Two Drug Interaction Studies of Sirolimus in Combination with Sorafenib or Sunitinib in Patients with Advanced Malignancies


Abstract

**Purpose:** Sirolimus is the prototypical mTOR inhibitor. Sorafenib and sunitinib are small molecule inhibitors of multiple kinases including VEGF receptor (VEGFR) kinases. These agents have different mechanisms of action, providing a strong rationale for combination.

**Experimental Design:** Patients with advanced cancer were assigned to receive either sirolimus or the VEGFR inhibitor alone for a 2-week lead-in period, followed by combination therapy. The primary end point of each trial was to determine whether a drug interaction exists between sirolimus and either sorafenib or sunitinib, as defined by a difference in $C_{\text{max}}$ for each drug alone compared with its $C_{\text{max}}$ during combination therapy.

**Results:** The sorafenib and sunitinib trials enrolled 34 and 23 patients, respectively. There were no clinically significant differences in $C_{\text{max}}$ for any of the drugs alone compared with the $C_{\text{max}}$ during combination therapy. Toxicity profiles were similar to those expected for each drug alone. One patient with adrenal cortical cancer had a partial response to sirolimus and sunitinib.

**Conclusions:** Sirolimus can be safely combined with sorafenib or sunitinib. Our trial design is feasible and informative in screening for potential drug–drug interactions, using a relatively small number of patients and limited pharmacokinetic sampling.

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study, patients were excluded if their corrected QT interval was greater than 500 milliseconds.

For the trial of sirolimus and sorafenib, prior treatment with sorafenib or an mTOR inhibitor (including sirolimus) was allowed; prior therapy with both sorafenib and an mTOR inhibitor was not allowed. In the trial of sirolimus and sunitinib, no prior VEGFR or mTOR inhibitors were permitted.

For the sunitinib trial, patients on a stable therapeutic dose of warfarin with an international normalized ratio (INR) less than 3 and no thromboembolic event within 6 months were eligible. For the sunitinib trial, a normal INR and no thrombotic or thromboembolic events for 1 year were required.

The protocol was reviewed by the Institutional Review Board, and all patients provided written informed consent.

Study design and treatments

The primary end point of each trial was to determine whether a drug interaction exists between sirolimus and either sorafenib or sunitinib, as indicated by a clinically significant change in PK parameters on coadministration compared with those of either drug alone. Within each trial, patients were sequentially assigned to 1 of 2 treatment arms. During the initial 2-week lead-in period, patients received either sirolimus or a VEGFR inhibitor alone, according to their assigned treatment arm within each trial. Starting on day 15, all patients on both trials received combination therapy (Table 1 and Fig. 1). The dose of sirolimus in both trials was selected as one half (sorafenib trial) or two thirds (sunitinib trial) of the maximally tolerated dose of 6 mg previously reported (4). PK sampling was done as described in the following text.

After PK and safety data from the initial treatment groups were reviewed, the sirolimus and sorafenib protocol was amended and an additional 14 patients were enrolled on a third treatment arm to test the tolerability of twice-daily sirolimus dosing. Patients on this expansion arm received sirolimus 2 mg twice daily along with sorafenib 400 mg twice daily on days 1 to 28 of each 28-day cycle.

Patients remained on study until radiographic or clinical disease progression, unacceptable toxicity, or withdrawal of consent. Full supportive care was provided as indicated. If a patient experienced any grade 3 or greater toxicity per the National Cancer Institute (NCI) Common Toxicity Criteria, version 3.0, both drugs were interrupted until the toxicity resolved to grade 1 or less. Patients were removed from the study for any dose interruption of greater than 3 weeks.

Assessments

Evaluations before and during treatment consisted of a complete medical history, physical examinations, hematologic and metabolic laboratory profiles, and toxicity assessments according to the NCI Common Toxicity Criteria, version 3.0. Complete and partial responses and progressive disease were defined and assessed according to the RECIST (Response Evaluation Criteria in Solid Tumors; ref. 12).

PK studies

Sorafenib trial. Samples for PK analysis of sorafenib were collected on day 1 at 1, 4, and 8 hours after sorafenib administration and on day 14 at 0.5 and 6 hours after administration. Sorafenib treatment was started on day 15. Samples for sorafenib pharmacokinetics were collected again on day 15 at 3 and 8 hours after both sorafenib and sorafenib administrations and on day 29 at 20 minutes and 5 hours after administration. For patients assigned to receive sirolimus alone for the initial 2-week lead-in period, samples for PK analysis of sirolimus were collected after sirolimus administration on day 1 at 40 minutes, 3 hours, and 5 hours after administration. For patients assigned to receive sirolimus alone for the initial 2-week lead-in period, samples for PK analysis of sirolimus were collected after sirolimus administration on day 1 at 40 minutes, 3 hours, and 8 hours and on day 8 at 20 minutes and 5 hours. Sorafenib treatment was started on day 15. Samples for sirolimus pharmacokinetics were collected again on days 15 and 22 at 20 minutes and 5 hours after drug administration.

Sunitinib trial. Samples for PK analysis of sunitinib and its metabolite, SU12662, were collected on day 14 before sunitinib administration and at 1, 4, 6, 8, and 24 hours after administration. Sunitinib treatment was started on day 15. Samples for sunitinib pharmacokinetics were collected again on day 28 at the same time points. For patients assigned to receive sirolimus alone for the initial 2-week lead-in period, followed by combination therapy starting on day 15, samples for PK analysis of sirolimus were collected on days 14 and 28, according to the same collection schedule as for sunitinib.

Specimen collection

All patient samples were collected in tubes labeled with the patient's full name and the date, and time of sample collection.
After centrifugation and freezing, sorafenib plasma concentrations were determined by Bayer Pharmaceuticals, using a high-performance liquid chromatographic mass spectrometric method for the determination of sorafenib in human plasma (13).

Sunitinib concentrations were determined after centrifugation, plasma separation, and freezing by BASi laboratories, using mass spectrometry. SU011248 and SU012662 were extracted from human plasma by liquid/liquid extraction at alkaline pH with ethyl acetate. Before the extraction, a deuterated internal standard of SU011248 was added. The organic layer was collected and evaporated to dryness. The residue was reconstituted with an ammonium formate/acetonitrile mixture and injected on a Betasil C18 column of a liquid chromatography/tandem mass spectrometer using an ammonium formate/acetonitrile mobile phase. Calibration standards for sunitinib and its metabolite were used for quality control within each analytic run to form a calibration curve based on mass spectrometer signal responses. A sirolimus interference assessment was done for the sunitinib and SU012662 assays as well. The presence of sirolimus in quality control standard samples does not have a significant effect on the accuracy and precision of sunitinib or SU012662 concentrations determined.

Sunitinib concentrations were measured using a validated method involving centrifugation, addition of internal standard (triamcinolone, 1 µg/mL), and injection into an API 2000 mass spectrometer (Applied Biosystems) equipped with an ESI source. Seven calibration standards and 3 quality control samples (at low, medium, and high concentrations) were included in each run. Intra-assay precision [coefficient of variation (CV) = 7%–11%] and accuracy (range: 90%–108%) were determined by carrying out 3 measurements of the same 7 standards on the same day. Interassay precision (CV = 7%–13%) and accuracy (range: 98%–103%) were determined by assays of the same set of standards in triplicate on 3 days.

Pharmacokinetic analysis

PK analyses for the trial of sirolimus and sorafenib were carried out using NONMEM (version VI, level 1; ICON Development Solutions, ref. 14) and PDx-Pop (version 3.0; ICON Development Solutions) in conjunction with a G95 Fortran compiler. A one-compartment open model with first-order absorption and elimination was used to fit the concentration data. Interindividual and residual unexplained variabilities were described by exponential and combined proportional and additive error models, respectively.

Each patient's individual PK parameters [Ka, apparent clearance (CL/F), and central volume of distribution (V/F)] were calculated by the population typical value and their

<table>
<thead>
<tr>
<th>Drug dosing and schedules</th>
<th>Cycle 1, days 1–14 (initial 2-wk lead-in period)</th>
<th>Cycle 1, day 15 and onward</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sorafenib trial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirolimus pharmacokinetics arm</td>
<td>3 mg po daily</td>
<td>3 mg po daily</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>–</td>
<td>400 mg po bid</td>
</tr>
<tr>
<td><strong>Sorafenib pharmacokinetics arm</strong></td>
<td>–</td>
<td>3 mg po daily</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>400 mg po bid</td>
<td>400 mg po bid</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>–</td>
<td>25 mg po daily</td>
</tr>
<tr>
<td><strong>Sunitinib trial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirolimus pharmacokinetics arm</td>
<td>4 mg po daily</td>
<td>4 mg po daily</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>–</td>
<td>25 mg po daily</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>25 mg po daily</td>
<td>25 mg po daily</td>
</tr>
</tbody>
</table>

Table 1. Drug dosing and schedules

Figure 1. Study design and PK data collection for each treatment arm. For each study arm, PK data for the initial drug were collected during the 2-week lead-in period on day 14. PK data for the same drug were again collected after introduction of the interacting drug. After cycle 1, all patients on both trials were treated with combination therapy.
interindividual variability. Next, area under the concentration–time curve from time zero to infinity (AUC_{0–\infty}) and \( C_{\text{max}} \) were calculated for patients by their individual parameters.

For the sirolimus and sunitinib trial, AUC_{0–\infty} was estimated by noncompartmental PK analysis, using PK Solutions software (version 2.0; Summit Research Services). \( C_{\text{max}} \) is the observed maximum concentration.

Statistical methods

The primary objective was to determine whether there was a drug interaction between sirolimus and either sorafenib or sunitinib. For both trials, changes in steady-state PK parameters of each drug resulting from the initiation of the interacting drug were analyzed separately in each arm.

A signed rank test was used to determine whether the \( C_{\text{max}} \) or AUC of each drug changed significantly after the addition of the interacting drug. Because we were interested only in large effects, we used a sample size of 6 and 8 patients per arm for the sorafenib and sunitinib trials, respectively. This sample size yielded 80% power to detect a 99% change in sirolimus \( C_{\text{max}} \) and a 82% change in sorafenib \( C_{\text{max}} \) assuming within-patient correlation \( \rho = 0.50 \) and \( C_{\text{max}} \) variability as previously reported (15). Similar power was assumed for the sunitinib trial.

Results

Patient characteristics

The baseline demographics and disease characteristics of the patients are presented in Table 2.

Pharmacokinetic data

Table 3 summarizes the population PK parameter estimates for the sirolimus and sorafenib trial.

Baseline \( C_{\text{max}} \) and AUC values for each drug and relative differences after initiation of combination therapy for both trials are reported in Table 4. There were no clinically significant (2-fold, or 100%) changes in \( C_{\text{max}} \) or AUC in the sirolimus and sorafenib trial. The median relative change in sirolimus \( C_{\text{max}} \) was 3.9% \( (p = 0.03) \), which was not clinically significant, even though it was statistically significantly different from 0%. The median relative change in sorafenib \( C_{\text{max}} \) was \(-40.7\% \) and was not statistically significant \( (p = 0.60) \).

There were no clinically significant differences in \( C_{\text{max}} \) or AUC in the sirolimus and sunitinib trial. The median changes in \( C_{\text{max}} \) for sirolimus, sunitinib, and SU12662 were 29.8%, 19.6%, and 35.3%, respectively \( (p > 0.05 \) for all three values; Table 4).

Toxicity

Table 5 summarizes the clinically relevant grade 3 to 5 adverse events for each arm. Combination therapy was well tolerated in both trials and did not result in unexpected toxicities. In the sirolimus and sorafenib trial, one patient died from infection and a second patient died of an arrhythmia. In the sirolimus and sunitinib trial, 2 patients died because of disease progression while on study. A third patient died of disease progression within 30 days of going off the study. None of the deaths on either of the trials were thought to be therapy related.

For the sirolimus and sorafenib expansion study arm of 14 patients treated with twice-daily sirolimus and sorafenib, there were again no unexpected toxicities noted compared with those expected for either drug alone.

Efficacy

In the sirolimus and sorafenib trial, seventy-nine 4-week cycles were administered (median: 2; range: 1–16). There were no responses. Of the 9 patients with stable disease, 2 patients with sarcoma and 1 patient with metastatic urothelial carcinoma had prolonged periods of stable disease with 6, 10, and 16 cycles of treatment, respectively.

In the sirolimus and sunitinib trial, eighty-four 4-week cycles were administered (median: 2; range: 1–14). The best response was a partial response in a patient with

![Table 2. Baseline demographics and disease characteristics](image-url)
adrenal cortical carcinoma who remained on study for 11 cycles. Of the 10 patients with stable disease, one patient with hepatocellular carcinoma received 12 cycles before stopping treatment to pursue an alternative therapy and another patient with non–small cell lung cancer received 14 cycles of treatment before coming off study due to progressive disease.

Fourteen additional patients were treated with twice-daily sirolimus and sorafenib, receiving a cumulative total of thirty-eight 4-week cycles (median: 2; range: 1–6). There were no responses.

Discussion

We conducted 2 drug interaction studies of sirolimus in combination with either sorafenib or sunitinib. Combined mTOR and VEGFR pathway inhibition is a feasible and potentially effective treatment option in patients with advanced malignancies. Currently, several trials examining the combination of sorafenib and the mTOR inhibitor everolimus are ongoing in patients with renal cell carcinoma (16), hepatocellular carcinoma (17, 18), neuroendocrine tumors (19), thyroid cancer (20), and other tumors.
Trials of sorafenib and temsirolimus are also ongoing in patients with similar malignancies (23–25), as well as in melanoma (26) and glioblastoma (27). Trials of sunitinib in combination with both everolimus and with temsirolimus are ongoing in patients with renal cell carcinoma (28, 29).

At the time we designed these studies, we chose to study sirolimus because of its commercial availability, oral formulation, and long safety record. Preclinical evidence for mTOR activity in decreasing tumor proliferation and angiogenesis made sirolimus a strong option to test the feasibility of combining mTOR- and VEGFR-targeted therapies. Given our limited national and global health care resources, the repositioning of drugs previously approved for other indications reduces both drug development costs in oncology and costs to the health care system. Taking into consideration these issues of limited resources and the high costs associated with oncology drug development, a feasible future clinical trial would randomize patients with renal cell carcinoma to VEGF inhibition with or without the addition of sirolimus.

We did not expect any major drug interactions in either trial. Although all 3 drugs are substrates for CYP3A4, none have been shown to be inhibitors of this enzyme. Furthermore, the pharmacokinetics of sunitinib and sorafenib are only modestly affected by inhibitors of CYP3A4 (30, 31).

As expected, we did not observe any clinically significant drug interactions between sirolimus and either sorafenib or sunitinib. However, our trials were powered to detect only large (2-fold or 100%) PK interactions and thus we cannot Table 5. Summary of grade 3 to 5 adverse events

<table>
<thead>
<tr>
<th></th>
<th>Sirolimus/sorafenib (n = 20)</th>
<th>Sirolimus/sorafenib twice daily (n = 14)</th>
<th>Sirolimus/sunitinib (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>5 (25%)</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Hand-and-foot syndrome</td>
<td>2 (10%)</td>
<td>3 (21%)</td>
<td>–</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (5%)</td>
<td>1 (7%)</td>
<td>–</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>2 (10%)</td>
<td>7 (50%)</td>
<td>–</td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>–</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>–</td>
<td>–</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>–</td>
<td>1 (7%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>–</td>
<td>–</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1 (5%)</td>
<td>2 (14%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Gastrointestinal bleed</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Infection</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1 (5%)</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Pain</td>
<td>2 (10%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Elevated aspartate aminotransferase</td>
<td>1 (5%)</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Elevated alanine aminotransferase</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Elevated bilirubin</td>
<td>–</td>
<td>1 (7%)</td>
<td>–</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>1 (5%)</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Superior vena cava syndrome</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Weight loss</td>
<td>1 (5%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (5%)</td>
<td>3 (21%)</td>
<td>–</td>
</tr>
<tr>
<td>Oral mucositis</td>
<td>–</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>–</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Small bowel obstruction</td>
<td>–</td>
<td>–</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>–</td>
<td>1 (7%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>–</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>–</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>–</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Kidney injury</td>
<td>–</td>
<td>–</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Confusion</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Pulmonary artery thrombus</td>
<td>–</td>
<td>1 (7%)</td>
<td>–</td>
</tr>
</tbody>
</table>

a Grade 5 toxicity.
exclude smaller interactions that could still have clinical sequelae.

Patients tolerated both regimens with toxicity profiles similar to those expected from either drug alone. There was notable grade 3 hypophosphatemia in the sirolimus and sunitinib study. Observed toxicities were similar to those seen in other early-phase trials combining mTOR and VEGF inhibition in patients with metastatic melanoma (37) and renal cell carcinoma (38, 39) and a trial in patients with glioblastoma (40), which also included PK data, suggesting no significant drug interaction between sorafenib and temsirolimus. In contrast, a phase I trial of sunitinib and temsirolimus in patients with renal cell carcinoma was terminated after 2 of 3 patients experienced grade 3 toxicities on the lowest dose level cohort (41).

Although other trials have examined the feasibility of combining mTOR- and VEGF-targeted agents using a traditional 2-drug dose escalation study design, our studies were designed with a more focused approach to detect potential drug interactions, emphasizing the importance of question-specific, drug-specific early-phase trial design (42). A previously reported phase I dose escalation trial of sirolimus defined 6 mg as the maximum tolerated dose of daily oral sirolimus, with 3 mg being well tolerated (4), whereas an ongoing study at the University of Chicago has administered 90 mg of sirolimus weekly without a dose-limiting toxicity (43). Therefore, we chose a conservative starting dose of 3 or 4 mg of sirolimus in the sorafenib and sunitinib trials, respectively. Furthermore, because the known pharmacology would have predicted no significant drug–drug interaction in either combination, the use of therapeutic dose levels of sorafenib and sunitinib was thought to be rational and acceptable, while also minimizing subtherapeutic dosing in any subject. Our 2-phase trial design allows for the direct comparison of PK parameters before and after the addition of a second drug, using treatment doses of both agents in all patients. In addition, by including a 2-week lead-in period, we were able to screen for potential drug interactions after achieving steady-state drug concentrations. We recognize that pharmacokinetic drug interactions represent only one of several effectors of treatment response and toxicity. We also note that a more formal drug–drug interaction study would be randomized and would also include statistical analysis of other end points, such as toxicity.

We have shown that our trial design is both feasible and informative in screening for potential drug interactions by using a relatively small number of patients and limited PK sampling. Ongoing clinical trials will further clarify the efficacy of combination mTOR and VEGF inhibition in specific tumors, such as renal cell carcinoma.

Disclosure of Potential Conflicts of Interest


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References


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