The Selective PI3K Inhibitor XL147 (SAR245408) Inhibits Tumor Growth and Survival and Potentiates the Activity of Chemotherapeutic Agents in Preclinical Tumor Models

Paul Foster, Kyoko Yamaguchi, Pin P. Hsu, Fawn Qian, Xiangnan Du, Jianming Wu, David J. Matthews, Peter Lamb, and A. Douglas Laird

Abstract

Dysregulation of PI3K/PTEN pathway components, resulting in hyperactivated PI3K signaling, is frequently observed in various cancers and correlates with tumor growth and survival. Resistance to a variety of anticancer therapies, including receptor tyrosine kinase (RTK) inhibitors and chemotherapeutic agents, has been attributed to the absence or attenuation of down-regulating signals along the PI3K/PTEN pathway. Thus, PI3K inhibitors have therapeutic potential as single agents and in combination with other therapies for a variety of cancer indications. XL147 (SAR245408) is a potent and highly selective inhibitor of class I PI3Ks (α, β, γ, and δ). Moreover, broad kinase selectivity profiling of >130 protein kinases revealed that XL147 is highly selective for class I PI3Ks over other kinases. In cellular assays, XL147 inhibits the formation of PIP3 in the membrane, and inhibits phosphorylation of AKT, p70S6K, and S6 in multiple tumor cell lines with diverse genetic alterations affecting the PI3K pathway. In a panel of tumor cell lines, XL147 inhibits proliferation with a wide range of potencies, with evidence of an impact of genotype on sensitivity. In mouse xenograft models, oral administration of XL147 results in dose-dependent inhibition of phosphorylation of AKT, p70S6K, and S6 with a duration of action of at least 24 hours. Repeat-dose administration of XL147 results in significant tumor growth inhibition in multiple human xenograft models in nude mice. Administration of XL147 in combination with chemotherapeutic agents results in antitumor activity in xenograft models that is enhanced over that observed with the corresponding single agents.

Introduction

There are three classes of PI3Ks, among which the class I kinases (subdivided into IA and IB subsets) convert phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3) in response to external cell stimuli (1–3). Activation of class IA PI3Ks (PI3Kα, β, and δ) is mediated by receptor tyrosine kinases (RTK). G-protein-coupled hormone receptors are implicated in activation of PI3Kβ and class IB PI3K (PI3Kγ; ref. 4). RAS can also directly bind to and activate p110α, the catalytic subunit of PI3Kα, and PI3Kα can mediate cellular transformation by RAS (2, 5). Similarly, p110β, the catalytic subunit of PI3Kβ, interacts with and serves as an effector of the GTPases RAC and cell division cycle 42 (CDC42; refs. 2, 6). Downstream effectors of PI3K signaling, such as phosphoinositide-dependent kinase-1 (PDK1) and AKT, bind to PIP3 at the cell membrane and are subsequently activated by phosphorylation, resulting in activation of signaling cascades regulating tumor proliferation, survival, angiogenesis, invasion, and dissemination (1, 2). TORC1, a multi-subunit complex, including the mTOR catalytic subunit and Raptor, acts downstream of AKT and is itself a notable target for therapeutic intervention in cancer and other diseases (7, 8).

Dysregulation of PI3K pathway components, resulting in upregulation of PI3K signaling, is observed in many cancers and is thought to promote tumor growth and survival (1, 2). PIK3CA, the gene encoding p110α, is the most frequently mutated kinase in human tumors, with mutations evident in approximately 10% of human cancers (9). In addition, the tumor suppressor PTEN, which is a critical negative regulator of PI3K signaling that converts PIP3 back to PIP2, is frequently deleted or downregulated in human tumors (1, 2, 10). PTEN-deficient tumors are believed to be highly PI3Kβ dependent, although it has recently been demonstrated that this dependence can be shifted to PI3Kα by concurrent mutations that activate p110α (11). In addition to PTEN, other tumor suppressor genes negatively regulating the PI3K pathway have been identified, including liver kinase B1 (LKB1), type II inositol polyphosphate-4-phosphatase, and tuberous sclerosis—these
are mutated/inactivated in a variety of familial and/or sporadic tumors (1, 2).

Resistance to therapy, both primary and acquired, is a persistent problem in cancer treatment. Activation of the PI3K pathway has been implicated in resistance to a variety of agents, including RTK inhibitors and chemotherapeutic agents (12). Consistent with this, blockade of the PI3K pathway has been shown to sensitize tumor cells to inhibitors of HER2, MET, and EGFR as well as to taxanes and platinum drugs (12). Selective PI3K inhibitors therefore have potential both as single agents and in combination with other therapies and a number of these agents have entered clinical testing in recent years (1, 2, 13).

Here, we describe the characterization of the preclinical pharmacology of the selective class I PI3K inhibitor XL147 (SAR245408). Collectively, these data demonstrate that PI3K pathway inhibition by XL147 inhibits proliferation, angiogenesis, cellular invasion, and tumor cell growth and survival in preclinical models, and has the potential to enhance the efficacy of chemotherapeutic agents.

Materials and Methods

**In vitro kinase inhibition assays and PIP3 mass balance assay**

Kinase inhibition assays and the PIP3 mass balance assay were performed as previously described (14).

pAKT and pS6 ELISA

ELISA for pAKT \(^{T_{308}}\) and total AKT were performed on PC-3 cell lysates and analyzed as previously described (14). The pS6 ELISA was performed as previously described (15) with minor modifications.

Cell-based assays

Cell lines were obtained from the ATCC in 2001–2005 and maintained in culture conditions at 37°C under 5% CO\(_2\) as previously described (14). For PI3K pathway status assessment following EGF treatment, the culture medium was replaced with test compounds dissolved in serum-free DMEM containing 0.3% DMSO. After incubation for 3 hours, cells were stimulated with 100 ng/mL of EGF (R&D Systems, 236-EG) for 10 minutes and Western immunoblot analysis of cell lysates was performed as previously described (14). Assessment of mTOR pathway status in Ramos cells was performed as previously described (14). Cellular proliferation was assessed as previously described (16) using the Cell Proliferation ELISA, bromodeoxyuridine (BrdUrd) chemiluminescence kit (Roche, Applied Science). Cytotoxicity, apoptosis (caspases-3/7), anchorage-independent growth, and PC-3 cell migration assays were performed as previously described (14).

Hepatocyte growth factor (HGF)-induced chemotaxis was assessed as previously described (16). The endothelial cell tube formation assay was performed as previously described (16), with minor modifications. Total tube length was quantified with Image Pro Plus software (Media Cybernetics).

For cell-cycle analysis by FACS, MCF7 cells were seeded at 1.2 \(\times\) 10\(^8\) cells per well in 12-well plates (Nunc) in DMEM containing 10% FBS, 1% NEAA, and 1% penicillin streptomycin (all from Cellgro). The cells were then incubated at 37°C, 5% CO\(_2\) for 24 hours. Serial dilutions of compounds in fresh growth medium at a final concentration of 0.3% DMSO (vehicle) were added to the cells and incubated for 48 and 72 hours. After treatment, medium was removed and cells were washed with cold PBS and immediately harvested in ice-cold PBS. Cells were fixed by adding ice-cold ethanol dropwise to the cell suspension to a final concentration of 75%. Cells were incubated in the fixative overnight at –20°C. The ethanol was washed off with PBS and cells were resuspended with propidium iodide (PI) staining solution to a final concentration of 5% PI (Molecular Probes), 10% RNase Cocktail (Ambion), and 85% PBS. Cells were stained for 1 hour at 37°C. Samples were run through the FACS Calibur instrument (Becton Dickinson) and data acquired by the CellQuest program (Becton Dickinson). FACS data were analyzed using the ModFit LT program (Verity Software).

**In vivo pharmacodynamic assays, efficacy studies, and tumor histologic assessment**

Female athymic nude mice and male nude mice were purchased from Taconic and housed according to the Exelixis Institutional Animal Care and Use Committee guidelines. Tumor cells were cultured and established as xenografts in mice, and body and tumor weights assessed as previously described (14). Statistical significance was determined using the two-tailed Student t test (significance defined as \(P \leq 0.05\)). XL147 was formulated in sterile water/10 mmol/L HCl or water and administered at the indicated doses and regimens by oral gavage at a dose volume of 10 mL/kg. Paclitaxel was purchased from MP Biomedicals and formulated for i.v. administration by dissolution of the dry powder into 1:1 EtOH/Cremophor mixture, with subsequent dilution (1:3) into normal saline. Carboplatin was purchased from PolyMed Therapeutics and formulated for i.v. administration by dissolution in normal saline containing 0.5% EtOH/0.5% CremophorEL.

For Western immunoblot assessment, tumors were collected at the indicated time points and tumor lysates were prepared, processed, and analyzed as previously described (14). For histologic assessment, after euthanasia, tumors from animals administered XL147 and/or other agents were excised and fixed in zinc fixative (BD Pharmingen) for 24 to 48 hours before being processed into paraffin blocks. Five \(\mu\)m thin sections were cut serially to represent the largest possible surface for each tumor and Ki-67 nuclear antigen, CD31-positive tumor vessels, or apoptotic cells (TUNEL) detected and analyzed as previously described (14).

**Results**

XL147 is a selective inhibitor of class I PI3Ks in biochemical assays

XL147 was identified following optimization of a quinoxaline scaffold for in vivo PI3K pathway inhibition and drug-like properties (XL147 structure is shown in Fig. 1A). In assays performed using purified proteins in a luciferase-coupled chemiluminescence format, XL147 displayed potent inhibitory activity against Class I PI3K isoforms p110\(\alpha\), p110\(\beta\), and p120\(\gamma\), with IC\(_{50}\) values of 39, 36, and 23 nmol/L, respectively (Table 1). XL147 was less potent against the remaining Class I isoform, p110\(\delta\), with an IC\(_{50}\) value of 383 nmol/L. The IC\(_{50}\) value for inhibition of PI3K\(\delta\) by XL147 was determined at various concentrations of ATP, revealing XL147 to be an ATP-competitive inhibitor with an equilibrium inhibition constant (K\(_{i}\)) value of 42 nmol/L.

XL147 had relatively weak inhibitory activity toward the class III PI3K vacular sorting protein 34 (VPS34; IC\(_{50}\) value of ~7.0 nmol/L) and the PI3K-related DNA-dependent protein
XL147 inhibits the PI3K pathway in multiple tumor cell models

MCF7 human mammary carcinoma cells and PC-3 human prostate adenocarcinoma cells were selected for the initial assessment of the effect of XL147 on PTEN/PI3K pathway signaling because they harbor, respectively, a heterozygous E545K activating mutation in the PIK3CA subunit of PI3Kα and a homozygous deletion of exons 3–9 of the PTEN tumor suppressor gene. PIP3 is the product of a class IA or IB PI3K acting on its physiologic substrate PIP2. Hence, PIP3 levels serve as a direct assessment of PI3K activity. Consistent with its inhibitory activity against purified PI3K proteins, XL147 inhibited EGF-induced PIP3 production in PC-3 and MCF7 cells in serum-free medium with IC50 values of 220 and 347 nmol/L, respectively (Table 2). The ability of XL147 to inhibit phosphorylation of key signaling proteins downstream of PI3K was examined by assessing its effects on EGF-stimulated phosphorylation of AKT and on nonstimulated phosphorylation of 4EBP1 in PC-3 cells in serum-free media by cell-based ELISA. XL147 inhibited these activities with IC50 values of 477 and 776 nmol/L, respectively (Table 2).

The effects of XL147 on the PI3K signaling pathway were examined by Western immunoblot analysis in MCF7 and PC-3 cells (see Fig. 1B and Supplementary Fig. S1). XL147 inhibited AKT phosphorylation at both activation sites (T308 and S473) at concentrations consistent with the IC50 values determined by ELISA in MCF7 and PC-3 cells stimulated with EGF following incubation for 3 hours in serum-free medium containing XL147. Inhibition of AKT substrate phosphorylation (PRAS40 and GSK3β) and inhibition of phosphorylation events downstream of mTOR (p70S6K, S6, and 4EBP1 phosphorylation) were also evident. XL147 induces a decrease in the levels of cyclin D1 protein, consistent with increased GSK3β activity as a result of inhibition of AKT leading to GSK3β-mediated phosphorylation and subsequent degradation of Cyclin D1 (Fig. 1B and Supplementary Fig. S1). In general, higher compound concentrations were required to inhibit phosphorylation events downstream of mTOR than more PI3K-proximal events such as AKT phosphorylation, consistent with selective inhibition at the PI3K level. XL147 does not inhibit nutrient-stimulated mTOR kinase activity in cells as assessed by S2481 autophosphorylation and p70S6K/4EBP1 phosphorylation, providing further evidence for lack of direct activity toward mTOR (Supplementary Fig. S2).

The control compound ZSTK474 (an inhibitor of PI3K; ref. 17) at 10 μmol/L robustly decreased the levels of all the phospho readouts assessed. The mTORC1 inhibitor rapamycin

Table 1. Kinase inhibition profile of XL147

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Abbreviations: DNAPK, DNA protein kinase; IC50, concentration required for 50% target inhibition; VPS34, vacuolar sorting protein 34.

XL147 inhibits the PI3K pathway in multiple tumor cell models

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demonstrated broad activity in these lines with no marked
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### Table 2. Effects of XL147 on PIP3 production and AKT and S6 phosphorylation

<table>
<thead>
<tr>
<th>Cell line</th>
<th>PIP3 IC50 (nmol/L)</th>
<th>pAKT IC50 (nmol/L)</th>
<th>pS6 IC50 (nmol/L)</th>
</tr>
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<tbody>
<tr>
<td>PC-3</td>
<td>220</td>
<td>477</td>
<td>776</td>
</tr>
<tr>
<td>MCF7</td>
<td>347</td>
<td>nd</td>
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Abbreviation: nd, not determined.

*IC50 values determined using ELISA assay.

Effects of XL147 on PIP3 production and AKT and S6 phosphorylation

At 0.1 μmol/L did not inhibit the phosphorylation of AKT or its
direct substrates PRAS40 and GSK3β, but in fact appeared to
stimulate phosphorylation of AKT. This is consistent with relief
of p70S6K-dependent negative feedback of PI3K (2). As
expected, rapamycin significantly decreased pp70S6K and pS6
levels, consistent with its well characterized ability to inhibit
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Effects of XL147 on proliferation in a panel of tumor cell lines

In MCF7 and PC-3 cells, XL147 inhibits proliferation (mon-
tored by BrdUrd incorporation) with IC50 values of 9,669
nmol/L and 16,492 nmol/L, respectively. When tested in a
broad panel of tumor cell lines with diverse origins and genetic
backgrounds, XL147 was found to inhibit proliferation with
IC50 values ranging from approximately 1200 nmol/L to
>30,000 nmol/L (Fig. 2 and Supplementary Table S3). In
general, PIK3A-mutant and, to a lesser extent, PTEN-mutant
cell lines tended to be relatively sensitive to XL147, whereas
RAS- or BRAF-mutant cell lines tended to be less sensitive.
Interestingly, several RAS-mutant cell lines were relatively
insensitive to XL147 despite their also harboring PI3CA muta-
tions (Fig. 2 and Supplementary Table S3). The micromolar
IC50 values demonstrated in these experiments may be attrib-
utable in part to the presence of 10% serum in these assays and
the consistently high in vitro plasma protein binding exhibited
by XL147, with 99.9% of this agent bound in mouse, rat, and
human plasma. Similarly, in vivo plasma protein binding,
quantified in plasma samples from mice and rats administered
XL147, showed plasma protein binding of 99.9% and 99.8% at
100 mg/kg dosages. All values were determined by an equi-
librium dialysis method. As discussed previously, the presence
of serum also causes a significant increase in the IC50 values for
XL147 inhibition of AKT and S6 phosphorylation.

Anchorage-independent growth in soft agar is considered the
most stringent assay for detecting malignant transformation of
cells. To further characterize the effects of XL147 on tumor cell
growth, an assay monitoring the anchorage-independent growth
of PC-3 and MCF7 cells in soft agar over a 14-day period was used.
XL147 inhibits colony growth with an IC50 of 3,996 nmol/L in
PC-3 cells and 2,730 nmol/L in MCF7 cells. These IC50 values are

![Figure 2](image-url)

Figure 2.
Relative sensitivity of tumor cells to XL147 as a function of genetic status. Cell proliferation IC50 values are presented normalized to that for BT474 (most sensitive cell line). See Materials and Methods and Supplementary Table S3 for details.
significantly lower than those required to inhibit growth of the cells in a monolayer, perhaps indicating an increased reliance on PI3K pathway signaling for growth in three dimensions.

To rule out direct cytotoxic effects of XL147 on tumor cells, its effects on cell viability were determined by bioluminescent measurement of cellular ATP. XL147 did not reduce ATP levels when incubated for 24 hours, indicating a lack of cytotoxicity (Supplementary Table S3, footnote). Induction of cytoplasmic caspases-3 and -7 was examined as an indication of apoptosis induction. XL147 did not affect the activity of these caspases at the compound doses and time point tested (Supplementary Table S3, footnote). In MCF7 cells, the antiproliferative effects of XL147 were associated with a specific block in the G1 phase of the cell cycle and an increase of sub-G1 cell population (Supplementary Table S4). Therefore, at least in MCF7 cells, the antiproliferative effects of XL147 in culture do not appear to be due to cytotoxic or proapoptotic effects, but rather reflect cytostasis.

XL147 inhibits tumor cell migration, invasion, and angiogenesis

One of the hallmarks of aggressive tumor cells is the ability to migrate in response to chemotactic stimuli and to invade surrounding tissue. HGF is one of the key stimulators of these behaviors, and cell lines expressing high levels of the HGF receptor MET, are highly invasive and metastatic in vitro. Because PI3K resides in the MET signaling pathway, the ability of XL147 to inhibit HGF-stimulated migration and invasion was tested using in vitro assays that measure these behaviors. Murine B16 melanoma cells express high levels of MET, which becomes highly phosphorylated when the cells are treated with HGF. In 10% serum, B16 cells plated in the top well of a Transwell chamber containing a barrier with 0.8-μm pores show very little ability to migrate to the lower chamber side. Addition of HGF to the lower Transwell chamber greatly increases migration through the barrier over a 24-hour period (Fig. 3A). XL147 blocked this effect with an IC50 value of 899 nmol/L. Inhibition of cell migration was observed at a 5-fold lower concentration than that associated with cytotoxicity (IC50 value of XL147 in B16 cells of 4,494 nmol/L). Therefore, the effects on inhibition of melanoma cell migration by XL147 are likely not due to cytotoxicity.

A second assay, the scratch assay, was utilized to test XL147 for activity against PC-3 tumor cell migration stimulated by EGF (Fig. 3B). In this assay, a cell-free zone was scratched into a monolayer of tumor cells, and the ability of XL147 to block EGF-stimulated migration of the cells into the cell-free zone was determined. In the absence of growth factors, migration of cells bordering the scratch into the cell-free space is minimal during the 18-hour time course of the experiment. The addition of EGF greatly stimulates migration, resulting in a nearly complete closure of the scratch over the same time course. XL147 inhibited cellular migration into the cell-free zone in response to EGF with an IC50 value of 394 nmol/L and exhibited no cytotoxicity (IC50 > 3,333 nmol/L).

PI3K plays an important role in angiogenesis as a central mediator of signaling downstream of angiogenic RTKs (18, 19). Endothelial tube formation was assayed to test the effect of XL147 in an in vitro model that reflects endothelial cell morphogenesis, a function thought to contribute to angiogenesis in vivo. When plated on a confluent layer of normal human diploid fibroblast cells, human microvascular endothelial cells (HMVEC) form extensive networks of tubules in response to VEGF over a 7-day period. Tubules are stained and quantitated using an antibody that recognizes the endothelial cell marker CD31, as illustrated in Fig. 3C. XL147 inhibited VEGF—induced tubule formation with an IC50 of 529 nmol/L, similar to the IC50 value of ZD6474 (646 nmol/L), an inhibitor of VEGFR2, PDGFRβ, FGFR1, and FLT4 that is known to inhibit angiogenesis (20).
XL147 strongly inhibits the PI3K pathway in tumor xenograft models and displays robust antitumor activity in tumor-bearing mice

Lysates of MCF7 xenograft tumors intradermally implanted in the hind flank of athymic nude mice contain high levels of constitutively phosphorylated AKT, p70S6K, and S6 proteins. The ability of XL147 to inhibit this endogenous phosphorylation of AKT, p70S6K, and S6 was examined following a single oral dose of 10, 30, 100, or 300 mg/kg. The tumors were harvested 4, 24, or 48 hours postdose and homogenized in lysis buffer. Tumor lysates from each animal (n = 4) were then pooled for each group and analyzed for levels of total and phosphorylated AKT, p70S6K, and S6 by Western immunoblotting (Fig. 4A).

Administration of XL147 caused a dose-dependent decrease in phosphorylation of AKT, p70S6K, and S6 in the tumors, reaching a maximum of 81% inhibition of AKT phosphorylation at 300 mg/kg at 4 hours. The dose–response relationships derived from the 4-hour time point predict 50% inhibition of AKT, p70S6K, and S6 phosphorylation at doses of 100 mg/kg (pAKTT308), 54 mg/kg (pAKTS473), 71 mg/kg (p-p70S6K), and 103 mg/kg (pS6; Supplementary Fig. S5). The inhibition of AKT, p70S6K, and S6 phosphorylation in MCF7 tumors following a 100 mg/kg dose of XL147 was maximal at 4 hours, reaching 55% to 75%; however, the level of inhibition decreased to 8% to 45% by 24 hours, and only minimal or no inhibition was evident by 48 hours (Fig. 3A). Following a 300 mg/kg dose of XL147, inhibition was also maximal at 4 hours (65%–81%). However, in contrast with the 100 mg/kg dose, inhibition at 24 hours (51%–78%) was almost comparable with that seen at 4 hours, and partial inhibition (25%–51%) persisted through 48 hours (Fig. 4A).

Similarly, administration of XL147 caused a dose-dependent decrease of phosphorylation of AKT, p70S6K, and S6 in PC-3 tumors in vivo, reaching a maximum of 74% inhibition of AKT phosphorylation at 300 mg/kg at 4 hours postdose (Fig. 4B). The dose–response relationships derived from the 4-hour time point predict 50% inhibition of AKT, p70S6K, and S6 phosphorylation to occur at doses of 64 mg/kg (pAKTT308), 100 mg/kg (pAKTS473), 95 mg/kg (p-p70S6K), and 99 mg/kg (pS6; Supplementary Fig. S5). Blood was collected at the same time that tumor tissue was harvested in both studies, and plasma concentrations of XL147 were assessed (Supplementary Table S5). On the basis of these data, the plasma concentrations required to inhibit phosphorylation of AKT, p70S6K, and S6 by 50% in these tumor models ranged from approximately 90 μmol/L to 180 μmol/L. Hence, XL147 exhibited comparable pharmacodynamic activity in PIK3CA-mutant MCF7 and PTEN-deficient PC-3 xenograft tumor models.
Multiple tumor models were utilized to explore the efficacy and potency of repeat-dose XL147 with regard to tumor growth inhibition in vivo. In addition to the previously described MCF7 and PC-3 models, the antitumor efficacy of XL147 was evaluated in xenograft models, including OVCAR-3 (human ovarian xenograft tumor model exhibiting PIK3CA amplification), U-87 MG (human glioblastoma xenograft tumor model harboring a deletion at codon 54 in the gene encoding PTEN, resulting in a frameshift), A549 (human NSCLC xenograft tumor model harboring a homozygous activating mutation in KRAS and a homozygous loss-of-function mutation in LKB1), Calu-6 (human NSCLC harboring mutationally activated KRAS Q61K), A2058 (PTEN-deficient human malignant melanoma harboring a homozygous V600E activating mutation in BRAF), and WM-266-4 (PTEN-deficient human malignant melanoma harboring a heterozygous V600D activating mutation in BRAF).

XL147 exhibited significant antitumor efficacy in vivo in all of these models (Figs. 5 and 6; and Supplementary Table S6) at doses that proved well tolerated as assessed by daily monitoring of mouse weights (Supplementary Fig. S6). The most efficacious once daily dose assessed was 100 mg/kg, suggesting that sustained pathway inhibition is required for maximal efficacy. This dose generally resulted in stasis or near-stasis of tumor growth, except in the case of the tumors harboring KRAS or BRAF mutations where tumors generally continued to grow although at a reduced rate (Fig. 6 and Supplementary Table S6). IHC analysis of MCF7 tumors collected at the end of the dosing period revealed significant, dose-dependent decreases in staining for Ki-67, a marker of cell proliferation (Table 3). Similarly, administration of XL147 at 100 mg/kg once daily for 2 weeks resulted in a 51% decrease in Ki-67 staining in PC-3 tumors. Moreover, decreased tumor vascularization was also observed in MCF7, PC-3, and Calu-6 tumors (Table 3 and Fig. 6). Thus, inhibition of PI3K by XL147 results in an antiproliferative effect, as well as a modest antivascular effect in xenograft tumors. In PC-3 and Calu-6 tumors, there was also evidence for increased apoptosis as judged by TUNEL staining (Fig. 6) although the absolute percentage of TUNEL-positive tumor cells remained small, suggesting that increased apoptosis was not a significant contributor to antitumor efficacy.

Plasma concentrations at the end of these efficacy studies were higher than those evident following single-dose administration, presumably reflecting accumulation. For example, in the MCF7 efficacy study, average plasma concentrations for XL147 administered at the 100 mg dose were 233, 329, and 112 μmol/L at the 1-, 4, and 24-hour time points, respectively (n = 3 per time point), compared with 179 and 86 μmol/L for the 4- and 24-hour time points for the same dose in the single dose MCF7 pharmacodynamic study shown in Fig. 4A (Supplementary Table S5).

In addition to its antitumor efficacy as monotherapy, XL147 potentiated the antitumor efficacy of the cytotoxic agents paclitaxel and carboplatin (Fig. 6). When XL147 was combined with paclitaxel or carboplatin, apoptosis was induced to an extent greater than that seen for either agent alone while there was also an enhanced reduction in tumor angiogenesis evident (Fig. 6). It is noteworthy that XL147 administered in these combinations proved generally well tolerated as assessed by daily monitoring of mouse body weights (Supplementary Fig. S6).

Discussion

To date, three PI3K pathway inhibitors have been approved by FDA for the treatment of cancer. These include the rapamycin analogs TORISEL (temsirolimus; Pfizer) for patients with advanced renal cell carcinoma (RCC) and Afinitor (everolimus; Novartis) for the treatment of patients with advanced RCC after failure of treatment with sunitinib or sorafenib, and for advanced estrogen receptor-positive breast cancer after failure of treatment with a nonsteroidal aromatase inhibitor. However, the efficacy of these agents may be limited by the fact that mTORC1 inhibition enhances tumor cell survival by upregulating PI3K/AKT signaling via inhibition of a mTORC1-dependent negative feedback loop acting through PI3K (2). In contrast, inhibition of PI3K offers the potential for concerted inhibition of both PI3K and mTORC1, while obviating this feedback loop. The PI3Kδ-specific inhibitor idelalisib has been approved by FDA for administration in combination with rituximab for patients with relapsed chronic lymphocytic leukemia for whom rituximab alone would be considered appropriate therapy, and as monotherapy for patients with relapsed follicular B-cell non-Hodgkin lymphoma and small
lymphocytic lymphoma who have received at least two prior systemic therapies, but selective inhibition of PI3Kδ has limited impact in solid tumor types. The results detailed above demonstrate that XL147 (SAR245408) is a potent and selective inhibitor of class I PI3Ks. In cellular assays, treatment with XL147 inhibits phosphorylation of proteins downstream of PI3K, including AKT and ribosomal protein S6, in multiple tumor cell lines with diverse molecular alterations impacting the PI3K pathway. In a broad panel of tumor cell lines, XL147 inhibits proliferation with a wide range of potencies, which appeared to be influenced by genetic background. In human xenograft tumor models in athymic nude mice, oral (PO) administration of XL147 results in dose-dependent inhibition of PI3K pathway components with a duration of action of approximately 24 hours. On repeat dosing, XL147 shows significant tumor growth inhibition in multiple human xenograft models at well-tolerated doses. When administered in combination with cytotoxic chemotherapy, XL147 showed antitumor efficacy which was enhanced over that seen with the corresponding monotherapies.
A major cause of PI3Kα activation in human tumors is gain-of-function mutations in PIK3CA, particularly in exons 9 and 20, which encode the helical and kinase domains of the p110α catalytic subunit of PI3K (3). Molecular alterations directly affecting PIK3CB are rare in human cancer (1), but PI3KB is considered to be the major driver of dysregulated PI3K pathway activity associated with PTEN deficiency, although PI3KB may be the more important isoform in PTEN-deficient tumors where p110α is concurrently activated by mutated RAS (11). Hence, coconcerted inhibition of PI3Kα and PI3KB is likely desirable in terms of broad potential utility in treating solid tumors. In biochemical assays, XL147 appears to be more potent against PI3Kα than against PI3Kβ (Table 1). However, in cellular assays, XL147 shows comparable activity versus PI3K pathway signaling in MCF7 breast (PIK3CA mutant) and PC-3 prostate (PTEN-deleted) tumor cells. Moreover, XL147 showed comparably pharmacodynamic activity against these cell lines when they were grown as xenograft tumors in mice. These data demonstrate that XL147 exhibits functionally equivalent activity against PI3Kα and PI3KB in cultured cells and preclinical tumor models.

XL147 exhibited a wide range of antiproliferative activity against tumor cells grown as monolayers. In MCF7 cells, these effects were associated with a G1 arrest, but not with acute cytotoxicity or induction of apoptosis. There was a trend suggesting enhanced sensitivity of cells exhibiting PIK3CA mutations to XL147, consistent with similar observations previously reported for the PI3K inhibitors GDC-0941 and CH5132799 (21, 22). Likewise, the relative insensitivity of RAS-mutant cell lines, regardless of PIK3CA status, to inhibition of proliferation by XL147 is consistent with preclinical observations with other PI3K pathway targeting agents (23).

When administered as monotherapy, XL147 showed efficacy in models with diverse genetic lesions activating the PI3K pathway, specifically a PIK3CA E545K mutation (MCF7), PIK3CA amplification (OVCar-3), PTEN deletion/deficiency (PC-3, U-87 MG, A2058, WM-266-4), KRAS mutation (A549 and Calu-6), and LKB1 mutation (A549). The fact that efficacy was observed in all these models suggests that XL147 may have broad utility in tumors with activation of the PI3K pathway. It is intriguing that, of these models, XL147 appeared to be least efficacious against Calu-6, a KRAS-mutant tumor lacking detectable genetic alterations in PIK3A, PTEN, or LKB1. On the basis of IHC/IF analyses, efficacy across models was likely mediated by a combination of antiproliferative and antiangiogenic effects (Table 3 and Fig. 6). The plasma concentrations associated with pharmacodynamic activity and antitumor efficacy in mice are comparable with plasma concentrations associated with PI3K pathway inhibition in a single-agent phase I clinical study (compare Supplementary Table S5 with clinical pharmacokinetics summarized in ref. 24).

Hence, XL147 pharmacokinetics and pharmacodynamic activities appear to translate well between mice and humans.

On the basis of the xenograft data presented here, and the clinical experience gained thus far with XL147 and other PI3K inhibitors in clinical development, it is not yet clear whether the presence of PIK3CA mutations or PTEN deficiency will be predictive of greater clinical responsiveness. In a single-agent phase I study, robust pharmacodynamic activity across diverse tumors was evident regardless of mutational status. For example, PI3K pathway inhibition was roughly comparable in a tongue squamous cell carcinoma harboring a PIK3CA E545K mutation with that seen in tumors lacking detectable PI3K pathway alterations (24). Moreover, in that study, a partial response was evident in a patient with non–small cell lung cancer, although no mutations affecting the PI3K pathway were detected in archival tumor tissue from the patient (24). It is also not yet clear whether the presence of PIK3CA mutations or PTEN deficiency will be predictive of greater clinical responsiveness to PI3K pathway inhibitors in general, although an analysis based on combining the results of multiple early-stage trials suggested that PIK3CA H1047R mutations are associated with response (25).

The ability of XL147 to be combined with cytotoxic agents in preclinical tumor models at well-tolerated doses is promising and consistent with encouraging early results in XL147-003, a single-arm, open-label, dose-escalation study of XL147 in combination with paclitaxel and carboplatin in subjects with refractory solid tumors (ref. 26; NCT00756847). Moreover, the enhanced efficacy evident when XL147 is combined with trastuzumab or lapatinib in mouse models (27, 28) provides strong support for exploring combinations of XL147 with RTK inhibitors. These and similar data provide a rationale supporting clinical studies where XL147 is combined with other targeted or cytotoxic agents (e.g., NCT01042925, NCT01082068, and NCT00692640).

### Disclosure of Potential Conflicts of Interest

P. Lamb has ownership interest (including patents) in Exelixis Inc. A.D. Laird has ownership interest (including patents) in Exelixis. No potential conflicts of interest were disclosed by the other authors.

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**Table 3.** IHC analyses of proliferation and vascularity in MCF7 xenograft tumors

<table>
<thead>
<tr>
<th>Group</th>
<th>% Positive cells</th>
<th>% Reduction</th>
<th>MVD</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle, 10 mL/kg PO qd</td>
<td>37 ± 4</td>
<td>na</td>
<td>78 ± 7</td>
<td>na</td>
</tr>
<tr>
<td>XL147, 30 mg/kg PO qd</td>
<td>30 ± 5</td>
<td>20</td>
<td>59 ± 9</td>
<td>25</td>
</tr>
<tr>
<td>XL147, 100 mg/kg PO qd</td>
<td>25 ± 5</td>
<td>32</td>
<td>49 ± 7</td>
<td>37</td>
</tr>
<tr>
<td>XL147, 300 mg/kg PO twice weekly</td>
<td>21 ± 5</td>
<td>43</td>
<td>39 ± 7</td>
<td>49</td>
</tr>
</tbody>
</table>

Abbreviation: na, not applicable.

*Values are the mean ± SD.

**Supplementary Table S5** with clinical pharmacokinetics summarized in ref. 24.

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