Antitumor Efficacy Profile of PKI-402, a Dual Phosphatidylinositol 3-Kinase/Mammalian Target of Rapamycin Inhibitor

Robert Mallon1, Irwin Hollander1, Larry Feldberg1, Judy Lucas1, Veronica Soloveva3, Aranapakam Venkatesan2, Christoph Dehnhardt2, Efren Delos Santos2, Zecheng Chen2, Osvaldo dos Santos2, Semiramis Ayral-Kaloustian2, and Jay Gibbons1

Abstract

PKI-402 is a selective, reversible, ATP-competitive, equipotent inhibitor of class I phosphatidylinositol 3-kinases (PI3K), including PI3K-α mutants, and mammalian target of rapamycin (mTOR; IC50 versus PI3K-α = 2 nmol/L). PKI-402 inhibited growth of human tumor cell lines derived from breast, brain (glioma), pancreas, and non-small cell lung cancer tissue and suppressed phosphorylation of PI3K and mTOR effector proteins (e.g., Akt at T308) at concentrations that matched those that inhibited cell growth. In MDA-MB-361 [breast: Her2+ and PIK3CA mutant (E545K)], 30 nmol/L PKI-402 induced cleaved poly(ADP-ribose) polymerase (PARP), a marker for apoptosis. In vivo, PKI-402 inhibited tumor growth in MDA-MB-361, glioma (U87MG), and lung (A549) xenograft models. In MDA-MB-361, PKI-402 at 100 mg/kg (daily for 5 days, one round) reduced initial tumor volume of 260 mm3 to 129 mm3 and prevented tumor regrowth for 70 days. In MDA-MB-361 tumors, PKI-402 (100 mg/kg, single dose) suppressed Akt phosphorylation (at T308) and induced cleaved PARP. Suppression of phosphorylated Akt (p-Akt) was complete at 8 hours and still evident at 24 hours. Cleaved PARP was evident at 8 and 24 hours. In normal tissue (heart and lung), PKI-402 (100 mg/kg) had minimal effect on p-Akt, with no detectable cleaved PARP. Preferential accumulation of PKI-402 in tumor tissue was observed. Complete, sustained suppression of Akt phosphorylation may cause tumor regression in MDA-MB-361 and other xenograft models. We are testing whether dual PI3K/mTOR inhibitors can durably suppress p-Akt, induce cleaved PARP, and cause tumor regression in a diverse set of human tumor xenograft models. Mol Cancer Ther; 9(4); 976–84. ©2010 AACR.

Introduction

Phosphatidylinositol 3-kinase (PI3K)-α plays a key role in the biology of human cancer. PI3K-α is a lipid kinase that is a central component in the PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway. This pathway regulates cell proliferation, growth, survival, and apoptosis (1, 2). The deregulated activation of PI3K-α and its downstream effectors, including Akt and mTOR, has been linked to tumor initiation and maintenance. PI3K/Akt/mTOR pathway activation can be caused by loss of PTEN (the phosphatase that regulates PI3K signaling), overexpression or activation of some receptor tyrosine kinases [e.g., epidermal growth factor receptor (EGFR) and HER-2], interaction with activated Ras, overexpression of the PI3K-α gene (PIK3CA), or mutations in PIK3CA that cause elevated PI3K-α kinase activity (1–3). Aberrantly elevated PI3K/Akt/mTOR pathway signaling has been implicated in poor prognosis and survival in patients with various lymphatic tumors, as well as breast, prostate, lung, glioblastoma, melanoma, colon, and ovarian cancers (1–4). Additionally, PI3K/Akt/mTOR pathway activation contributes to resistance of cancer cells to both targeted anticancer therapies and conventional cytotoxic agents (5, 6). An effective inhibitor of the PI3K/Akt/mTOR pathway could both prevent cancer cell proliferation and induce programmed cell death (apoptosis; refs. 1, 2, 5). Therefore, the quest of the Wyeth PI3K inhibitor discovery project was to identify potent small-molecule inhibitors of the PI3K/Akt/mTOR signaling pathway, which we hypothesized would exert antitumor activity in a broad array of preclinical tumor models.

PKI-402 is an example of small molecules we have identified as potent inhibitors of the PI3K/mTOR signaling pathway. PKI-402 is a reversible, ATP-competitive, and equipotent inhibitor of class I PI3Ks, including the E545K and H1047R PI3K-α mutants, and mTOR (IC50...
versus PI3K-α = 2 nmol/L). Selectivity of PKI-402 was established in a screen against 236 diverse human kinases. PKI-402 caused *in vitro* growth inhibition of human tumor cell lines derived from a diverse set of human tumor tissues, including breast, brain (glioma), pancreas, and non–small cell lung cancer (NSCLC) tissues. *In vitro*, PKI-402 suppressed phosphorylation of PI3K and mTOR effector proteins, particularly phosphorylated Akt (p-Akt) at T308, at concentrations that closely matched those that inhibited tumor cell growth. In MDA-MB-361, a breast tumor line with mutant PI3K-α (E545K) and elevated levels of Her2 receptor, PKI-402 induced cleaved poly(ADP-ribose) polymerase (PARP), a marker for apoptosis. *In vivo*, PKI-402 displayed antitumor activity (i.v. route) in breast [MDA-MB-361: Her2+ and PIK3-]/pancreas, and non–small cell lung cancer (NSCLC) xenograft models.

A key question about PI3K/mTOR signaling inhibitors is: can they cause tumor regression in preclinical models? Data we present here show that PKI-402 caused regression in the MDA-MB-361 xenograft model. PKI-402 effect was most pronounced at 100 mg/kg (daily for 5 days, one round), which reduced initial tumor volume and prevented tumor regrowth for 70 days. In MDA-MB-361 tumor tissue, PKI-402 at 100 mg/kg (single dose) fully suppressed p-Akt at both the T308 and the S473 sites at 8 hours and induced cleaved PARP. At 24 hours, p-Akt suppression was still evident, as was cleaved PARP. Biomarker analysis of heart and lung tissue showed minimal effect on p-Akt and no induction of cleaved PARP by PKI-402 at 100 mg/kg. Preferential accumulation of PKI-402 in tumor tissue was observed. Data presented here indicate that sustained suppression of p-Akt correlates with tumor regression in the MDA-MB-361 xenograft model.

**Materials and Methods**

**Enzyme assays**

Enzyme assays were done in fluorescent polarization (FP) format, adapted from the Echelon K-1100 PI3K FP assay kit protocol (7). Human class I PI3Ks and PI3K-α mutants (E545K and H1047R) were produced in Sf9 or purchased from Upstate Biotech. GST-GRP1 (murine) was produced in *Escherichia coli* or isolated by GST-Sepharose. Assay buffers were reaction buffer [20 mmol/L HEPES (pH 7.1), 2 mmol/L MgCl₂, 0.05% CHAPS, and 0.01% β-mercaptoethanol] and stop/detection buffer [100 mmol/L HEPES (pH 7.5), 4 mmol/L EDTA, 0.05% CHAPS]. FP reaction was run for 30 min at room temperature in 20 μL of reaction buffer containing 20 μmol/L phosphatidylinositol 4,5-bisphosphate (PIP2), 25 μmol/L ATP, and <4% DMSO (compound solvent). FP reaction was stopped with 20 μL of stop/detection buffer (10 nmol/L probe and 40 nmol/L GST-GRP), and after 2 h, data were collected using an Envision plate reader (Perkin-Elmer).

Selectivity of PKI-402 was evaluated in the Invitrogen 236 human kinase panel at [ATP] = Km for each enzyme.

**Cell culture, growth inhibition, and translocation assays**

Cell lines obtained from the American Type Culture Collection were MDA-MB-361, MDA-MB-468, T47D, MCF7, BT474, HT29, HCT116, DLD1, U87MG, H157, NCI-H460, A549, NCI-H1975, NCI-H1650, NCI-H2170, KB, 786-0, A498, MIA-PaCa-2, and PC3. Mutational status (http://www.sanger.ac.uk/genetics/CGP/cosmic/) of various oncogenes is listed in Table 1. U2OS cells engineered to monitor FOXO1–green fluorescent protein (GFP) cellular translocation were from Thermo Scientific. All cell lines were propagated at 37°C in 5% CO2 incubators in supplier-recommended growth medium.

Cell growth inhibition was determined using the CellTiter 96 AQueous proliferation assay from Promega. Manufacturer’s protocol was used, with adjustments for cell line growth characteristics. Data were collected

| Table 1. PKI-402 IC<sub>50</sub> values in human tumor cell line growth inhibition assays |
|-----------------------------------------------|-----------------|--------------|-------------|
| **Cell line**                                    | **Mutations**    | **IC<sub>50</sub> (μmol/L)** | **SD (n ≥ 3)** |
| Breast MDA-MB-361                               | PI3CA          | 0.006         | 0.001       |
| Breast MDA-MB-468                               | PTEN, RB, p53  | 0.009         | 0.001       |
| Pancreas T47D                                    | p53, PI3CA     | 0.013         | 0.002       |
| Pancreas BT474                                   | PI3CA, p53     | 0.026         | 0.006       |
| Prostate MCF7                                    | PI3CA          | 0.101         | 0.014       |
| Colon HCT116                                    | K-Ras, PI3CA   | 0.033         | 0.002       |
| Colon HT29                                      | B-Raf, PI3CA, p53 | 0.136       | 0.041       |
| Colon DLD1                                      | K-Ras, PI3CA   | 0.227         | 0.009       |
| Lung (NSCLC) NCI-H157                           | K-Ras, STK11, p53 | 0.036       | 0.004       |
| Lung (NSCLC) NCI-H460                           | K-Ras, PI3CA, STK11 | 0.089       | 0.003       |
| Lung (NSCLC) A549                               | K-Ras, STK11   | 0.116         | 0.031       |
| Lung (NSCLC) NCI-H2170                          | p53            | 0.127         | 0.027       |
| Lung (NSCLC) NCI-H1975                          | EGFR, PI3CA, p53 | 0.210       | 0.015       |
| Lung (NSCLC) NCI-H1650                          | EGFR, p53      | 0.349         | 0.061       |
| Pancreas MIA-PaCa-2                              | K-Ras, p53     | 0.051         | 0.003       |
| Brain (glioma)                                   | PTEN           | 0.077         | 0.017       |
| Brain (glioma)                                  | U87MG          | 0.016         | 0.002       |
| Prostate PC3                                     | PTEN, p53      | 0.016         | 0.002       |
| Epidermoid KB                                    | VHL            | 0.027         | 0.003       |
| Renal A498                                       | VHL, p53       | 0.268         | 0.002       |
| Renal 786-0                                     | PTEN, VHL, p53 | 0.334         | 0.060       |

www.aacrjournals.org Mol Cancer Ther; 9(4) April 2010

Published OnlineFirst April 6, 2010; DOI: 10.1158/1535-7163.MCT-09-0954
Tumor weight was calculated by the formula tumor = (d2 x D/2), where d and D are the shortest and longest diameters of the tumor, respectively, measured in millimeters. Significant (statistically, Student’s t test) reduction in the tumor growth of treated groups compared with controls (vehicle) was defined as P < 0.05.

Pharmacodynamic (biomarker) measurements were done on tumor-bearing female nude mice administered PKI-402. Tumor or normal tissue samples were collected from euthanized animals, homogenized, washed twice with cold (4°C) PBS, and then treated with cell lysis buffer (described above). Cell lysates were processed and probed with the various antibodies as described above.

**Results**

**Enzyme assays**

PKI-402 (Fig. 1) is a pan class I PI3K inhibitor with a 2 nmol/L IC50 against PI3K-α. IC50 determinations for PKI-402 against class I PI3Ks (α, β, γ, and δ) were inhibited by PKI-402 at concentrations equivalent to the IC50 for wild-type PI3K-α, PI3K-β, PI3K-γ, and PI3K-δ isoforms. Inhibition of protein phosphorylation was quantified from Western blots analyzed using Quantity One software.

**Cell growth inhibition assay**

<table>
<thead>
<tr>
<th>IC50 (nmol/L)</th>
<th>Human tumor cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>H1047R</td>
</tr>
<tr>
<td>20</td>
<td>H1650 (IC50 range)</td>
</tr>
<tr>
<td>268-349</td>
<td>A498</td>
</tr>
<tr>
<td>700-1500</td>
<td>786-0</td>
</tr>
<tr>
<td>2500</td>
<td>NCI-H1650</td>
</tr>
</tbody>
</table>

Pharmacodynamic (biomarker) measurements were done on tumor-bearing female nude mice administered PKI-402. Tumor or normal tissue samples were collected from euthanized animals, homogenized, washed twice with cold (4°C) PBS, and then treated with cell lysis buffer (described above). Cell lysates were processed and probed with the various antibodies as described above.

**Establishment of xenograft tumors, efficacy studies, and biomarker analysis**

Establishment of tumors, group randomization, tumor, and body weight recording during efficacy studies were described elsewhere (8). PKI-402 or vehicle was administered by i.v. route. All in vivo studies using nude mice were conducted under an approved Institutional Animal Care and Use Committee protocol. Tumor weight was calculated by the formula tumor weight (mg) = (d2 x D/2), where d and D are the shortest and longest diameters of the tumor, respectively, measured in millimeters. Significant (statistically, Student’s t test) reduction in the tumor growth of treated groups compared with controls (vehicle) was defined as P < 0.05.

Pharmacodynamic (biomarker) measurements were done on tumor-bearing female nude mice administered PKI-402. Tumor or normal tissue samples were collected from euthanized animals, homogenized, washed twice with cold (4°C) PBS, and then treated with cell lysis buffer (described above). Cell lysates were processed and probed with the various antibodies as described above.

**Results**

**Enzyme assays**

PKI-402 (Fig. 1) is a pan class I PI3K inhibitor with a 2 nmol/L IC50 against PI3K-α. IC50 determinations for PKI-402 against class I PI3Ks (α, β, γ, and δ) were inhibited by PKI-402 at concentrations equivalent to the IC50 for wild-type PI3K-α, PI3K-β, PI3K-γ, and PI3K-δ isoforms. Inhibition of protein phosphorylation was quantified from Western blots analyzed using Quantity One software.

**Cell growth inhibition assay**

<table>
<thead>
<tr>
<th>IC50 (nmol/L)</th>
<th>Human tumor cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>H1047R</td>
</tr>
<tr>
<td>20</td>
<td>H1650 (IC50 range)</td>
</tr>
<tr>
<td>268-349</td>
<td>A498</td>
</tr>
<tr>
<td>700-1500</td>
<td>786-0</td>
</tr>
<tr>
<td>2500</td>
<td>NCI-H1650</td>
</tr>
</tbody>
</table>

Pharmacodynamic (biomarker) measurements were done on tumor-bearing female nude mice administered PKI-402. Tumor or normal tissue samples were collected from euthanized animals, homogenized, washed twice with cold (4°C) PBS, and then treated with cell lysis buffer (described above). Cell lysates were processed and probed with the various antibodies as described above.
PKI-402 in vitro profile in biomarker, caspase activation, and FOXO1-GFP translocation assays

PKI-402 effect on phosphorylation of PI3K and mTOR effector proteins and activation of caspase-3/7 in MDA-MB-361 [Her2* and PIK3CA (E545K)]. The effect of PKI-402 on a representative group of PI3K/mTOR effector proteins in MDA-MB-361 is shown in Fig. 2A. This was done to link PKI-402 enzyme inhibition to cellular anti-proliferative effects. PKI-402 lipid kinase activity in cells was not directly measured. Class I PI3Ks convert PIP2 to PIP3 (phosphatidylinositol 3,4,5-trisphosphate) at the inner cell membrane (1, 2). PIP3 is bound by the pleckstrin homology domains of both PDK1 and Akt serine/threonine kinases, resulting in their close proximity at the inner cell membrane where PDK1 phosphorylates (at T308) and activates Akt. Suppression of cellular PIP3 by PKI-402 was indirectly shown by potent (IC50, <10 nmol/L) suppression of Akt phosphorylation at T308 (Fig. 2A). Full activation of Akt kinase occurs when the mTOR-containing TORC2 protein complex phosphorylates Akt at S473. Figure 2A shows that potent (IC50, <30 nmol/L) suppression of Akt phosphorylation at S473 was also caused by PKI-402. PKI-402 did not affect the overall Akt content in MDA-MB-361 cells at any concentration tested, indicating that p-Akt suppression was not an artifact of compound cytotoxicity.

TORC1 as well as TORC2 mTOR complexes were inhibited by PKI-402. Examples of PKI-402 effect on TORC1 were suppression of 4EBP1 and p70S6 kinase phosphorylation. Both p70S6K and 4EBP1 phosphorylation was inhibited at IC50 of <10 nmol/L (Fig. 2A).

PKI-402 suppression of Akt phosphorylation caused consequent effects on Akt effectors such as PRAS40, ENOS, and GSK3. PRAS40 regulates mTOR activity, ENOS generates nitrous oxide in blood vessels and is involved in regulation of vascular function and angiogenesis, and GSK3 is a serine/threonine protein kinase that regulates cell cycle progression and glucose metabolism (2, 11, 12). Akt phosphorylation of PRAS40 at T246 was suppressed at an IC50 of <30 nmol/L (Fig. 2A). Akt phosphorylation of ENOS at S1177 and GSK3α/GSK3β at S9/S21 was suppressed by PKI-402 at IC50 of <10 nmol/L (Fig. 2A).

An effect of PKI-402 particularly evident in MDA-MB-361 cells was induction of cleaved PARP, an indicator of cells undergoing apoptosis (13, 14). Complete PKI-402 suppression of Akt phosphorylation in MDA-MB-361 correlated with detectable cleaved PARP at 0.1 μmol/L PKI-402 (Fig. 2A). Cleaved PARP was detected in MDA-MB-361 within 4 hours after exposure to PKI-402.

We also measured PKI-402 activation of caspase-3 (Caspase-3/7 assay, Promega) in MDA-MB-361 because caspase-3 is a critical mediator of apoptosis, being either partially or totally responsible for the proteolytic cleavage of many key proteins including the nuclear enzyme PARP (14). Figure 2B shows that PKI-402 caused a dose-dependent increase in caspase-3/7 activity at 4 hours. MDA-MB-361 cells overexpress the Her2 receptor; therefore, we tested the ability of HKI-272 (irreversible Her2 kinase inhibitor; ref. 15) or PD0325901 [MAP/extracellular signal-regulated kinase kinase (MEK) inhibitor; ref. 16] to either induce caspase-3/7 activity alone or enhance PKI-402-induced caspase activity. Neither PD0325901 nor HKI-272 caused caspase-3/7 activity at concentrations tested (Fig. 2B). When PKI-402 was combined with 0.1 μmol/L (shown) or 1.0 μmol/L (data not shown) of either PD0325901 or HKI-272, caspase-3/7 activation in MDA-MB-361 cells was unaffected. These data indicate the significant addiction of MDA-MB-361 to PI3K/mTOR signaling to maintain cell viability. Less than 10% of MDA-MB-361 cells exposed to PKI-402 at 0.3 μmol/L (or higher) for 24 hours remained viable (Supplementary Data).

PKI-402 effect on FOXO1(FKHR)-GFP translocation in U2OS. Forkhead proteins (e.g., FOXO1) comprise a...
highly conserved family of transcription factors that control genes encoding proteins regulating insulin production, apoptosis, and cell cycle (17). FOXO1 activity is regulated by Akt-mediated phosphorylation. Akt-phosphorylated FOXO1 is sequestered in the cytosol by 14-3-3 protein, and unphosphorylated FOXO1 locates to the cell nucleus. Figure 2B shows that PKI-402, which suppressed Akt phosphorylation (T308 and S473; Fig. 2A), caused translocation of FOXO1(FKHR)-GFP to cell nuclei in U2OS cells engineered (Thermo Scientific) to monitor compound-mediated FOXO1-GFP nuclear translocation. The IC$_{50}$ value for PKI-402 effect on FOXO1-GFP nuclear translocation was 21 nmol/L (Fig. 2C). Figure 2D shows FOXO1-GFP (green) cytoplasmic distribution in untreated U2OS cells (left), with nuclear translocation evident (at right) after 1-hour exposure to PKI-402.

**PKI-402 induction of cleaved PARP and activation of caspase-3/7 in HCT116 cells.** To determine whether the PKI-402 effect on cell survival observed in MDA-MB-361 breast tumor cells was unique, or also evident in other tumor cells, we evaluated the sensitivity of the colon tumor line HCT116 (K-Ras and PIK3CA) to this compound. In vitro growth inhibition IC$_{50}$ of HCT116 by PKI-402 was 0.033 μmol/L. Suppression of p-Akt-T308 was observed at ≥0.1 μmol/L after 4-hour exposure to PKI-402. However, no cleaved PARP was evident after 4-hour exposure to PKI-402 up to 3 μmol/L. Longer exposure (24 hours) of HCT116 to PKI-402 again showed suppression of p-Akt-T308 (IC$_{50}$ <0.1 μmol/L; Fig. 3A, top), with only a trace cleaved PARP band at 3.0 μmol/L.

Because HCT116 cells have both PIK3CA and K-Ras mutations, we tested whether a MEK inhibitor, PD0325901, could influence PKI-402 effect on cleaved

---

**Figure 2. In vitro profile of PKI-402.** MDA-MB-361 [Her2+/PIK3CA (E545K)] cells were exposed to PKI-402 (0.003–0.3 μmol/L) for 4 h. A, PKI-402 suppression of p-Akt (both T308 and S473) and suppression of phosphorylation of Akt effectors [GSK3 (at S9/21), PRAS40 (at T246), and ENOS (at S1177)] and mTOR effectors [p70S6K (at T389) and p-4EBP1 (at T37/46)]. PKI-402 induction of cleaved PARP (cPARP) at 0.1 and 0.3 μmol/L. B, caspase-3/7 activity in MDA-MB-361 after a 4-h exposure to PKI-402, HKI-272 (Her2 inhibitor), or PD0325901 (MEK inhibitor) at 0.03 to 3.0 μmol/L. (◊), maximal caspase-3/7 activity was caused by 1.0 to 3.0 μmol/L PKI-402. Neither HKI-272 (□) nor PD0325901 (Δ) induced caspase-3/7 activity. C and D, PKI-402 combined with 0.1 μmol/L of either HKI-272 (×) or PD0325901 (+) did not increase caspase-3/7 activity. U2OS (FOXO1-GFP) cells were exposed to PKI-402 (0.003–10.0 μmol/L) for 1 h. C, nuclear to cytoplasmic ratio of FOXO1-GFP in U2OS cells after 60-min incubation with PKI-402 (IC$_{50}$, 21 nmol/L). D, translocation of FOXO1-GFP (green) in U2OS from the cytoplasm (left) to the nucleus (right) after 60-min exposure to PKI-402. Nuclei (blue) were stained with Hoechst 33342.
PKI-402, a Dual PI3K/mTOR Inhibitor

...activity at PKI-402 concentrations from 0.03 to 3.0 μmol/L (5-fold increase at 3.0 μmol/L). This effect will be investigated in future in vivo efficacy studies with HCT116.

**In vivo efficacy and biomarker profile of PKI-402 in MDA-MB-361 (breast) tumor xenografts**

PKI-402 at 25, 50, and 100 mg/kg caused regression of MDA-MB-361 tumors (Fig. 4A). PKI-402 effect was most pronounced at 100 mg/kg (daily for 5 days, one round), which reduced an initial tumor volume of 260 mm³ to 129 mm³ and prevented tumor regrowth for 70 days. PKI-402 given at 100 mg/kg daily for 5 days was well tolerated; however, continuous exposure at this level for longer than 7 days did cause weight loss. Tumor regrowth occurred between days 16 and 20 when PKI-402 was administered at 25 and 50 mg/kg (daily for 5 days, two rounds; both regimens well tolerated). However, when PKI-402 was readministered at 100 mg/kg, large tumors were significantly (P < 0.02) reduced in volume. In the 25 mg/kg treatment group, PKI-402 re-administered at 100 mg/kg (daily for 5 days, one round) on day 37 caused ~650 mm³ tumors to shrink to ~200 mm³, a ~69% reduction in tumor volume. In the 50 mg/kg treatment group, PKI-402 re-administered on day 45 (100 mg/kg, daily for 3 days, two rounds) caused ~600 mm³ tumors to ultimately shrink to ~200 mm³, a ~58% reduction in tumor volume. In Fig. 4B, photographic evidence of PKI-402 effect on MDA-MB-361 tumors treated at 100 mg/kg (daily for 5 days, one round) is shown.

Biomarker evaluation was done on MDA-MB-361 tumor tissue from mice treated with 25, 50, and 100 mg/kg (single dose) of PKI-402. Figure 4C shows the complete suppression of p-Akt (at T308 and S473) and p-p70S6K at 8 hours after administration of PKI-402 at 100 mg/kg. Distinct PKI-402 effect on this set of PI3K/mTOR effector proteins was also evident at the 50 and 25 mg/kg dosing levels. Cleaved PARP was evident when PKI-402 was given at 50 and 100 mg/kg. Table 2 lists serum and tumor tissue concentrations after 50 or 100 mg/kg single-dose administration of PKI-402. At 50 mg/kg PKI-402, preferential accumulation in tumor tissue relative to serum was evident at 4 hours and persisted up to 8 hours. Tumor/serum ratio (6.2) peaked at 6 hours. Tumor/serum ratio for PKI-402 at 100 mg/kg was 8.9 at 24 hours.

The effect of PKI-402 on PI3K/mTOR effector protein phosphorylation and cleaved PARP in both tumor and normal (e.g., heart and lung) tissue was assessed. In MDA-MB-361 tumor tissue, PKI-402 (100 mg/kg, single dose) effect on p-Akt (both T308 and S473), p-p70S6K, and p-pS6 was reduced (compared with 8 hours) but still evident at 24 hours (Fig. 4D, left). Cleaved PARP signal, however, was equal if not greater than that observed at 8 hours (Fig. 4C). In both heart (Fig. 4D, right) and lung tissue (data not shown) from nude mice bearing MDA-MB-361 tumors, PKI-402 (single dose, 100 mg/kg)
reduced p-Akt (T308 and S473) at 24 hours, but there was no detectable cleaved PARP.

In vivo efficacy of PKI-402 in A549 (NSCLC) and U87MG (glioma) tumor xenografts

PKI-402 was tested for antitumor efficacy in the A549 (NSCLC) and U87MG (glioma) xenograft models. A549 cells contain mutant K-Ras and STK11. Because these mutations influence both PI3K and mTOR activity, we tested PKI-402 in vivo growth-inhibitory effects on A549 tumors. Figure 5A shows that PKI-402 significantly ($P < 0.02$) inhibited the growth of A549 tumors in nude mice at 25 and 50 mg/kg (daily for 5 days, for three rounds).

PKI-402 inhibition of U87MG tumor growth showed efficacy in a PTEN-negative glioblastoma multiforme tumor line. PKI-402 was given at 100 mg/kg (single dose) and tissue was harvested at 24 h. Note the lack of cleaved PARP in the heart tissue.

### Table 2. Concentration (ng/mL) of PKI-402 in serum and tumor following a single dose

<table>
<thead>
<tr>
<th>Pharmacokinetic summary</th>
<th>ng/mL</th>
<th></th>
<th></th>
<th>8 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKI-402 i.v. 50 mg/kg Saline/lactic acid</td>
<td>6,228</td>
<td>1,215</td>
<td>527</td>
<td>549</td>
<td></td>
</tr>
<tr>
<td>PKI-402 i.v. 100 mg/kg Saline/lactic acid</td>
<td>8,319</td>
<td>6,576</td>
<td>3,265</td>
<td>2,219</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKI-402 i.v. 50 mg/kg Saline/lactic acid</td>
<td>814</td>
<td>132</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKI-402 i.v. 100 mg/kg Saline/lactic acid</td>
<td>2,813</td>
<td>1,184</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: PKI-402 accumulation in tumor tissue was observed at 4 to 8 h (50 mg/kg) and at 8 to 24 h (100 mg/kg).
round) caused significant ($P < 0.01$) reduction in tumor growth relative to untreated controls (Fig. 5B). Rapid growth of U87MG tumors in this model was evident by the $\sim 5$-fold increase in control tumor size over the 7-day study period. PKI-402 clearly attenuated this growth, permitting only a $\sim 2.3$-fold increase in tumor size.

**Discussion**

We identified PKI-402 in enzyme assays that measured activity of class I PI3Ks and mTOR. PKI-402 was a potent inhibitor of these enzymes, including the most frequently occurring mutant forms of PI3K-$\alpha$ (8). Analysis of PKI-402 against a panel of 236 human kinases showed a highly selective profile. *In vitro*, PKI-402 inhibition of cell growth and suppression of Akt phosphorylation correlated with induction of apoptosis in MDA-MB-361 (breast) human tumor cells. The proapoptotic effects of PKI-402 alone or in combination (i.e., PD0325901) on other cell lines (HCT116; Fig. 3) suggest that PKI-402 could have a broad range of activity and possibly more than a cytostatic effect on xenograft tumor models.

A key question about PI3K/mTOR signaling inhibitors is: can they cause tumor regression in preclinical models? Based on current data disclosed about other PI3K/mTOR inhibitors (e.g., BEZ235, PI-103, and SF1126; refs. 18, 19), PKI-402 shows a distinctive single-agent profile in that it can potently induce cell death *in vitro* and cause tumor regression *in vivo* (MDA-MB-361 model).

Indeed, at 25, 50, and 100 mg/kg, PKI-402 caused regression of MDA-MB-361 tumors (Fig. 4A). PKI-402 effect was most pronounced at 100 mg/kg (daily for 5 days, one round), which reduced an initial tumor volume (260 mm$^3$ to 129 mm$^3$) and prevented tumor regrowth for 70 days. Tumor regrowth occurred between days 16 and 20 when PKI-402 was administered at 25 and 50 mg/kg (daily for 5 days, two rounds). However, these enlarged tumors remained sensitive to PKI-402. For example, when PKI-402 was readministered at 100 mg/kg (daily for 3 days, one round) at day 37 (25 mg/kg group), large tumors (\$ \sim 600 \text{ mm}^3 \$) were reduced in volume by 69%.

Biomarker analysis done with MDA-MB-361 tumor tissue from mice given PKI-402 at 100 mg/kg (single dose) showed sustained suppression of Akt phosphorylation at both the T308 and the S473 sites and induction of cleaved PARP. Suppression of p-Akt was complete at 8 hours and still evident at 24 hours, whereas cleaved PARP was evident at both 8 and 24 hours. Biomarker analysis of heart and lung tissue from MDA-MB-361 tumor-bearing mice given PKI-402 at 100 mg/kg showed minimal effect on p-Akt and no induction of cleaved PARP (Fig. 4D). Preferential accumulation of PKI-402 in tumor tissue was observed (Table 2). It may be that complete and sustained suppression of p-Akt is needed to cause tumor regression in the MDA-MB-361 and other tumor xenograft models. We are doing detailed pharmacokinetic, pharmacodynamic, and antitumor efficacy studies to confirm this hypothesis. If this link is established, it could become a key criterion for advancing compounds to clinical development.

Finally, we established PKI-402 antitumor efficacy in additional *in vivo* models. These were A549 (NSCLC; K-Ras/STK11) and U87MG (glioma, PTEN). Clinical outcome for NSCLC is especially bleak (20). PKI-402 efficacy against A549 tumors (Fig. 5) supports the concept that inhibition of the K-Ras effector PI3K is an effective antitumor strategy (1, 5). In cells lacking functional STK11,
such as A549, defective AMPK regulation of mTOR, and in turn deregulation of mTOR-controlled cell metabolism, suggests possible sensitivity to PKI-402 mTOR-inhibitory effects. PKI-402 significantly inhibited the growth of A549 tumors in nude mice at both 25 and 50 mg/kg (daily for 5 days, three rounds).

As with NSCLC, patients with PTEN-negative glioblastoma multiforme tumors generally have a poor prognosis (21). In clinical settings, such tumors are resistant to EGFR inhibitors, radiotherapy, and most alkylating agents (22). PKI-402 (100 mg/kg, daily for 5 days) inhibited U87MG tumor growth, with significant reduction in tumor growth relative to untreated controls. Despite the high dosing level needed for efficacy, these data suggest that compounds such as PKI-402 may be useful either as a single agent or in combination with cytostatic or cytotoxic (e.g., temozolomide; ref. 23) drugs in treatment of glioblastoma multiforme.

Disclosure of Potential Conflicts of Interest

All authors are employees of Wyeth Research. No other potential conflicts of interest were disclosed.

Acknowledgments

We thank Ker Yu and Lourdes Toral-Barza for evaluating PKI-402 in their mTOR kinase assay.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 10/13/2009; revised 12/30/2009; accepted 01/14/2010; published OnlineFirst 04/06/2010.

References

Molecular Cancer Therapeutics

Antitumor Efficacy Profile of PKI-402, a Dual Phosphatidylinositol 3-Kinase/Mammalian Target of Rapamycin Inhibitor


Mol Cancer Ther 2010;9:976-984. Published OnlineFirst April 6, 2010.

Updated version

Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-09-0954

Supplementary Material

Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2010/04/05/1535-7163.MCT-09-0954.DC1

Cited articles

This article cites 23 articles, 6 of which you can access for free at:
http://mct.aacrjournals.org/content/9/4/976.full.html#ref-list-1

Citing articles

This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/9/4/976.full.html#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.