980 was evident across all evaluated tumor tissue types (p < 0.001) and all genotypes except those that are HER2 amplified (Supplemental Figure 1).

Cell viability IC\textsubscript{50} values and half-maximal effective concentrations (EC\textsubscript{50}) values for rapamycin are different due to the shallow dose-titration curves (Supplemental Figure 2). Consistent with previous reports (21), we found that direct inhibition of the mTOR kinase and PI3K was more effective than rapamycin treatment alone.

To investigate potential molecular predictors of response to GDC-0980, we examined whether key alterations in the PI3K and MAPK pathways such as HER2 amplification, PIK3CA mutations, loss of PTEN protein, LKB1 inactivating mutations, deletions or loss, EGFR activating mutations, RAS hotspot mutations, or BRAF hotspot mutations were associated with increased or decreased
sensitivity to GDC-0980 (Supplemental Tables 2-8). Across all tumor types hotspot mutations within BRAF or RAS proteins were negative predictors of GDC-0980 potency \( (p = 0.01) \), while no significant positive predictors of potency were identified. Across all lines a trend was observed for PTEN loss (PTEN (-)) association with GDC-0980 potency, but this was not statistically significant \( (p = 0.07) \). Sensitivity of breast tumor cell lines to GDC-0980 was similar to what has been previously described for the class I PI3K inhibitor GDC-0941 \( (22) \). Breast tumor cell lines harboring mutations in the \textit{PIK3CA} gene exhibit increased sensitivity to GDC-0980 \( (p = 0.01) \). In NSCLC lines, mutations in BRAF or RAS proteins approached significance \( (p = 0.07) \) as negative predictors of GDC-0980 sensitivity.

We next examined signaling components of the PI3K signaling network (figure 2A) in cell lines representing each of these six tumor tissue types. In each cell line treated with GDC-0980 for 4 hours, phosphorylation of all downstream markers was reduced (figure 2B). These included signaling markers downstream of PI3K, such as phospho-Akt (Thr308), which is phosphorylated by PDK1, and biomarkers downstream of mTOR such as phospho-Akt (Ser473) and phospho-S6 (Figure 2A). We also assessed phospho-ERK (Thr202/Tyr204) in GDC-0980 treated samples and observed a slight reduction of this marker in two of six cell lines.

To determine the downstream molecular consequences of PI3K pathway inhibition by GDC-0980 on cancer cells, we assessed markers of cell cycle, cyclin D1, and apoptosis, cleaved-poly (ADP-ribose) polymerase (cleaved-
PARP), 24 hours after GDC-0980 treatment (Figure 2C). Cyclin D1 is expressed in proliferating cells and helps control progression of cells through the cell cycle (23). A dose dependent reduction in cyclin D1 levels in response to GDC-0980 was observed in most cancer lines, with the exception of the pancreatic cell line KP4 and the melanoma line A375 which were both less responsive to GDC-0980 in the viability assay (Figure 1). PARP is one of the main cleavage targets of caspase-3 and cleaved PARP serves as a marker for apoptotic cells (24). Cleaved PARP was detected following GDC-0980 treatment in both the KPL4 (HER2+, PI3K^{H1047R}) and LoVo (KRas^{G13D}) cell lines, indicating that an apoptotic response had been triggered in these cells as early as 24 hours.

**Inhibition of mTOR does not augment apoptotic responses**

We utilized inhibitors with different target specificities to investigate the signaling and apoptotic consequences of solely blocking PI3K, solely blocking mTOR, or dual blockade of both. In these studies, GDC-0980 was compared to GDC-0941 PI3K inhibitor as well as an mTOR kinase inhibitor. To evaluate the effects of inhibition of mTOR kinase we used mTOR kinase inhibitor, an ATP-competitive selective small molecule inhibitor of TORC1 and TORC2 (25). The potency (Ki) against mTOR kinase for GDC-0980, GDC-0941 and mTOR1/2 inhibitor is 17 nM, 580 nM, and 5 nM, respectively. For these studies, single agent treatments with the three inhibitors were used in the KPL4 PI3K mutant breast cancer cell line, at concentrations ranging from 78 nM to 5 μM (Figure 3). At four hours of treatment, all three inhibitors had reduced phospho-Akt (Ser473) levels. The
phospho-S6 (Ser235/236) reduction was strongest for GDC-0980. GDC-0941 resulted in a reduction of both phospho-S6 sites at 4 hours, but the pS6 decrease was not as significant as phospho-Akt. By 24 hours phospho-S6 (Ser240/242) levels were strongly decreased with GDC-0941 treatments. GDC-0980 was most effective against all four phosphorylation markers of pathway activity, indicating a more complete blockade was quickly achieved and sustained with the PI3K/mTOR inhibitor.

To investigate the consequences of inhibiting the pathway at different nodes, we looked at the marker of apoptosis cleaved-PARP at 4 and 24 hours post-treatment in the KPL4 (PI3K<sup>H1047R</sup>) cell line and at 24 hours in EVSA-T (PTEN (-)) and HDQ-P1 cell lines. We detected a difference between mTOR inhibitor and PI3K inhibitors in their ability to induce apoptosis (Figure 3). An increase in cleaved PARP was minimally detected with mTOR inhibition at any concentration, but an increase of cleaved-PARP was observed in a dose-dependent manner with GDC-0941 or GDC-0980 treatments in all three lines, regardless of genotype. Thus mTOR kinase inhibition alone was not enough to induce apoptosis, while PI3K inhibition was sufficient.

**Oral dosing of GDC-0980 results in significant antitumor responses in xenograft models on daily or intermittent schedules**

Pharmacokinetic (PK) studies indicated that GDC-0980 has excellent PK properties in mouse, with high oral bioavailability, low to moderate clearance and low plasma protein binding, consistent with its solubility and pharmaceutical
properties (Supplemental Table 1, Supplemental Figure 4, and (19)). The in vivo anti-tumor activity of GDC-0980 was investigated in 20 xenograft tumor models representative of six different human tumor types (Figure 4A). For these studies, mice bearing established tumors (7-10 days post-implantation, tumor volume 150-250 mm$^3$) were dosed orally with GDC-0980 at 5 mg/kg GDC-0980 daily for 21 days. The 5 mg/kg dose was approximately 70% of the maximum tolerated dose based on body weight loss. A low dose of GDC-0980 was sufficient to generate significant efficacy because of the high exposure achieved following oral dosing and the high free fraction. The data in figure 4A is represented as percent tumor growth inhibition (%TGI) at the end of the dosing period, with 100% TGI indicating tumor stasis. Overall, dosing GDC-0980 at 5 mg/kg daily resulted in greater than 50% TGI in 15 of the 20 xenograft models. The weakest in vivo effects were observed in the melanoma A375 (BRAF$^{V600E}$) model, triple negative breast cancer MX-1 (PTEN (-)) model, and two pancreatic KRAS mutant xenograft models, KP4 (KRAS$^{G12C}$) and MiaPaCa2 (KRAS$^{G12C}$). Consistent with in vitro data in other cell lines, we detected cleaved-PARP in two MCF7-neo/HER2 xenograft tumors 4 hours after the last dose (Figure 2B).

The possibility of changing dose and schedule may enhance the ability to balance tolerability and activity in the clinic. In the PC3 xenograft model dose-dependent tumor growth inhibition resulted from GDC-0980 administered orally either daily, every four days, or on a once weekly schedule (Figure 4C). Tumor growth delay was observed on each schedule, and was dose-dependent.
The PTEN null PC3 prostate cancer xenograft was used to investigate the relationship of pharmacokinetic (PK), pharmacodynamic (PD), and efficacy of GDC-0980 (Figure 5). In mice that were administered GDC-0980 daily, tumor growth delay occurred at 2.5 mg/kg, tumor stasis at 5 mg/kg, and tumor regression at 10 mg/kg (Figure 5A). Single dose GDC-0980 PK/PD relationships were evaluated in animals with established PC3 tumors. In these studies, GDC-0980 was dosed at 2.5, 5, or 10 mg/kg and plasma concentrations of GDC-0980 and tumor levels of pAkt were evaluated over 48 hours. As expected, an increase in unbound GDC-0980 was detected in the plasma, and drug concentration decreased over time (Figure 5B). Phosphorylated Akt at the Ser473 site was evaluated in tumors and normalized with total Akt protein (pAkt/tAkt) at the same timepoints. All three doses of GDC-0980 reduced pAkt/tAkt levels significantly for the first 8 hours, and an unbound GDC-0980 concentration of at least 0.2 μM was required to decrease this marker by at least 75%. By 48 hours unbound GDC-0980 was not detected in any of the dose groups. Relationships between efficacy, GDC-0980 plasma levels and tumor pAkt/tAkt levels can be described by assessing the data at 24 hours. In the 2.5 mg/kg cohort, a dose that results in tumor growth delay in this xenograft model, GDC-0980 plasma levels were undetectable and pAkt/tAkt levels had returned to baseline at 24 hours. At the 5 mg/kg dose, which causes tumor stasis, unbound GDC-0980 was minimally measurable in the plasma at the 24 hour timepoint, indicating the molecule was clearing. As a consequence, the pAkt/tAkt signal in the xenograft tumor was starting to return to baseline, with approximately 60%
reduction at 24 hrs. At a GDC-0980 dose of 10 mg/kg, which causes tumor regression, GDC-0980 was measureable in the plasma at 24 hours and pAkt/tAkt levels were decreased by 75%. Thus, the difference in tumor response to GDC-0980 treatment correlated with the duration of knockdown of pAkt/tAkt, and is most evident when assessing plasma PK and tumor PD effects at 24 hours after drug dosing.

**GDC-0980 enhances the antitumor activity of docetaxel in vivo.**

Resistance to anti-cancer agents has been attributed to increased PI3K/PTEN pathway signaling to promote cell survival, hence inhibitors of the pathway have potential utility in combination with these therapies (26). The chemotherapeutic docetaxel (DTX) is an established treatment for a variety of cancers, and interferes with cell division by binding to microtubules (27, 28). The in vitro combination of GDC-0980 and DTX at their IC\textsubscript{50} concentrations caused an increase in apoptosis compared to either agent alone (Supplemental Figure 3). Anti-tumor efficacy of GDC-0980 was investigated in vivo in three xenograft models in combination with DTX: 1) MX-1 is a breast cancer model that lacks PTEN protein expression, 2) A549 is a NSCLC model that contains an activating K-RAS mutation and is negative for LKB1 protein expression, 3) MCF7-neo/HER2 is a breast cancer model that overexpresses HER2 and harbors the PIK3CA E545K hot-spot mutation. In these in vivo studies DTX was administered intravenously once per week (QW) for three weeks while GDC-0980 was dosed orally daily (QD). Single agent GDC-0980 and single agent
DTX treatments caused tumor growth inhibition characterized by either stasis or tumor growth delay in all xenograft models tested. However, the combination of GDC-0980 and DTX resulted in tumor regressions in all 3 xenograft model evaluated (Figure 6A-C). Most notably, the combination treatment of GDC-0980 and DTX in the MCF7-neo/HER2 xenograft model resulted in a 90% objective response rate based on the number of partial and complete regressions (Figure 6C). This enhanced efficacy in combination occurred at well-tolerated doses with no significant weight loss in all 3 xenograft models tested (Figure 6A-C).

Discussion

The PI3K/PTEN pathway is a central signaling network that is perturbed in a number of cancers, thus PI3K pathway inhibitors are currently being evaluated in clinical trials in patients with advanced solid tumors (16). GDC-0980 is a novel, potent small molecule inhibitor with selectivity for class I PI3K and mTOR kinase, and has recently entered clinical trials. In the preclinical studies presented here, GDC-0980 strongly inhibited the PI3K pathway and decreased tumor cell viability in a broad set of cancer cell lines. Across all tumor types mutations in BRAF or RAS were negative predictors of GDC-0980 potency. Cell lines that have BRAF or RAS mutations may be more resistant to GDC-0980 because they are more dependent on MAPK pathway signaling. The additional property of inhibiting mTOR kinase in addition to PI3K may be responsible for the greater cell potency of GDC-0980 relative to GDC-0941. Recent studies have shown that inhibition of mTOR kinase can result in strong anti-proliferative effects in cancer lines (29).
Viability changes were reflected with both cell cycle and apoptotic effects produced by GDC-0980, while apoptosis was not observed with an mTOR kinase inhibitor, indicating that PI3K is sufficient for cell survival. Interestingly, despite the trend for mutations in BRAF or RAS as negative predictors for response to GDC-0980 potency, there are certain KRAS mutant lines (e.g. LoVo) where GDC-0980 induces apoptosis in cultured cells that correspond to strong tumor growth inhibition in vivo. Thus the negative predictive value may lie in cancer types such as pancreatic or melanoma or other predictors of sensitivity may be required in addition to RAS and RAF status. GDC-0980 has dose-dependent exposure that is consistent with good solubility and absorption. In xenograft models the knockdown of pathway signaling for 8-24 hours resulted in the strongest efficacy, and PD biomarker knockdown was inversely correlated with plasma concentrations of GDC-0980. The dual nature of GDC-0980 as a blockade of two nodes in the PI3K signaling network resulted in the strongest knockdown of phospho-protein markers when compared to either a Class I PI3K inhibitor GDC-0941, or an mTOR kinase inhibitor. This effective dual blockade of the pathway by GDC-0980 also resulted in compelling potency of <200 nM in 25% of cancer cell lines, and significant in vivo efficacy of >50% TGI in 15 of 20 xenograft tumor models. In addition, GDC-0980 and docetaxel combined to enhance the anti-tumor efficacy in tumor xenograft models. In summary, GDC-0980 is a potent small molecule inhibitor of class I PI3K and mTOR kinase with promise for clinical trials in cancer as either a single agent or in combination with anti-mitotic agents.
Acknowledgements

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References

FIGURE LEGENDS:

**Figure 1.** Potency of GDC-0980 PI3K/mTOR inhibitor in biochemical and cell based assays. (A) GDC-0980 IC50 for PI3K alpha, beta, delta, and gamma isoforms, and Ki for mTOR kinase. (B) GDC-0980 and GDC-0941 (class I PI3K inhibitor) IC50 values were determined in a 96-hour viability assay in breast, NSCLC, pancreatic, colon, melanoma and prostate cancer lines. Cell lines have been categorized into the tumor tissue types from which they were derived. Any detected mutation for PIK3CA, RAS, BRAF, EGFR or LKB1 is indicated by a colored square. PTEN (-) indicates a non-detectable signal for this protein by Western blot.

**Figure 2.** Cell pathway and mechanistic effects of GDC-0980 treatment in tumor cell lines. (A) GDC-0980 decreases PI3K pathway signaling in all tumor cell lines. Immunoblots from 4-hour-treated samples showing protein concentrations for phospho-AktThr308 (pAkt<sup>Thr308</sup>), phospho-AktSer473 (pAkt<sup>Ser473</sup>), Akt, phospho-PRAS40Thr246 (pPRAS40<sup>Thr246</sup>), phospho-S6RPSer235/236 (phospho-S6<sup>Ser235/236</sup>) and S6. (B) Disparate apoptosis and cell cycle effects with GDC-0980 treatment. Protein concentrations of apoptosis marker cleaved PARP and cell cycle marker cyclin D1 after 24 hours. (C) PI3K pathway signaling and GDC-0980 treatment.

**Figure 3.** Pharmacodynamic and apoptotic responses to PI3K pathway node targeted small molecule inhibitors. (A) Immunoblots from 4-hour and 24-hour-treated KPL4 cell samples for PI3K pathway proteins phospho-Akt (pAkt<sup>Ser473</sup>), Akt, phospho-S6 (phospho-S6<sup>Ser235/236</sup> and phospho-S6<sup>Ser240/242</sup>), S6 and the apoptotic marker cleaved PARP. Small molecule inhibitors started at 5 μM and
were dose-titrated with two-fold dilutions. (B) Immunoblots from 24-hour-treated EVSA-T and HDQ-P1 cell samples. Treatments are identical to figure 3A.

**Figure 4.** Single agent efficacy of GDC-0980 in human xenograft tumor models. (A) Daily dosing of GDC-0980 in multiple tumor xenograft models. Tumor-bearing mice were dosed orally with GDC-0980 (5 mg/kg) or vehicle each day for 21 days. Data is represented as %TGI (percent tumor growth inhibition) and is a comparison of the 5 mg/kg GDC-0980 treatment groups to vehicle at day 21. The higher the %TGI value, the more significant level of efficacy that was achieved in the study. One hundred %TGI is indicative of complete tumor stasis. Tumor growth delay increases from zero to one hundred %TGI. (B) Tumors treated with GDC-0980 undergo apoptosis. MCF7-neo/HER2 tumor bearing mice were treated 4 hours with vehicle or GDC-0980 at 7.5 mg/kg. Four xenograft tumors are represented in each group. (C) GDC-0980 is efficacious when dosed intermittently. PC3 tumor-bearing mice were dosed orally on different treatment schedules with vehicle or different amounts of GDC-0980 each day for 21 days. Data is represented as %TGI as in figure 4A.

**Figure 5.** GDC-0980 efficacy is linked to pharmacodynamic and pharmacokinetic readouts. (A) Dose proportional response to GDC-0980 treatment in PC3 human xenograft tumors. Tumor-bearing mice were dosed orally with vehicle or GDC-0980 daily (QD) for 14 days at the concentrations indicated. (B) Increases in GDC-0980 free drug concentration are linked to reductions in PI3K pathway signaling. Tumor-bearing mice were dosed orally with vehicle or GDC-0980 at the concentrations indicated. Phospho-AktSer473 and total Akt protein concentrations were determined by ELISA in xenograft tumors in a timecourse after dosing. Data for proteins is shown as a ratio of phospho-AktSer473 to total
Akt (pAkt/tAkt, left axis, gray bars). Free drug concentration for GDC-0980 (GDC-0980 Unbound, right axis, blue triangles) is also indicated.

**Figure 6.** Efficacy of GDC-0980 with docetaxel in human tumor xenograft models. (A) Increases in efficacy in MX-1 xenografts when GDC-0980 is dosed in combination with docetaxel (DTX). GDC-0980 was dosed orally at 5 mg/kg daily (QD) as a single agent or in combination with DTX. In this study DTX was dosed weekly (QW) at 5 mg/kg. (B) A549 xenograft study with single agent GDC-0980 at 4 mg/kg QD, DTX at 10 mg/kg QW, and the combination. (C) MCF7-neo/HER2 tumors treated with GDC-0980 at 4 mg/kg QD, DTX at 7.5 mg/kg QW, or the drugs in combination.
A.

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B.

- **Breast**
- **Pancreatic**
- **NSCLC**
- **Colon**
- **Prostate**
- **Melanoma**

- **GDC-0941**
- **GDC-0980**

**PIK3CA Mutation**
- **PTEN (-)**
- **HER2**
- **RAS or BRAF Mutation**

**LKB1 Mutation/Deletion**
- **BRAF Mutation**
- **PTEN (-)**
A.

Growth factor receptors

PI3K

PIP

PIP

PTEN

GDC-0980 (mM): 0 0.1 0.5 0 0.1 0.5

Proliferation

Survival

Protein Synthesis and Growth

B.

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<tr>
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<th>KPL4</th>
<th>H2122</th>
<th>KP4</th>
<th>LoVo</th>
<th>PC3</th>
<th>A375</th>
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<td>KRAS G12D</td>
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<td>PTEN (-)</td>
<td>BRAF V600E</td>
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C.

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</tbody>
</table>
3.

A. KPL4 (PI3K H1047R), 4 Hours

B. EVSA-T (PTEN-)

KPL4 (PI3K H1047R), 24 Hours

HDQ-P1
A.

![Graph showing GDC-0980 %TGI for various cell lines with different mutations and treatments.]

- **Mutation Types:**
  - PI3K Mutation
  - PTEN (-)
  - HER2
  - RAS or BRAF Mutation
  - LKB1 Mutation/Deletion

- **Cell Lines:**
  - MX-1
  - MDA-MB-231
  - Cal51
  - MCF-7-neo/HER2
  - Fo5
  - MAX:1102
  - KP4
  - H1299
  - H2122
  - A549
  - A375
  - MiaPaca2
  - Cal51
  - KPL4
  - LuCap35V
  - PC3

B.

![Image showing Cleaved PARP and β-Actin with Treatment and Tumor conditions.]

- **Treatment:**
  - Vehicle
  - GDC-0980

- **Cell Lines:**
  - 1
  - 2
  - 3
  - 4

- **Conditions:**
  - Tumor
  - Vehicle
  - GDC-0980

C.

![Bar graph showing GDC-0980 %TGI across different doses and schedules.]

- **Doses:**
  - 0.25 mg/kg
  - 0.5 mg/kg
  - 1.0 mg/kg
  - 2.5 mg/kg
  - 5.0 mg/kg
  - 10 mg/kg
  - 15 mg/kg

- **Schedules:**
  - Daily
  - Every 4 Days
  - Weekly
A.

![Graph A](image1.png)

B.

![Graph B](image2.png)
Molecular Cancer Therapeutics

GDC-0980 is a novel class I PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway

Jeffrey J. Wallin, Kyle A Edgar, Jane Guan, et al.

Mol Cancer Ther  Published OnlineFirst October 13, 2011.

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