A preclinical therapeutic schedule optimizing docetaxel plus estramustine administration in prostate cancer

Ahmed Dahmani[a], Ludmilla de Plater[a], Charlotte Guyader[a], Jean-Jacques Fontaine[d], Aurélie Berniard[a], Franck Assayag[a], Philippe Beuzeboc[b], Elisabetta Marangoni[a], Fariba Némati[a], Marie-France Poupon[a], Christophe Pasik[e], Stéphane Oudard[c] and Didier Decaudin[a,b]

Androgen-dependent and castration-resistant prostate cancer (PC) is usually sensitive to docetaxel chemotherapy. Nevertheless, docetaxel resistance frequently appears after several cycles of treatment, raising the problem of salvage treatment for docetaxel-resistant PC patients. Although the combination of docetaxel and estramustine prolongs metastasis-free and overall survival of patients with androgen-independent PC, the use of this modality remains limited in elderly patients or patients with several comorbidities, especially vascular disease or gastrointestinal toxicity, because of unacceptable toxicity including venous thrombosis. The aims of this study were therefore (i) to evaluate the in-vivo efficacy of estramustine combined with docetaxel since initial tumor growth and following the appearance of docetaxel resistance in the androgen-dependent human PC xenograft PAC120, and (ii) to evaluate the efficacy of estramustine in six human androgen-independent PC models derived from PAC120. In docetaxel-resistant tumor-bearing mice, estramustine alone induced a TGD_2 of 18 days, whereas the combination of docetaxel and estramustine induced a TGD_2 of 50 days ($P < 0.05$) with no significantly different overall survival of mice treated by docetaxel and estramustine since day 1 or since the onset of resistance to docetaxel. Among the six human androgen-independent tumors treated with estramustine alone, two highly sensitive models, two intermediate responding tumors, and two resistant models were observed. Altogether, these results suggest that estramustine should be combined with docetaxel in PC patients, but the use of this treatment could be limited, particularly in elderly patients, to docetaxel-resistant cases. *Anti-Cancer Drugs* **21:927–931 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Anti-Cancer Drugs 2010, **21**:927–931

Keywords: docetaxel, estramustine, prostate cancer, resistance, xenografts

[a]Department of Translational Research, Laboratory of Preclinical Investigation, [b]Department of Medical Oncology, Institut Curie, [c]Department of Medical Oncology, Hôpital Européen Georges Pompidou, Paris, [d]National Veterinary School, Maisons-Alfort and [e]Keocyt, Malakoff, France

Correspondence to Dr Didier Decaudin, MD, PhD, Service d'Hématologie, Institut Curie, 26 rue d'Ulm, 75.248 Paris Cedex 05, France
Tel: +33 1 44 32 46 90; fax: +33 1 53 10 40 11;
e-mail: didier.decaudin@curie.net

Received 4 June 2010 **Revised form accepted** 30 June 2010

Introduction

Advanced prostate cancers (PC) are usually sensitive to docetaxel chemotherapy [1,2]. Moreover, docetaxel-based chemotherapeutic regimens, particularly the docetaxel and estramustine combination [3–7], have been shown to be more effective than docetaxel alone. Although the combination of estramustine and docetaxel prolongs metastasis-free and overall survival rates of patients with hormone-refractory PC, the use of this modality remains limited in elderly patients because of unacceptable toxicity including venous thrombosis [8]. This safety profile therefore suggests the need for delayed administration of estramustine, alone or combined with docetaxel, in docetaxel-resistant PC patients. Docetaxel reintroduction appears to be effective in patients with progressive metastatic castration-resistant PC, who initially responded to a first-line docetaxel-based regimen [9]. Re-treatment using docetaxel and estramustine is also a potentially effective option. Using a well-characterized human preclinical model of androgen-dependent PC and various androgen-independent variants [10,11], the aims of this study were to evaluate the *in vivo* efficacy of (i) estramustine combined with docetaxel since initial tumor growth and after the appearance of docetaxel resistance, and (ii) estramustine alone in various androgen-independent PC models.

Materials and methods

In-vivo models

The preclinical models used included the androgen-dependent PAC120 xenograft PC and six hormone-independent variants. PAC120 has a Gleason score 9 (5 + 4) and a nonmutated and functional androgen receptor [10]. Six hormone-independent xenografts were obtained from PAC120 after long-term androgen deprivation and were transplanted in the castrated male mice [11,12].

0959-4973 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e32833db887

In-vivo tumor growth and antitumor efficacy of estramustine ± docetaxel

Nude mice, 4–6 weeks old, bred at the Institut Curie, were used for in-vivo experiments. Fragments measuring 30–60 mm^3 were grafted subcutaneously into the interscapular fat pad. When tumors reached a size of 60–200 mm^3, the mice were randomly assigned to the control or treatment groups and treatment was started on day 1. Tumor growth was evaluated by measuring two perpendicular tumor diameters with a caliper, twice a week. Individual tumor volume, relative tumor volume (RTV), and tumor growth inhibition (TGI) were calculated according to standard methodology [13]. The statistical significance of TGI was calculated by the paired Student's *t*-test. Xenografted mice were sacrificed when their tumor reached a volume of 2500 mm^3.

Docetaxel was administered at a dosage of 20 mg/kg every 3 weeks by intraperitoneal injection for a maximum of six cycles, and estramustine was administered intraperitoneally at a dosage of 12 mg/kg on days 1–5 every 3 weeks until the mice were killed. In the PAC120 model, escape from docetaxel was defined at the beginning of each subsequent cycle $(n + 1)$ as an RTV_{n+1}/RTV_n ratio ≥ 2 (Fig. 1). Resistant mice were then randomized to two groups, one treated with estramustine alone, and the other treated with a combination of docetaxel and estramustine with a final evaluation of tumor growth delay for a twofold increase in tumor size (TGD_2).

Comparison of the survival of mice treated with docetaxel and estramustine since day 1 or since the onset of docetaxel resistance was measured at 4 months using a χ^2 test.

Immunohistochemistry

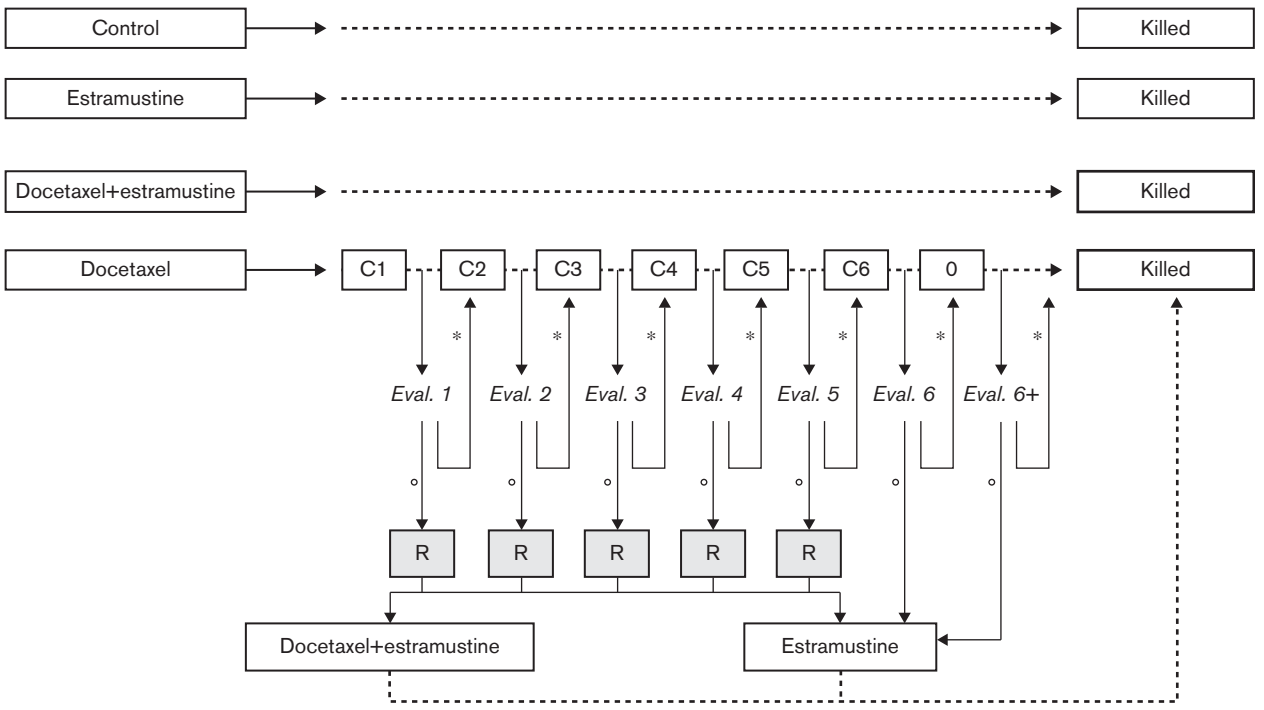
Lung metastases and vascular thromboses of mice bearing PAC120 tumors included in the various groups (controls and treatments) were evaluated on paraffin-embedded sections according to standard histological examination protocols.

Results

In-vivo efficacy of various therapeutic schedules combining docetaxel and estramustine in hormone-dependent PC PAC120 xenograft

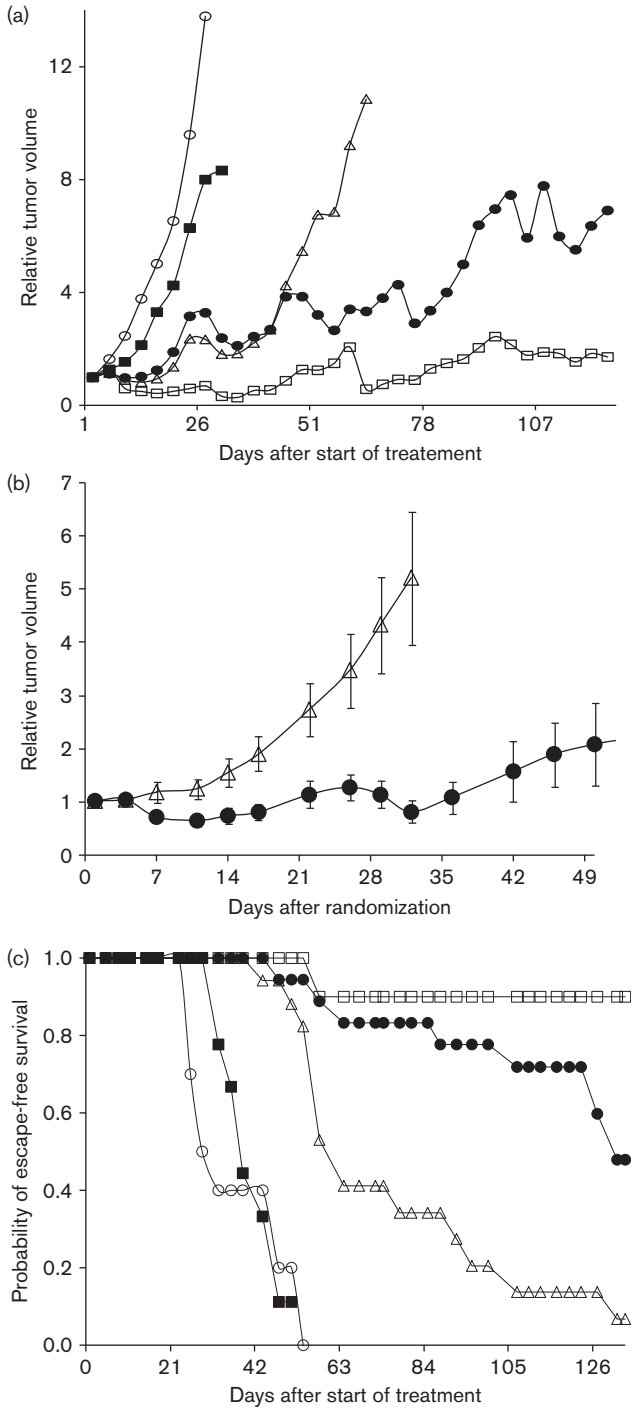
Estramustine alone had no effect on PAC120 tumor growth. In contrast, docetaxel alone or combined with estramustine induced a TGI of 81% and 95% on day 26, respectively ($P < 0.05$; Fig. 2a). In docetaxel-resistant tumor-bearing mice, estramustine alone induced a TGD_2 of 18 days, whereas the combination of docetaxel and estramustine induced a TGD_2 of 50 days ($P < 0.05$) (Fig. 2b). The 4-month survival of mice treated with

Fig. 1



Preclinical therapeutic schedule of docetaxel and estramustine administration in hormone-dependent PAC120 xenograft. R means randomization between docetaxel-responding tumors (*) ($RTV_{t+1}/RTV_t < 2$) and docetaxel-resistant tumors (°) ($RTV_{t+1}/RTV_t \geq 2$).

Fig. 2



In-vivo efficacy of various therapeutic schedules combining docetaxel and estramustine in hormone-dependent prostate cancer PAC120 xenograft. (a) Effect of docetaxel ± estramustine on PAC120 xenograft tumor growth. (b) Effect of estramustine alone or docetaxel plus estramustine in docetaxel-resistant tumors. (c) Overall survival of PAC120-bearing mice according to treatments. Xenografted mice were treated with estramustine alone (■), docetaxel plus estramustine since D1 (□), docetaxel then estramustine in docetaxel-resistant tumors (△), or docetaxel then docetaxel plus estramustine in docetaxel- resistant tumors (●). Mice in the control group (○) received 0.3 ml of the drug-formulating vehicle with the same schedule as the treated animals.

docetaxel and estramustine since day 1 or since onset of docetaxel resistance was 89 and 67%, respectively (P = not significant; Fig. 2c).

In-vivo efficacy of estramustine in hormone-independent PC xenografts

Six tumors were treated with estramustine alone. Various responses were observed: three intermediate sensitive models [human androgen-independent (HID)101, HID115, and HID119; TGI of 57, 60, and 48%, respectively], one moderately responding tumor (HID110, TGI of 27%), and two resistant models (HID28 and HID126, TGI of 0) (Fig. 3).

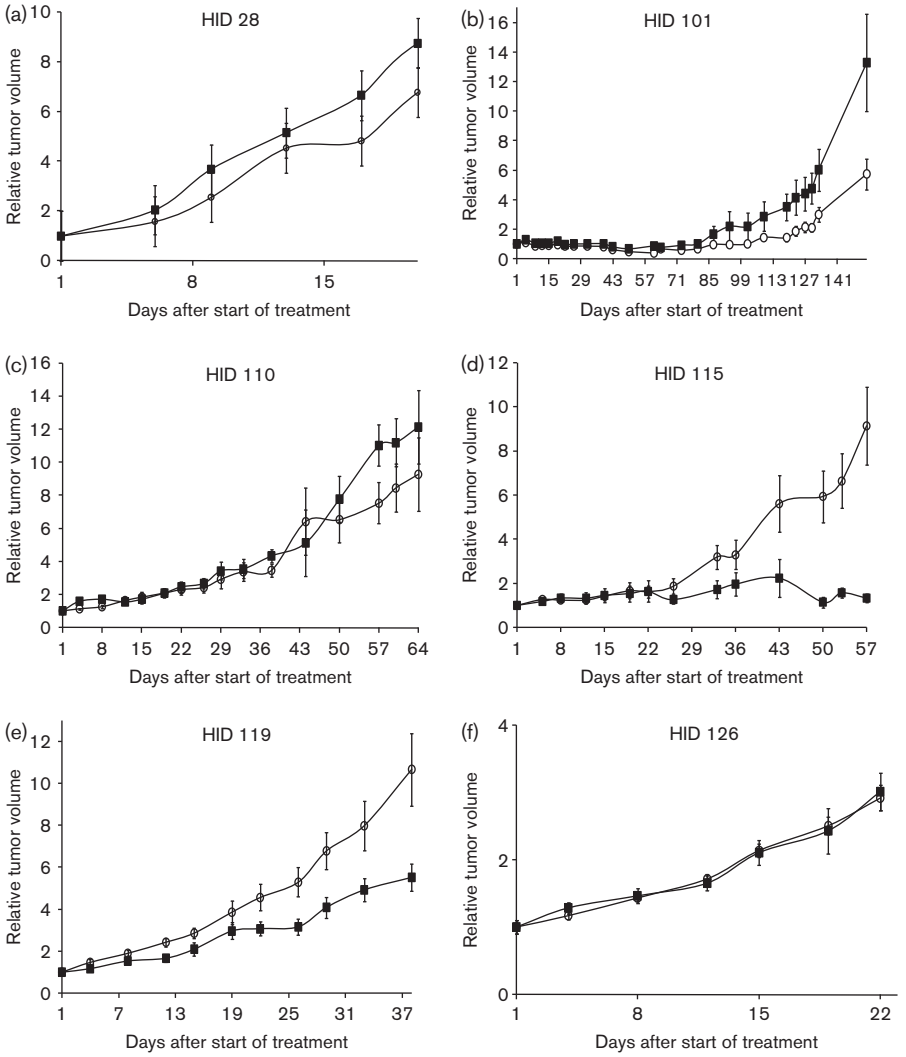
Evaluation of lung metastases and vascular thromboses in PAC120-bearing mice

One acute pulmonary artery thrombosis was observed in one estramustine-treated mouse (data not shown). Several acute and organized (older) thrombi were also detected in one mouse treated with estramustine and docetaxel (data not shown). Finally, the incidence of lung metastases in mice treated with docetaxel and estramustine since day 1 or since docetaxel resistance was 20% and 12%, respectively (P = not significant).

Discussion

Estramustine-induced response rates range between 5 and 48% depending on the criteria used to define therapeutic response; that is, decrease of serum PSA levels or bone pain, or improvement of the patient's performance status [14–16]. In contrast, estramustine combined with docetaxel induced $\geq$ 50% PSA responses, and prolonged median progression-free and/or overall survival of castration-resistant PC patients [2–4,6,7]. However, the combination of these two chemotherapeutic agents is associated with gastrointestinal toxicity and venous and/or arterial thromboses with a risk ratio of about 5% [5]. One alternative to optimizing the benefit/risk ratio of this combination would be delayed adjunction of estramustine to docetaxel in the case of docetaxel-resistant or slowly responding disease. Using a well-characterized human PC xenograft, estramustine alone was shown to have a slight and brief efficacy in docetaxel-resistant tumor-bearing mice, whereas estramustine combined with docetaxel induced strong and prolonged TGI. Moreover, the overall survival of mice treated with docetaxel and estramustine since day 1 or since the onset of docetaxel resistance was not significantly different. These results therefore suggest that the administration of estramustine can be delayed and can be combined with docetaxel in docetaxel-resistant PCs with no impact on outcome. These data also show that estramustine phosphate possesses a therapeutic potential in PC patients and that the optimal modalities of administration have yet to be defined; that is, combined treatment in docetaxel-resistant tumors, as in our study, or discontinuous induction treatments, or maintenance therapy after

Fig. 3



In-vivo efficacy of estramustine in hormone-independent prostate cancer xenografts. Six models, that is, human androgen-independent (HID) 28 (a), HID 101 (b), HID 110 (c), HID 115 (d), HID 119 (e), HID 126 (f) were treated with estramustine alone (■). Mice in the control group (○) received 0.3 ml EPPI with the same schedule as the treated animals.

the first chemotherapy-induced remission. Moreover, new innovatively targeted treatments, and new treatment combinations, particularly estramustine, could also be used to improve response rates and overall survival of PC patients. New drugs including antiandrogens (Abiraterone, MDV3100) [17], immunotherapy (ipilimumab) [18], antiangiogenic therapy (sunitinib, sorafenib, thalidomide) [19], or new chemotherapeutic agents could be used before the administration of docetaxel, in combination with or after the failure of docetaxel. The appropriate timing of the administration of these drugs could be determined by evaluating these therapies in preclinical models. Finally, further studies are required to improve our knowledge of taxane (docetaxel and cabazitaxel) resistance (MDR1, tubulin mutation) and to more clearly define the place of estramustine. The availability of well-characterized and relevant preclinical models would be a useful tool to define optimal schedule(s) of administration, to identify predictive markers for response and/or resistance, and to avoid unexpected and unacceptable toxicities in cancer patients [20].

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