Cancer Chemother Pharmacol (2016) 78:101–109
DOI 10.1007/s00280-016-3064-0

ORIGINAL ARTICLE

A phase I study of mTOR inhibitor everolimus in association with cisplatin and radiotherapy for the treatment of locally advanced cervix cancer: PHOENIX I

Andréia Cristina de Melo[1] · Rachele Grazziotin-Reisner[1] · Felipe Erlich[1] · Mariane S. Fontes Dias[1] · Giulliana Moralez[1] · Michel Carneiro[1] · Álvaro Henrique Ingles Garces[1] · Flávia Vieira Guerra Alves[1] · Bruna Novaes Neto[1] · Mateus Fuchshuber-Moraes[1] · Juliane Morando[2] · Guilherme Suarez-Kurtz[1] · Carlos Gil Ferreira[3]

Received: 18 April 2016 / Accepted: 11 May 2016 / Published online: 20 May 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract
Background Cervix cancer (CC) represents the fourth most common cancer in women. Treatment involving cisplatin and radiotherapy has been the standard for locally advanced disease. Everolimus inhibits the aberrant activity of mTOR that is part of carcinogenesis in CC. Further everolimus inactivates the HPV E7 oncoprotein and inhibits its proliferation. Preclinical models have suggested that everolimus sensitizes tumoral cells and vasculature to cisplatin and radiotherapy.
Methods In a 3 + 3 design, the trial aimed to treat three dose levels of at least three patients with daily doses of everolimus (2.5, 5 and 10 mg/day), cisplatin and radiotherapy delivered in a 9-week interval in CC patients, stage IIB, IIIA or IIIB. Patients received everolimus from day −7 up to the last day of brachytherapy. Primary objective was to evaluate safety, toxicity and the maximum-tolerated dose (MTD) of everolimus in association with cisplatin and radiotherapy. Pharmacokinetic (PK) parameters and response rates were analyzed as secondary objectives.
Results Thirteen patients were enrolled, 6 at 2.5 mg, 3 at 5 mg and 4 at 10 mg. Four patients did not complete the planned schedule, 1 at 2.5 mg presented grade 4 acute renal failure interpreted as dose-limiting toxicity (DLT) and 3 at 10 mg: 1 with disease progression, and 2 with DLTs—1 grade 3 rash and 1 grade 4 neutropenia. PK results were characterized by dose-dependent increases in AUC and C_{max}.
Conclusions The MTD of everolimus in combination with cisplatin and radiotherapy has been defined as 5 mg/day. The data regarding safety and response rates support further studies.

Keywords Cervix cancer · Phase I · Everolimus · Cisplatin · Radiotherapy

Introduction

Cervix cancer (CC) is a public health problem representing the fourth most commonly diagnosed cancer and the fourth cause of cancer death in women worldwide [1].

Several studies have demonstrated the superiority of platinum-based chemotherapy combined with radiotherapy compared to radiotherapy alone for patients with locally advanced CC [2–9]. However, the results are still disappointing, with a 5-year survival rate lower than 50 % [8]. Novel strategies to improve the prognosis of these patients are needed.

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that is a central regulator of growth, cellular proliferation and survival in the phosphatidylinositol 3-kinase (PI3K)/AKT pathway and is present in two intracellular complexes, namely mTOR complex 1 (mTORC1) and mTORC2. Downstream targets of mTORC1 are the protein translation regulator 4E-BP1 and the ribosomal S6 protein kinase (p70S6K) [10]. The high aberrant activity of mTOR complexes seems to be the underlying cause of carcinogenesis in gynecological cancers, and its inhibition represents a promising treatment strategy [11].

✉ Andréia Cristina de Melo
andreia.melo@inca.gov.br

[1] Brazilian National Cancer Institute, Rio de Janeiro, Brazil

[2] Novartis, São Paulo, Brazil

[3] Brazilian Clinical Cancer Research Network (RNPCC) - INCA/Decit/MS, D'or Institute of Research and Education (IDOR), Rio de Janeiro, Brazil

Virtually all CCs (more than 99 %) are caused by high-risk human papillomavirus (HPV) [12]. The HPV E7 oncoprotein is essential for CC carcinogenesis. The AKT phosphorylation demonstrated in samples of CC suggests a constitutive activation of the PI3K/AKT pathway in patients [13]. Moreover, mTOR inhibitors block the 4E-BP1-protein phosphorylation and significantly reduce the level of E7 protein on in vitro models, leading to an accumulation of cells on G1 phase and thereby inducing apoptosis [14]. In addition, it is established that radiation activates the PI3K/AKT pathway and that mTOR inhibitors sensitize tumor and endothelial cells to cisplatin and radiotherapy effects [15].

Everolimus, a mTOR inhibitor, is a derivative of rapamycin for oral administration. It has been tested and approved by the FDA for adult patients with advanced hormone receptor-positive, HER2-negative breast cancer in combination with exemestane, progressive neuroendocrine tumors of pancreatic origin, advanced renal cell carcinoma after failure of treatment with sunitinib or sorafenib, and pediatric and adult patients with subependymal giant cell astrocytoma.

Based on the mechanisms of carcinogenesis in CC [12], preclinical data [13–15] and the results of everolimus use in different tumors [16–18], a regimen adding everolimus to cisplatin and pelvic radiotherapy for locally advanced CC was set out. The primary objective was to evaluate the safety, toxicity and the maximum-tolerated dose (MTD) of everolimus in combination with chemo-radiotherapy. Pharmacokinetic parameters and response rates were analyzed as secondary objectives.

Patients and methods

Study design and statistics

This was a single-institution, phase I study to determine the safety, tolerability and the MTD of everolimus in combination with pelvic radiotherapy and cisplatin for locally advanced CC.

The local ethics committee and the national ethics committee approved the protocol and its subsequent amendments. The study was done in accordance with the ethical principles from the Declaration of Helsinki and with Good Clinical Practice as defined by the International Conference on Harmonization. All participating patients gave written informed consent.

In a modified Fibonacci schedule, three cohorts (from 3 to 6 patients) were included with progressive increments of the everolimus dose (2.5 mg, 5 mg and 10 mg/day). Intrapatient dose escalation was not permitted. Patients were followed up for 14 weeks (10 weeks of treatment plus 4 weeks of observation). After this period, the absence of dose-limiting toxicity (DLT) allowed progression to the next dose level until the maximum planned dose (10 mg). If 1 out of 3 patients experienced a DLT, 3 additional patients would be enrolled at the same dose level. If two patients presented DLT at 2.5 mg, the study should be interrupted. If two patients presented DLT at 5 or 10 mg dose levels, the MTD would be defined as the previous dose level.

Toxicity analysis was conducted in all included participants. The efficacy analysis was conducted, and descriptive statistics on patient characteristics were done.

Eligibility criteria

Patients with pathologically confirmed squamous cell carcinoma of the uterine cervix were eligible if they were older than 18 years old, had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, an International Federation of Gynecology and Obstetrics (FIGO) stage of IIB, IIIA or IIIB, a measurable lesion according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1, an adequate renal, hepatic, and bone marrow function, and a normal lipid profile. For women at reproductive age, a serum-negative β-human chorionic gonadotropin assay was required and a contraceptive method along the entire study was recommended.

Exclusion criteria included a previous malignancy in the last 5 years, positive para-aortic lymph nodes in the imaging evaluation, uncontrolled diabetes, pulmonary and cardiovascular disease, psychiatric disorders, and known HIV, hepatitis B and C infection.

Study assessment and follow-up

Patient demographics and medical history were assessed; all participants underwent a physical examination (including gynecological exam), complete laboratory review, pulmonary function test, thorax and abdominal computed tomography (CT), pelvic magnetic resonance imaging (MRI) and positron emission tomography with CT fusion (PET/CT) before enrollment. Physical examination, vital signs, PS, laboratory panel and toxicities were reviewed weekly during the entire treatment. Systemic toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

Baseline biopsies, within 1 week of treatment and 12 weeks post-treatment, as well as blood samples were collected for molecular analysis according to the Brazilian National Tumor Bank guidelines. Blood samples for everolimus pharmacokinetics analysis were also collected according to a specified protocol. Morphologic (pelvic MRI) response assessment according to RECIST 1.1 and metabolic (PET/CT) response assessment using visual

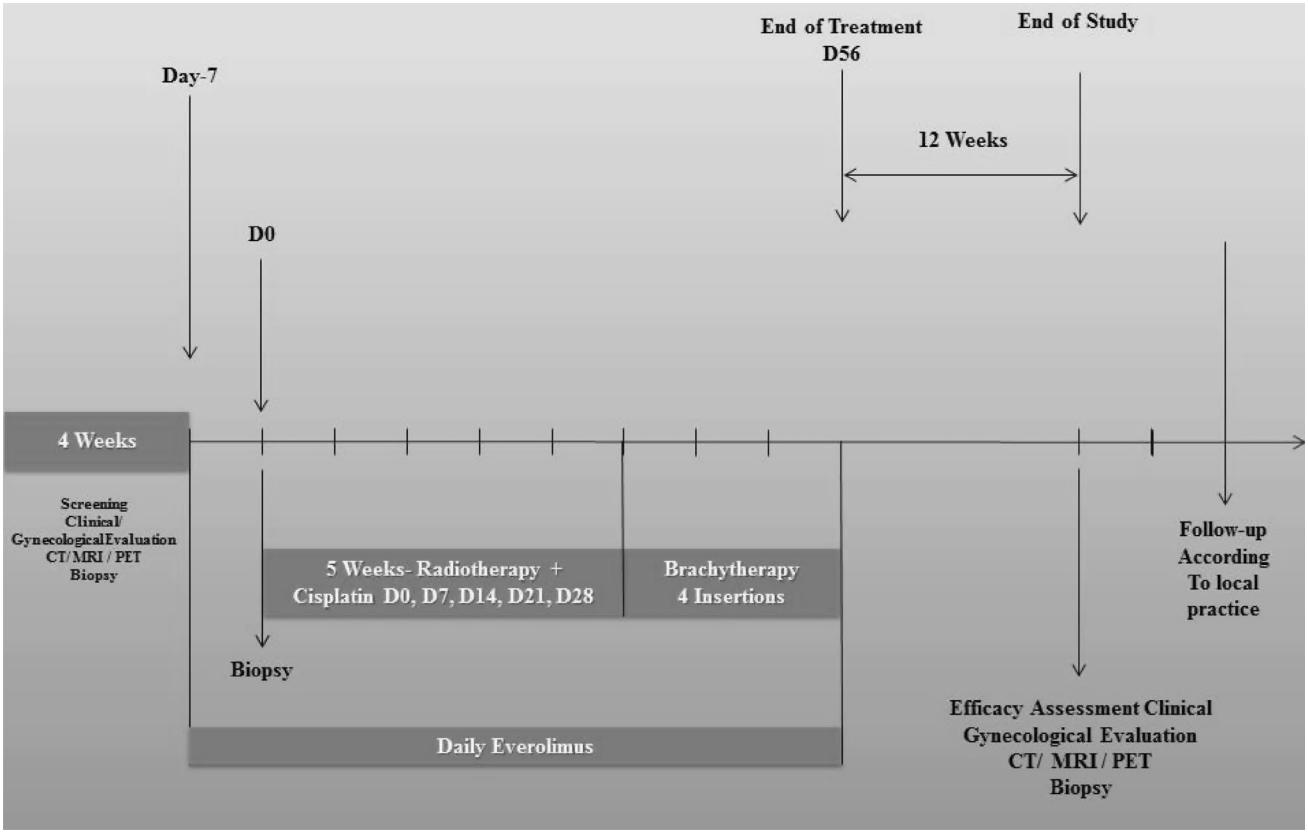

Fig. 1 Therapeutic plan

Table 1 Dose escalation plan

Dose level	Everolimus (day −7 until last day of brachytherapy) (mg)	Cisplatin (days 0, 7, 14, 21 and 28) (mg/m^2)	Radiotherapy	Brachytherapy
1	2.5	40	4500 cGy, 25 fractions	2400 cGy, 4 insertions
2	5	40	4500 cGy, 25 fractions	2400 cGy, 4 insertions
3	10	40	4500 cGy, 25 fractions	2400 cGy, 4 insertions

analysis took place on week 12 after the end of treatment. Afterward, follow-up visits were performed following local practice.

Therapeutic plan

Three dose-level escalations were planned in consecutive cohorts of patients receiving everolimus orally in doses of 2.5, 5 and 10 mg. Radiotherapy and cisplatin doses were fixed. Everolimus was started 7 days before chemo-radiation to allow stable blood levels and was continued daily until the last day of brachytherapy (Fig. 1).

Chemotherapy was started concurrently with radiotherapy. Planned chemotherapy consisted of weekly cisplatin 40 mg/m^2 (maximum dose of 70 mg) on days 0, 7, 14, 21, and 28 (Table 1).

Radiotherapy was delivered during 9 weeks—teletherapy with 4500 cGy divided into 25 daily fractions, 5 days a week, followed by brachytherapy in 4 weekly insertions. During the brachytherapy, cisplatin was not administered. Teletherapy with megavoltage photons energy was planned according to CT using four isocentric fields (anteroposterior × posteroanterior—AP × PA and lateral × lateral—LAT × LAT). Treatment volume was based on ICRU 50 with the gross tumor volume corresponding to the primary tumor, and the clinical target volume embracing the pelvic lymphatic draining (paracervical, internal and external iliac, obturator, presacral, and part of common iliac lymph

nodes), the whole uterus, bilateral parametria, presacral nodes, uterosacral ligaments and any other paracervical tissue involved. The first insertion of brachytherapy with iridium-192 was done on the last teletherapy week, after its end. Individual planning was designed for 600 cGy prescribed at point A each insertion, as per ICRU Report 38 recommendations. The maximum delay allowed for total dose administration was 2 weeks for teletherapy and 2 weeks for brachytherapy.

Criteria to define dose-limiting toxicity

In general terms, DLT was defined as a single grade 4 toxicity related to the use of everolimus, except for rash, or any persisting grade 3 toxicity. For example, neutrophils lower than 500/mm^3 (grade 4) or lower than 1000/mm^3 with fever; platelets count lower than 25 $\times$ 10^3/mL (grade 4) or grade 3 thrombocytopenia (<50 to 25 $\times$ 10^3/mL) lasting more than 2 weeks; creatinine clearance remaining lower than 45 mL/min for two consecutive weeks, or for three non-consecutive weeks; grade 3 or 4 ototoxicity or neuropathy; grade 4 diarrhea or grade 3 diarrhea persisting for more than two consecutive weeks, or for three non-consecutive weeks; grade 4 rash or grade 3 rash persisting for more than 1 week; grade 4 fatigue; grade 3 or 4 pneumonitis and grade 3 or 4 non-hematologic toxicity (not including those specified above) were DLTs. Patients with grade 2 or 3 hypophosphatemia, hyperglycemia, or hypercholesterolemia/hypertriglyceridemia without clinical symptoms, were allowed to continue the treatment without interruption. Grade 4 lymphopenia was considered DLT if associated with an opportunistic infection. Once a DLT was detected, everolimus was permanently discontinued and the treatment with radiotherapy and cisplatin was administered according to physician's discretion.

Pharmacokinetics

Sequential blood samples for pharmacokinetic (PK) analysis were collected on days −7 and 0, immediately before, and 1, 2, 4, 8 and 24 h after administration of everolimus. Additional samples were collected at 24 h after everolimus administration on treatment days 7, 14, 21, 28, 35, 42, 49 and 56. Blood specimens were collected in plastic tubes containing potassium EDTA, frozen at −20 °C within 60 min and stored. The quantification of everolimus in plasma by LC–MS/MS was performed at DASA (Diagnósticos da América S.A., Rio de Janeiro, RJ, Brazil), according to the procedures previously described [19], using everolimus-d4 as the internal standard. After chromatographic separation, the species were monitored at the transitions ($m > z$) 975.6 → 908.5 for everolimus (ammonium adduct) and 979.6 → 912.15 (everolimus-d4). The quantification limit for everolimus was 1.0 ng/mL; the intra- and inter-day CVs were 11.7 and 12.8 %, respectively.

The PK parameters C_{max} (maximal everolimus plasma concentration) and T_{max} (time of occurrence of C_{max}) on days −7 and zero were obtained from the measured everolimus plasma concentrations. Non-compartmental analysis of the plasma concentration data points on days −7 and 0 provided the area under the concentration–time curve from zero time to 24 h (AUC). Estimates of the everolimus AUC after administration on treatment days 7, 14, 21, 28, 35, 42, 49 and 56 were based on the reported tight correlation between the everolimus plasma concentration at 24 h (C_{24h}) and the respective AUC. To validate this approach, we first examined the correlation between C_{24h} and AUC after everolimus administration on days −7 and 0 to all our patients and obtained a correlation coefficient $R^2 = 0.82$ ($p < 0.0001$). The corresponding linear regression equation (AUC = 30.9 + 28.5 $\times$ C_{24h}) was then applied to estimate the AUC corresponding to the samples collected at 24 after everolimus administration on treatment days 7, 14, 21, 28, 35, 42, 49 and 56.

Results

Patient characteristics

From December 2011 to April 2014, 15 patients were screened, being 13 patients included and two screening failures. All 13 patients deemed eligible were included in the safety population and 12 patients in the response evaluation. At baseline, the median age was 43 years (range 30–63), the FIGO stage was IIB in 53.9 %, IIIA in 7.7 %, and IIIB in 38.4 % of patients; and the PS was 0 in 15.4 % and 1 in 84.6 % of patients. Patients were categorized according to the Brazilian census as white (38.4 %), brown ("pardo" in Brazilian Portuguese) (38.4 %) and black (23.2 %) (Table 2). The median duration of therapy for patients who completed the planned schedule was 72 days (range 64–77).

Toxicity

Dose escalation proceeded through to cohort 3 (everolimus, 10 mg/day); six patients were included at dose level 2.5 mg, 3 at dose level 5 mg and 4 at dose level 10 mg. Four patients did not complete the planned schedule, one patient at dose level 10 mg was included with an estimated creatinine clearance lower than 60 mL/min and during the first week of everolimus presented clinical disease progression—for this reason, she was discontinued from the study and was treated according to the standard of care,

being replaced by another patient included at the same dose level; other three patients experienced DLTs and did not complete the planned schedule—one patient in the lowest dose level and 2 in the highest dose level due to renal failure, grade 3 rash lasting more than one week and grade 4 neutropenia, respectively. All DLTs were completely reversible after discontinuation from the trial; thereafter, patients were treated according to local guidelines. No DLTs were reported in the intermediate dose level (Table 3).

All patients had at least 1 adverse event (AE). Most frequent AEs (>30 % of occurrence, all grades) were as follows: diarrhea (76.9 %), nausea (76.9 %), anemia (61.6 %), leukopenia (61.6 %), dysuria (61.6 %), lymphopenia (53.9 %), neutropenia (53.9 %), vomiting (53.9 %), radiodermitis (38.5 %), systemic rash (38.5 %), increased levels of alanine aminotransferase (ALT) (38.5 %), aspartate aminotransferase (AST) (30.8 %) and dysgeusia (30.8 %). Other class AEs as mucositis, fatigue, pneumonitis, hypophosphatemia, hyperglycemia and hypercholesterolemia were less frequent in the treated population. There were no treatment-related deaths. The most common grade 3 or 4 AEs were lymphopenia (53.9 %), leukopenia (30.7 %), neutropenia (30.7 %) and anemia (15.4 %) (Table 4).

Table 2 Patients characteristics

Characteristics	Patients [*n* (%)]
Median age (years)	43 (range 30–63)
ECOG PS	
0	2 (15.4)
1	11 (84.6)
2	0
Stage	
IIB	7 (53.9)
IIIA	1 (7.7)
IIIB	5 (38.4)
Race	
White	5 (38.4)
Black	3 (23.2)
Brown	5 (38.4)
Total	13 (100)

Efficacy assessment

Response assessment was done 12 weeks after the end of treatment. Twelve patients were evaluable for response. Eleven out of 12 evaluable patients (91.6 %) experienced complete response (CR), 1 (8.4 %) partial response (PR) and objective response rate (ORR = CR + PR) of 100 %

Table 3 Treatment delivery

Dose level	Subject number	Everolimus (days)	Cisplatin (weeks)	Radiotherapy (fractions delivered)	Brachytherapy (insertions delivered)	Treatment duration (since day −7)[a]
1	1	74	5	25	4	74
	2	64	5	25	4	64
	3*	35	4	25	4	94
	4	73	5	25	4	76
	5[b]	NA	NA	NA	NA	NA
	6	66	5	25	4	66
	7	73	5	25	4	73
2	8[b]	NA	NA	NA	NA	NA
	9	66	5	25	4	66
	10	77	5	25	4	77
	11	70	5	25	4	69
3	12*	57	5	25	4	70
	13	72	5	25	4	72
	14[c]	7	NA	NA	NA	NA
	15[d]	49	5	25	4	88

NA not applicable

[a] Planned treatment—cisplatin 40 mg/m^2/week for 5 weeks, radiotherapy 4500 cGy in 25 fractions, brachytherapy 2400 cGy in 4 insertions and everolimus for 63 days if no radiotherapy/brachytherapy delay or toxicity

[b] Screening failure

[c] Patient excluded due to clinical disease progression

[d] Patient experienced DLT

Table 4 Most frequent adverse events

Adverse event	Any grade (>30 % of occurrence)—[*n* (%)]	Grade ≥ 3—[*n* (%)]
Diarrhea	10 (76.9)	1 (7.7)
Nauseas	10 (76.9)	1 (7.7)
Anemia	8 (61.6)	2 (15.4)
Leukopenia	8 (61.6)	4 (30.7)
Dysuria	8 (61.6)	0
Lymphopenia	8 (61.6)	7 (53.9)
Neutropenia	7 (53.9)	4 (30.7)
Vomiting	7 (53.9)	1 (7.7)
Radiodermatitis	5 (38.5)	0
Systemic rash	5 (38.5)	1 (7.7)
Constipation	5 (38.5)	0
ALT increased	5 (38.5)	0
AST increased	4 (30.8)	0
Dysgeusia	4 (30.8)	0
Renal failure	0	1 (7.7)

at the end of treatment according to RECIST 1.1. Using the metabolic response assessment (PET/CT), 9 (75 %) patients had CR and 3 (25 %) had PR.

Pharmacokinetics

The everolimus concentration in plasma versus time curves following administration of 2.5, 5 or 10 mg on days −7 (first dose) and 0 (after 7 daily doses) is shown in Fig. 2, and the pharmacokinetic data are summarized in Table 5. Despite considerable inter-individual PK variability, there were dose-dependent increases in the median values for C_{max} and AUC at both days -7 and 0. The AUC, but not C_{max}, increased significantly ($p < 0.0001$, paired t test for combined three dose levels) between days −7 and 0. The estimated AUC for the doses administered on days 7, 14, 21, 28, 35, 42, 49 and 56 (Table 1) did not differ significantly from the AUC measured at day zero ($p = 0.61$, ANOVA test for combined three dose levels).

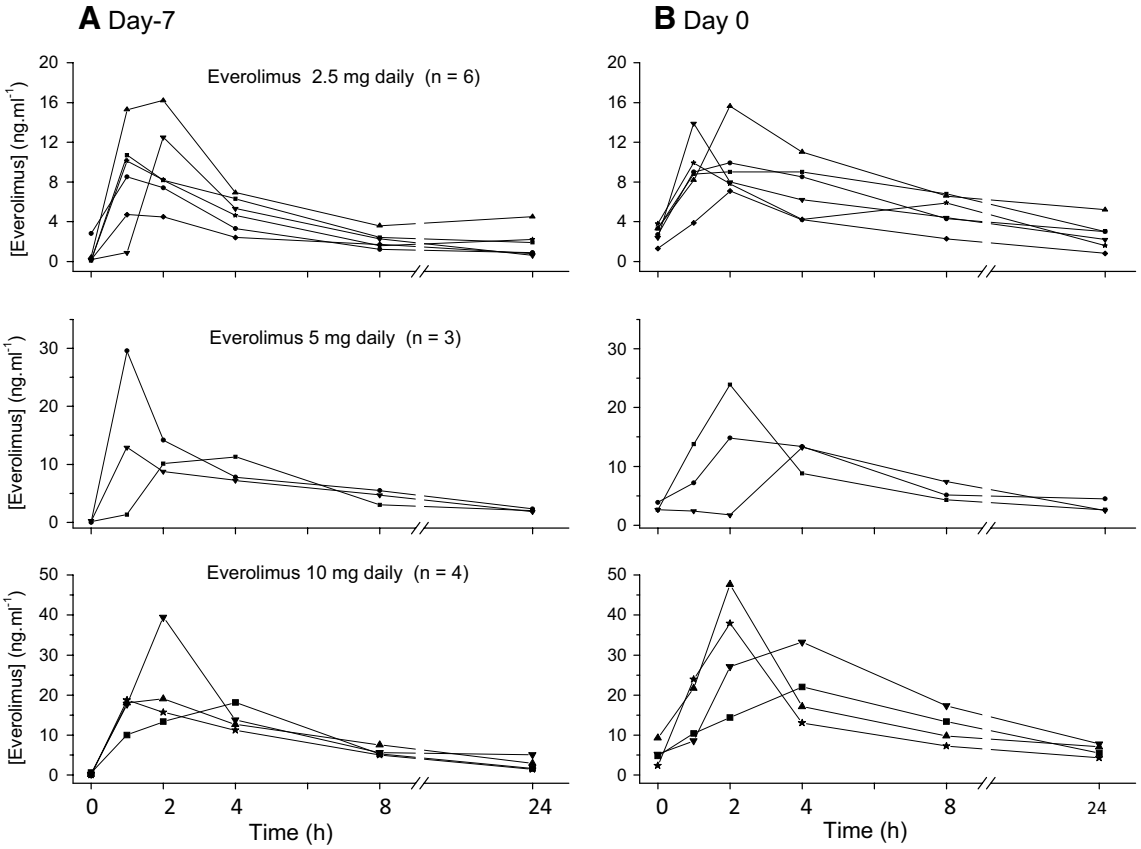

Fig. 2 Everolimus concentration in plasma versus time curves following administration of 2.5, 5 or 10 mg. **a** Day −7 (first dose). **b** Day 0 (after 7 daily doses)

Table 5 Pharmacokinetic parameters of daily everolimus

Days	AUC (ng h/mL)[a]		
	2.5 mg/daily	5 mg/daily	10 mg/daily
−7	66.6 (42.1–132.5), 6	109.2 (96.4–147.7), 3	168.0 (137.9–215.1), 4
0	112.8 (57.2–172.8), 6	141.1 (139.8–158.5), 3	291.2 (228.6–386.8), 4
7	171.4 (46.3–211.8), 6	119.4 (54.0–154.1), 3	227.2 (61.7–327.3), 3
14	121.4 (38.6–338.9), 6	127.1 (127.1–288.8), 3	250.3 (92.5–616.1), 3
21	115.6 (46.3–254.2), 6	104.0 (69.4–208.0), 3	192.6 (42.4–200.3), 3
28	159.9 (50.1–288.8), 6	138.7 (50.1–188.7), 3	196.4 (65.5–369.7), 3
35	127.3 (38.6–323.5), 5	146.4 (46.3–181.0), 3	142.5 (34.7–208.0), 3
42	104.1 (34.7–308.1), 5	119.4 (50.1–219.5), 3	194.5 (54.0–335.1), 2
49	92.5 (34.7–161.8), 5	92.5 (34.7–157.9), 3	256.1 (80.9–431.3), 2
56	88.7 (30.9–181.0), 5	127.1 (42.4–131.0), 3	136.8 (34.7–238.8), 2
Days	C_{max} (ng/mL)		
	2.5 mg/daily	5 mg/daily	10 mg/daily
−7	10.4 (4.7–16.2), 6	12.9 (11.3–29.6), 3	18.9 (18.1–39.4), 4
0	9.9 (7.1–15.6), 6	14.8 (13.2–23.9), 3	35.5 (22–47.7), 4

Data for AUC and C_{max} expressed as median (range), number of patients

[a] AUC for days 7–56 estimated as described in text

Discussion

The carcinogenesis of CC is complex and driven by HPV [12]. Different alterations on cell signaling pathways are induced by HPV and may represent potential targets for more rational therapeutic approaches [11, 20].

In that line, we have previously explored the overexpression of the EGFR pathway in both preclinical and clinical models [21–24]. In this HPV-driven context, the mTOR pathway emerged as another potential target through distinct alterations [13–15]. In our hands, the addition of everolimus on the top of cisplatin-based chemo-radiation for CC patients was feasible at a MTD of 5 mg.

Everolimus has been already tested in combination with radiotherapy, cisplatin or both in phase I trials for different indications. The use of everolimus given with weekly cisplatin (30 mg/m^2 for 6 weeks) and concurrent intensity modulated radiotherapy for patients with locally and/or regionally advanced head and neck cancer was tested, and 5 mg/day was the recommended phase II dose; the DLTs appeared to be primarily related to intensification of local toxicities in the radiation field and were reversible with discontinuation of study drug [25]. Everolimus was also explored in 2 phase I trials in combination with concurrent radiation and temozolomide in newly diagnosed glioblastoma patients, and everolimus was successfully escalated to 10 mg daily and well tolerated with an acceptable toxicity profile [26, 27]. Recently, everolimus combined with concurrent radiotherapy followed by chemotherapy with vinorelbine and cisplatin in unresectable non-pretreated stage III/IV non-small cell lung cancer was published, and the recommended phase II dose was 50 mg/week [28].

Any grade of diarrhea was described in 55 % of patients treated with platinum-based chemotherapy and radiotherapy in a phase III trial and grades 3 or 4 diarrhea in 9.8 % [4]. Grades 1–4 anemia (48 %), leukopenia (37.5–87.7 %), neutropenia (63.9 %), vomiting (62.3 %), radiodermatitis (5.7–22.5 %) were also frequent AEs in phase III trials of chemo-radiation [2, 4–6]. Phase III studies employing everolimus have shown as AEs: systemic rash (29–49 %), diarrhea (30–34 %), nausea (20–26 %), dysgeusia (10–17 %), anemia (17–92 %), vomiting (15–20 %) [17, 18] and increased AST (21 %) and ALT (25 %) levels [18]. Paralleling our data to the above-mentioned studies, the most commonly reported AEs in the current phase I were hematological (anemia, leucopenia, lymphopenia, and neutropenia), gastrointestinal (diarrhea, nausea, and vomiting), dermatological (radiodermatitis and systemic rash), hepatic (increased levels of ALT and AST) and urinary (dysuria), in accordance with AEs from phase III trials formerly reported. In-field toxicity was a major concern, once pelvic radiotherapy alone may induce diarrhea and radiodermatitis [2–7] and the combination with everolimus could overlap the toxicity profile within irradiated areas, though unexpected pelvic AEs were not detected.

The DLTs in the present study were renal failure, neutropenia and rash. Neutropenia is consistent with prior observations regarding the additive myelosuppressive effects of mTOR inhibitors when combined with platinum-based

chemotherapy [29]. Rash is an expected class effect of mTOR inhibitors [18]; renal failure is an event related to the natural history of CC [2–7] and is a frequent side effect of cisplatin, especially when combined with diarrhea and dehydration.

Focusing on metastatic CC patients, some trials have evaluated the effect of treatment with mTOR inhibitors. A phase 1 trial has evaluated 74 heavily pretreated patients with gynecologic and breast malignancies treated with liposomal doxorubicin, bevacizumab and temsirolimus, an intravenous mTOR inhibitor. Thirteen CC patients were included with 2 PRs observed [30]. Forty-one patients with advanced gynecologic malignancies were treated with bevacizumab and temsirolimus in a phase I trial including six patients with CC. Among all patients, 20 % had stable disease (SD) lasting more than 6 months [31]. A non-randomized phase II trial included 38 patients with CC and up to 1 prior line of chemotherapy for metastatic or recurrent disease, treated with temsirolimus. The median duration of SD was 6.5 months (range 2.4–12.0), and 28 % (95 % CI 14–43) of patients had SD lasting for 6 months or more. The toxicities were manageable and main grade 3 and 4 toxicities included hematologic and hepatic side effects [32].

Interestingly, most of the influential trials using platinum-based chemotherapy and radiotherapy for locally advanced CC have not reported efficacy assessment in terms of response rate [2, 3, 5, 7]. In the current trial, 12 patients were evaluable for response and 11 (91.6 %) experienced CR at the end of treatment. Despite the restrictions of comparing results across different studies, these responses are remarkable when in parallel with historical data, where CR was observed in 38–75 % of patients [33–36].

When analyzing our phase 1 data, some caveats must be considered. Radiotherapy regimen encompassed 5 weeks of chemo-radiation and 4 weeks of brachytherapy (total treatment time of 63 days without interruptions, considering the first week of everolimus alone). In our trial, all patients completed the planned radiation therapy—except the one who was discontinued due to disease progression—some patients were treated in longer periods due to holiday breaks, preventive radiotherapy device maintenance and treatment interruptions related to toxicities. We have included only patients with the histological diagnosis of squamous cell carcinoma; nevertheless, adenocarcinoma accounts for approximately 20 % of CC worldwide. Whether the data generated on adenocarcinoma would be similar is yet to be tested. It must be considered, though, that previous data from our group suggest that adenocarcinomas are, in general, treated in the same manner and have similar therapeutic response rates [37]. Because of the dismal prognosis, we have also excluded IVA stage CC.

PK results were characterized by dose-dependent increases in AUC and C_{max}, but dose proportionality was not clear due to large inter-individual variability at each dose level. The PK results are consistent with those observed in subjects with advanced solid tumors treated with everolimus monotherapy [38], and no significant changes were observed in the PK profile of everolimus due to concurrent administration of cisplatin as previously described [29]. Samples of blood and tumor tissue were collected at different stages of the treatment, aiming for future genomic and proteomic studies.

In conclusion, in combination with cisplatin and radiotherapy the MTD of everolimus has been defined as 5 mg/day. To the best of our knowledge, this is the first report combining everolimus, cisplatin and pelvic radiotherapy for locally advanced CC. These safety data may be applicable to other pelvic tumors. The data regarding safety and response rates support further studies with everolimus and may shed light on other combinations tackling the same signaling pathway including PI3K inhibitors.

Acknowledgments This phase I trial was funded by Novartis; Phoenix ClinicalTrials.gov identifier NCT01217177.

Compliance with ethical standards

Conflict of interest Juliane Morando is a Novartis employee. All other authors have no conflicts of interest to declare.

References

1. Forman DBF, Brewster DH, Gombe Mbalawa C et al (2012) GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012. http://globocan.iarc.fr. Accessed 15 June 2015
2. Whitney CW, Sause W, Bundy BN et al (1999) A randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stages IIB–IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecology Oncology Group and Southwest Oncology Group Study. J Clin Oncol 17:1339–1348
3. Morris M, Eifel PJ, Lu J et al (1999) Pelvic radiation with concurrent chemotherapy versus pelvic and para-aortic radiation for high-risk cervical cancer: a randomized Radiation Therapy Oncologic Group clinical trial. N Engl J Med 340:1137–1143
4. Peters WA III, Liu PY, Barret RJ et al (2000) Cisplatin and 5-fluorouracil plus radiation therapy are superior to radiation therapy as adjunctive in high-risk early stage carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: report of a phase III intergroup study. J Clin Oncol 18:1606–1613
5. Rose PG, Bundy BN, Watkins EB et al (1999) Concurrent cisplatin based chemoradiation improves progression free and overall survival in an advanced cervical cancer: results of a randomized Gynecologic Oncology Group Study. N Engl J Med 340:1144–1153
6. Keys HM, Bundy BM, Stehman FBV et al (1999) Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. N Engl J Med 340:1154–1161

7. Pearcey R, Brundage M, Drouin P et al (2002) Phase III trial comparing radical radiotherapy with and without cisplatin chemotherapy in patients with advanced squamous cell cancer of the cervix. J Clin Oncol 20:966–972
8. Tierney JF, Vale C, Symonds P (2008) Concomitant and neoadjuvant chemotherapy for cervical cancer. Clin Oncol 20:401–416
9. National Cancer Institute (1999) NCI Clinical Announcement. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD, USA
10. Meric-Bernstam F, Gonzalez-Angulo AM (2009) Targeting the mTOR signaling network for cancer therapy. J Clin Oncol 27(13):2278–2287
11. Husseinzadeh N, Husseinzadeh HD (2014) mTOR inhibitors and their clinical application in cervical, endometrial and ovarian cancers: a critical review. Gynecol Oncol 133(2):375–381
12. Bosch FX, Lorincz A, Muñoz N et al (2002) The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 55(4):244–265
13. Bertelsen BI, Steine SJ, Sandvei R et al (2006) Molecular analysis of the PI3K-AKT pathway in uterine cervical neoplasia: frequent PI3KCA amplification and AKT phosphorylation. Int J Cancer 118:1877–1883
14. Oh KJ, Kalinina A, Park NH et al (2006) Deregulation of eiF4E: 4E-BP1 in differentiated human papillomavirus-containing cells leads to high levels of expression of the E7 oncoprotein. J Virol 80:7079–7088
15. Beuvink I, Boulay A, Fumagallo S et al (2005) The mTOR inhibitor RAD001 sensitizes tumor cells to DNA-damaged induced apoptosis through inhibition of p21 translation. Cell 120:747–759
16. Baselga J, Campone M, Piccart M et al (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366(6):520–529
17. Yao JC, Shah MH, Ito T et al (2011) Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 364(6):514–523
18. Motzer RJ, Escudier B, Oudard S et al (2010) Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. Cancer 116(18):4256–4265
19. Brignol N, McMahon LM, Luo S et al (2001) High-throughput semi-automatic 96-well liquid/liquid extraction and liquid chromatography/mass spectrometric analysis of everolimus (RAD001) and cyclosporine A (CsA) in whole blood. Rapid Commun Mass Spectrom 15:898–907
20. Hu G, Liu W, Mendelsohn J et al (1997) Expression of epidermal growth factor receptor and human papillomavirus E6/E7 proteins in cervical carcinoma cells. J Natl Cancer Inst 89:1271–1276
21. Meira DD, de Almeida VH, Mororó JS et al (2009) Combination of cetuximab with chemoradiation, trastuzumab or MAPK inhibitors: mechanism of sensitisation of cervical cancer cells. Br J Cancer 101:782–791
22. Meira DD, Nóbrega I, de Almeida VH et al (2009) Different antiproliferative effects of matuzumab and cetuximab in A431 cells are associated with persistent activity of the MAPK pathway. Eur J Cancer 45:1265–1273
23. Nogueira-Rodrigues A, Carmo CC, Viegas C et al (2008) Phase I trial of erlotinib combined with cisplatin and radiotherapy for patients with locally advanced cervical squamous cell cancer. Clin Cancer Res 14:6324–6329
24. Nogueira-Rodrigues A, Moralez G, Grazziotin R et al (2014) Phase 2 trial of erlotinib combined with cisplatin and radiotherapy in patients with locally advanced cervical cancer. Cancer 120(8):1187–1193
25. Fury MG, Lee NY, Sherman E et al (2013) A phase 1 study of everolimus + weekly cisplatin + intensity modulated radiation therapy in head-and-neck cancer. Int J Radiat Oncol Biol Phys 87:479–486
26. Chinnaiyan P, Won M, Wen PY et al (2013) RTOG 0913: a phase 1 study of daily everolimus (RAD001) in combination with radiation therapy and temozolomide in patients with newly diagnosed glioblastoma. Int J Radiat Oncol Biol Phys 86:880–884
27. Sarkaria JN, Galanis E, Wu W et al (2011) North Central Cancer Treatment Group Phase I trial N057 K of everolimus (RAD001) and temozolomide in combination with radiation therapy in patients with newly diagnosed glioblastoma multiforme. Int J Radiat Oncol Biol Phys 81:468–475
28. Deutsch E, Le Péchoux C, Faivre L et al (2015) Phase I trial of everolimus in combination with thoracic radiotherapy in non-small-cell lung cancer. Ann Oncol 26(6):1223–1229
29. Fury MG, Sherman E, Haque S et al (2012) A phase I study of everolimus plus low-dose weekly cisplatin for patients with advanced solid tumors. Cancer Chemother Pharmacol 69:591–598
30. Moroney JW, Schlumbrecht MP, Helgason T et al (2011) A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies. Clin Cancer Res 17(21):6840–6846
31. Piha-Paul SA, Wheler JJ, Fu S et al (2014) Advanced gynecologic malignancies treated with a combination of the VEGF inhibitor bevacizumab and the mTOR inhibitor temsirolimus. Oncotarget 5(7):1846–1855
32. Tinker AV, Ellard S, Welch S et al (2013) Phase II study of temsirolimus (CCI-779) in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC Clinical Trials Group (NCIC CTG IND 199). Gynecol Oncol 130(2):269–274
33. Buxton EJ (1992) Experience with bleomycin, ifosfamide, and cisplatin in primary and recurrent cervical cancer. Semin Oncol 19:9–17
34. Kumar L, Kaushal R, Nandy M et al (1994) Chemotherapy followed by radiotherapy versus radiotherapy alone in locally advanced cervical cancer: a randomized study. Gynecol Oncol 54:307–315
35. Leborgne F, Leborgne JH, Doldan R et al (1997) Induction chemotherapy and radiotherapy of advanced cancer of the cervix: a pilot study and phase III randomized trial. Int J Radiat Oncol Biol Phys 37:343–350
36. Classe JM, Rauche P, Rodier JF et al (2006) Surgery after concurrent chemoradiotherapy and brachytherapy for the treatment of advanced cervical cancer: morbidity and outcome: results of a multicenter study of the GCCLCC (Groupe des Chirurgiens de Centre de Lutte Contre le Cancer). Gynecol Oncol 102:523–529
37. Nogueira-Rodrigues A, Ferreira CG, Bergmann A et al (2014) Comparison of adenocarcinoma (ACA) and squamous cell carcinoma (SCC) of the uterine cervix in a sub-optimally screened cohort: a population-based epidemiologic study of 51,842 women in Brazil. Gynecol Oncol 135(2):292–296
38. O'Donnell A, Faivre S, Burris HA et al (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. J Clin Oncol 26:1588–1595