Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of SAR245409 (XL765), a Novel, Orally Administered PI3K/mTOR Inhibitor in Patients with Advanced Solid Tumors

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Abstract

Purpose: This phase I, first-in-human study evaluated the safety, maximum-tolerated dose (MTD), pharmacokinetics, pharmacodynamics, and preliminary efficacy of SAR245409, an inhibitor of pan-Class I phosphoinositide 3-kinase (PI3K) and mTOR, administered orally once or twice daily in patients with advanced solid tumors.

Experimental Design: Eighty-three patients received SAR245409. Doses ranged from 15 to 120 mg twice daily, and 70 to 100 mg once daily. A 3+3 dose-escalation design was used to determine the MTD. Patients were evaluated for adverse events and response. Assessments included pharmacokinetic, pharmacodynamic impact of SAR245409 on PI3K pathway signaling in hair sheath cells, skin and tumor, and characterization of tumor molecular alterations.

Results: The MTDs were 50 mg twice daily and 90 mg once daily. The most frequent treatment-related adverse events were nausea (36.1%), diarrhea (21.7%), vomiting (19.3%), and decreased appetite (16.9%). The most frequent treatment-related grade 3/4 adverse events were increases in alanine aminotransferase (6.0%) and aspartate aminotransferase (4.8%). SAR245409 had a relatively short plasma half-life (2.96–7.52 hours). At MTDs, once- and twice-daily regimens yielded similar mean steady-state plasma exposure. A reduction in PI3K and mTORC1/mTORC2 pathway signaling was observed in serial hair sheath cells, skin, and tumor samples. Best response was stable disease in 48% of evaluable patients; seven patients had minor tumor regression. Twelve patients with stable disease were treated for ≥16 weeks. No trend was observed correlating tumor molecular alteration with antitumor activity.

Conclusion: SAR245409 had a manageable safety profile, demonstrated reduced PI3K and mTORC1/mTORC2 pathway signaling and was associated with clinically relevant stable disease. Clin Cancer Res; 1–12. ©2014 AACR.

Introduction

Phosphoinositide 3-kinase (PI3K) signaling is involved in essential cellular functions, including cell metabolism, growth, survival, and motility (1). Molecular alterations in components of the PI3K pathway are frequently observed in human cancers. These include gain-of-function mutations or amplifications of oncogenes and loss or downregulation of negative regulators of the pathway (2–4). Alterations in the PI3K pathway are implicated in resistance to receptor tyrosine kinase antagonists/inhibitors (5) and a number of cytotoxic anticancer therapies (6, 7). Therefore, treatment with PI3K inhibitors as single agents may reduce tumor cell proliferation and possibly promote tumor cell death, whereas their use in combination with other anticancer therapies may sensitize cancer cells to such therapies (6–8).

Inhibitors of the PI3K pathway in clinical development include agents targeting PI3K (either pan-Class I or isoform-specific inhibitors), AKT, mTOR, and molecules with multiple
Molecular alterations in the phosphoinositide 3-kinase (PI3K) pathway are frequently observed in human cancer and have been implicated in resistance to anticancer therapies. In this regard, PI3K has been established as a valid target for anticancer therapy, and several PI3K inhibitors are under clinical evaluation. However, agents that inhibit both PI3K and mTOR are able to suppress mTORC1 and mTORC2 downstream of PI3K, which may result in more complete pathway inhibition and attenuation of PI3K activation triggered by inhibition of mTOR-dependent negative-feedback mechanisms.

This article reports the results of a first-in-human study of SAR245409 (XL765), a specific pan-Class I PI3K inhibitor and mTORC1/mTORC2 inhibitor, in patients with advanced tumors. PI3K and mTORC1/mTORC2 pathway inhibition by SAR245409 was observed in serial hair sheath cells, skin, and tumor tissue. Preliminary evidence of antitumor activity and a manageable toxicity profile supports further development of SAR245409 as monotherapy or in combination with other agents.

targets such as dual PI3K/mTOR inhibitors (9, 10). Dual PI3K/mTOR inhibitors are able to suppress the mTORC1 and mTORC2 protein complexes downstream of PI3K, resulting in more complete pathway inhibition, and should attenuate PI3K activation triggered by inhibition of mTOR-dependent negative-feedback mechanisms (1, 11).

SAR245409 (XL765; Sanofi) is a pyridopyrimidone-derivative, highly selective, potent ATP-competitive, reversible pan-Class I PI3K inhibitor and mTORC1/mTORC2 inhibitor (9, 12, 13). SAR245409 inhibits the phosphorylation of multiple PI3K/Phosphatase and tensin homolog (PTEN) pathway proteins (AKT, p70S6K, and S6) in cell lines in vitro and in human xenograft tumor tissue (14). The pharmacodynamic activity of SAR245409 is associated with inhibition of tumor cell proliferation, inhibition of tumor angiogenesis, and induction of apoptosis in xenograft tumors, which results in substantial tumor growth inhibition in multiple human tumor models (9, 14).

In preclinical toxicology studies for up to 6 months in rats and dogs, findings of potential clinical relevance included reversible toxicity to the hematopoietic tissues, gastrointestinal tract, and liver. In a 6-month study in rats, possible SAR245409-related ophthalmologic findings included focal cataracts in both eyes of 2 rats (2%), with a correlative microscopic finding of unilateral lenticular degeneration in one animal (data on file). Based on preclinical data, the potential for SAR245409 to affect the QTc interval and cause torsade de points seemed to be low. In vivo results from rat and dog demonstrated that the major circulating metabolite was a glucuronide of a hydroxylated SAR245409.

The primary objective of this phase I, first-in-human study (NCT00485719) was to evaluate the safety and tolerability and to determine the maximum tolerated dose (MTD) of SAR245409 administered orally once or twice daily in fasting patients with solid tumors. Secondary objectives included evaluation of pharmacokinetics, pharmacodynamic impact, and preliminary efficacy data. Pharmacodynamic impact on the PI3K pathway was explored using plasma biomarkers, hair sheath cells, skin, and tumor tissue.

**Patients and Methods**

**Study population**

Eligible patients were aged ≥18 years, with histologically confirmed metastatic or unresectable solid tumors, Eastern Cooperative Oncology Group (ECOG) performance status ≤2 and adequate hematologic and organ function. Additional requirements were fasting plasma glucose <160 mg/dL and glycosylated hemoglobin <8%. Patients were excluded if they had received prior treatment with a PI3K inhibitor. Patients with brain metastases were considered eligible if they had not received radiation therapy within 2 weeks of enrollment and had been on a stable dose of steroids for ≥2 weeks.

The protocol was approved by all relevant Independent Ethics Committees and Institutional Review Boards, and complied with the recommendations of the Helsinki Declaration. Informed consent was obtained from each patient before any study-related procedure.

**Study design**

This multicenter, phase I, nonrandomized, open-label, first-in-human study evaluated SAR245409 as a single agent in patients with solid tumors. Two oral dosing regimens, continuous once- and twice-daily administration of SAR245409 capsules for 28-day cycles, were investigated sequentially. Patients fasted for 2 hours before and 1 hour after each dose of SAR245409.

A standard 3 + 3 dose-escalation design was used to determine the MTD for each dose schedule, with starting doses of 15 mg twice daily, based on preclinical in vivo studies, and 100 mg once daily. The starting dose for the once-daily dosing schedule was based on the preliminary MTD determined for the preceding twice-daily dosing schedule. The preliminary MTD for each dose schedule was defined as the highest dose level in which ≤1 in 6 patients experienced a dose-limiting toxicity (DLT), based on drug-related toxicities that occurred during cycle 1; at subsequent cycles, cumulative toxicity observed at lower doses, and adverse events in all patients enrolled were taken into consideration in assigning the final MTD and recommended phase II dose. A DLT was defined as any National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0 (15) grade ≥3 hematologic or nonhematologic toxicity, or any toxicity requiring dose reduction/discontinuation, including grade ≥3 hyperglycemia despite treatment with an oral hypoglycemic at standard doses and

**Translational Relevance**

Molecular alterations in the phosphoinositide 3-kinase (PI3K) pathway are frequently observed in human cancer and have been implicated in resistance to anticancer therapies. In this regard, PI3K has been established as a valid target for anticancer therapy, and several PI3K inhibitors are under clinical evaluation. However, agents that inhibit both PI3K and mTOR are able to suppress mTORC1 and mTORC2 downstream of PI3K, which may result in more complete pathway inhibition and attenuation of PI3K activation triggered by inhibition of mTOR-dependent negative-feedback mechanisms.

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grade €3 nausea, vomiting, and diarrhea despite adequate management. Once the preliminary MTD was determined for each schedule, an additional 9 to 15 patients with selected solid tumors considered likely to harbor PI3K pathway alterations, or patients with tumors with documented molecular alterations affecting the PI3K pathway, were enrolled into the expanded MTD cohorts.

Safety assessments
Safety evaluations were performed at baseline, days 1, 8, 15, 22, 27, and 28/29 of cycle 1, days 1, 8, 15, 22, and 23 of cycle 2, weekly in cycle 3 and bi-weekly thereafter. Variables included clinical findings, adverse events, electrocardiogram (ECG), vital signs, and laboratory assessments. Ophthalmic evaluation was performed at baseline and at day 1 of cycles 2 and 4, and every 3 subsequent cycles.

Pharmacokinetic assessments
Blood samples for pharmacokinetic analyses were collected predose and 0.25, 0.5, 1, 2, 3, 4, 6, 8, approximately 10 to 12 hours, and 24 hours postmorning dose on day 1 and 27 of cycle 1, and on day 22 of cycle 2. Single predose and 2-hour postmorning dose samples were taken on additional days (see Supplementary Material). To investigate drug washout, no doses were given following the morning dose on day 27 of cycle 1 and pharmacokinetic samples were taken at 24 and 48 hours postdose before cycle 2. Plasma concentrations of SAR245409 were measured using a validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. NONcompartmental pharmacokinetic analysis and calculation of descriptive statistics were performed using WinNonlin Professional 5.2 (Certara). Pharmacokinetic plasma concentrations and pharmacokinetic parameters were based on measured free-base SAR245409 whereas doses were expressed as the amount of SAR245409 HCl salt for this capsule formulation (e.g., a 50-mg dose contains 44 mg of SAR245409 free-base). The amount and percentage of SAR245409 excreted unchanged in urine were also determined (see Supplementary Methods).

Pharmacodynamic assessments
The pharmacodynamic effects of SAR245409 were explored using plasma biomarkers, hair sheath cells, skin, and tumor tissue collected at prespecified time points. In addition, archival and/or freshly collected tumor tissues from patients were subjected to molecular profiling for alterations in genes encoding PI3K pathway components and/or modulators. Additional tumor molecular alteration data were provided by the trial sites (see Supplementary Materials for pharmacodynamic methods).

Efficacy measurements
Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST v1.0; ref. 16). Patients with measurable lesions were assessed using computed tomography scan at baseline and approximately every 8 weeks.

Statistical analysis
Continuous and categorical data were summarized with descriptive statistics or frequencies and percentages, respectively. The safety population included all patients who received at least one dose of study drug; the efficacy population included all patients in the safety population with a baseline assessment and at least one postbaseline tumor assessment.

Results
Patient population
A total of 83 patients were enrolled between June 6, 2007, and July 15, 2010. Of these, 52 patients received SAR245409 orally twice daily (15, 30, 50, 60, and 120 mg twice-daily cohorts) and 31 received treatment once daily (70, 90, and 100 mg once-daily cohorts). Patient demographics are summarized in Table 1. All patients discontinued treatment; 61 (73.5%) had disease progression, 11 (13.3%) had an adverse event requiring discontinuation, 9 (10.8%) withdrew consent, one (1.2%) discontinued because of investigator decision and one (1.2%) died. The median duration of exposure for all patients was 41.0 days (range: 4–371 days).

Dose-limiting toxicities and MTD
DLTs occurred in 6 patients. With twice-daily dosing, dose escalation from 15 mg (n = 3) to 30 mg (n = 3), 60 mg (n = 3), and 120 mg (n = 4) proceeded without DLT. One patient receiving 120 mg developed grade 3 liver enzyme elevation during cycle 2, prompting expansion to 3 additional patients at this dose level. One of these patients experienced a DLT of grade 3 decreased appetite and hypophosphatemia, and 2 patients developed grade 3 liver enzyme elevation during cycle 2. Consequently, the 60-mg dose level was expanded, with no DLT in 3 additional patients. With further safety expansion at this dose level in 4 patients, there was 1 DLT of intolerable grade 2 pruritic rash and 1 patient developed grade 2 liver enzyme elevation in cycle 2 requiring dose interruption. An intermediate dose of 50 mg was tested in 8 patients, without occurrence of DLT. A further 17 patients were treated at 50 mg, 2 of whom developed grade >2 liver enzyme elevation in cycle 2. Four additional patients with tumor genetic alterations of the PI3K pathway were permitted to enroll at a dose level one lower than the MTD (30 mg); none experienced DLT or grade >2 adverse events. Although most cases of treatment-related liver function abnormalities did not meet DLT criteria and often occurred after cycle 1, concerns existed that liver toxicity would be cumulative and therefore limit dosing at and above the 60-mg twice-daily dose level. Thus, 50 mg was declared the MTD for twice-daily dosing.

With once-daily dosing, 2 of 7 patients treated at 100 mg had DLTs of grade 3 rash and grade 2 dyskinesia. Dose de-escalation to 70 mg in 5 patients showed no DLTs. A dose of
90 mg once daily was next explored: no DLTs occurred in 6 patients and the cohort was expanded to include an additional 13 patients. There was 1 DLT each of grade 3 rash and grade 3 ECG abnormalities (asymptomatic T-wave inversion) in 1 patient. The 90-mg dose was declared the MTD for the once-daily dosing schedule.

Dose delays or reductions related to adverse events occurred in 7 of 25 patients in the 50-mg twice-daily cohort and 7 of 19 patients in the 90-mg once-daily cohort.

Safety and tolerability

All 83 patients were evaluable for safety and all experienced ≥1 adverse event; the most frequently reported adverse events regardless of causality are shown in Supplementary Tables S1 and S2. Sixty-one (73.5%) patients had ≥1 adverse event considered related or possibly related to study treatment; the most frequently reported treatment-related adverse events were nausea (36.1%), diarrhea (21.7%), vomiting (19.3%), and decreased appetite (16.9%; Table 2). Gastrointestinal events occurred less frequently in the 50-mg twice-daily cohort than in the 90-mg once-daily cohort (nausea 16.0% vs. 47.4%; diarrhea 12.0% vs. 31.6%; vomiting 12.0% vs. 31.6%), but differences were not clinically meaningful overall.

The most common treatment-related grade 3/4 adverse events, occurring in 5 patients, were reversible increases in alanine aminotransferase (ALT; 6.0%) and aspartate aminotransferase (AST; 4.8%; Table 2). A total of 16 (19.3%) patients experienced at least one incidence of elevated liver enzymes because of SAR245409.

Treatment-related skin events, most commonly rash and dry skin, were experienced by 10 (12.0%) patients, of which 2 (2.4%) had a grade 3/4 event. Rashes were most commonly pruritic and/or maculopapular. Treatment-related ocular adverse events were experienced by 4 (4.8%) patients; cataracts occurred in 3 (3.6%) patients and grade 1 reversible decreased visual acuity in 1 patient. Drug attribution for cataracts was based on preclinical findings of focal cataracts following prolonged exposure to SAR245409. Consistent with preclinical data, no patients experienced QTcF prolongation.

Grade 1/2 hyperglycemia was reported as an adverse event in 11 (13.3%) patients with no apparent dose effect; only one of these events was treatment related. No patient had grade ≥3 hyperglycemia.

A total of 11 (13.3%) patients discontinued treatment because of an adverse event, most commonly because of increases in the liver enzymes AST and ALT, each in 3 (3.6%) patients. Two (2.4%) patients discontinued because of rash.

Six treatment-related serious adverse events (diarrhea [grade 2], oral mucosa erythema [grade 3], rash generalized [grade 3], ALT increased [grade 4], AST increased [grade 4], and ECG abnormal T-wave inversion [grade 3]) occurred in 4 (4.8%) patients. Ten patients died during the study, 9 because of disease progression and 1 of stroke; no deaths were attributed to SAR245409.

Pharmacokinetic analysis

The median time to maximum plasma concentration (t_{max}) of SAR245409 ranged from 1 to 4 hours, and the geometric mean terminal half-lives ranged from 2.96 to 7.52 hours (Table 3; Supplementary Fig. S1). Neither parameter showed a clear dependence on dosing regimen or dose level. Steady state was achieved by day 8. At day 27, the 90-mg once-daily and 50-mg twice-daily regimens showed approximately 1.1- and 2.6-fold accumulation of SAR245409 in plasma, respectively. The majority of SAR245409 was cleared from plasma over the dosing interval (12 or 24 hours); geometric mean values of the minimum observed plasma concentration (C_{min}) on day 27 were 3.3% and 5.4% of the maximum observed plasma concentration (C_{max}) for the 90-mg once-daily and 50-mg twice-daily regimens, respectively.
### Table 2. Treatment-related adverse events that occurred in ≥5.0% of patients (all grades) and ≥2.0% of patients (grade 3/4)

<table>
<thead>
<tr>
<th>Dose</th>
<th>15 mg twice daily</th>
<th>30 mg twice daily</th>
<th>60 mg twice daily</th>
<th>120 mg twice daily</th>
<th>50 mg once daily</th>
<th>100 mg once daily</th>
<th>70 mg once daily</th>
<th>90 mg once daily</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>25</td>
<td>7</td>
<td>5</td>
<td>19</td>
<td>83</td>
<td>63</td>
</tr>
<tr>
<td>n (%)</td>
<td>4.0 (2, 8)</td>
<td>2.0 (1, 6)</td>
<td>2.0 (1, 10)</td>
<td>2.0 (1, 12)</td>
<td>1.0 (1, 6)</td>
<td>1.0 (1, 2)</td>
<td>2.0 (1, 13)</td>
<td>2.0 (1, 13)</td>
<td></td>
</tr>
</tbody>
</table>

Number of cycles of study treatment administered per patient, median (range)

| Number of patients who received | 3 (100.0) | 6 (85.7) | 7 (70.0) | 6 (85.7) | 15 (60.0) | 7 (100.0) | 1 (20.0) | 16 (84.2) | 61 (73.5) |

Number of patients with at least 1 adverse event related to study treatment

<table>
<thead>
<tr>
<th>Nausea</th>
<th>1 (33.3)</th>
<th>1 (14.3)</th>
<th>3 (30.0)</th>
<th>4 (57.1)</th>
<th>4 (16.0)</th>
<th>7 (100.0)</th>
<th>1 (20.0)</th>
<th>9 (47.4)</th>
<th>30 (36.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>2 (28.6)</td>
<td>0</td>
<td>5 (71.4)</td>
<td>3 (12.0)</td>
<td>1 (14.3)</td>
<td>1 (20.0)</td>
<td>6 (31.6)</td>
<td>18 (21.7)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>0</td>
<td>2 (20.0)</td>
<td>3 (42.9)</td>
<td>3 (12.0)</td>
<td>2 (28.6)</td>
<td>0</td>
<td>6 (31.6)</td>
<td>16 (19.3)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>1 (33.3)</td>
<td>2 (28.6)</td>
<td>0</td>
<td>4 (57.1)</td>
<td>3 (12.0)</td>
<td>2 (28.6)</td>
<td>1 (20.0)</td>
<td>1 (5.3)</td>
<td>14 (16.9)</td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>0</td>
<td>2 (20.0)</td>
<td>0</td>
<td>5 (71.4)</td>
<td>3 (12.0)</td>
<td>0</td>
<td>0</td>
<td>1 (5.3)</td>
<td>11 (13.3)</td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
<td>0</td>
<td>1 (10.0)</td>
<td>0</td>
<td>5 (71.4)</td>
<td>4 (16.0)</td>
<td>0</td>
<td>0</td>
<td>1 (5.3)</td>
<td>11 (13.3)</td>
</tr>
<tr>
<td>γ-Glutamyltransferase increased</td>
<td>0</td>
<td>1 (10.0)</td>
<td>0</td>
<td>5 (71.4)</td>
<td>4 (16.0)</td>
<td>0</td>
<td>0</td>
<td>1 (5.3)</td>
<td>11 (13.3)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>1 (14.3)</td>
<td>0</td>
<td>1 (14.3)</td>
<td>1 (4.0)</td>
<td>1 (14.3)</td>
<td>1 (20.0)</td>
<td>0</td>
<td>5 (6.0)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0</td>
<td>1 (14.3)</td>
<td>0</td>
<td>1 (14.3)</td>
<td>1 (4.0)</td>
<td>1 (14.3)</td>
<td>1 (20.0)</td>
<td>0</td>
<td>5 (6.0)</td>
</tr>
</tbody>
</table>

NOTE: Reported event terms were coded using MedDRA dictionary version 15.0. Adverse events were defined according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Treatment-related adverse events were defined as judged possibly or probably related by the investigator.
Table 3. Summary of pharmacokinetic parameters of SAR245409

<table>
<thead>
<tr>
<th></th>
<th>15 mg twice daily</th>
<th>30 mg twice daily</th>
<th>50 mg twice daily</th>
<th>60 mg twice daily</th>
<th>70 mg once daily</th>
<th>90 mg once daily</th>
<th>100 mg once daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 n</td>
<td>3</td>
<td>7</td>
<td>25(^a)</td>
<td>10</td>
<td>7(^b)</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>(t_{\text{max}}), h, median (range)</td>
<td>1.92 (1.00, 2.00)</td>
<td>2.00 (1.00, 3.00)</td>
<td>1.03 (0.250, 8.00)</td>
<td>2.04 (1.00, 4.00)</td>
<td>0.583 (3.08)</td>
<td>2.08 (2.00, 6.00)</td>
<td>2.00 (0.500, 6.08)</td>
</tr>
<tr>
<td>(C_{\text{max}},\ \text{ng/mL},\ \text{geometric mean, mean (CV%)})</td>
<td>43.5 (29.7)</td>
<td>105 (62.7)</td>
<td>148 (72.1)</td>
<td>235 (92.5)</td>
<td>277 (85.5)</td>
<td>241 (42.7)</td>
<td>212 (70.6)</td>
</tr>
<tr>
<td>(AUC_{0-\text{h}}\times\text{ng/mL, geometric mean, mean (CV%)})</td>
<td>155 (82.2)</td>
<td>387 (56.6)</td>
<td>556 (72.1)</td>
<td>818 (1.00, 3.07)</td>
<td>675 (1.00, 3.00)</td>
<td>277 (110.0)</td>
<td>212 (124.0)</td>
</tr>
</tbody>
</table>

Day 2 n

|                | 2                | 4\(^c\)          | 16\(^d\)         | 5                | 3\(^e\)         | 3              | 14\(^f\)         |
| \(t_{\text{max}}\), h, median (range) | 1.50 (1.00, 2.00) | 2.50 (1.02, 3.07) | 1.00 (1.00, 3.00) | 2.00 (1.00, 6.00) | 2.00 (1.00, 2.07) | 2.00 (2.00, 6.00) | 2.00 (1.00, 24.0) | 4.00 (2.00, 6.00) |
| \(C_{\text{max}},\ \text{ng/mL},\ \text{geometric mean, mean (CV\%)}\) | 126 (44.0) | 151 (59.1) | 176 (63.1) | 407 (60.6) | 437 (77.6) | 410 (39.5) | 385 (68.3) | 285 (28.5) |
| \(C_{\text{min}},\ \text{ng/mL},\ \text{geometric mean, mean (CV\%)}\) | 5.40 (122.2) | 10.7 (122.3) | 7.43 (146.1) | 7.39 (90.1) | 8.40 (43.3) | 4.39 (70.3) | 4.39 (70.3) | 5.93 (160.3) |
| \(AUC_{0-\text{h}}\times\text{ng/mL, geometric mean, mean (CV\%)}\) | 472 (49.4) | 504 (87.1) | 410 (76.0) | 616 (60.9) | 601 (50.3) | 813 (50.3) | 2,120 (31.0) | 1,720 (38.7) |
| \(t_{1/2,Z},\ \text{h, geometric mean, mean (CV\%)}\) | 2.96 (48.7) | 3.15 (87.8) | 4.09 (75.2) | 5.19 (61.7) | 5.92 (55.3) | 5.73 (54.8) | 5.82 (79.1) | 7.34 (19.8) |
| \(AUC_{0-\text{h}}\ \text{day 27/day 1 ratio, geometric mean, mean (CV\%)}\) | 2.03 (17.7) | 2.05 (44.2) | 0.93 (88.2) | 2.04 (118.9) | 1.03 (39.1) | 2.03 (20.6) | 1.28 (117.6) | 1.10 (113.2) |

NOTE: \(AUC_{0-\text{h}}\), the area under the plasma concentration–time curve, integrated from time 0 to end of dosing interval (>9 and ≤14 hours for twice daily; >20 and ≤28 hours for once daily) on day 1 and integrated from time 0 to exactly 12 hours (twice daily) or to exactly 24 hours (once daily) on day 27; \(C_{\text{max}}\), observed maximum plasma concentration over dosing interval; \(C_{\text{min}}\), observed minimum plasma concentration over dosing interval; CV, coefficient of variation; \(t_{1/2,Z}\), terminal phase half-life; \(t_{\text{max}}\), time to reach the maximum plasma concentration.

\(^a\)Except for \(AUC_{0-\text{h}}\) (\(n = 22\)).
\(^b\)Except for \(AUC_{0-\text{h}}\) (\(n = 6\)).
\(^c\)Except for \(t_{1/2,Z}\) (\(n = 3\)).
\(^d\)Except for \(t_{1/2,Z}\) (\(n = 15\)), and for \(AUC_{0-\text{h}}\) day 27/day 1 ratio (\(n = 14\)).
\(^e\)Except for \(AUC_{0-\text{h}}\) day 27/day 1 ratio (\(n = 2\)).
\(^f\)Except for \(t_{1/2,Z}\) (\(n = 13\)).
\(^g\)The geometric mean value for this parameter could not be calculated using all available data because 2 patients had concentrations below the lower limit of quantitation (set to 0) over the dosing interval. When these 2 patients were excluded, the geometric mean \(C_{\text{min}}\) value was 9.43 ng/mL (\(n = 12\)).
Exposure following single and repeated doses of SAR245409 was variable between patients; the CV% for the 50-mg twice-daily and 90-mg once-daily dose groups ranged from 76% to 91% for the area under the plasma concentration–time curve (AUC). Because of this interpatient variability, dose proportionality could not be definitively established, although a nonstatistically significant trend toward a less than dose-proportional increase in plasma exposure was observed. Similarly, a nonstatistically significant trend toward a higher steady-state plasma $C_{\text{min}}$ ($\sim 1.8$-fold) was seen for the twice-daily regimen. The percentage of the SAR245409 dose excreted unchanged in urine was low and independent of dose ($<1\%$; data not shown).

**Pharmacodynamic analysis**

A significant augmentation in plasma insulin was observed at 4 hours postdose on days 1, 8, and 27/28, coinciding with the end of fasting. In contrast with day 1, at the 2-hour time point on days 8 and 27/28, insulin levels were modestly but significantly elevated above baseline, suggesting hyperinsulinemia (Supplementary Fig. S2A). A minimal increase in plasma glucose was observed, which was transient and reversible with no evidence of hyperglycemia (Supplementary Fig. S2B). 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).

During dose escalation, limited surrogate tissue analyses were performed to confirm pharmacodynamic activity, with a focus on tumor pharmacodynamics at MTD. Four sets of serial hair sheath cells and 12 sets of serial skin biopsies were analyzed (see Supplementary Materials) and time-dependent inhibition of PI3K and mTORC1/mTORC2 pathways was evident (Fig. 1). Pathway inhibition on day 27 to 29 reached 53% to 90% in hair sheath cells (in 4 patients receiving SAR245409 15, 60, and 120 mg twice daily) and 42% to 92% in skin biopsies (in 12 patients receiving SAR245409 50, 60, and 120 mg twice daily and 70 and 100 mg once daily).

Paired baseline and day 27/28 tumor biopsies were collected from 12 patients receiving either the twice-daily MTD of 50 mg ($n = 6$), the once-daily MTD of 90 mg ($n = 5$), or 60 mg twice daily ($n = 1$). Inhibition of PI3K and mTORC1/mTORC2 pathways in tumor was evident in 11 patients, with reductions of 45% to 88% for $p$AKT$^{T308}$, 45% to 94% for $p$AKT$^{S473}$, and 55% to 89% for $p$4EBP1$^{T70}$ at day 27/28 compared with baseline (Fig. 2 and Supplementary Table S3). A moderate ($20\%$–$53\%$) postdose reduction in the proliferation marker Ki67 in tumor was evident for 9 patients. However, a more pronounced reduction in tumor Ki67 was observed for a patient with PTEN-deficient chondrosarcoma and a patient with breast cancer with an AKT1 mutation (96% and 68% reduction, respectively). Changes in the apoptotic marker TUNEL were minimal, suggesting that the effect of SAR245409 as monotherapy in patients with solid tumors is primarily antiproliferative, not pro-apoptotic. In addition, SAR245409 had an impact on tumor extracellular-signal-regulated kinase (ERK) with reductions in phosphorylated extracellular-signal-regulated kinase (pERK)$^{T202/Y204}$ (42%–88%) and phosphorylated mitogen-activated protein kinase kinase (pMEK)$^{S217/S221}$ (45%–62%; Fig. 2 and Supplementary Fig. S3 and Table S3). As surrogate tissue analyses were generally limited to the dose-escalation phase, tumor/surrogate tissue comparisons were not possible.

Of the 12 tumors assessed, 6 had no detectable molecular abnormalities leading to PI3K/mTOR pathway activation, whereas 1 patient with chondrosarcoma, 1 patient with colorectal mucinous tumor, and 3 patients with breast cancer exhibited either PI3K catalytic subunit $\alpha$ (PIK3CA) mutation, AKT1 mutation, PTEN deficiency, or HER2 amplification. Hence, pharmacodynamic modulation and impact on cell proliferation was not confined to tumors with molecular alterations predicted to result in PI3K pathway activation (Supplementary Table S3). The lack of pathway inhibition observed in the patient with BRAF-mutant/PTEN-deficient colorectal cancer may reflect constitutive activation of both the PI3K and ERK pathways.

**Efficacy**

The best overall response was stable disease, occurring in 24 of 50 evaluable patients (48%), with 7 patients showing some evidence of tumor regression (Fig. 3). Of these 24 patients, 15 received the twice-daily schedule and 9 received the once-daily schedule. Twelve patients (24%) had documented stable disease (SD) and were treated for $\geq 16$ weeks. Three patients with KRAS-mutant tumors remained on study for 24 to 33 weeks. Overall, no correlation between molecular alterations and clinical outcomes was observed. However, consistent with observed pathway inhibition and decreased tumor cell proliferation, improvement in skin lesions was noted in a patient with AKT1-mutant ductal breast carcinoma following initiation of SAR245409 (Supplementary Fig. S4).

**Discussion**

This phase I, first-in-human, dose-escalation study of the orally administered pan-Class I PI3K and mTOR inhibitor SAR245409 in patients with advanced solid tumors determined the MTD, and recommended doses for phase II development, to be 50 mg for the twice-daily and 90 mg for the once-daily dosing schedules.

The safety profile of SAR245409, with the exception of hyperglycemia, was comparable to other PI3K inhibitors in clinical development, for which fatigue, gastrointestinal and cutaneous adverse events, and biochemical changes such as liver enzyme abnormalities and hyperglycemia have been commonly reported (17–21). Overall, the incidence of adverse events did not differ markedly between once-daily and twice-daily dose cohorts at the MTDs. Drug-related increases in liver enzymes were frequently observed, but were reversible with dose reduction or discontinuation. Efficacy was an exploratory objective in the study; best overall response of stable disease was achieved in 48% of patients, which is consistent with responses in phase I.
studies of other oral PI3K and PI3K/mTOR inhibitors in solid tumors (17–19, 21). Interestingly, prolonged objective responses with SAR245409 50 mg twice daily were observed in patients with relapsed lymphoma (ongoing complete response of more than 125 weeks in a patient with transformed lymphoma, and partial responses in a patient with diffuse large B-cell lymphoma and a patient with mantle cell lymphoma of 15 and 111 weeks, respectively; 22).

SAR245409 had a relatively short plasma terminal half-life with no clear dose dependence. At the MTD, once-daily and twice-daily regimens seemed to yield similar average plasma exposures at steady state within the context of interpatient variability. Because of interpatient variability...
in exposure, a definitive assessment of dose proportionality could not be conducted.

Novel immunofluorescence staining protocols with pixel- and intensity-based quantitative readouts were developed to investigate pathway modulation by SAR245409. PI3K and mTORC1/mTORC2 pathway inhibition was observed in serial hair sheath cells and skin (below MTD), and in tumor tissue (at MTD). In skin and tumor tissue, similar degrees of pathway inhibition were observed with once-daily and twice-daily regimens. Whether more complete pathway inhibition might translate into improved single-agent efficacy in solid tumor patients is conjectural. Of note, comparable depth of PI3K/mTOR pathway inhibition (−60% to −88%) in tumor samples from a patient with mantle cell lymphoma treated with the twice-daily MTD of 50 mg was sufficient to block cell proliferation and demonstrate a partial response following 2 cycles of treatment (22). Similar levels of pathway inhibition were observed in glioblastoma tumor tissue using quantitative immunochemistry (23).

Figure 2. Reduction in PI3K and mTORC1/mTORC2 signaling by SAR245409 in paired tumor biopsies. A, effect of SAR245409 on the PI3K/mTOR signaling pathway and tumor proliferation. Inhibition of PI3K/mTOR pathway signaling was documented by immunofluorescence staining of cryopreserved biopsy samples collected from a patient with ductal breast carcinoma at baseline and cycle 1, day 27 postdose. Representative fields from each time point were captured per readout [red for pAKT<sup>Thr308</sup>, pAKT<sup>Ser473</sup>, or phosphorylated eIF4E-binding protein-1 (p4EBP1)<sup>Ser70</sup>, blue for 4′,6-diamidino-2-phenylindole (DAPI) and magenta for Ki67] at ×400 magnification. Reductions in immunofluorescence of 64% (pAKT<sup>Thr308</sup>), 53% (pAKT<sup>Ser473</sup>), 55% (p4EBP1<sup>Ser70</sup>), and 68% (Ki67) were observed at cycle 1 day 27 compared with baseline. B, quantification of immunofluorescence staining at baseline and on cycle 1 day 27 or 28 of individual patient samples (each line representing an individual patient) and box plot representations (inset plot) for pAKT<sup>Thr308</sup>, pAKT<sup>Ser473</sup>, p4EBP1<sup>Ser70</sup>, and pERK<sup>Thr202/Tyr204</sup> levels in tumor biopsies for 12 patients receiving twice-daily and once-daily MTD of SAR245409.
Although SAR245409 has no direct inhibitory activity on ERK pathway enzymes (12), a reduction in ERK signaling in tumor tissues was observed at tolerated doses. The mechanism underlying this modulation of the RAS/ERK pathway in tumor tissue is unclear, but may reflect diminished cross-talk between the PI3K and RAS/ERK pathways, or changes in tumor biology secondary to PI3K pathway inhibition. The reduction in tumor pERK associated with SAR245409 administration contrasts with the increase in tumor pERK associated with administration of the rapamycin analog everolimus (24). Prolonged stable disease observed in 3 patients with KRAS-mutant colorectal cancer suggests attenuation of the effect of upstream hyperactivated RAS (25).

Importantly, effects on PI3K pathway signaling and on proliferation were not confined to tumors exhibiting molecular alterations in the PI3K pathway. Based on the limited characterization performed, no correlation between molecular alterations and clinical outcome was identified, perhaps reflecting the diverse phase I population, the multiple doses administered, and the modest degree of clinical benefit evident in patients with solid tumors. Similar results were reported in a phase I trial of the PI3K inhibitor BKM120 (Novartis Pharma AG; ref. 18). Identification of predictive biomarkers for patient selection, elucidation of optimal depth and duration of target inhibition, and rational drug combinations will help maximize the antitumor potential of this class of agents. Exploration of predictive biomarkers of response to SAR245409 in archival tumor tissue and in circulating tumor DNA is ongoing in phase II studies in solid tumors and in relapsed or refractory lymphoma. In addition, the relationship of SAR245409 exposure to glucose homeostasis is being further explored in ongoing phase II studies.

In summary, this phase I, first-in-human study demonstrated a favorable safety profile, promising pharmacodynamic effects and preliminary antitumor activity of SAR245409 in patients with advanced solid tumors, supporting its further development. Rational combination studies of SAR245409 with other targeted and cytotoxic agents are ongoing or completed, including with the MEK inhibitor pimasertib in solid tumors (NCT01390818) and ovarian cancer (NCT01936363), erlotinib (NCT00777699) and temozolomide (in anaplastic gliomas/glioblastomas; NCT00704080). Both once-daily and twice-daily MTDs of SAR245409 are being explored in phase II combination and single-agent studies. Encouraging objective partial and
complete responses in patients with lymphomas (22) have prompted studies of twice-daily SAR245409 in combination with rituximab and/or bendamustine (NCT01410513) and as monotherapy in a phase II trial in lymphoma and leukemia (NCT01403636).

Disclosure of Potential Conflicts of Interest
A.D. Laird and D. Miles are employees of and have ownership interest (stocks and shares) in Exelixis Inc. C. Egle and W. Shi are employees of Sanofi. R. Ruiz-Soto is an employee of and has ownership interest (stocks and shares) in Sanofi. A. Patnaik reports receiving research funding from Sanofi. J. Tabernero is a consultant/advisory board member for Amgen, Boehringer, Bristol-Myers Squibb, Genentech, Imclone, Lilly, and Merck, and has received speakers bureau honoraria from Amgen, Merck KGaA, Novartis, Roche and Sanofi. No potential conflicts of interest were disclosed by the other authors.

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