Phase Ib study of weekly mammalian target of rapamycin inhibitor ridaforolimus (AP23573; MK-8669) with weekly paclitaxel

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Background: The additive cytotoxicity in vitro prompted a clinical study evaluating the non-prodrug rapamycin analogue ridaforolimus (AP23573; MK-8669; formerly deforolimus) administered i.v. combined with paclitaxel (PTX; Taxol).

Materials and methods: Patients with taxane-sensitive solid tumors were eligible. The main dose escalation foresaw 50% ridaforolimus increments from 25 mg with a fixed PTX dose of 80 mg/m², both given weekly 3 weeks in a 4-week cycle. Collateral levels with a lower dose of either drug were planned upon achievement of the maximum tolerated dose in the main escalation. Pharmacodynamic studies in plasma, peripheral blood mononuclear cells (PBMCs) and skin biopsies and pharmacokinetic (PK) interaction studies at cycles 1 and 2 were carried out.

Results: Two recommended doses were determined: 37.5 mg ridaforolimus/60 mg/m² PTX and 12.5 mg/80 mg/m². Most frequent toxic effects were mouth sores (79%), anemia (79%), fatigue (59%), neutropenia (55%) and dermatitis (48%). Two partial responses were observed in pharyngeal squamous cell and pancreatic carcinoma. Eight patients achieved stable disease ≥4 months. No drug interaction emerged from PK studies. Decrease of eukaryotic initiation factor 4E-binding protein1 (4E-BP1) phosphorylation was shown in PBMCs. Similar inhibition of phosphorylation of 4E-BP1 and mitogen-activated protein kinase was present in reparative epidermis and vascular tissues, respectively.

Conclusion: Potential antiangiogenic effects and encouraging antitumor activity justify further development of the combination.

Key words: angiogenesis, mTOR inhibitor, paclitaxel, phase I, ridaforolimus

introduction

The mammalian target of rapamycin (mTOR) is at the crossroads of different intracellular signals elicited by mitogens, growth factors and nutrients that interact with the PI3K pathway and lead to regulation of cell cycle progression, cell growth and metabolism and vascular endothelial growth factor (VEGF)-dependent angiogenesis [1–3]. Many tumors carry gene mutations that result in the hyperactivation of phosphatidylinositol 3-kinase/protein kinase B and mTOR signaling pathways. Overall, these data point to mTOR as a relevant target for antitumor treatment [4, 5].

Ridaforolimus (AP23573; MK-8669; formerly deforolimus) is a non-prodrug analogue of mTOR that has antitumor activity in preclinical models of colon, lung, breast and pancreatic carcinomas [6]. Ridaforolimus was tested as daily i.v. infusion for 5 days every 2 weeks in a phase I trial that reported antitumor activity [7]. Weekly i.v. ridaforolimus [8, 9] and different oral regimens were tested in separate phase I trials [10].

The in vivo antitumor activity of mTOR inhibitors including ridaforolimus is in part attributed to antiangiogenic effects [11]. Evidence links mTOR to regulation of angiogenesis through hypoxia-inducible factor 1α (HIF1α), integrin-linked kinase (ILK) and 2-deoxy-D-ribose (dRib) [11–13]. Production of dRib and the subsequent angiogenesis is linked to thymidine phosphorylase activity that is induced by taxanes in tumor models [12, 13]. These observations support the combined use of ridaforolimus and paclitaxel (PTX) [14]. In vitro, several
cytotoxic agents, including taxanes, exhibit at least additive antiproliferative activity in combination with ridaforolimus [15, 16]. In view of the ability of PTX to be cytotoxic on the tumor and to trigger antiangiogenic effects through interference with microtubule dynamics in the endothelial cells [17], we designed a combination study of ridaforolimus and PTX. We here report the results of the phase 1 study in which both drugs were administered i.v. weekly for 3 weeks in a 4-week cycle with associated characterization of the PKs of the drugs in combination and of relevant pharmacodynamic markers.

materials and methods

eligibility
Eligibility required was as follows: progressive solid tumor amenable to therapy with taxanes, measurable/evaluable disease according to RECIST [18], ≤2 prior chemotherapies for advanced disease, Eastern Cooperative Oncology Group performance status of zero to one, hemoglobin level ≥9 g/dl, absolute neutrophil count (ANC) ≥1.5 x 10⁹/l, platelets ≥100 x 10⁹/l, bilirubin upper normal limit (UNL), alkaline phosphatase ≤1.5 x UNL, aspartate aminotransferase and alanine aminotransferase ≤UNL or ≤2.5 x UNL in case of liver metastases and creatinine ≤UNL.

Excluded were patients with brain metastases, prior exposure to mTOR inhibitors or to anticancer treatment within 4 weeks or clinical resistance to taxanes (i.e. progression during or within 6 months from last administration).

The study was conducted in three centers, coordinated by the Southern Europe New Drugs Organization, and approved by local ethics committees. All patients signed written informed consent.

study design and treatment
Primary objectives were as follows (i) definition of the maximum tolerated doses (MTD) of weekly ridaforolimus and PTX (Taxol; PTX was obtained from standard commercial sources as the licensed product) and (ii) characterization of the safety and PKs of the combination. Secondary objectives were efficacy (tumor response according to RECIST [18], time to progression, progression-free survival and duration of response) and pharmacodynamics (see below).

A first dose-escalation part was followed by an expansion at the recommended dose (RD). If during escalation, more than two of six patients experienced a dose-limiting toxicity (DLT), the dose one level below was defined as RD. First-cycle DLT was defined as any of the following: ANC ≤500 x 10⁹/l for ≥5 days; febrile neutropenia; grade ≥3 thrombocytopenia; grade ≥2 neurological, cardiac, renal and skin toxicity; grade ≥3 mucosal and other non-hematological toxic effects including nausea and vomiting refractory to standard antiemetic therapy; and failure of delivering therapy for two consecutive weeks.

The escalation design is illustrated in Figure 1A and included two main steps with a starting dose of ridaforolimus at 25 mg (i.e. 50% of the single-agent RD) to maximum 37.5 mg/week in combination with 80 mg/m² PTX and collateral levels in case of excessive DLTs. The possibility of PK interaction was investigated by varying the sequence of drug administration in cycle 1: ridaforolimus was followed by PTX 2 h later and the sequence reversed on days 8 and 9 (see below and Figure 1B). From day 15, cycle 1 onward, study drugs were administered concomitantly. Ridaforolimus was infused i.v. over 30 min and PTX over 1 h after standard premedication (days 1, 8 and 15 every 4 weeks), except for cycle 1 (see below). Safety assessments included weekly physical examination, chemistry, hematology and urinalysis. Toxicity was recorded according to the National Cancer Institute—Common Terminology Criteria for Adverse Events Version 3.0 scale [7]. Efficacy was assessed every other cycle. Patients were assessable for safety if they received at least one dose of study treatment; patients were assessable for DLT if they missed no more than one dose of PTX or ridaforolimus for reasons other than toxicity, within the first cycle.

pharmacokinetics
As illustrated in Figure 1B, whole blood samples were drawn to measure ridaforolimus on days 0 and 9 of cycle 1 and on day 1 of cycle 2 at the following times: before therapy, just before the end of infusion (28 min) and then 0.25, 0.5, 1, 2, 4, 7, 24 and 48 h after infusion. Another sample was collected between days 6 and 8 after each administration. Ridaforolimus was measured by a liquid chromatography/tandem-mass spectrometry method validated according to current Food and Drug Administration guidelines [8] and over a concentration range of 0.5–100 ng/ml.

Samples for PTX were collected for 24 h on days 1 and 8 of cycle 1 and on day 1 of cycle 2 at the following times: before therapy, just before the end of infusion (58 min) and then 0.5, 1, 3, 6 and 23 h after infusion (Figure 1B). PTX concentrations were measured by an high-pressure liquid chromatography-described or UV-described method characterized by a lower limit of quantitation of 5 nM [19].

PK parameters were calculated by noncompartmental methods. Student’s t-tests for paired or unpaired data (two-tailed) were used to test for differences in PK parameters between sequences of administration.

peripheral blood mononuclear cells
Peripheral blood mononuclear cells (PBMCs) were isolated by Vacutainer Cell Preparation Tube (VACUTAINER CPT; Becton Dickinson, Franklin Lakes, NJ) from blood collected on days 0 (baseline), 24 h after ridaforolimus, 8 (before PTX), 9 (24 h after PTX) and 10 (24 h after ridaforolimus) of cycle 1 and on day 1 (baseline) and on day 2 (24 h after ridaforolimus and PTX) of cycle 2 (Figure 1B).

PBMCs lysates were analyzed by western blot using antibodies specific for total Eukaryotic Initiation Factor 4E-binding protein1 (4E-BP1) (Cell Signaling Technology, Beverly, MA) or phosphorylated 4E-BP1 (p4E-BP1, Ser65/Thr70; Santa Cruz Biotechnology, Santa Cruz, CA) [7, 20]. p4E-BP1 levels were normalized to total levels in each sample and expressed relative to baseline.

vascular endothelial growth factor
Blood samples for plasma VEGF were collected at the same times as PBMCs (Figure 1B) and analyzed in triplicate by an enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, MN) with a lower limit of 31 pg/ml.

skin biopsies
Skin biopsies were collected from all patients and analyzed for several biomarkers (see below) in epidermal and vascular tissue. Since skin’s adult endothelial cells are not proliferating, a wound tissue assay evaluated skin granulation tissue, which contains proliferating endothelial cells. A punch biopsy (4 mm diameter) from a normal skin area was carried out on day 7 and the same area was biopsied again on day 0 (pretherapy sample); another skin biopsy from a normal area was carried out on day 0 and rebiopsied before dosing on reparative tissue on day 7 or 8 (on-therapy) (Figure 1B). Immunohistochemical analysis of total mitogen-activated protein kinase (MAPK), phosphorylated MAPK (pMAPK) (phospho-extracellular signal-regulated kinase 1/2 at Thr202/Tyr204), total 4E-BP1, p4E-BP1 at Thr70, total 56, p56 at Ser235/236 and Ki67 were carried out on 4-µm formalin-fixed paraffin-embedded sections [21]. A histo-score (H-score) was calculated by counting the percentage of keratinocytes from reparative epidermis or endothelial cells from vascular tissue that positively stained with low, medium or high intensity. The final score ranged from 0 to 300 and was calculated by the formula: H-score = (low%) x 1 + (medium%) x 2 + (high%) x 3, where the constants 1, 2 and 3 were the weighting factors for low, medium and high intensity, respectively [22]. Changes between pre and on-therapy expression in paired samples were 1316 | Sessa et al.

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original article
results

patient characteristics

Main patient characteristics are illustrated in Table 1. Twenty-nine patients were enrolled and were assessable for safety; 23 were assessable for first-cycle DLT (Table 1). Six patients were not assessable for DLT: five because two ridaforolimus and PTX doses were missed due to tumor-related disorders or due to intercurrent illnesses and one because of incorrect ridaforolimus dosing. All patients were pretreated with one or more chemotherapies except one with clear-cell carcinoma of the kidney.

dose escalation and patient disposition

Two of three patients treated at the starting doses (25 mg of ridaforolimus, 80 mg/m² of PTX) experienced DLT consisting of grade 2 functional stomatitis and grade 3 thrombocytopenia, respectively, that missed retreatment on days 8 and 15 (Table 2). To rule out that the unexpected toxicity was due to drug interaction dependent on the sequence of administration planned for PKs, the same doses of the drugs were administered to three additional patients in whom PTX was given the day before ridaforolimus. Also with the reversed sequence, two patients had DLT consisting of stomatitis grade 1 and 2 lasting for 16 and 19 days, respectively, that did not allow for delivery of planned therapy on days 8 and 15. The MTD was therefore exceeded. At the same time, according to the study design, the collateral level 1b was tested with a total of six patients evaluated due to the occurrence of one DLT (dose delay of >2 weeks due to grade 2 mouth sores) in the first three patients (Table 2). At this point, the collateral level 1a (ridaforolimus 12.5 mg, PTX 80 mg/m²) was opened and concurrently level 2a (ridaforolimus 37.5 mg, PTX 60 mg/m²) was tested. This strategy aimed at defining the highest dose of ridaforolimus which could be combined with a tolerable dose of PTX, on the
basis of proven tolerability of PTX 60 mg/m² and ridaforolimus 25 mg (level 1b) and the excessive toxicity of PTX 80 mg/m² and ridaforolimus 25 mg (level 1). At each level, six patients were enrolled and one DLT was observed at level 1a (grade 2 skin rash). On the basis of tolerability, the doses that could be recommended for phase II testing were ridaforolimus 37.5 mg with PTX 60 mg/m² and ridaforolimus 12.5 mg with PTX 80 mg/m².

**safety**

Safety was assessed in 29 patients and 87 cycles (Table 3). Adverse events were mostly of grade 1 or 2 intensity and manageable. The most frequent ones were: mouth sores (79% of patients, n = 23, including under this term stomatitis, cheilitis, gingival pain and swelling, lip and mouth ulceration and mouth discomfort), fatigue (59%, n = 17, including asthenia), dermatitis (48%, n = 14, including skin rash, acneiform dermatitis, eczema, erythema and skin exfoliation and fissures), neutropenia (55%, n = 16), anemia (79%, n = 23), anorexia (31%, n = 9) and diarrhea (21%, n = 6). The only treatment-related grade 3 or 4 event occurring in >5% of patients was neutropenia (17%, n = 5). As expected, there was some increase in triglycerides. Importantly, we did not observe any pulmonary toxicity.

**efficacy**

Twenty-five patients were assessable for response. Two partial responses, one in a patient with squamous cell carcinoma of the pharynx previously irradiated (70 Gy) and pretreated with methotrexate and one in a patient with pancreatic cancer who previously had received capcitabine and 5-fluorouracil were observed at levels 1 and 1b, respectively. The decrease of tumor size and the symptomatic improvement occurred immediately after or even during the first cycle. Responses lasted 156 and 267 days, respectively. Stabilization of disease lasting ≥4 months was observed in eight patients (one each with breast, ovarian and colon cancer, mesothelioma, melanoma and thymoma and two with gastric cancer) at all tested dose levels.

**pharmacokinetics**

The PKs of ridaforolimus administered alone (day 0 of cycle 1; Figure 1B) was characterized by a terminal half-life (t½) of 47 ± 8 h (mean ± standard deviation). The drug was still detectable on day 8. The clearance total body (CL TB) tended to increase with increasing doses as already observed [8, 9] (supplemental Figure 1, available at Annals of Oncology online). The limited range of doses did not allow to explore for a correlation between dose and area under the curve (AUC) or maximum concentration.

The effect of PTX on whole blood ridaforolimus disposition was minimal overall (Figure 2A). However, the analysis of paired data showed that the AUC of ridaforolimus was significantly (P < 0.05; t-test for paired data) lower at 12.5 and 25 mg doses when PTX was given 24 h before ridaforolimus than in the opposite sequence (supplemental Table 4, available at Annals of Oncology online), and the CL TB resulted significantly increased. These differences were negligible at 37.5 mg and when the mTOR inhibitor was administered concomitantly with PTX (data not shown). Ridaforolimus had no effect on the disposition of PTX irrespective of the sequence of administration (Figure 2B).

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**Table 1.** Characteristics of treated patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>All dose levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of treated patients</td>
<td>29</td>
</tr>
<tr>
<td>N, n (%)</td>
<td>29</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>20 (60)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>58 (32–71)</td>
</tr>
<tr>
<td>ECOG PS = 0, n (%)</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Tumor type, n (%)</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>9 (31)</td>
</tr>
<tr>
<td>Gastric</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Ovary</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Breast</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Other*</td>
<td>13 (45)</td>
</tr>
<tr>
<td>Prior chemotherapy, n (%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (3)</td>
</tr>
<tr>
<td>1 line</td>
<td>15 (52)</td>
</tr>
<tr>
<td>≥2 lines</td>
<td>13 (45)</td>
</tr>
<tr>
<td>Prior taxanes</td>
<td>5 (17)</td>
</tr>
</tbody>
</table>

*Other tumor types are represented by one case each.
ECOG PS, Eastern Cooperative Oncology Group performance status.

**Table 2.** Number of patients, cycles and occurrence of DLT per dose level

<table>
<thead>
<tr>
<th>Dose level no.</th>
<th>Dose level ridaforolimus (mg)/PTX (mg/m²)</th>
<th>Number of treated/assessable*</th>
<th>Number of patients with DLT</th>
<th>DLT type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25/80</td>
<td>7/6</td>
<td>4 (67%)</td>
<td>Days 8 and 15 withhold due to stomatitis G2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thrombocytopenia G3 and days 8 and 15 withhold due to neutropenia G2</td>
</tr>
<tr>
<td>1b</td>
<td>25/60</td>
<td>8/5</td>
<td>1 (20%)</td>
<td>Days 8 and 15 withhold due to stomatitis G1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Days 8 and 15 withhold due to stomatitis G2</td>
</tr>
<tr>
<td>1a</td>
<td>12.5/80</td>
<td>8/6</td>
<td>1 (17%)</td>
<td>Delayed recovery from stomatitis G2</td>
</tr>
<tr>
<td>2a</td>
<td>37.5/60</td>
<td>6/6</td>
<td>0</td>
<td>Skin rash G2</td>
</tr>
</tbody>
</table>

* Patients were not assessable for DLT if two ridaforolimus and PTX doses had been missed at cycle 1 due to reasons unrelated to the study treatment.

DLT, dose-limiting toxicity; PTX, paclitaxel; G, National Cancer Institute—Common Terminology Criteria for Adverse Events severity grade.
Table 3. Main drug-related toxic effects by patient (all cycles) of ridaforolimus (AP23573) and PTX by dose level

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>No. of patients with toxicity</th>
<th>Dose level rida 12.5 (mg)/PTX 80 (mg/m²), N = 8</th>
<th>Dose level rida 25 (mg)/PTX 60 (mg/m²), N = 8</th>
<th>Dose level rida 25 (mg)/PTX 80 (mg/m²), N = 7</th>
<th>Dose level rida 37.5 (mg)/PTX 60 (mg/m²), N = 6</th>
<th>Any dose level, N = 29</th>
<th>Any G (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G1–2 (%) G3–4 (%)</td>
<td>G1–2 (%) G3–4 (%)</td>
<td>G1–2 (%) G3–4 (%)</td>
<td>G1–2 (%) G3–4 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouth sores</td>
<td>62.5</td>
<td>12.5</td>
<td>100</td>
<td>–</td>
<td>86</td>
<td>50</td>
<td>– 79</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>50</td>
<td>12.5</td>
<td>75</td>
<td>–</td>
<td>86</td>
<td>50</td>
<td>– 69</td>
</tr>
<tr>
<td>Cheilitis</td>
<td>–</td>
<td>–</td>
<td>12.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 3</td>
</tr>
<tr>
<td>Gingival pain</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td>– 3</td>
</tr>
<tr>
<td>Gingival swelling</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 3</td>
</tr>
<tr>
<td>Lip ulceration</td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 7</td>
</tr>
<tr>
<td>Mouth ulceration</td>
<td>–</td>
<td>–</td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 7</td>
</tr>
<tr>
<td>Oral discomfort</td>
<td>–</td>
<td>–</td>
<td>12.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 3</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>50</td>
<td>–</td>
<td>50</td>
<td>–</td>
<td>14</td>
<td>83</td>
<td>– 48</td>
</tr>
<tr>
<td>Rash</td>
<td>37.5</td>
<td>–</td>
<td>37.5</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td>33</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>–</td>
<td>–</td>
<td>12.5</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td>– 7</td>
</tr>
<tr>
<td>Dermatitis acneiform</td>
<td>12.5</td>
<td>–</td>
<td>12.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 7</td>
</tr>
<tr>
<td>Eczema</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td>Erythema</td>
<td>12.5</td>
<td>–</td>
<td>12.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>– 7</td>
</tr>
<tr>
<td>Skin exfoliation</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td>Skin fissures</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td>Asthenia/fatigue</td>
<td>50</td>
<td>–</td>
<td>75</td>
<td>–</td>
<td>43</td>
<td>–</td>
<td>67</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>12.5</td>
<td>–</td>
<td>37.5</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td>Anorexia</td>
<td>37.5</td>
<td>–</td>
<td>37.5</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td>33</td>
</tr>
<tr>
<td>Triglyceride increase</td>
<td>25</td>
<td>–</td>
<td>62.5</td>
<td>–</td>
<td>29</td>
<td>–</td>
<td>67</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>12.5</td>
<td>37.5</td>
<td>37.5</td>
<td>–</td>
<td>71</td>
<td>14</td>
<td>33</td>
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<td>Anemia</td>
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<td>–</td>
<td>87.5</td>
<td>–</td>
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<td>–</td>
<td>83</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>37.5</td>
<td>–</td>
<td>37.5</td>
<td>–</td>
<td>43</td>
<td>14</td>
<td>83</td>
</tr>
</tbody>
</table>

*aThe overall incidence of adverse reactions affecting the oral mucosa is presented using the general term ‘mouth sores’; the percentages refer to the number of patients experiencing at least one of the following adverse reactions: stomatitis, cheilitis, gingival pain, gingival swelling, lip ulceration, mouth ulceration and oral discomfort. Incidence of individual adverse reactions is also presented in the rows immediately below.

*bThe overall incidence of dermatitis-like adverse reactions is presented using the general term ‘dermatitis’; the percentages refer to the number of patients experiencing at least one of the following adverse reactions: rash, dermatitis, dermatitis acneiform, eczema, erythema, skin exfoliation and skin fissures. Incidence of individual adverse reactions is also presented in the rows immediately below.

rida, ridaforolimus; PTX, paclitaxel; G, National Cancer Institute—Common Terminology Criteria for Adverse Events severity grade.
The phosphorylation of 4E-BP1 in PBMCs was measured in 25 patients. Phosphorylation was inhibited by at least 70% (median value) at 24 h after ridaforolimus at all doses. Some degree of inhibition was maintained until day 8 and was not affected by PTX (data not shown). Figure 3A shows a representative course of p4E-BP1 inhibition in PBMCs from a patient receiving 37.5 mg of ridaforolimus and 60 mg/m² of PTX.

The phosphorylation of 4E-BP1 as well as of MAPK was also assessed by immunohistochemistry in paired skin biopsies obtained before and after ridaforolimus treatment. Supplemental Figure 2 (available at Annals of Oncology online) shows the immunohistochemical analysis of p4E-BP1 in reactive epidermis and of pMAPK in wound vascular tissue in patients treated with 12.5 or 37.5 mg of ridaforolimus, respectively. Figure 3B shows a ridaforolimus dose-dependent inhibition of the mTOR pathway in reparative epidermis in 22 patients. A dose of 37.5 mg produced a larger and statistically significant (P < 0.05) decrease of p4E-BP1 in keratinocytes than doses of 12.5 or 25 mg. The decreased phosphorylation of MAPK in endothelial cells had a similar dose-dependent pattern (Figure 3B). The effects on protein phosphorylation were not due to changes in protein expression because total 4E-BP1 and MAPK levels were unchanged. Ki67 staining as a marker of proliferation in reparative epidermis and endothelial cells in wound tissue, or phosphorylation of ribosomal S6 protein as a marker of messenger RNA recruitment to the ribosome, were unaffected by ridaforolimus. Finally, plasma VEGF initially decreased and later rebounded at each dose of the combination (data not shown). The average decrease was about 50% of baseline level and the fluctuation of the levels was not statistically significant.

**discussion**

This is the first phase Ib study reporting the combined use of a cytotoxic drug with the novel agent ridaforolimus for patients with solid tumors. The combination was designed to exploit the inhibitory effects of ridaforolimus at the mTOR regulation of growth and survival with the cytotoxicity of PTX. There also was the aim of exploiting the antiangiogenic effects exerted by weekly PTX through impairment of microtubule functions in endothelial cells [17] and by ridaforolimus through blockade of HIF1α, ILK and dRib [11].

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**Figure 2.** Pharmacokinetics of ridaforolimus (A) and paclitaxel (PTX) (B). The figure shows whole blood disposition of ridaforolimus and plasma disposition of PTX in eight patients treated with 12.5 mg ridaforolimus and 80 mg/m² PTX. In figure 2A, the inset box shows the disposition of ridaforolimus up to 8 days (192 h). Concentration data are mean ± standard deviation.
are expressed as mean mg). The similar trend for pMAPK in keratinocytes is not reported. Data
0 (pretherapy) at three different ridaforolimus doses (12.5, 25 and 37.5
epidermis, quantified as H-score ratio between day 8 (on-therapy) and day
kinase 1/2 at Thr202/Tyr204) level in endothelial cells from reparative
Blue boxes represent the pMAPK (phospho-extracellular signal-regulated
bars represent the range for duplicate determinations. (B) Dose-dependent
expression of target tissues including PBMCs [7–9, 24]. Both markers were
phosphorylation in target tissues including PBMCs [7–9, 24]. In addition, phosphorylation of MAPK is a distinctive marker
of cell growth and it also is inhibited by drugs affecting the
PI3K–AKT–mTOR pathway [25]. Both markers were
consistently inhibited in keratinocytes and in endothelial cells
of reparative epidermis. The effect on 4E-BP1 in PBMCs was
maintained for the week-long interval between ridaforolimus
doses. A possible explanation of the discrepancy observed in the
effect of ridaforolimus on pMAPK between PBMCs and
keratinocytes is not reported. Data are expressed as mean ± standard error.

DLT was observed at the starting dose level (25 mg of
ridaforolimus, 80 mg/m² of PTX) in two of the first three
patients. The PK study involved the sequential
administration of ridaforolimus and PTX in two consecutive
days. To probe whether sequence had any role in causing the
unexpected DLT’s at entry level, four additional patients were
reversed order of PTX before ridaforolimus. The same pattern of stomatitis lasting 2–3 weeks and
neutropenia was observed ruling out that sequence
influenced tolerability. The observation prompted the test of
two collateral levels with reduced dose of PTX (60 mg/m²) or
ridaforolimus (12.5 mg). At these levels and at the
subsequently tested doses of PTX 60 mg/m² and
ridaforolimus 37.5 mg, the combination was feasible for
repeated cycles and mostly associated with mild to moderate
neutropenia and mouth sores.

The effects on the mouth mucosa were characteristically long
lasting, more severe and functionally more limiting than what
the morphological appearance would indicate. The features
were similar to those reported with other mTOR inhibitors and
with single-agent ridaforolimus itself [7, 23]. On the basis of
tolerability findings, two RDs could be proposed for further
testing: ridaforolimus 37.5 mg with 60 mg/m² weekly PTX and
ridaforolimus 12.5 mg with PTX 80 mg/m².

The limiting toxicity at the entry level prompted an in-depth
PK analysis to explore whether a drug interference were
responsible for the clinical observation. As reported in other
studies, the whole blood PKs of ridaforolimus was
characterized by increasing CLTB with increasing dose [9]. The
observed t1/2 (47 ± 8 h) was consistent with maintenance of
effective concentrations with a weekly schedule. Ridaforolimus
did not influence the PKs of PTX. However, a statistically
significant lower whole blood exposure to ridaforolimus was
observed when PTX was administered before rather than after
the mTOR inhibitor. The effect was small and magnified by the
different sequence of administration, while it was almost
undetectable when both drugs were given concomitantly.

Therefore, ridaforolimus and PTX at the doses of this study are
not associated with significant PK interference of potential
clinical consequence.

mTOR leads to phosphorylation of 4E-BP1 [5]. Several
studies documented that ridaforolimus inhibits 4E-BP1
phosphorylation in target tissues including PBMCs [7–9, 24].
In addition, phosphorylation of MAPK is a distinctive marker
cell growth and it also is inhibited by drugs affecting the
P3K–AKT–mTOR pathway [25]. Both markers were
consistently inhibited in keratinocytes and in endothelial cells
of reparative epidermis. The effect on 4E-BP1 in PBMCs was
maintained for the week-long interval between ridaforolimus
doses. A possible explanation of the discrepancy observed in the
effect of ridaforolimus on pMAPK between PBMCs and
granulation tissues could be the higher concentration achieved
in blood cells as compared with the skin. The administration of
PTX had no measurable effect.

Blockade of mTOR also affects angiogenesis via HIF1α,
VEGF and endothelial cell proliferation [1, 5]. In the present
study, the plasma levels of VEGF initially decreased after each
dose to later undergo a rebound. VEGF levels were never
reduced to >50% of pretreatment and as such were not
informative about the antiangiogenic effects. However, the
inhibition pMAPK in the endothelial cells of the wound healing
epidermis indicated a clear effect consistent with the expected
angiogenesis of mTOR inhibitors. The effect was dose
dependent with paradoxical stimulation at 12.5 mg, no effect at
25 mg and highest inhibition at 37.5 mg of ridaforolimus. The
data do not allow to ascertain whether the combination
obtained addition of antiangiogenesis or whether the two drugs
acted synergistically. However, the weekly doses of 37.5 mg
of ridaforolimus and 60 mg/m² of PTX might have a more
pronounced antiangiogenic effect and could be the preferred RD of the two that emerged from the tolerability analysis. It is known that some mTOR inhibitors can up-regulate pMAPK in tumors also in the clinical setting; however, we did not carry out tumor biopsies in this study and we cannot comment on the effect of ridaforolimus and PTX on this signaling pathway in human tumors.

The patients enrolled in the study had tumors potentially sensitive to PTX but received doses of PTX or ridaforolimus that were lower than recommended for each drug as single agent. In spite of this limitation, the combination had encouraging antitumor activity consisting of objective partial responses and in stabilization of disease for >4 months in cases with different types of tumor.

In summary, the study successfully defined the RDs of weekly ridaforolimus and PTX. There was not a significant PK interference that could account for the recording of DLT at the entry level, while the pharmacodynamic assessment showed antiangiogenesis that would support the use of weekly PTX at 60 mg/m² with weekly ridaforolimus at 37.5 mg. On the basis of initial antitumor activity, the combination should be further tested in studies better suited to probe its antitumor properties.

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disclosure
FH is Vice-President, Clinical Research and has stock ownership interests at Ariad Pharmaceuticals Inc.

VMR is Senior Director, Biology and has stock ownership interests at Ariad Pharmaceuticals Inc.

LB is Research Scientist II and has stock ownership interests at Ariad Pharmaceuticals Inc.

LG has been advisor for Ariad Pharmaceuticals Inc.

references