CLINICAL STUDY

Phase II study of weekly paclitaxel and sorafenib as second/third-line therapy in patients with adrenocortical carcinoma

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Abstract

Background: There is a strong rationale in the use of antiangiogenic therapy in the management of adrenocortical carcinoma (ACC). Metronomic administration of chemotherapy and antiangiogenic drugs can be synergistic in targeting endothelial cells.

Objective: We assessed the activity of sorafenib plus metronomic paclitaxel as second/third-line therapy in advanced ACC patients. We also tested the activity of sorafenib and paclitaxel against NCI-H295R *in vitro*.

Design: Multicenter, prospective phase II trial.

Setting: Referral centers for ACC.

Methods: Twenty-five consecutive metastatic ACC patients who progressed after mitotane plus one or two chemotherapy lines were planned to be enrolled. The patients received a combination of i.v. paclitaxel (60 mg/m² every week) and oral sorafenib (400 mg twice a day) till progression. The primary aim was to measure the progression-free survival rate after 4 months and the secondary aims were to assess the objective response rate and toxicity.

Results: Tumor progression was observed in nine evaluable patients at the first assessment. These results led to the premature interruption of the trial. The treatment was well tolerated. The most relevant toxicities were fatigue, being grade 2 or 3 in four patients, and hypophosphatemia, being grade 3 in three patients. In the *in vitro* study, sorafenib impaired the viability of H295R cells with dose–response and time–response relationships. The *in vitro* sorafenib activity was not increased in combination with paclitaxel.

Conclusions: Despite the *in vitro* activity, sorafenib plus weekly paclitaxel is an inactive salvage treatment in patients with advanced ACC and should not be recommended.

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Introduction

Adrenocortical carcinoma (ACC) is a highly malignant disease that is resistant to medical treatment. Surgery is the only treatment potentially able to cure ACC patients (1), but when ACC cannot be surgically removed, or the tumor recurs after surgery, prognosis is dismal (1, 2, 3).

The efficacy of the currently used chemotherapy regimens is modest at best, and most patients are destined to relapse. Therefore, there is an urgent need for new treatment strategies (4).

Metronomic chemotherapy is the administration of antineoplastic drugs at low doses, on a frequent or continuous schedule, with no extended interruptions (5). This treatment modality targets tumor cells indirectly

via inhibiting angiogenesis and vasculogenesis by continuously exposing the more slowly proliferating tumor endothelial cells to cytotoxic therapy (5, 6). Low-dose metronomic chemotherapy may offer several advantages, including low toxicity and treatment response irrespective of the resistance profile of the tumor cell population (5, 7, 8).

Paclitaxel is an anticancer agent that has been found to be effective in the treatment of several malignancies (9). It promotes polymerization of tubulin dimers to form microtubules and stabilizes microtubules by preventing depolymerization (9). Preclinical studies have shown that paclitaxel is active against ACC cell lines (10). Paclitaxel administered on a weekly metronomic schedule was found to be more effective than the

3-week administration in two randomized prospective clinical trials involving breast cancer patients either as a neoadjuvant approach before primary surgery (11) or in advanced disease (12). Paclitaxel, therefore, seemed to be a good candidate to be tested in a metronomic schedule in advanced ACC patients.

Since ACC is highly vascularized (13) and shows high levels of vascular endothelial growth factor (VEGF) (14), we believed that the introduction of a small molecule targeting the angiogenesis pathways at different levels could be a good strategy to be used in ACC treatment.

Sorafenib is an inhibitor of several receptor tyrosine kinases involved in neovascularization, including VEGFR2, VEGFR3, and platelet-derived growth factor. Sorafenib also inhibits RAF-1, a key enzyme in the RAS/RAF/MEK/ERK signaling pathway leading to cell proliferation (15). Sorafenib was approved by the FDA for the treatment of advanced renal cell cancer and hepatocellular cancer and shows good clinical activity in thyroid cancer (16). Sorafenib showed a broad spectrum of antitumor activity in preclinical studies (15), so multiple clinical trials are undertaken to further investigate the role of sorafenib alone or in combination with paclitaxel for the treatment of various tumor entities.

As suggested by Kerbel & Kamen (5), there is a strong rationale for combining metronomic chemotherapy with antiangiogenic drugs. Before preclinical metronomic chemotherapy, studies have shown that the combination of antiangiogenic drugs with metronomic chemotherapy can increase the antitumor efficacy compared with either agent alone (17, 18). Two nonrandomized phase II trials have tested the association of metronomic chemotherapy plus bevacizumab in advanced ovarian and breast cancers, respectively, and promising results have been obtained (19, 20).

On these bases, a prospective, open-label, single-arm, multicenter study was planned with the aim to determine the activity and toxicity of sorafenib plus weekly paclitaxel as a second/third-line approach in patients with advanced/metastatic ACC.

The objective of the primary study was to assess the clinical benefit as measured by the nonprogressing rate after 4 months of the combination regimen. The secondary aims were to assess the objective response rates and toxicity. In order to obtain information on the interaction between the two drugs, an *in vitro* study was also performed to test the antiproliferative effect of either drug, alone or in combination, against NCI-H295R ACC cells.

Materials and methods

Patients and methods

To be included in this trial, the patients had to meet the following inclusion criteria: age ≥ 18 years, histologically confirmed diagnosis of ACC, locally advanced or metastatic disease not amenable to surgery,

progressive disease after one or two cytotoxic chemotherapy regimens (including a cisplatin-based protocol), Eastern Cooperative Oncology Group (ECOG) performance status 0–2, life expectancy ≥ 3 months, and at least one unidimensional (RECIST criteria) measurable lesion, adequate bone marrow reserve (neutrophils $\geq 1500/\text{mm}^3$ and platelets $\geq 100\,000/\text{mm}^3$), hemoglobin ≥ 9.0 g/dl, total bilirubin ≤ 1.5 times the upper limit of normal, PT-INR/PTT $< 1.5 \times$ upper limit of normal, serum creatinine $\leq 1.5 \times$ upper limit of normal, effective contraception in premenopausal female and male patients, written informed consent, and ability to comply with the protocol procedures.

Exclusion criteria were history of prior malignancy, except for cured nonmelanoma skin cancer, cured *in situ* cervical carcinoma, or other treated malignancies with no evidence of disease for at least 3 years, active clinically serious infections (greater than grade 2 National Cancer Institute - Common Toxicity Criteria (NCI-CTC) version 3.0), symptomatic metastatic brain or meningeal tumors, seizure disorder requiring medication (i.e. steroids or antiepileptics), concomitant rifampicin, concomitant St. John's Wort (*Hypericum perforatum*), decompensated heart failure (ejection fraction $< 45\%$), myocardial infarction or revascularization procedure during the last 6 months, unstable angina pectoris, uncontrolled cardiac arrhythmia, hypertension that was not controlled by medications ($> 160/100$ mmHg despite optimal medical therapy), patients with recent or active bleeding diathesis, pregnant or breast-feeding patients, previous treatment with sorafenib, other anticancer chemotherapy or immunotherapy during the study or within 4 weeks of study entry, radiotherapy during study or within 3 weeks of study start (palliative radiotherapy was allowed), major surgery within 4 weeks of start of study, current treatment with another investigational drug, any other severe acute or chronic medical or psychiatric condition, or laboratory abnormality which, in the judgment of the investigator, would have made the patient inappropriate for entry into this study. The investigation was approved by the Local Ethics Committee and was conducted according to Good Clinical Practice. Written consent was obtained from each patient after full explanation of purpose and nature of all procedures used.

Treatment schedule Sorafenib was administered orally at the dose of 400 mg twice a day and paclitaxel was administered i.v. at the dose of $60\text{ mg}/\text{m}^2$ every week. The treatment was planned to be continued until disease progression. Mitotane was withdrawn 1 month before sorafenib initiation till the end of the study.

In order to prevent major hypersensitivity reactions, a premedication with corticosteroids (dexamethasone, 4 mg i.v.), H1-receptor antagonist (diphenhydramine, 50 mg i.v.), and H2-receptor antagonists (cimetidine, 300 mg or ranitidine, 150 mg i.v.) was given before paclitaxel administration.

Recommended treatment modifications for hematological toxicities were as follows: if granulocyte counts were $<1.5 \times 10^9/l$ and/or platelet counts were $<100 \times 10^9/l$ 1 week, a delay of paclitaxel was introduced while sorafenib was decreased by one dose level. In case of lower hematological values, paclitaxel was delayed until resolution and sorafenib delayed until recovery of at least grade 2 toxicity. Then, sorafenib was reduced by one dose and paclitaxel administered at 60% of dose.

As far as nonhematological toxicities were concerned, in case of grade 4 toxicities, both drugs were stopped permanently and for grade 3 toxicity (except for skin toxicity), sorafenib was delayed until less than or equal to grade 2 and then restarted at a dose decreased by one level. In case of grade 2 and 3 skin toxicity, paclitaxel was not interrupted while sorafenib was interrupted till resolution to grade 1 or less. The subsequent dose level of the drug was administered depending on its first, second, or more appearances. For grade 2 neurological toxicity, paclitaxel was administered at 80% of the dose, and in case of grade 3 toxicity, the drug was interrupted until return to baseline then restarted with 60% of planned dose.

Treatment evaluation The following tests were evaluated before entry onto the study: medical history and physical examination; performance status; weight; and routine laboratory tests: echocardiography, chest radiography, and chest and abdominal CT scan. Bone scan and brain CT (or magnetic resonance imaging) were performed if clinically indicated. An endocrine workup was also performed including measurement of serum and urinary cortisol, serum 17-hydroxyprogesterone, DHEAS, as well as testosterone in women and estradiol in men. Physical examination and hematological profile were repeated every week before starting paclitaxel administration and CT scan and endocrine workup were planned to be repeated every 8 weeks for the first 4 months and then every 12 weeks. In case of documented disease progression, the subjects were excluded from the study. Disease response was assessed using the RECIST criteria (21), while toxicity was graded using the NCI criteria.

Progression-free survival (PFS) was measured from the beginning of the treatment until the criteria for disease progression were met or last follow-up in nonprogressing patients. Overall survival was defined as the time interval between the date of beginning of treatment and the date of death from any cause or the last known alive date.

Plasma samples were collected for determining mitotane concentration at baseline and 3 and 6 weeks after starting paclitaxel and sorafenib treatment. Plasma mitotane was measured in-house by an HPLC assay, as described previously (22).

Cell culture and chemical reagents NCI-H295R cell line was supplied by the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in a 1:1 mixture of DMEM and Ham's F-12 nutrient mixture (DMEM/F12; Sigma) supplemented with 1% L-glutamine (Sigma) and 2.5% Nu-Serum (Becton Dickinson, San Jose, CA, USA) and enriched with 1% of ITS+Premix (Becton Dickinson) at 37 °C in 5% CO₂ and 95% humidity. Sorafenib tosylate was a gift from Bayer Schering Pharma and paclitaxel was purchased from Calbiochem (Darmstadt, Germany); both drugs were dissolved in 100% DMSO (Sigma).

Treatment and cell viability assay H295R cells were seeded at a density of 15×10^3 cells/100 µl in 96-well flat-bottomed plates (Corning, New York, NY, USA) in culture medium plus 1% ITS+Premix and 2.5% Nu-Serum. After 48 h, the medium was changed, and the cells were then cultured in medium alone (untreated conditions) or in medium containing different concentrations of drugs for different times. Cells were treated for 24 and 48 h with sorafenib (1, 5, 10, 25, 50 µM) alone and with paclitaxel alone (5, 10, 50, 100, 500, 1000 nM) respectively. After incubation time, 10 µl Cell Proliferation Reagent WST-1 (Roche Applied Science) were added to each well and incubated for 1 h at 37 °C in order to measure cell proliferation. The absorbance was determined using a microplate (ELISA) reader at a test wavelength of 450 nm and reference wavelength of 630 nm. Three replicate wells were used to determine each data point. The basal conditions were normalized to 100% for each assay and cell viability was expressed as a percentage of the untreated control. The mean percentage of inhibition of proliferation was calculated with respect to the control for each sample. To evaluate the possible synergic effect of sorafenib and paclitaxel on cell viability, H295R cells were exposed to fixed concentrations of sorafenib (10 or 50 µM) plus paclitaxel (5, 10, 50, 100, 500, 1000 nM) for 24 and 48 h respectively. After incubation time, cell viability was assessed as described above and the mean percentage of inhibition of proliferation was calculated with respect to treatment with 10 or 50 µM sorafenib for each sample.

Statistical analysis

The primary end point of the study was to estimate PFS at a fixed time point of 4 months. PFS rate was planned to be evaluated in all the patients registered in the study, according to the intent to treat principle. The size of this trial was determined using the optimal two-stage phase II study design by Simon (23). Accordingly, the sample size was assessed in order to refuse a PFS rate of 15% (p0) after 4 months and to provide a statistical power of 80% in assessing the activity of the regimen as a PFS rate after 4 months of 40%. The upper limit for the first-stage drug rejection was one nonprogressing patient after 4 months out of the first consecutive seven

patients. The upper limit of the second-stage rejection was six nonprogressing patients after 4 months out of 25 consecutively enrolled patients. All survival functions were computed using the Kaplan–Meier method.

In vitro changes of proliferative activity induced by sorafenib and paclitaxel on ACC cells were analyzed by paired *t*-test. Data were expressed as mean $\pm$ s.d. Statistical analysis was performed using SPSS for Windows Software (version 17; SPSS Inc., Casalecchio di Reno, Italy). Levels of statistical significance were set at *P* value <0.05.

Results

This open-label, single-arm, multicenter phase II study was planned to be conducted in Italy, at the Medical Oncology Unit of the Azienda Ospedaliero Universitaria San Luigi di Orbassano, and in France, at the Service de Médecine Nucléaire et de Cancérologie Endocrinienne, Institut Gustave Roussy, Villejuif Cedex.

The study was open for enrollment in Italy in November 2008 and was closed to accrual in February 2009 due to inefficacy. According to the stopping rules adopted in this trial, the enrollment was interrupted when the first seven patients had been evaluated and disease progression observed in all of them at first radiological evaluation. At that time, ten patients had been enrolled.

Patients and tumor characteristics

Table 1 shows the patient characteristics of this series. Eight patients have been previously treated with surgery, five of them have been admitted to more than one surgical approach (median 3 (range 2–5)). Post-surgery radiation therapy was given to two patients. Two patients had metastatic disease at diagnosis and were treated with chemotherapy only. All patients had previously received mitotane therapy, five of them for advanced disease only, while in five patients mitotane was administered either in an adjuvant setting or for advanced disease. All patients did receive at least one chemotherapy line administered in association with mitotane. First-line chemotherapy consisted of cisplatin plus etoposide, plus/minus adriamycin. Four patients also received a second-line therapy consisting of streptozotocin plus mitotane (one patient), gemcitabine plus capecitabine plus mitotane (two patients), and carboplatin plus mitotane (one patient). Serum mitotane levels at baseline were <1 mg/ml in three patients, between 3 and 4.5 ng/ml in four patients, around 7.5 ng/ml in two patients, while in the remaining patient, it was 18.7 ng/ml.

Treatment received

Eight patients had received 8 weeks of treatment, one patient interrupted the treatment after 5 weeks due to

deterioration of his performance status, and one patient interrupted the treatment after 2 weeks due to an acute abdomen. This adverse event resolved completely within 15 days, but then the patient refused further treatment. Both sorafenib and paclitaxel were interrupted in one patient for 1 week due to lung infection.

Toxicity

The treatment was well tolerated and the worst toxicities encountered are summarized in Table 2. None of them were dose limiting. The most relevant toxicity was fatigue that was observed in eight patients, being grade 2 or 3 in four patients. Three patients had severe hypophosphatemia (grade 3), accompanied by hypocalcemia in one of them (grade 2). Hematological and hepatic toxicities were mild. No patients had renal or skin toxicity.

Table 1 Patient characteristics.

Characteristics	Value
Total number	10
Males	4
Females	6
Age (median (range); years)	46 (30–69)
Performance status (ECOG)	
0	2
1	8
Mitosis count ^a (median (range))	22 (10–40)
Stage at diagnosis	
Locoregional	8
Metastatic	2
Secretory status at the study entry	
Cortisol	4
Cortisol + androgens	2
Estradiol	1
Primary treatment at diagnosis	
Surgery	8
Chemotherapy plus mitotane	2
Number of surgical operations	
1	5
2	2
≥3	3
Adjuvant mitotane	5
Chemotherapy for advanced disease	
First line	
EDP + mitotane	7
CDDP + VP16	2
CDDP + VP16 + mitotane	1
Second line	
Streptozotocin + mitotane	1
Gemcitabine + capecitabine	2
Carboplatin	1
Radiation therapy	2
Sites of disease	
Lung	8
Liver	5
Lymph nodes	4
Abdomen	7
Bone	1
Number of sites involved	
2	6
3	4

^aPer 50 high power field.

Table 2 Toxicity according to the NCI criteria. For each patient, the worst instance of toxicity was recorded.

	Grades				
	0	1	2	3	4
Leukocytes (total WBC)	9	1			
Platelets	10				
Hemoglobin	8		2		
Creatinine	10				
Nausea/vomiting	9	1			
Diarrhea	9	1			
Stomatitis	8	2			
Gamma-GT	8			2	
AST/ALT	9		1		
Bilirubin	10				
Alkaline phosphatase	9	1			
Cardiovascular	10				
Neurology	9	1			
Fever	8	1	1		
Alopecia	10				
Myalgia	10				
Skin toxicity	10				
Asthenia	2	4	1	3	
Hypophosphatemia	7			3	
Hypocalcemia	9		1		

Treatment activity

Disease response was assessable in nine patients; the patient who interrupted the treatment after 2 weeks was not evaluable for response. All evaluable patients showed a disease progression on target lesions at the first evaluation performed after 8 weeks. As shown in the waterfall diagram depicted in Fig. 1, in this short period of time, the tumor increase was dramatic: median 60% (range 26–152%). In two patients, the progressive disease on target lesions was associated with the appearance of new lesions: mediastinal lymph nodes in one patient and lung metastases in one patient. Tumor progression was associated with a deterioration of performance status in eight patients. At the last follow-up, in December 2010, all but one patient was deceased.

Cytotoxic activity of sorafenib and paclitaxel in vitro

Sorafenib impaired cell viability of H295R cells either at 24 h (data not shown) or 48 h (Fig. 2a). A time course of sorafenib effects on cell growth and survival showed dose–response and time–response relationships. Conversely, treatment with paclitaxel alone showed a slight effect on cell viability (Fig. 2b). Moreover, H295R cells were treated with two fixed concentrations of sorafenib (10 and 50 μ M) and a wide range of paclitaxel concentration (Fig. 2c and d). The overall effect of two drugs was similar to treatment with sorafenib alone at the same times and concentrations; indeed, no significant changes in viability ratio were detected with the

combination of drugs with respect to sorafenib alone. A 24-h preincubation with paclitaxel, before treatment with sorafenib, showed the same response of concurrent administration (data not shown).

Discussion

ACC is a highly vascularized disease (24) and metronomic chemotherapy is thought to exert its anticancer activity mainly by inhibiting tumor angiogenesis. Moreover, the antiangiogenic activity of metronomic chemotherapy can be theoretically increased by the concomitant administration of an antiangiogenic drug (4). Therefore, there is a strong rationale for testing the combination of metronomic chemotherapy plus antiangiogenic drugs in the management of ACC patients.

Despite this sound biological basis, the combination of weekly paclitaxel and sorafenib was totally ineffective in ACC patients progressing after one or two chemotherapy lines. A clear progressive disease was observed in nine consecutive patients at the first restaging done after 2 months, and this led to interruption of the clinical trial.

It is noteworthy that in many patients, the tumor progression was dramatic and the increase in tumor lesions was greater and faster than that in the months before the trial. These data suggest that this combination therapy may have paradoxically favored tumor progression.

This treatment was well tolerated, although safety data are limited due to the short study duration.

In this population of heavily pretreated patients, some of them had grades 2–3 asthenia, anemia, and hypophosphatemia.

In a multicenter phase II trial previously done in Germany, ten patients who were heavily pretreated with three or more therapeutic lines received a combination of bevacizumab and capecitabine administered on a metronomic schedule (25). Similar to our data, all patients showed a disease progression (25).

Figure 1 Waterfall diagram of percentage change in tumor size at first disease assessment.

Figure 2 Cytotoxic effects of sorafenib, paclitaxel, and the combination of both drugs. The antiproliferative effect of sorafenib and paclitaxel alone and the combination of both drugs was assessed *in vitro* on H295R adrenocortical carcinoma cells. (A) H295R cells were incubated for 48 h with different concentrations of sorafenib (1, 5, 10, 25, and 50 μ M) and (B) paclitaxel (5, 10, 50, 100, 500, 1000 nM). In addition, H295R cells were incubated for 48 h (C and D) with two fixed concentrations of sorafenib (10 and 50 μ M) and increasing concentrations of paclitaxel. Cell viability was determined by the WST-1 method and expressed as ratio between treated cells and untreated controls (basal); data are expressed as mean $\pm$ S.D., $n=3$. Significance vs untreated cells, * $P<0.05$.

However, our *in vitro* study showed that ACC cells are sensitive to sorafenib administration. Paclitaxel, conversely, failed to be active against H295R ACC cells at variance with previous results (10). Paclitaxel, however, did not impair the antiproliferative effect of sorafenib, so the combination maintained an activity against ACC cells *in vitro*.

How can we explain our disappointing results? Of course, the great tumor burden and the multiple previous treatment lines could have been contributory. It should be noted, however, that both metronomic chemotherapy and sorafenib target angiogenesis and the combination of the two treatments may have induced tumor hypoxia in the cellular microenvironment leading to accelerated tumor growth (26, 27). It is known that tumor hypoxia is associated with a more aggressive phenotype in cancers. Tumor hypoxia occurs by stimulation of the hypoxia-inducible factor 1 transcription factor, which in turn stimulates a number of genes involved in either angiogenesis or tumor cell motility and invasion (28).

The finding of a U-shaped dose-response curve of antiangiogenic drugs activity provides support to this hypothesis (26).

A better understanding of how antiangiogenic drugs modify biology of ACC is therefore mandatory before further testing these drugs in ACC patients.

Our study, however, cannot exclude that sorafenib could be active if combined with other chemotherapeutic agents with known efficacy in ACC such as cisplatin (4).

We have recently shown that the combination of gemcitabine and metronomic capecitabine was active in heavily pretreated ACC patients (29). These findings suggest that a metronomic approach deserves interest despite the negative results of the present trial.

It should be noted, however, that mitotane was not withdrawn in the gemcitabine and capecitabine trial as in the present one, and mitotane serum levels within the therapeutic range (14–20 ng/ml) were associated with a prolonged PFS (29). These data suggest that mitotane may potentiate chemotherapy activity even in refractory disease. In the present study, all patients but one had mitotane levels consistently below the therapeutic range. On the other hand, it has been shown that sorafenib metabolism is altered by drugs able to increase hepatic p450 enzymes such as mitotane (30).

In conclusion, sorafenib plus weekly paclitaxel is a well-tolerated but inactive treatment in patients with advanced ACC and should not be recommended.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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