Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of SAR245408 (XL147), an Oral Pan-Class I PI3K Inhibitor, in Patients with Advanced Solid Tumors


Abstract

Purpose: SAR245408 is a pan-class I phosphoinositide 3-kinase (PI3K) inhibitor. This phase I study determined the maximum tolerated dose (MTD) of two dosing schedules [first 21 days of a 28-day period (21/7) and continuous once-daily dosing (CDD)], pharmacokinetic and pharmacodynamic profiles, and preliminary efficacy.

Experimental Design: Patients with refractory advanced solid malignancies were treated with SAR245408 using a 3 + 3 design. Pharmacokinetic parameters were determined after single and repeated doses. Pharmacodynamic effects were evaluated in plasma, hair sheath cells, and skin and tumor biopsies.

Results: Sixty-nine patients were enrolled. The MTD of both schedules was 600 mg; dose-limiting toxicities were maculopapular rash and hypersensitivity reaction. The most frequent drug-related adverse events included dermatologic toxicities, diarrhea, nausea, and decreased appetite. Plasma pharmacokinetics showed a median time to maximum concentration of 8 to 22 hours, mean terminal elimination half-life of 70 to 88 hours, and 5- to 13-fold accumulation after daily dosing (first cycle). Steady-state concentration was reached between days 15 and 21, and exposure was dose-proportional with doses up to 400 mg. SAR245408 inhibited the PI3K pathway (~40%-80% reduction in phosphorylation of AKT, PRA540, 4EBP1, and S6 in tumor and surrogate tissues) and, unexpectedly, also inhibited the MEK/ERK pathway. A partial response was seen in one patient with advanced non–small cell lung cancer. Eight patients were progression-free at 6 months. Pharmacodynamic and clinical activity were observed irrespective of tumor PI3K pathway molecular alterations.

Conclusions: SAR245408 was tolerable at doses associated with PI3K pathway inhibition. The recommended phase II dose of the capsule formulation is 600 mg administered orally with CDD. Clin Cancer Res; 20(1); 233–45. ©2013 AACR.

Introduction

The phosphoinositide 3-kinase (PI3K) pathway regulates essential cellular functions, including proliferation, apoptosis, protein synthesis, and metabolism (1, 2). Class I PI3K enzymes convert phosphatidylinositol 4,5-bisphosphate (PIP$_2$) into phosphatidylinositol 3,4,5-trisphosphate (PIP$_3$) in response to external cell stimuli (3, 4). Activation of class IA PI3Ks (PI3Kα, -β, and -δ) is mediated by receptor tyrosine kinases (RTKs). In addition, PI3Kβ and PI3Kδ are activated by G-protein–coupled receptors (5). PIP$_3$ production promotes membrane localization and activation of several downstream effectors, such as phosphoinositide-dependent kinase-1 (PDK1) and AKT, leading to cell-cycle progression and inhibition of apoptosis through phosphorylation of their respective substrates (3).

Hyperactivation of PI3K signaling in cancer cells occurs through molecular alterations of PI3K pathway components and RTKs. The PI3KCA gene, encoding the...
The phosphoinositide 3-kinase (PI3K) pathway is heavily implicated in tumor cell growth, proliferation, and survival and contributes to resistance to chemotherapy and targeted agents. SAR245408 (XL147) is a novel orally bioavailable pan-class I PI3K inhibitor with potent antitumor activity in xenograft models. This first-in-human study established the maximum tolerated dose of the capsule formulation (administered daily either for the first 21 days of a 28-day period or continuously for 28 days), and showed a manageable toxicity profile and favorable pharmacokinetic parameters. SAR245408 demonstrated a pharmacodynamic effect on fasting insulin levels, as well as PI3K pathway modulation in hair, skin, and tumor tissue. Pathway inhibition and clinical activity were observed in tumors with and without apparent molecular alterations of PI3K pathway components. Overall, 25 (43.9%) patients had stable disease as the best response; eight were progression-free at 6 months. These results lay the groundwork for additional studies of SAR245408 either as monotherapy or in combination regimens.

Translational Relevance
The phosphoinositide 3-kinase (PI3K) pathway is heavily implicated in tumor cell growth, proliferation, and survival and contributes to resistance to chemotherapy and targeted agents. SAR245408 (XL147) is a novel orally bioavailable pan-class I PI3K inhibitor with potent antitumor activity in xenograft models. This first-in-human study established the maximum tolerated dose of the capsule formulation (administered daily either for the first 21 days of a 28-day period or continuously for 28 days), and showed a manageable toxicity profile and favorable pharmacokinetic parameters. SAR245408 demonstrated a pharmacodynamic effect on fasting insulin levels, as well as PI3K pathway modulation in hair, skin, and tumor tissue. Pathway inhibition and clinical activity were observed in tumors with and without apparent molecular alterations of PI3K pathway components. Overall, 25 (43.9%) patients had stable disease as the best response; eight were progression-free at 6 months. These results lay the groundwork for additional studies of SAR245408 either as monotherapy or in combination regimens.

Materials and Methods
Patient population
Eligible patients were aged ≥18 years, with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2 and histologically confirmed metastatic or unresectable solid tumors for which standard curative or palliative measures were unavailable or ineffective. Adequate organ and bone marrow function, fasting plasma glucose < 160 mg/dL, HbA1c < 8%, and the presence of disease evaluable by tumor markers or physical or radiologic means, were required. Patients who had previously received PI3K inhibitor treatment or had received chemotherapy, a biologic agent, or an investigational agent within 4 weeks, a small-molecule kinase inhibitor or radiotherapy within 2 weeks or 3 half-lives, or hormonal therapy within 1 week of the first dose of SAR245408, were excluded. Furthermore, patients with ongoing toxicity (grade ≥1) due to prior therapy or uncontrolled intercurrent illness (e.g., infection or heart disease) were excluded. In vitro inhibition and induction studies with SAR245408 using human liver material showed the potential of SAR245408 to inhibit cytochrome P450 (CYP)3A4 and CYP2C9 and induce CYP1A2 and CYP3A4. Therefore, concomitant use of drugs with narrow therapeutic indices that are substrates for CYP1A2, CYP2C9, and CYP3A4 were avoided unless considered clinically necessary.

Study populations included the safety, pharmacokinetic, pharmacodynamic, and efficacy (exploratory) populations. The safety and pharmacokinetic populations were defined as all patients who received at least one dose of study drug; the pharmacodynamic population was defined as patients receiving at least one dose of study drug and from whom tumor or non-tumor tissue samples were collected. The efficacy population encompassed all patients in the safety population evaluable for response (i.e., patients who had a baseline tumor assessment and at least one post-baseline tumor assessment).

Study design and dose escalation
This was a phase I, open-label, single-arm, dose-escalation study (NCT00486135). Patients received SAR245408 (capsule formulation) using 1 of 2 regimens: either once daily for the first 21 of every 28 days (21/7) or continuous once-daily dosing (CDD) in each 28-day cycle. For each schedule, a standard 3 + 3 design was used for dose escalation, with dose-limiting toxicities (DLT) defined during the first 28-day cycle. Starting doses were 30 mg for the 21/7 regimen, calculated on the basis of results from preclinical in vivo studies, and 100 mg for the CDD cohort, chosen once safety and PK data from the 21/7 regimen were available. A DLT was defined as the occurrence during the study treatment period (cycle 1) of specific events considered
related to the study drug, including: grade 4 neutropenia lasting ≥4 days; grade 4 febrile neutropenia; grade 3 febrile neutropenia lasting ≥3 days, or any other grade 4 hematologic adverse event (AE). In addition, grade ≥3 nonhematologic events that occurred despite prophylaxis or were not easily managed or corrected by medical intervention, and grade ≥3 hyperglycemia not controlled with oral hypoglycemic agents at standard doses, were also considered dose limiting. Drug-related AEs that prevented the start of cycle 2 within 14 days of the planned start date, or prevented ≥75% of the planned dose being taken in cycle 1, were considered DLTs. Any toxicity-related dose delay of >21 days (21/7 regimen) or >28 days (CDD regimen) resulted in patient withdrawal from the study, unless the patient was deriving clinical benefit from study treatment per investigator judgment.

The preliminary maximum tolerated dose (MTD) was defined as the highest dose level below the maximum administered dose at which ≤1 of 6 patients experienced a DLT. After the MTD was identified, additional patients were enrolled in MTD expansion cohorts to further assess safety, pharmacokinetic and pharmacodynamic effects. For the CDD regimen, a proportion of the additional patients enrolled was required to have solid tumors amenable to biopsy, and the rest were required to have non–small cell lung cancer (NSCLC). Information collected beyond cycle 2 and in MTD expansion cohorts was used to determine the recommended phase 2 dose.

To explore the hypothesis that tumors with molecular alterations affecting PI3K pathway components would be more sensitive to SAR245408, a cohort of patients with tumors harboring molecular alterations affecting PI3K pathway components and modulators, such as PI3KCA mutation or PTEN deficiency, was enrolled to a dose level one below the MTD.

Approval was obtained from the ethics committees at the participating institutions and from regulatory authorities. All patients provided informed consent. The study followed the Declaration of Helsinki and good clinical practice guidelines.

Safety assessments
Safety was assessed using standard clinical findings, AEs, echocardiograms, ECOG PS, physical examination, vital signs, concomitant medications, and laboratory assessments. Safety evaluations were conducted during the screening period, at set times during each cycle and during follow-up. Drug-related AEs occurring within 30 days after the last dose were followed until resolution, stabilization, or initiation of new treatment. AEs were graded according to National Cancer Institute Common Terminology Criteria for AEs version 3.0. Safety findings were reviewed on an ongoing basis.

Pharmacokinetic assessments
Whole blood (for plasma) was collected pre-dose on days 1, 2, 8, 15, and 21 (day 28 for CDD regimen) during cycle 1, on day 1 of every cycle for cycles 2–4 (both regimens), and on day 1 of every fourth cycle thereafter (both regimens). During cycle 1, post-dosing blood samples were collected at 0.5, 1, 2, 4, and 8 hours on day 1 (both regimens), at 4 hours on day 8 (both regimens), and at 0.5, 1, 2, 4, and 8 hours on day 21, and on days 22 and 23 or 24 (21/7 regimen) or at 0.5, 1, 2, 4, and 8 hours on day 28 (CDD regimen). During cycles 2–4, post-dosing blood samples were collected at 4 hours on day 1 of every cycle and every 4 cycles thereafter (both regimens). Urine samples were collected within 2 hours pre-dose on day 1 of cycle 1, with an additional single sample collected during the 24-hour period following the cycle 1 day 20 dose (21/7 regimen) or the cycle 1 day 28 dose (CDD regimen).

Pharmacokinetic parameters assessed included: terminal elimination half-life (t1/2,z), time to maximum concentration (tmax), maximum concentration (Cmax), area under the concentration–time curve up to the last measurable concentration (AUClast), area under the concentration–time curve up to 24 hours (AUC24), area under the concentration–time curve from time 0 to infinity (AUCinf), accumulation ratio (AR), and apparent clearance (Cl/F). The amount and percentage of SAR245408 excreted unchanged in urine were also assessed.

Pharmacodynamic bioanalytical assays
Bioanalysis of human plasma (separated from whole blood by centrifugation) and urine samples was conducted by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following a solid-phase extraction (SPE). K2EDTA anticoagulant was removed from plasma samples using an SPE cartridge (Waters Oasis HLB, 30 mg). SAR245408 was eluted from the SPE cartridge using methanol/acetonitrile solution (1:1, v/v), and 10 µL of solution was injected into the LC-MS/MS system. The standard curve covered a linear range of 1.00 to 2,000 ng/mL in human plasma and urine with 100 µL of matrix. The lowest detection limit of the method for plasma and urine was 1 ng/mL. Extracted samples were analyzed using a Shimadzu LC-20AD integrated HPLC system and an Applied Biosystems/MD Sciex API 4000 mass spectrometer with an APCI interface. An isocratic mobile phase containing acetonitrile/water (65/35, v/v) with 1% formic acid at a flow rate of 0.40 mL/min and an HPLC analytical column (Thermo Electron Betasil CN, 2.1 × 50 mm², 5 µm) were used. A positive ion multiple-reaction-monitoring mode was used to detect analyte (m/z 541 → 456) and internal standard (D₆-SAR245408, m/z 546 → 456).

Pharmacodynamic and molecular profiling assessments
Detailed biomarker procedures are provided in the Supplementary section. Briefly, the pharmacodynamic effects of SAR245408 were evaluated in plasma, peripheral blood mononuclear cells (PBMCs), hair sheath cells, buccal mucosa, and skin biopsies from patients in the dose escalation cohort, and in paired tumor biopsies (and skin biopsies in several instances) from patients in the expanded MTD and the tumor molecular alteration cohorts. In addition, molecular profiling was conducted in archival and/or baseline fresh tumor tissue to identify molecular alterations of...
PI3K pathway components and/or modulators that could affect the efficacy of or resistance to SAR245408.

**Efficacy assessments**

Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (19). Patients with measurable disease were assessed using computed tomographic (CT) scans conducted within 21 days before the initial dose and approximately every 8 weeks thereafter. In patients with nonmeasurable lesions, tumor response was assessed (as feasible) using physical examination, radiographic methods, or tumor markers.

**Statistical analyses**

The study used a conventional 3 + 3 dose-escalation design and had no formal sample size calculation or hypothesis testing. The total sample size was dependent on the number of dose levels required to determine the MTD. Safety assessment was the primary objective and efficacy assessment was an exploratory objective. All data were summarized using descriptive statistics within each dose level and/or dosing schedule, and overall in all treated patients unless stated otherwise. For efficacy data, 90% confidence intervals (CI) were constructed on the basis of an exact binary distribution. Pharmacokinetic parameters ($t_{1/2}$, $t_{\text{max}}$, $C_{\text{max}}$, $AUC_{\text{last}}$, $AUC_{0-24}$, $AUC_{\text{inf}}$, AR, CI/F) and the amount and percentage of SAR245408 excreted unchanged in urine were calculated from individual concentration-time data using a noncompartmental method. Further details on the calculation of each of these parameters are provided in the Supplementary Methods.

**Results**

**Study population**

Between July 2007 and February 2011, 68 patients with advanced solid tumors and 1 patient with small lymphocytic lymphoma (SLL) were enrolled and treated ($n = 41$, 21/7 and $n = 28$, CDD). Fifty-seven patients ($n = 34$, 21/7 and $n = 23$, CDD) were evaluable for response. Patient characteristics are summarized in Table 1. Forty-three

| Table 1. Patient baseline and disease characteristics |
|------------------------------------------|------------------|------------------|
| All 21/7 combined ($n = 41$) | All CDD combined ($n = 28$) | Total ($n = 69$) |
| Age, y | 57.5 (13.68) | 60.8 (9.03) | 58.8 (12.05) |
|       | Median (range) | 62.0 (25–86) | 60.0 (43–84) | 60.0 (25–86) |
| Sex, n (%) | 23 (56.1) | 13 (46.4) | 36 (52.2) |
| Male | 18 (43.9) | 15 (53.6) | 33 (47.8) |
| Female | 23 (56.6) | 8 (28.6) | 23 (33.3) |
| ECOG performance status, n (%) | 24 (58.5) | 20 (71.4) | 44 (63.8) |
| 0 | 2 (4.9) | 0 | 2 (2.9) |
| 1 | 0 | 1 (3.6) | 1 (1.4) |
| 2 | 0 | 1 (3.6) | 1 (1.4) |
| Race, n (%) | 2 (4.9) | 2 (7.1) | 4 (5.8) |
| American Indian or Alaska Native | 39 (95.1) | 25 (89.3) | 64 (92.8) |
| Black or African American | 12 (29.3) | 12 (42.9) | 24 (34.8) |
| White | 5 (12.2) | 3 (10.7) | 8 (11.6) |
| NSCLC | 5 (12.2) | 3 (10.7) | 8 (11.6) |
| Prior therapy, n (%) | 19 (46.3) | 10 (35.7) | 29 (42.0) |
| Years since diagnosis | 2 (4.9) | 2 (7.1) | 4 (5.8) |
| Mean (SD) | 2.78 (0.4–17.1) | 2.46 (0.6–22.4) | 2.62 (0.4–22.4) |
| Median (range) | 4.04 (3.8) | 4.07 (4.5) | 4.05 (4.1) |
| Prior systemic cancer therapy only | 14 (34.1) | 10 (35.7) | 24 (34.8) |
| Prior radiation therapy only | 1 (2.4) | 0 | 1 (1.4) |
| Prior systemic therapy and radiation | 25 (63.4) | 18 (64.3) | 43 (63.2) |
| No prior therapy reported | 1 (2.4) | 0 | 1 (1.4) |

Abbreviations: 21/7 = 21 consecutive days on treatment followed by 7 days off treatment; CDD, continuous once-daily dosing; NSCLC, non-small cell lung cancer; SD, standard deviation.
patients (62.3%) had received both radiation and a systemic anticancer therapy before the trial; the median number of prior regimens was 4 (maximum 11).

**Dose-escalation and MTD**

Seven dose levels were evaluated in the 21/7 administration schedule (30, 60, 120, 225, 400, 600, and 900 mg) and 3 were evaluated in the CDD administration schedule (100, 400, and 600 mg). The median overall duration of exposure was 50 days [range, 8–721 days; 21/7 regimen: 49 days (range, 8–616 days); CDD regimen: 56 days (range, 10–721 days)]. Twenty-eight patients (41%) completed 2 treatment cycles; 13 patients (18.8%) received more than 5 cycles.

In the 21/7 group, dose-escalation proceeded to 600 mg without DLT. In the first 600 mg cohort, one patient experienced drug-related grade 3 rash, but no other dose-limiting events occurred in another cohort of 3 patients. At 900 mg, 2 of 3 patients experienced grade 3 rash during the first cycle, defining this dose level as the maximum administered dose and 600 mg as the preliminary MTD. Another 10 patients were enrolled at 600 mg in an expanded cohort without further DLT, so that only 1 of 16 patients enrolled at the 600 mg dose level of the 21/7 schedule experienced a DLT. Six additional patients with PI3K pathway molecular alterations were enrolled at 400 mg. One of the additional patients experienced grade 2 rash (Supplementary Table S1).

With the CDD schedule, no DLTs occurred in the 100 and 400 mg cohorts. At 600 mg, there was one potential DLT of a grade 3 hypersensitivity reaction. A patient with penicillin allergy and an ongoing mild rash developed a diffuse blanching patchy maculopapular erythematous rash over the entire skin surface on day 8. Intravenous fluids, steroids, and antihistamines were administered and the patient discontinued the study on day 11. The rash resolved by day 18. The event was considered to be unlikely related to SAR245408 and more likely related to the patient’s history of allergy and ongoing rash at baseline. No further DLTs were observed in 20 additional patients treated at 600 mg. Further escalation was not pursued because of the intolerability of 900 mg on the 21/7 schedule. Therefore, for SAR245408 capsules, 600 mg was the formally defined MTD on the 21/7 dosing schedule and the recommended dose for the CDD schedule (Supplementary Table S1).

**Safety findings**

As shown in Table 2, 44 (63.8%) patients experienced a drug-related AE (all grades), most commonly (all cohorts) skin toxicities (26%; including events such as macular or generalized rash, erythema, dry skin, and pruritus), nausea (21.7%), diarrhea (20.3%), and decreased appetite (11.6%). Drug-related laboratory abnormalities were uncommon and included 2 events of anemia and 5 instances of hyperglycemia, for which the highest severity was grade 2. Nine patients (13.0%) reported grade 3/4 drug-related AEs, the most frequent being rash and diarrhea. Drug-related AEs (all grades) appeared to be more frequent in the CDD cohort than in the 21/7 cohort (75.0% vs. 56.1%). One (1.4%) occurrence of grade 3/4 γ-glutamyltransferase was considered treatment-related.

Of the 18 patients with drug-related skin toxicities (rash group), 5 patients reported events of grade 3 severity, 2 of which occurred outside of the DLT observation period. Severe rashes were typically generalized, macular, erythematous, and pruritic, with nonspecific biopsy findings.

![Table 2. All-grade and grade ≥3 treatment-related adverse events occurring in at least 2 patients (21/7 and CDD capsule regimens, n = 69)](image-url)
suggested of either folliculitis or perivascular dermatitis. SAR245408 was discontinued for grade 3 events, and patients received oral steroids, with or without topical steroids. Resolution was variable and was documented within 4 to 50 days. After resolution, several patients resumed with dose reduction (e.g., 600 mg reduced to 400 mg) without recurrence of rash. However, one patient on the 600 mg CDD dose level, who also had mild eosinophilia (13.6%) at the time of the initial grade 3 presentation, developed a recurrent rash in areas of sun exposure when he resumed at 400 mg. SAR245408 was discontinued with gradual improvement, although leathery, cracking skin persisted. Grade 1 and 2 events often resolved with topical steroids, antihistamines, or, in some cases, no intervention, developed a recurrent rash in areas of sun exposure when he resumed at 400 mg. SAR245408 was discontinued for grade 3 events, and resolution was variable and was documented during the rest week of one of the first 2 cycles.

Overall, 22 patients (31.9%) experienced serious AEs (SAEs); of these, 3 (4.3%) patients reported an SAE possibly related to SAR245408 (grade 1 pyrexia; grade 3 full-body rash; grade 4 arterial thrombosis). No notable trends in related toxicity were reported. Eight patients died within 30 days of treatment discontinuation between treatment cohorts. SAR245408 was discontinued for grade 3 events, and continued because of AEs, of which 2 AEs (grade 3 rash and grade 4 arterial thrombosis) were considered related to SAR245408. There were no apparent differences in reasons for discontinuation between treatment cohorts.

Pharmacokinetic analysis

Single-dose PK data are shown in Table 3. Single-dose exposure (C_{max} and AUC_{last}) for the 21/7 regimen showed high interpatient variability across cohorts. C_{max} and AUC_{last} appeared to increase dose proportionally in the dose range of 30 to 400 mg, but values at 400 and 600 mg appeared similar. C_{max} and AUC_{last} values were approximately 2-fold higher in the 900 mg cohort than in the 400 and 600 mg dose cohorts. Within the CDD cohort, C_{max} and AUC_{last} values showed high interpatient variability across cohorts. Mean total Cl/F values were 2-fold higher at the 900 mg dose level cohorts. Mean total Cl/F values were 2-fold higher at the 900 mg dose level; consistent with the 21/7 schedule cohorts, exposure values for the 400 and 600 mg dose levels were comparable. The median T_{max} occurred at 4 to 22 hours post-dose across all cohorts.

Repeated-dose PK data at steady state were analyzed at cycle 1, day 21 for the 21/7 schedule and at cycle 1, day 28 for the CDD schedule (Table 4 and Supplementary Fig. S1). For the 21/7 schedule, the mean terminal half-life (t_{1/2}) of SAR245408 ranged from 70 to 103 hours across the dose cohorts. Steady-state plasma levels were achieved after 15 to 21 days of drug administration. Interpatient variability was observed, with the AUC coefficient of variation percentage ranging 5% to 89%. On both schedules, C_{max} and AUC_{last} values increased approximately 2-fold proportionately up to the 400 mg dose levels, whereas values were similar for the 400 and 600 mg dose level cohorts. Mean total Cl/F values were 2-fold higher for the 600 mg dose than for doses ranging 30 to 400 mg (Supplementary Fig. S1). For all dose levels, high accumulation of SAR245408 was observed; the mean AR

### Table 3. Pharmacokinetic results from single-dose analysis

<table>
<thead>
<tr>
<th>Dose, mg (range)</th>
<th>Median T_{max}, h</th>
<th>C_{max}, ng/mL</th>
<th>C_{max}/D, ng/mL/mg</th>
<th>Median T_{last}, h</th>
<th>AUC_{last}, ng/mL</th>
<th>AUC_{last}/D, ng/mL/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>21/7 30 (22.8–23.8)</td>
<td>2.2</td>
<td>1,500 (815) (40.9)</td>
<td>50.1 (20.5) (40.9)</td>
<td>23.2 (0.853) (3.7)</td>
<td>27,600 (12,800) (46.5)</td>
<td>919 (427) (46)</td>
</tr>
<tr>
<td>60 (8.0–21/7)</td>
<td>3</td>
<td>830 (810) (92.3)</td>
<td>51.7 (20.5) (92.3)</td>
<td>24.2 (0.347) (1.4)</td>
<td>17,790 (5,200) (29.7)</td>
<td>649 (409) (83.0)</td>
</tr>
<tr>
<td>120 (4.1–20/8–6.0)</td>
<td>4</td>
<td>8,960 (2,300) (36.1)</td>
<td>74.7 (26.9) (36.1)</td>
<td>24.1 (0.199) (0.8)</td>
<td>1,030 (7,000) (34.0)</td>
<td>592 (409) (83.0)</td>
</tr>
<tr>
<td>225 (3.0–8.0–8.1)</td>
<td>5</td>
<td>8,260 (4,810) (55.9)</td>
<td>36.7 (20.5) (55.9)</td>
<td>23.3 (1.29) (6.5)</td>
<td>1,550 (8,400) (54.8)</td>
<td>687 (377) (55.0)</td>
</tr>
<tr>
<td>400 (6.5–24/5–4.5)</td>
<td>6</td>
<td>17,300 (14,000) (80.9)</td>
<td>43.3 (35.1) (80.9)</td>
<td>23.7 (0.748) (3.2)</td>
<td>3,010 (2,500) (33.2)</td>
<td>752 (414) (55.0)</td>
</tr>
<tr>
<td>600 (8.0–24/3–8.9)</td>
<td>7</td>
<td>15,600 (8,560) (54.7)</td>
<td>26.1 (14.2) (54.7)</td>
<td>21.7 (6.08) (28.1)</td>
<td>2,740 (1,860) (67.9)</td>
<td>456 (209) (88.0)</td>
</tr>
<tr>
<td>900 (8.0–24/3–23.7)</td>
<td>8</td>
<td>28,100 (19,100) (68.0)</td>
<td>31.2 (21.2) (68.0)</td>
<td>24.0 (0.376) (1.56)</td>
<td>5,900 (4,200) (71.2)</td>
<td>656 (467) (71.0)</td>
</tr>
<tr>
<td>CDD 100 (2.0–24/0–4.0)</td>
<td>9</td>
<td>5,200 (1,730) (33.2)</td>
<td>52.0 (17.3) (33.2)</td>
<td>24.4 (0.692) (0.69)</td>
<td>1,030 (5,500) (34.4)</td>
<td>1,030 (353) (34.0)</td>
</tr>
<tr>
<td>400 (3.0–24/2–24.0)</td>
<td>10</td>
<td>12,500 (4,340) (34.8)</td>
<td>31.1 (10.8) (34.8)</td>
<td>24.2 (0.337) (1.4)</td>
<td>2,500 (7,000) (30.1)</td>
<td>816 (713) (31.0)</td>
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<tr>
<td>600 (8.0–24/2–4.4)</td>
<td>11</td>
<td>13,900 (10,800) (77.9)</td>
<td>23.2 (18.1) (77.9)</td>
<td>23.3 (1.52) (15.1)</td>
<td>2,510,000 (2,500,000) (76.9)</td>
<td>418 (322) (77.0)</td>
</tr>
</tbody>
</table>

Abbreviations: 21/7, 21 consecutive days on treatment followed by 7 days off treatment; AUC_{last}, area under the concentration–time curve up to the last measurable concentration; C_{max}, maximum concentration; CDD, continuous once-daily dosing; CV, coefficient of variation; D, dose normalized; D_{CDD}, dose normalized; t_{1/2}, terminal elimination half-life.
Table 4. Pharmacokinetic results from repeated-dose analysis

<table>
<thead>
<tr>
<th>Dose, mg</th>
<th>$t_{1/2}$, h</th>
<th>$T_{max}$, h</th>
<th>$C_{max}$, ng/ml</th>
<th>$C_{max}/D_{1}$, ng/ml</th>
<th>$T_{last}$, h</th>
<th>AUC$_{0-24}$, h ng/ml</th>
<th>AUC$_{0-24}$, h ng/ml</th>
<th>CI/F, ml/h</th>
<th>AR C$_{max}$</th>
<th>AR AUC</th>
<th>AUC$_{last}$, h ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 (NC)</td>
<td>2.69 (1.13)</td>
<td>12,300 (5,510)</td>
<td>411 (184)</td>
<td>143 (82.7)</td>
<td>2,58,000 (1,12,000)</td>
<td>8,589 (3,743)</td>
<td>140 (82)</td>
<td>10.34 (7.98)</td>
<td>11.91 (8.31)</td>
<td>9.44 (6.30)</td>
<td>9.44 (6.30)</td>
</tr>
<tr>
<td>60 (NC)</td>
<td>4.04 (0.589)</td>
<td>10,200 (5,140)</td>
<td>169 (85.7)</td>
<td>192 (1.77)</td>
<td>2,29,000 (1,22,000)</td>
<td>3,808 (2,039)</td>
<td>307 (164)</td>
<td>4.76 (1.04)</td>
<td>5.01 (0.46)</td>
<td>13,40,000 (5,84,000)</td>
<td></td>
</tr>
<tr>
<td>120 (NC)</td>
<td>14.0 (14.2)</td>
<td>44,000 (919)</td>
<td>366 (7.68)</td>
<td>179 (14.9)</td>
<td>9,82,000 (50,200)</td>
<td>8,192 (436)</td>
<td>122 (7.5)</td>
<td>6.02 (0.14)</td>
<td>6.42 (0.28)</td>
<td>50,10,000 (88,400)</td>
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<td>225 (NC)</td>
<td>6.69 (2.29)</td>
<td>45,700 (39,803)</td>
<td>203 (177)</td>
<td>137 (77.8)</td>
<td>3,60,000 (9,39,000)</td>
<td>4,699 (4,185)</td>
<td>334 (213)</td>
<td>5.25 (2.26)</td>
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<td>51,80,000 (61,10,000)</td>
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<tr>
<td>400 (NC)</td>
<td>3.63 (3.25)</td>
<td>79,900 (36,600)</td>
<td>249 (91.4)</td>
<td>109 (9.78)</td>
<td>22,60,000 (2,35,000)</td>
<td>5,642 (586)</td>
<td>178 (18)</td>
<td>10.98 (6.62)</td>
<td>10.64 (5.80)</td>
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<tr>
<td>600 (NC)</td>
<td>4.22 (2.74)</td>
<td>95,200 (43,600)</td>
<td>159 (72.7)</td>
<td>156 (8.23)</td>
<td>20,60,000 (9,38,000)</td>
<td>3,429 (1,562)</td>
<td>462 (484)</td>
<td>8.05 (4.98)</td>
<td>8.98 (7.07)</td>
<td>1,02,00,000 (59,40,000)</td>
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<tr>
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<td>0.59 (NC)</td>
<td>1,36,000 (NC)</td>
<td>151 (NC)</td>
<td>217 (NC)</td>
<td>29,40,000 (NC)</td>
<td>3,267 (NC)</td>
<td>306 (NC)</td>
<td>12 (NC)</td>
<td>13 (NC)</td>
<td>2,04,00,000 (NC)</td>
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Abbreviations: 21/7, 21 consecutive days on treatment followed by 7 days off treatment; AUC$_{last}$, area under the concentration–time curve up to the last measurable concentration; AUC$_{0-24}$, area under the concentration–time curve up to 24 hours; AR, accumulation ratio; C$_{max}$, maximum concentration; CDD, continuous once-daily dosing; CL, apparent clearance; CV, coefficient of variation; D, dose normalized; NC, not calculated; $t_{last}$, time to last measurable concentration; $t_{max}$, time to maximum concentration; $t_{1/2}$, terminal elimination half-life.
Figure 1. Reduction of PI3K signaling by SAR245408 in xerial hair cells and in skin biopsies. Pharmacodynamic inhibition by SAR245408 on the PI3K pathway was documented by immunofluorescence in hair sheath cells and skin biopsies. A, effect of SAR245408 on the PI3K/mTOR pathway in hair sheath cells, assessed by immunofluorescence staining of phosphorylated eIF4E-binding protein-1 (p4EBP1)T70, pAKTT308, phosphorylated proline-rich AKT1 substrate (pPRAS40)T246, and pS6S240/244 in cross-sections of baseline and post-dose hair collected from a patient who received SAR245408 225 mg daily on the 21/7 schedule. A representative field was captured per readout at ×400 magnification. (Continued on the following page.)
Pharmacodynamic analysis

Repeated dosing of SAR245408 caused a minor increase in fasting plasma insulin 2 hours post-dose on days 8 and 21, and a significant food-induced insulin increase at 4 hours post-dose on days 8 and 21, indicative of hyperinsulinemia (Supplementary Fig. S2) and consistent with the known role of PI3K in insulin signaling. In contrast, minimal to no effect on plasma glucose concentrations was evident. No consistent modulation of VEGF-A, insulin-like growth factor binding protein-2 (IGFBP-2), or regulated and normal T-cell expressed and secreted (RANTES) levels was observed (data not shown).

The impact of SAR245408 on the PI3K pathway was assessed in paired surrogate tissues (hair sheath cells and skin) from a limited number of patients during dose escalation and in tumor biopsies (and in some instances skin biopsies) from patients treated at the MTD. Novel immunofluorescence staining methods providing more quantitative readout than traditional immunohistochemistry were developed to investigate PI3K pathway modulation (20). Analysis of hair sheath sets collected from four patients treated with 120, 225, and the 600 mg MTD dose in the 21/7 cohort showed a time-dependent pathway inhibition, with pronounced effects at 120 and 225 mg (Fig. 1A and B; Supplementary Table S2) for PI3K proximal biomarkers (pAKT <i>S</i>473, pPRAS40 <i>T</i>246 and pAKT <i>T</i>308 with 51%–80%, 59%–80%, and 48%–75% reduction, respectively), and downstream biomarkers (p4EBP1 <i>T</i>70 and pS6 <i>S</i>240/244 with 59%–82% and 68%–82% reduction, respectively). No obvious dose–response relationship was observed but pathway inhibition was more pronounced at higher plasma concentrations of SAR245408. Analysis of paired skin biopsies collected from 9 patients treated with doses ranging from 30 to 900 mg showed moderate pathway inhibition, with maximum inhibition ranging from 30% to 55% for proximal and distal biomarkers (Fig. 1C and D and Supplementary Table S2). Pharmacodynamic effects were also explored in PBMC lysates and buccal mucosal smears; however, because of technical challenges, limited data were obtained (data not shown).

Analysis of paired tumor biopsies collected from 9 patients enrolled in the 600 mg MTD cohort (including a patient enrolled in the 900 mg cohort who underwent dose reduction to 600 mg) showed robust but partial PI3K pathway inhibition, with >60% pAKT <i>T</i>308 reduction in 5 of the 9 tumor sets (range, 41%–82%; Fig. 2A–C and Supplementary Table S3). Downstream inhibition of the PI3K pathway was evident with reduction of TORC1 biomarkers (p4EBP1 <i>T</i>70 and pS6 <i>S</i>240/244 of 39%–73% and 68%–70%) and TORC2 biomarkers (pAKT <i>S</i>477 and pPRAS40 <i>T</i>246 of 55%–61% and 50%–68%). Interestingly, SAR245408 also had an effect on the RAS/MEK/ERK pathway in tumor tissue, with pERK <i>T</i>202/204 and pMEK <i>T</i>217/221 reductions of 42% to 70% and 46% to 59%, respectively (Fig. 2 and Supplementary Fig. S3). The interpatient variability in exposure observed did not account for observed differences in pathway inhibition. The impact on cell proliferation was modest (15%–49% reduction of Ki67) and induction of apoptosis was minimal or not apparent (Fig. 2C). Inhibition of the PI3K pathway occurred in tumors with and without molecular alterations in PI3K pathway components/modulators (Fig. 2 and Supplementary Table S3).

Pathway modulation by SAR245408 was more pronounced in tumor tissue compared with normal skin in 3 patients receiving the 600 mg MTD dose for whom both tumor and normal skin samples were available (Figs. 2C and 2B; Supplementary Tables S2 and S3). Similarly, when tumor and adjacent normal skin were collected in the same biopsy from either a patient with hamartoma (Cowden syndrome) or one with Merkel cell carcinoma, pathway modulation was more pronounced in tumor tissue than in normal skin adjacent to tumor tissue (Fig. 2D and Supplementary Table S3).

Antitumor activity

Twenty-five patients (43.9%) had stable disease as the best response, and 8 patients were progression-free at 6 months, including patients with NSCLC, prostate, and head and neck cancer, as well as the patient with SLL (Supplementary Table S4). Three patients were on study for ≥12 months. Prolonged stable disease was observed among patients regardless of the mutational status of components of the PI3K pathway. Three of 33 patients evaluated had tumors with PIK3CA mutation, 6 of 33 patients had tumors harboring PTEN deletion or mutation, and 5 of 14 patients had tumors with TP53 mutation, all consistent with frequencies expected for this phase I population. In the one patient with NSCLC with a partial response, no mutations in PIK3CA, PTEN, KRAS, MET, EGFR, or LKB1 genes were detected in archival tumor tissue (Supplementary Table S4). There were also no apparent differences in efficacy between the 2 dosing regimens.

(Continued.)
Figure 2. Reduction in PI3K signaling by SAR245408 in paired tumor biopsies. Pharmacodynamic inhibition by SAR245408 of the PI3K and ERK pathways, documented by immunofluorescence. A, effect of SAR245408 on the PI3K pathway and tumor proliferation in 3 patients treated with SAR245408 at 600 mg daily. Inhibition of the PI3K pathway was assessed by immunofluorescence staining of cryopreserved biopsy samples collected from patients with NSCLC or leiomyosarcoma treated with the 21/7 schedule, and a patient with tongue SCC treated with the CDD schedule. (Continued on the following page.)
Discussion

We report here the safety, tolerability, and preliminary efficacy results of the pan-class I PI3K inhibitor SAR245408. The MTD in the capsule formulation was determined to be 600 mg, both for the 21/7 and for the CDD schedules. Although the number of dose reductions was higher in the 21/7 cohort and treatment-related AEs were slightly more common in the CDD cohort, differences between the schedules appeared to be of limited importance, such that SAR245408 had a manageable toxicity profile, with drug-related severe AEs being infrequent. Of note is the relatively low incidence of hyperglycemia, a secondary effect of PI3K pathway inhibition observed with some PI3K pathway inhibitors. In this study, 5 patients (7.2%) presented with drug-related hyperglycemia (all grades). In a recent study of BKM120, a pan-class I PI3K inhibitor, 37% of patients experienced drug-related hyperglycemia, including 9% with grade 2–3 severity (21). In contrast, minimal and/or transient hyperglycemia was observed with the irreversible pan-class I PI3K inhibitor PX-866 (22) and the AKT inhibitor MK-2206 (23), probably due to compensatory mechanisms.

The drug exposure parameters presented here show that pharmacokinetic variables were similar between the 21/7 and CDD cohorts and did not change with duration of dosing. The plasma concentrations associated with robust pharmacodynamic impact and antitumor efficacy in mice (17) are comparable to plasma concentrations associated with PI3K pathway inhibition in this clinical study. Pharmacodynamic analyses showed evidence of PI3K pathway inhibition in tumor tissue and hair sheath cells and, to a lesser extent, in skin tissue.

PI3K pathway modulation was observed in all 9 tumor biopsy sets evaluated (Supplementary Table S3). Furthermore, PI3K pathway inhibition was still apparent at day 28 in a patient with ovarian leiomyosarcoma enrolled to the 21/7 schedule, suggesting that pathway modulation persisted through the 7-day break in dosing. This observation may be attributed to the long half-life and high accumulation of SAR245408.

PI3K pathway inhibition in tumor was partial (pAKT^T308 range in reduction of 41%–82%, including four tumor sets with >60% reduction). The degree of inhibition required for antitumor activity in patients is unknown and may vary depending on tumor addiction to the pathway and/or the presence of molecular alterations causing resistance. PI3K pathway modulation has also been documented to achieve greater pathway inhibition at doses associated with more pronounced than in adjacent tumor tissue, suggesting some degree of tumor-cell selectivity and hence the potential to minimize AEs in clinical practice. Such selectivity may stem from differences in pathway activation state or local exposure to SAR245408 due to altered vascular integrity in tumors.

Because mTOR inhibition by rapalogs has been associated with ERK pathway activation in both preclinical models and patients (24), we examined the impact of SAR245408 on the RAF/MEK/ERK pathway in tumor tissue. A clear inhibition of the MEK/ERK pathway was observed. SAR245408 is highly selective for class I PI3K isoforms, with IC_{50} values for BRAF, CRAF, and MEK >10 μmol/L, and no effect on the MAPK pathway was observed in preclinical models (18, 19). The mechanisms underlying SAR245408 inhibition of the ERK pathway in tumor tissue are unclear; PI3K inhibition may disrupt positive crosstalk between the PI3K and ERK pathways, or alternatively, the effect may be indirect and mediated by changes in tumor biology caused by PI3K inhibition that are not yet elucidated. This observation is of substantial interest given the potential importance of simultaneously inhibiting the PI3K and MAPK pathways in tumor cells. These data may explain the efficacy of SAR245408 against KRAS-mutant xenograft models (18).

Modest effects on proliferation and apoptosis were observed, suggesting that with the exposure achieved in the study population, SAR245408 effects are primarily cytostatic and not pro-apoptotic. As such, it is not surprising that stable disease was the best response seen in 44% of patients, of which 8 maintained their response for >6 months. These results are comparable to those reported for the pan-class I PI3K inhibitor BKM120 (21), the irreversible pan-class I PI3K inhibitor PX-866 (22), and the AKT inhibitor MK-2206 (23). Several PI3K isoform-specific inhibitors are in development, and these agents may have the potential to achieve greater pathway inhibition at doses associated with minimal AEs. The data here show that the pan-PI3K inhibitor SAR245408 administered at biologically active doses is associated with a manageable safety profile that compares favorably with other pan-PI3K inhibitors (21).

Frequencies of molecular alterations in PIK3CA, PTEN, and TP53 were consistent with those expected for this phase I population. Inhibition of the PI3K pathway occurred in tumors with and without alterations in PI3K pathway components/modulators, and no correlation between efficacy and molecular alterations was identified. Some
preclinical studies have suggested that PIK3CA mutations might predict sensitivity to pan-PI3K and dual PI3K/mTOR inhibitors (25); however, these results have not yet been clinically validated. In contrast, studies with α-isomser-selective and β-isomser-sparing PI3K inhibitors have produced responses among patients with tumors harboring PIK3CA mutations (26, 27); in addition, the presence of a PIK3CA mutation-related gene signature may identify patients with breast cancer who may benefit from the addition of everolimus to letrozole (28), although clinical benefit from everolimus is not restricted to patients with PIK3CA-mutant tumors. The molecular profiling conducted here was limited to analysis of a few candidate genes and evaluation of PTEN protein levels in a heterogeneous population. Further extensive next-generation molecular profiling analyses in homogenous patient populations with agents targeting the PI3K/mTOR pathway will be required to better define signatures of response, clinical benefit, and resistance.

In summary, this first-in-human phase I study showed a favorable safety profile, demonstrable pharmacodynamic effects and preliminary antitumor activity of SAR245408 in patients with advanced solid tumors, supporting its further development. Evaluation of SAR245408 as monotherapy is ongoing in endometrial cancer, glioblastoma, and lymphoma. In addition, a tablet formulation of SAR245408 is being evaluated in patients with advanced solid tumors. Rational combination studies of SAR245408 with other targeted or cytotoxic agents are ongoing or completed, which should help define the role of PI3K inhibition in the anticancer armamentarium.

Disclosure of Potential Conflicts of Interest
A.D. Laird, L.T. Nguyen, and C. Scheffold are employees of Exelixis Inc. as Senior/Executive Directors (A.D.L., L.T.N.) and Medical Director (C.S.). A.D. Laird and C. Scheffold hold stocks and shares in Exelixis Inc. C. Egile and Y. Xu are employees of Sanofi as Directors. E.L. Kwak declares financial support from Sanofi (paid to the place of employment) related to the study reported here. C. Bedell has Honoraria from Speakers Bureau of Colgene and is a Consultant/Advisory Board member of Teva. No potential conflicts of interest were disclosed by the other authors.

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Writing, review, and/or revision of the manuscript: G.I. Shapiro, J. Rodon, E.L. Kwak, I. Braia, S.S. Pandya, C. Scheffold, A.D. Laird, C. Egile
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G.I. Shapiro, E.L. Kwak, A.D. Laird, C. Egile, G. Edelman

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References


Correction: Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of SAR245408 (XL147), an Oral Pan-Class I PI3K Inhibitor, in Patients with Advanced Solid Tumors

In this article (Clin Cancer Res 2014;20:233–45), which was published in the January 1, 2014, issue of Clinical Cancer Research (1), the affiliation for Dr. Jordi Rodon was listed incorrectly. Dr. Rodon’s affiliation should read as follows: Vall d’Hebron University Hospital and Universitat Autonoma de Barcelona, Barcelona, Spain. The publisher regrets this error.

Reference

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Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of SAR245408 (XL147), an Oral Pan-Class I PI3K Inhibitor, in Patients with Advanced Solid Tumors

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