Phase I study and preclinical efficacy evaluation of the mTOR inhibitor sirolimus plus gemcitabine in patients with advanced solid tumours

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Background: We conducted a phase I study in patients with advanced solid tumours to identify the recommended dose, assess pharmacokinetics (PK), pharmacodynamic activity and preclinical antitumour efficacy of the combination of sirolimus and gemcitabine.

Methods: Nineteen patients were treated with sirolimus 2 or 5 mg daily and gemcitabine 800 or 1000 mg m⁻² on days 1 and 8. Dose escalation depended on dose-limiting toxicity (DLT) rate during the first 3-week period. Paired skin biopsies were evaluated for phosphorylated S6 (pS6) as marker of mTOR (mammalian target of rapamycin) inhibition. Pharmacokinetics and preclinical evaluation of efficacy using two different sarcoma cell lines and leiomyosarcoma xenografts were also conducted.

Results: Three DLTs were observed: grade 3 transaminitis, grade 3 thrombocytopenia and grade 4 thrombocytopenia. Common treatment-related adverse events included anaemia, neutropenia, thrombocytopenia and transaminitis. Pharmacodynamic analyses demonstrated mTOR inhibition with sirolimus 5 mg and PK showed no influence of sirolimus concentrations on gemcitabine clearance. In vitro and in vivo studies suggested mTOR pathway hyperactivation by gemcitabine that was reversed by sirolimus. Tumour growth in leiomyosarcoma xenografts was dramatically inhibited by the treatment.

Conclusions: Recommended dose was sirolimus 5 mg per 24 h plus gemcitabine 800 mg m⁻². Antitumour activity in preclinical sarcoma models and mTOR signalling inhibition were observed. A phase II study is currently ongoing.

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that plays a central role in the phosphatidyl inositol 3'-kinase (PI3K)-AKT signalling pathway (Aoki et al, 2001; Sabatini, 2006). Activation of mTOR by different environmental and nutritional stimuli triggers transduction of proliferative signals by the phosphorylation of two key downstream effectors, the p70 S6
kinase and the eukaryotic initiation factor 4E binding protein 1 (4EBP-1; Janus et al, 2005). These proteins are involved in the biosynthesis of ribosomes and translation of mRNA necessary for normal cell-cycle regulation (Mamane et al, 2006). The correlation between mTOR pathway abnormalities and carcinogenesis has been extensively reported (Shaw and Cantley, 2006; Hernando et al, 2007). Indeed, up to half of all human tumours have been found to be somehow driven by alterations in the mTOR pathway (Vivanco and Sawyers, 2002; Xu et al, 2004). In addition, it is also critical in some tumour microenvironment processes such as angiogenesis (Vífals et al, 1999; Guba et al, 2002; Hudson et al, 2002; Humar et al, 2002; Mayerhofer et al, 2002; Land and Tee, 2007). Therefore, targeting mTOR is a rational therapeutic approach in human cancer. Sirolimus, also known as rapamycin, was one of the first compounds able to inhibit mTOR (Wiederecht et al, 1995). It is a macrolide that prevents the phosphorylation of S6 and 4EBP-1 and therefore their activation (Brown et al, 1994; Fairev et al, 2006). Some of its derivatives, namely everolimus, temsirolimus and ridaforolimus, have been successfully assessed in phase III trials in different malignancies (Hudes et al, 2007; Motzer et al, 2008; Yao et al, 2011; Baselga et al, 2012; Demetri et al, 2013).

Gemicitabine is a pyrimidine analogue that targets cells undergoing DNA synthesis and blocks progression of cells from G1 to S-phase (Elagger et al, 2012). It is currently in use in a vast spectrum of tumours either alone or in combination thanks to its favourable toxicity profile (Gesto et al, 2012).

Combination of sirolimus with gemicitabine has been reported to increase apoptosis in vitro and enhance antitumoural activity in vivo on different epithelial tumours (Gru¨nwald et al, 2002; Mondsire et al, 2004). Specifically in sarcomas, an in vitro study in leiomyosarcoma cell lines has shown that this combination has a synergistic effect in extracellular-signal-regulated kinases (ERK 1/2) inhibition, producing a dramatic effect in cell cycle (Merimsky et al, 2007). However, no studies in xenograft sarcoma models have been published to date. Nevertheless, response in a patient affected by leiomyosarcoma has been reported (Merimsky, 2004) suggesting that this combination may have profound effects on these malignancies.

This phase I trial was designed to determine the recommended dose (RD), safety profile, pharmacokinetic (PK) parameters and pharmacodynamic activity of the combination of sirolimus and gemicitabine. Preclinical antitumour efficacy both in vitro and in vivo was also evaluated.

**Patient selection.** To be enrolled in this study, patients had to meet the following eligibility criteria: diagnosis of advanced solid tumour that have progressed or are ineligible for standard treatment, no prior treatment with mTOR inhibitors or gemicitabine, Eastern Cooperative Oncology Group performance status (ECOG PS) 0–1, either measurable or evaluable disease and age ≥18 and ≤70 years. The upper limit of age was established due to the increased risk of toxicity often seen in elderly patients. Adequate bone marrow, hepatic and renal function were mandatory and were defined as: absolute neutrophil count ≥1.5 x 10^9/l, platelets ≥100 x 10^9/l, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase and creatinine ≤1.5 x upper limit of normal and creatinine clearance ≥60 ml min⁻¹. Patients with a history of other previous malignancies diagnosed or treated in the past 5 years (except basal cell skin carcinoma, adenoacarcinoma in situ of the uterine cervix and superficial bladder cancer) and known central nervous system metastases were considered ineligible. Other exclusion criteria were treatment with experimental drugs within 30 days prior, pregnancy or lactancy, presence of active infection or any concomitant serious disease.

All patients signed written informed consent and the study was conducted according to local and national ethical review board approval, the Declaration of Helsinki and standards of Good Clinical Practice.

**Study design and drug dosage, escalation and administration.** Sirolimus was administered as a continuous daily oral dose (2 or 5 mg) starting on day 2 of cycle 1 until progression or intolerance. Gemicitabine was administered intravenously at a fixed-dose rate of 10 mg m⁻² min⁻¹ on days 1 and 8 of each cycle. The duration of each cycle was 21 days. A maximum of six cycles of gemicitabine per patient were allowed. Single agent sirolimus was continued after six planned cycles of gemicitabine in the absence of progressive disease (PD) and good tolerance. Protocol was amended according to pharmacodynamic results and a new dose level was added (Table 1).

The trial was performed using a standard 3+3 dose-escalation phase I design with cohorts of 3–6 patients. If less than one-third of patients at a dose level experienced a dose-limiting toxicity (DLT), dose escalation continued. If more than one-third but less than two-thirds of patients at a dose level had a DLT, three additional patients were enrolled at that same dose level. If two-thirds or more of patients at a dose level experienced a DLT, the dose was considered toxic and the next cohort of patients was included at the next lower dose level. Dose escalation within a patient was not permitted. Patients were withdrawn from study treatment when there was evidence of PD, unacceptable toxicity or consent withdrawal.

Routine clinical and laboratory assessments were conducted on a weekly basis. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria version 3.0 (NCI-CTCAE v3.0). Dose-limiting toxicity was defined as any of the following within 3 weeks after the administration of the first cycle: absolute neutrophil count <0.5 x 10^9/l, over ≥5 days or associated with fever ≥38.5°C, platelets <50 x 10^9/l, any grade 3–4 non-haematological toxicity (excluding nausea and vomiting non-refractory to antiemetic treatment) or skin rash grade 2 related to treatment and not controlled with support medication. Maximum tolerated dose (MTD) was defined as the highest dose level in which two or more patients experienced DLT. Next lower dose level was considered as RD.

In order to assess tumour response to treatment, thorax–abdomen–pelvis CT scans were performed every 6 weeks and Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST v1.0) were used (Therasse et al, 2000).

**Pharmacokinetics.** Gemicitabine concentrations were measured at days 1 and 24 of the study and PK sampling was performed at 0.5, 1, 2.5, 4, 8, 10 and 24 h after the start of the infusion, which was started on day 2 of cycle 1 until progression or intolerance. Gemcitabine was administered intravenously at a fixed-dose rate of 10 mg m⁻² min⁻¹ on days 1 and 8 of each cycle. The duration of each cycle was 21 days. A maximum of six cycles of gemicitabine per patient were allowed. Single agent sirolimus was continued after six planned cycles of gemcitabine in the absence of progressive disease (PD) and good tolerance. Protocol was amended according to pharmacodynamic results and a new dose level was added (Table 1).

| Dose level | Sirolimus (mg per 24 h) orally | Gemcitabine (mg m⁻² intravenously) | DLT/patients | Toxicity |
|------------|-------------------------------|-----------------------------------|--------------|---------|
| 1          | 1                             | 800                               | 0/3          |         |
| 2          | 2                             | 1000                              | 1/6          | Transaminitis G3 |
| 2.5        | 5                             | 800                               | 0/6          |         |
| 3          | 5                             | 1000                              | 2/4          | Thrombocytopenia G3: Thrombocytopenia G4 |
**Pharmacodynamics.** Paired skin biopsies were planned for every patient: at baseline and 21 days after first dose administration. In order to assess mTOR pathway inhibition, immunohistochemistry of phosphorylated S6 at Ser235/236 (pS6) #4858 was performed in formalin-fixed paraffin-embedded sections of skin samples using a 1:50 dilution of a rabbit polyclonal antibody (from Cell Signaling Technology). Blots were then incubated at room temperature for 1h with a horseradish peroxidase-conjugated secondary antibody and the peroxidase activity was detected by enhanced chemiluminescence (Pierce, Rockford, IL, USA) following the instructions of the manufacturer. Immunodetection of α-tubulin was used as a loading reference.

**In vitro study.** Two sarcoma cell lines acquired from Cell Lines Service (CLS, Eppelheim, Germany) were used to assess the in vitro efficacy of the treatment: SKLMS-1 and SW982 ( leiomyosarcoma and synovial sarcoma, respectively). Both cell lines were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) and were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air.

**Cell proliferation assay.** Sirolimus and gemcitabine were diluted in cell medium at 20 ng ml⁻¹ and 100 nm, respectively and then cells were treated with both drugs separately, sequentially and in combination for 48 h. Dimethyl sulfoxide (DMSO) was added to cultures as control. Cell proliferation and cell death were determined by the trypan blue exclusion assay.

**Western blot.** SKLMS-1 and SW982-treated cells were lysed with radioimmunoprecipitation assay buffer containing protease inhibitors (1 mmol l⁻¹ phenylmethylsulfonyl fluoride, 10 mg ml⁻¹ aprotonin, and 10 mg ml⁻¹ leupeptin) and the lysates were centrifuged at 13 000 × g, at 4°C, for 30 min. Lysate aliquots (50 µg) were resolved by 10% SDS–PAGE and transferred onto nitrocellulose membranes. After blocking with 5% skimmed milk in PBS containing 0.2% Tween 20 (Dallas, TX, USA) at room temperature for 1 h, membranes were incubated overnight at 4°C with the appropriate primary antibody (cleaved caspase 3 #9661, native S6 #2217, and pS6 #4858 from Cell Signaling Technology). Blots were then incubated at room temperature for 1h with a horseradish peroxidase-conjugated secondary antibody and the peroxidase activity was detected by enhanced chemiluminescence (Pierce, Rockford, IL, USA) following the instructions of the manufacturer.

**In vivo study.** An in vivo xenograft model was established by subcutaneous injection of 3.5 × 10⁶ SKLMS-1 cells suspended in 100 µl of saline in athymic nude mice (BALB/cnu/nu) from Harlan (Indianapolis, IN, USA). Animal care and procedures were followed according to the Institutional Guidelines for the Care and Use of Laboratory Animals. Once tumours reached 100 mm³, groups of five mice were treated with sirolimus 2.5 mg kg⁻¹ and gemcitabine 60 mg kg⁻¹ followed by sirolimus 2.5 mg kg⁻¹ after 24 h. All treatments were administered in intraperitoneal manner for 2 weeks (sirolimus once daily and gemcitabine once weekly). An additional group of five mice were treated with DMSO as controls. Tumours were measured every 2 days with calipers, and toxicity was monitored by weight loss. Mice were killed once tumours reached 2500 mm³ (or after manifestation of morbidity) and tumours were removed and stored in 4% paraformaldehyde. Immunohistochemistry was performed in formalin-fixed paraffin-embedded sections from tumour samples. Phosphorylated S6 was detected with a 1:50 dilution of a rabbit polyclonal antibody #4858 (from Cell Signaling Technology).

### RESULTS

**Patient characteristics.** From June 2010 to September 2011, 19 patients were enrolled in a single centre. All patients were assessable for toxicity and efficacy. Demographics characteristics are shown in Table 2. All patients except one had received prior chemotherapy treatment. Median number of previous lines was 2.5 (range 0–6) and 7 (37%) patients had radiation therapy before enrolment in the study. A total of 77 cycles of the study regimen were administered. Median number of cycles per patient was 4 (range 1–6).

**Safety.** All 19 patients were evaluable for DLT. Initially, the three dose levels planned were evaluated. One patient experienced DLT consisting in grade 3 transaminitis at dose level 2 and two patients experienced DLT at dose level 3 consisting in grade 3...
thrombocytopenia and grade 4 thrombocytopenia, respectively. Thus, MTD was reached at dose level 3. However, the pharmacodynamic analysis performed in the 13 patients treated at those dose levels revealed poor mTOR pathway inhibition at doses <5 mg of sirolimus. Therefore, an amendment was performed including a new dose level under the reached MTD consisting of sirolimus 5 mg and gemcitabine 800 mg m⁻² (dose level 2.A). At this dose level, no DLT was observed and it was established as the RD (Table 1).

The majority of side effects reported were grade 1–2. The most commonly observed treatment-related events were haematological: anaemia (84%; n = 16), neutropenia (68%; n = 13) and thrombocytopenia (68%; n = 13). The most frequent non-haematological toxicities were raised AST (58%; n = 11), raised GGT (47%; n = 9), hypercholesterolaemia (47%; n = 9), anorexia (47%; n = 9) and mucositis (42%; n = 8). In general, toxicity was mild and easily manageable. No pulmonary toxicity was reported. Three patients required dose reduction of sirolimus, being grade 3 thrombocytopenia the reason in two cases and grade 2 fever in one case. Gemcitabine dose reduction was required in two patients due to grade 4 anaemia and grade 2 transaminitis, respectively. Toxicity is summarised in Table 3.

**Pharmacokinetics and pharmacodynamics.** Since gemcitabine is a drug with well-known activity against a large number of malignancies, we designed the study to determine whether the addition of sirolimus has any influence on its PK. Data from all 19 patients were used in the PK analysis. The effects of gender, age, weight (WGT), body surface area (BSA) and sirolimus through concentrations were assessed on gemcitabine PK at day 21. Demographic characteristics and sirolimus trough concentrations are summarised in Table 4. Correlation between WGT/BSA and height (HGT) was found. The plasma concentration vs time profiles of gemcitabine at days 1 and 21 are displayed in Figure 1. It should be noted that quantifiable gemcitabine concentrations were found up to 2.5–4 h post administration in both occasions. The PK of gemcitabine after intravenous infusion of 10 mg m⁻² min⁻¹ in the target population was best described by a two-open-compartment model with first-order elimination. All recorded covariates were tested in the PK parameters, plasma clearance (CL) and central compartment distribution volume (Vc), with NONMEM, but no statistically significant relationship could be identified in any case. No statistically significant effect of anthropometric covariates (WGT, HGT and BSA) and age on the PK parameters was found (P > 0.05) and no specific trends were observed between CL or Vc values and sirolimus concentrations (Supplementary Figure 1). The estimated PK parameters with final model (NONMEN) listed in Supplementary Table 1 were in agreement with those previously reported in the literature (Keith *et al.*, 2003; Lin *et al.*, 2004). Between-patient variability could be associated to CL (14.6%) and Vc (98.2%), meanwhile between-occasion variability could be to Vc (47.1%).

Immunohistochemistry of pS6 in patients’ paired skin biopsies showed significant inhibition of mTOR at RD (Supplementary Figure 2). Weaker staining of pS6 was achieved with 5 mg (dose levels 2.A and 3) compared to 2 mg.

**Efficacy.** Two patients achieved partial response (PR): one patient at dose level 2.A (colon adenocarcinoma) and the other one at dose level 3 (uterine cervix cancer). Nine patients experienced stable disease (SD) as best response that lasted >12 weeks and in three cases, the duration of the stabilisation was at least 6 months.

**In vitro study results**

**Cell proliferation assay results.** Both cell lines were sensitive to gemcitabine and sirolimus. Interestingly, higher cell death rate was observed in both cell lines with the sequential treatment administering first gemcitabine and 24 h later sirolimus than with the inverse order or with the administration of both drugs at the same time (data not shown).

**Western blot results.** We used cleaved caspase 3 as apoptosis marker to assess the in vitro efficacy of the combination. Results showed that the greatest activation of apoptosis was achieved with the sequential treatment administering gemcitabine first followed by sirolimus 24 h later (Figure 2A).

We assessed by western blot phosphorylation of S6 as a marker of mTOR activity. Although the non-phosphorylated forms had no relevant changes with the treatment, pS6 was highly induced when cells were treated with gemcitabine alone. This induction was clearly reversed when sirolimus was added (Figure 2B).

**In vivo study results.** Xenograft model was established using SKLMS-1 cells. According to in vitro results, treatment was administered in a sequential fashion (first gemcitabine and 24 h later sirolimus). Tumour growth was strongly inhibited with the sequential combination of the two drugs compared to Control and to each drug alone (Figure 3).
Immunohistochemistry results. Strong pS6 staining in tumours treated with gemcitabine alone was observed. In contrast, that staining was dramatically absent in tumours treated with the combination, indicating that the addition of sirolimus is able to reverse pS6 induction also in vivo (Figure 4).

**Table 3. Toxicity**

| Toxicity               | Total (n = 19) | Dose level 1 (n = 3) | Dose level 2 (n = 6) | Dose level 2.A (n = 6) | Dose level 3 (n = 4) | All grades | Grade 3–4 |
|-----------------------|---------------|---------------------|---------------------|------------------------|---------------------|------------|-----------|
|                       |               | All grades          | Grade 3–4           | All grades             | Grade 3–4           | All grades  | Grade 3–4 |
| Anorexia              | 2             | 2                   | 3                   | 2                      | 9                    | 47         |
| Mucositis             | 2             | 2                   | 3                   | 1                      | 8                    | 42         |
| Fever                 | 3             | 3                   | 3                   | 1                      | 7                    | 37         |
| Nausea/vomiting       | 1             | 3                   | 2                   | 1                      | 7                    | 37         |
| Fatigue               | 3             | 3                   | 3                   | 6                      | 32                   |
| Rash                  | 2             | 3                   | 1                   | 6                      | 32                   |
| Diarrhoea             | 1             | 2                   | 2                   | 1                      | 16                   | 84         | 1         |
| Anaemia               | 2             | 4                   | 6                   | 1                      | 4                    | 16         | 84        | 1         | 5         |
| Neutropenia           | 2             | 1                   | 3                   | 1                      | 5                    | 3          | 1         | 13        | 68        | 6         | 32        |
| Thrombocytopenia      | 1             | 1                   | 4                   | 5                      | 3                    | 2          | 13        | 68        | 3         | 16        |
| Leukopenia            | 1             | 1                   | 3                   | 3                      | 8                    | 42         |
| Raised AST            | 1             | 4                   | 1                   | 4                      | 2                    | 11         | 58        | 2         | 11        |
| Raised GGT            | 3             | 1                   | 2                   | 1                      | 4                    | 9          | 47        | 2         | 11        |
| Hypercholesterolaemia | 1             | 4                   | 1                   | 3                      | 1                    | 9          | 47        | 1         | 5         |
| Raised ALT            | 1             | 3                   | 2                   | 2                      | 1                    | 7          | 37        | 2         | 11        |
| Hyperglycaemia        | 1             | 2                   | 2                   | 5                      | 26                   |
| Raised creatinine     | 1             |                     |                     |                        |                      |            | 1         | 5         |

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase. Toxicities reported at any time from first treatment administration to 30 days of last treatment administration are included.

**Table 4. Demographic characteristics of patients of the study population**

| Patients’ characteristics | Mean (RSE%) | Median | Minimum | Maximum |
|---------------------------|-------------|--------|---------|---------|
| Patients (n)              | 19          |        |         |         |
| Age (years)               | 54.1 (18.6) | 54.5   | 36      | 70      |
| Gender (n), male          | 13          |        |         |         |
| Female                    | 6           |        |         |         |
| Height (cm)               | 166.9 (10.6)| 167    | 151     | 184     |
| Body weight (kg)          | 73.0 (22.0) | 75     | 44.2    | 107     |
| Body surface area (m²)    | 1.81 (1.22) | 1.90   | 1.40    | 2.30    |
| Sirolimus concentrations (µg l⁻¹) | 9.05 (7.78) | 7.60   | 0.90    | 28.50   |

Abbreviation: RSE% = relative standard error.

**DISCUSSION**

This study demonstrates that the combination of sirolimus and gemcitabine is feasible and safe, allowing administration of active doses of both agents and achieving mTOR pathway inhibition even in heavily pretreated patients. The most common adverse events registered were haematological, but they were generally mild and easily manageable. Other mild toxicities observed were raised liver enzymes, hypercholesterolaemia, anorexia and mucositis, all of them usually related to either sirolimus or gemcitabine in monotherapy, but modifications in the treatment schedule or dose were not necessary in almost any case. Furthermore, the toxicity profile showed no synergistic effects in these adverse events with the combination of the two drugs. Transaminitis grade 3 and thrombocytopenia grades 3 and 4 where the DLTs found, all of them are relatively common and expected in patients treated with gemcitabine. No unexpected toxicity appeared with the treatment. Moreover, PK showed no effects of sirolimus concentrations on gemcitabine clearance. This favourable profile leads us to
recommend dose level 2A (sirolimus 5 mg per 24 h plus gemcitabine 800 mg m\(^{-2}\)) as the optimal dose due to its well-proved safety record.

In addition, the preclinical study also showed encouraging results. Thus, the in vitro study showed that caspase 3 cleavage was more evident when cells were treated sequentially (gemcitabine before sirolimus) than administering both drugs simultaneously. Therefore, a clear pro-apoptotic induction as a result of this combination is responsible for the dramatic effect on tumour survival. Sequential administration of drugs, including sirolimus, as a cancer therapeutic strategy has been used elsewhere (Iacovelli et al, 2013; Rosa et al, 2013). mTOR inhibition results in downregulation of several antiapoptotic proteins such as Bcl-xL and Mcl-1 (Tirado et al, 2005; Faber et al, 2014). Thus, sirolimus addition sequentially after gemcitabine may prevent resistance to this drug through antiapoptotic pathway activation. In agreement with this hypothesis, several reports demonstrate that inhibition of antiapoptotic bcl-2 family members sensitises tumour cells to gemcitabine (Schniewind et al, 2004; Zhang et al, 2011). In contrast, one of the main effects of mTOR inhibition is G1 arrest (Carew et al, 2011) that makes cells less prone to be damaged by gemcitabine. This hypothesis is being currently tested in the laboratory. On the other hand, we found both in vitro and in vivo that S6 was activated when cells were treated with gemcitabine alone but such activation dramatically reversed when sirolimus was added, correlating with the efficacy of the combinatory treatment. These interesting data suggest hyperactivation of mTOR pathway as a cellular mechanism of defence triggered by gemcitabine that can be reversed with the addition of sirolimus. This brand new finding opens an exciting line of investigation worth exploring. Furthermore, xenograft tumour growth was dramatically reduced with the combined treatment and pharmacodynamic analysis showed an effective mTOR inhibition at RD, making this therapeutic strategy even more promising.

Combination of an mTOR inhibitor with conventional chemotherapy with gemcitabine could be a way to improve the efficacy of either of the agents alone in different tumour types such as pancreatic cancer, renal cell cancer or sarcomas. Specifically, in sarcomas, positive results with mTOR inhibitors have been reported. Thus, sirolimus and its derived temsirolimus have shown activity in perivascular epithelioid cell tumours (PEComas), a specific subtype of mesenchymal tumour (Italiano et al, 2010; Wagner et al, 2010). Moreover, it has been recently published in a positive phase III trial in sarcomas with the mTOR inhibitor ridaforolimus. This double-blind, placebo-controlled phase III trial randomised 702 sarcoma patients who had achieved CR, PR, or SD after 1, 2, or 3 lines of chemotherapy to receive placebo or ridaforolimus as maintenance treatment. Ridaforolimus showed signs of activity, inducing a mean 1.3% decrease in target lesion size vs a 10.3% increase with placebo. In addition, it achieved a statistically significant improvement in PFS compared to placebo in both independent and per investigator assessment. However, the magnitude of that improvement was very modest (median PFS 17.7 weeks vs 14.6 weeks per independent review; Demetri et al, 2013). These results, positive but excessively limited, suggest some important conclusions: mTOR inhibitors are active in sarcomas but the best therapeutic strategy is still unknown. Therefore, combination treatments with mTOR inhibitors and cytotoxic drugs...
(like the one assessed in this study) is a promising alternative that
deserve further investigation.

In conclusion, this phase I trial of the combination of sirolimus and
gemcitabine demonstrated that this regimen is feasible and
safe. Moreover, it showed signs of activity both in vitro and in vivo.
In addition, mTOR inhibition was achieved at RD and PK analysis
showed no influence of sirolimus on gemcitabine clearance.
Further studies to assess the activity of this combination are
warranted and a phase II trial in sarcomas is ongoing (Clinical-
Trials.gov identifier NCT01684449).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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