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# Etoposide in prostatic cancer: experimental studies and phase II trial in patients with bidimensionally measurable disease*

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**Summary.** Etoposide, a semisynthetic derivative of podophyllotoxin, was evaluated concurrently in vitro against a human derived hormone-resistant cell line, PC-3, and in vivo in bidimensionally measurable hormone-resistant human prostatic cancer. In vitro, a dose-response relationship was observed, with 74% inhibition at 10 μg/ml (1 h incubation) and >99% inhibition at 90 μg/ml, both in the range of clinically achievable concentrations. In vivo, 1 PR (5%, 95% confidence limits 0–12%) of 18+ months was observed in 20 adequately treated patients. The results confirm the limited role of etoposide in hormone-refractory disease and the need for new model systems for evaluation of potential chemotherapeutic compounds in this disease.

## Introduction

Despite extensive evaluation, few agents have shown significant antitumor activity in patients with hormone-resistant prostatic cancer [2, 11, 14]. In vitro models have been sought that might allow more specific selection of agents for evaluation in clinical trial [1, 7, 10]. Metcalf et al. evaluated eight chemotherapeutic agents against four prostatic cancer cell lines, and reported modest correlation between in vitro and in vivo response [8]. PC-3 is a human bone marrow-derived hormone-resistant cell line of prostatic cancer, originally derived in 1979. In our laboratory, adriamycin and cisplatin showed minimal activity (WDW Heston, personal communication, 1985), which correlated with a clinical evaluation in our Center [12, 17]. Etoposide is a seminsynthetic derivative of podophyllotoxin that has activity against a number of tumor types [4, 9]. Following the demonstration by Mador et al. of in vitro activity against the Dunning R3327H prostatic adenocarcinoma [6], a concurrent clinical and laboratory evaluation of this agent was initiated in patients with prostatic cancer. As with our previous studies, entry was restricted to patients with bidimensionally measurable disease in whom a clear end-point of response could be elicited [12, 16, 17].

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## Materials and methods

### *Laboratory investigations*

*Cell lines.* The PC-3 cell line was derived from a bony deposit of a metastatic prostatic carcinoma and obtained from the American Type Culture Collections, Rockville, Md. [5].

*Clonogenic assay.* Liquid clonogenic assays were performed as previously described [3]. At 48 h after plating of $10^3$ PC-3 cells per 60 mm petri dish, the medium (RPMI 1640, 10% FCS) was aspirated and replaced with either medium alone or medium containing etoposide at 90 μg/ml, 30 μg/ml, or 10 μg/ml. Following a 1-h incubation the medium was aspirated and replaced with fresh medium and the incubation continued for 5 more days. The medium was then aspirated and rinsed with HBSS, and the cells fixed with methanol, stained with Harris's hematoxylin, and rinsed in cold tapwater. The number of colonies was enumerated by counting at 12× magnification those areas with greater than 50 cells per colony with an American Optical Stereo Star Zoom microscope. All experiments were performed in triplicate.

*Clinical trial.* Patients with hormone-resistant metastatic prostatic adenocarcinoma histologically confirmed by the Department of Pathology were considered. Entry requirements included a Karnofsky performance status (KPS) ≥50, adequate hematologic status (WBC ≥3000 cells/μl, platelets ≥150000 cells/μl), serum bilirubin ≤1.5 mg%, creatinine ≤1.5 mg%, BUN≤30 mg%, and bidimensionally measurable disease. Measurable disease included lymph nodes palpable by physical examination, pulmonary nodules, and retroperitoneal and pelvic masses on CT scan or ultrasound of the liver. Palpable tumor masses were measured in the largest perpendicular diameter by two independent observers, and all roentgenographic material was evaluated independently (RCW). When final review did not confirm bidimensionally measurable disease, patients were reclassified as evaluable (osseous metastases, unidimensional pleural based masses, or prostatic enlargement) and considered separately.

Patients were required to have recovered from the myelosuppressive effects of prior chemotherapy and radia-

tion therapy (3–6 weeks). Laboratory evaluation included an automated blood and platelet count, 12-channel screening profile, serum acid phosphatase (SAP), 5'nucleotidase, and serum creatinine determinations. A chest roentgenogram and, when clinically indicated, computed tomography (CT) scan of the abdomen and pelvis were performed. Roentgenograms and scans were repeated at 4- to 6-week intervals when used to evaluate measurable disease. Radionuclide bone scans with roentgenographic evaluation of abnormal areas was also obtained, and repeated at 3-month intervals if clinically indicated. Informed consent was obtained prior to initiation of therapy.

The starting dose of etoposide was 100 mg/m$^2$ i. v., on days 1, 2, and 3 given i. v. over 1 h for patients with a history of no (7 cases) or minimal (7 cases) radiation therapy (prostate, pelvis or ribs) and 80 mg/m$^2$ for patients (6 cases) with moderate (pelvis and axial skeleton) radiation therapy. An additional four patients with evaluable disease were treated at 80 (2 cases) and 100 (2 cases) mg/m$^2$. Cycles were repeated every 21–28 days, depending on count recovery. Automated blood and platelet counts were obtained 10–14 days after treatment. When no myelosuppression (WBC $<3000$ cells/mm$^3$, platelets $<125000$ cells/mm$^3$) was observed, subsequent doses were increased by 20 mg/m$^2$ (total 60 mg/m$^2$). If an interim WBC $<2500$ cells/mm$^3$ or platelet count $<75000$ cells/mm$^3$ was observed, doses were decreased by 20 mg/m$^2$ (total 60 mg/m$^2$). An adequate trial was defined as one cycle (3 doses), with evidence of myelosuppression and/or disease progression.

Standard phase II response criteria were used. Complete remission (CR) denoted complete disappearance of all measurable, radiological, and biochemical abnormalities. Partial remission (PR) was defined as a greater than 50% decrease in the summed products of two or more diameters of all measurable soft tissue lesions revealed by physical examination and by CT scan for a minimum of 1 month, a $\geq 50\%$ decrease in malignant hepatomegaly revealed by physical examination and in all hepatic-associated biochemical abnormalities and filling defects on CT scan or hepatic ultrasound for more than 1 month, and a $\geq 50\%$ decrease of abnormaly elevated SAP and CEA levels. Minor response (MR) was a 25%–49% decrease in tumor size or biochemical abnormalities for more than 1 month or a $\geq 50\%$ decrease for less than 1 month. Stabilization of disease (STAB) was a $<25\%$ decrease or increase in tumor size and/or biochemical abnormalities for a minimum of 3 months. Progression (PROG) was a $<25\%$ increase in tumor size or biochemical abnormalities. Mixed responses, such as a decrease in the size of lymph nodes with increasing osteolytic lesions on bone roentgenograms or in biochemical abnormalities (SAP) were classified as PROG. Patients who required radiation therapy for symptomatic bony metastases were considered to have progressed at the start of radiation therapy. Duration of response was measured from the initiation of therapy until the documentation of progression.

**Table 1.** Clonogenic survival after one hour incubation with etoposide at different concentrations

| | Clonogenic survivors | % of control |
|---|---|---|
| Control | 589 ± 94 | 100% |
| Etoposide 10 μg/ml | 152 ± 32 | 26% |
| Etoposide 30 μg/ml | 25 ± 10 | 4% |
| Etoposide 90 μg/ml | <1 | <0.2% |

All samples were evaluated in triplicate and are expressed as the mean ± SD

## Results

### *In vitro trial*

The results of the in vitro evaluation for a 1-h incubation are listed in Table 1. A dose-response relationship was noted. At 10 μg/ml a 74% inhibition and at 90 μg/ml >99% inhibition of clonogenic survival relative to nontreated controls was observed.

### *Clinical trial*

After review of all available data, 4/24 patients were reclassified as evaluable. The clinical characteristics of patients with bidimensionally measurable disease are listed in Table 2. The median age was 66 years and median PS (Karnofsky) 80%. Four patients had received prior chemotherapy (three with 1 agent, one with 3 agents), and all had progressed during hormonal therapy. Thirteen patients had prior radiation therapy including $^{125}$I implantation in two, 1+ sites (pelvis, prostate or ribs) in five and 2+ (pelvis plus other sites) in six. The primary measurable indicator lesion included lymph nodes in ten patients (7 measured by CT scan); hepatomegaly in six; prostatic masses detected by CT scan in three; and subcutaneous masses in one patient. Elevated acid phosphatase was noted in 60% (12/20) and increased alkaline phosphatase in 75% (15/20). Of four patients with evaluable disease, two had had prior chemotherapy, two had elevated alkaline phosphatase, and two, elevated acid phosphatase.

Of 20 patients with bidimensionally measurable disease, 1 (5%, 95% confidence limits 0–12%) achieved a PR in hepatic metastases of 18+ months' duration. A second

**Table 2.** Clinical characteristics of 20 evaluable patients with measurable disease

| | | |
|---|---|---|
| Age | 66 (57–85) | |
| KPS | 80 (60–90) | |
| Prior chemotherapy | 4 (3, 1 agent, 13 or more) | |
| Prior radiation | None | 7 |
| | $^{125}$I | 2 |
| | 1+ | 5 |
| | 2+ | 6 |
| Prior hormonal therapy | Orch | 6 + DES 4 |
| | DES | 11 |
| | Lupron | 1 |
| | Flut/DES | 2 |
| Primary indicator lesions | Nodes | 10 |
| | Liver | 6 |
| | Prostate | 3 |
| | SUB Q | 1 |
| Alkaline phosphatase (increased) | | 15/20 |
| Acid phosphatase (increased) | | 11/19 |

achieved an MR in hepatic disease on physical examination, with a decrease in acid phosphatase from 14 to 4.9 units for 3 months. One additional patient had a decrease in acid phosphatase from 10 units to 1 unit and a decrease in alkaline phosphatase from 1640 to 730 units, but died of intestinal obstruction 30 days after the initiation of therapy. No change in measurable disease was noted, and the patient was not listed in a response category. Of the evaluable cases, 2 were inevaluable and 2 showed progression of disease. Of the inevaluable cases, 1 developed an allergic reaction with a flare in bone pain after one dose of treatment and received no further therapy. The second received an inadequate dose (due to myelosuppression) and did not respond.

The median number of cycles was three (range 1–14). Six dose escalations and two, dose reductions were required for myelosuppression. Toxicity included granulocytopenia ($<2500$ cells/mm$^3$) in five cases (24%), thrombocytopenia (125 000 cells/mm$^3$) in four (20%), and an allergic reaction with a flare in bone pain in 1 patient. In general, the drug was well tolerated and administered safely on an outpatient basis.

## Discussion

A 1-h serum concentration ($c \times t$) for etoposide would be about 100 μg/ml (concentration). Agents tested at 1/10 their $c \times t$ level and which produce a greater than 70% decrease in clonogenic survival would be predicted to yield a clinical response [1, 10] For etoposide, 1/10 the $c \times t$ (10 μg/ml) produced 74% inhibition of clonogenic survival. This falls in the realm of clinically achievable concentrations in vivo [13]. Thus it would be anticipated from this prostate-derived cell line, that human prostatic cancer would be sensitive to etoposide. However, unlike primary prostatic cancer, which has been difficult to grow in vitro, the PC-3 line has a rapid growth rate and high cloning efficiency. Thus, PC-3 may not be truly representative of the majority of clinical prostatic cancers.

The response rate of 5% was disappointing and emphasizes the need for appropriate model systems and new classes of agents. Prostatic cancer continues to be a disease refractory to therapy. The findings are consistent with those of Trump et al. [15], who noted no responses in 19 adequately treated patients using a continuous infusion schedule (50 mg/m$^2$ over 24 h for 5 days). The combined response rate of 1/39 (95% confidence limits 0–6%) confirms the limited role of etoposide in the treatment of prostatic cancer.

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