Cancer Chemother Pharmacol (2003) 52: 119–124
DOI 10.1007/s00280-003-0644-6

ORIGINAL ARTICLE

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A phase I trial of weekly paclitaxel, 13-*cis*-retinoic acid, and interferon alpha in patients with prostate cancer and other advanced malignancies

Received: 13 January 2003 / Accepted: 9 April 2003 / Published online: 24 May 2003

Abstract *Purpose*: Based on prior studies demonstrating the effect of 13-*cis*-retinoic acid and interferon alpha (CRA/IFN) in decreasing the expression of the antiapoptotic protein bcl-2, our prior clinical study of CRA/IFN with paclitaxel (TAX) administered every 3 weeks, and data demonstrating increased activity of weekly TAX against prostate cancer, we designed a phase I study of weekly TAX in combination with CRA/IFN in patients with prostate cancer and other advanced malignancies. To develop a marker of drug effect, we assessed bcl-2 downregulation in patient peripheral blood mononuclear cells (PBMC). *Methods*: Enrolled in the study were 14 patients with prostate cancer or other advanced malignancies, and 13 were treated with 1 mg/kg CRA on days 1 and 2, 6 MU/m^2 IFN subcutaneously on days 1 and 2, and TAX at increasing doses on day 2 each week for 6 weeks out of an 8-week cycle. The effect of CRA/IFN on bcl-2 expression was assessed in PBMCs by immunoblotting. *Results*: The combination of CRA/IFN and TAX was well tolerated. Dose-limiting toxicities (DLT) in the first cycle of therapy included one patient with fever and neutropenia, and one patient with grade 4 hypertriglyceridemia. The recommended phase II dose of TAX in this combination was 80 mg/m^2. Of 13 patients assessable by tumor markers or scans, 5 had stable disease and 2 had a biochemical partial response including a patient with a decrease in PSA of $>50\%$ while on study. The assessment of patient PBMC bcl-2 was feasible in ten patients. *Conclusions*: This is the first study in which the safety and clinical activity of weekly TAX combined with CRA/IFN has been demonstrated. The assessment of PBMC bcl-2 is feasible in this weekly chemotherapy schedule.

Keywords Prostate cancer · Bcl-2 · Paclitaxel · 13-*Cis*-retinoic acid · Interferon alpha

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Introduction

Current chemotherapy regimens used for prostate cancer and other advanced malignancies are only temporarily effective because of the development of molecular mechanisms of resistance [17]. Novel approaches to overcome specific mechanisms of drug resistance are clearly needed. The overexpression of the antiapoptotic protein bcl-2 is one such important mechanism of chemotherapy resistance [2, 10, 17]. Our group and others have studied agents that abrogate bcl-2-mediated resistance [2, 3, 4, 11, 12, 13]. Multiple laboratory studies have demonstrated that 13-*cis*-retinoic acid (CRA) can reduce the expression of bcl-2, and cytotoxicity is enhanced in combination with interferon alpha (IFN) [1, 4, 5, 7, 8, 9]. Our group has completed a phase I trial with CRA/IFN and paclitaxel (TAX) administered every 3 weeks and have found that the regimen is safe and active in patients with advanced malignancies [4]. More recently, clinical trials of TAX in patients with hormone-refractory prostate cancer (HRPC) have demonstrated greater activity of a weekly schedule of TAX, in contrast to a schedule of TAX administered every 3 weeks [15, 18]. Given our initial experience with CRA/IFN, and the more recent clinical data on the weekly schedule of TAX, we conducted a phase I study of CRA/IFN in combination with a weekly schedule of TAX. Additionally, we assessed peripheral blood mononuclear cells (PBMC) for bcl-2 expression for use in further studies that could validate this as a marker of bcl-2 modulation in the clinic.

Patients and methods

Patients

Enrolled in this trial were 14 patients with prostate cancer or refractory metastatic malignancy. One patient decided not to start therapy after registration. Eligibility criteria included histologically confirmed metastatic solid tumors that had failed to respond to standard treatment, Eastern Cooperative Oncology Group (ECOG) performance status 0–2, and a life expectancy of at least 8 weeks. Requirements also included age greater than 18 years, adequate bone marrow function, granulocytes greater than 2000/mm^3, platelets greater than or equal to 100,000/mm^3, hemoglobin greater than or equal to 9 g/dl, adequate renal function, serum creatinine level less than or equal to 1.5 mg/dl, adequate hepatic function, bilirubin level less than or equal to 1.5 mg/dl, and serum glutamic oxaloacetic transferase (SGOT) less than or equal to twice the upper limit of normal. All patients were required to give written informed consent, which was approved by the Institutional Review Board of Robert Wood Johnson University Hospital.

Treatment plan

Patients were treated with all three drugs delivered weekly for 6 weeks in an 8-week schedule. All patients received CRA 1 mg/kg per day orally on days 1 and 2, IFN 6 MU/m^2 on days 1 and 2, and TAX on day 2 of each week at escalating doses. The initial dose of TAX chosen was 70 mg/m^2 based on prior studies of weekly TAX and our prior data demonstrating that CRA increases the area under the curve (AUC) of TAX [4]. Response was followed by assessment of measurable lesions if applicable or by tumor markers (PSA and CA-125). Complete response was defined as the complete disappearance of all clinical signs of active disease. Partial response was defined as at least 50% decrease in the sum of products of the longest perpendicular diameters of all measured lesions. Stable disease was defined as less than 25% increase in tumor size or less than 50% decrease in tumor size with no new lesions. Biochemical partial response in patients with prostate cancer or ovarian cancer was defined as a decrease in tumor marker of more than 50% maintained for at least 1 month.

Dose-limiting toxicity

Dose-limiting toxicity (DLT) was defined only during the first cycle of therapy. The following was considered DLT: grade 3/4 non-hematologic toxicities (excluding nausea/vomiting); grade 4 hematologic toxicity occurring during treatment, or within 1 week of treatment completion and lasting >7 days; or omission or delay for toxicity of two or more doses in a cycle, or delay in a cycle's initiation beyond the mandated 2 weeks because of toxicity. If none of the first three patients in a cohort experienced DLT, the next three patients were planned to be started at the next higher level. If one of the first three patients at a cohort level experienced DLT, then two additional patients were planned to be added to that dose level. If more than two of the first five patients experience DLT, then the previous cohort was the maximum tolerated dose (MTD). If only one of five patients experience DLT, then the next patient was to be started at the next higher dose level. Once the MTD was identified, additional patients were added at the MTD, so that a total of at least eight were entered to more precisely define the risk of toxicity of this regimen.

Immunoblot analysis of patient mononuclear cells

The assessment of bcl-2 was made at baseline, and on day 2 and day 4, based on our prior study showing reduction in PBMC bcl-2 over the first 4 days of therapy [4]. Patient blood was centrifuged at 1500 *g* for 30 min in CPT mononuclear cell isolation tubes (Becton Dickinson, Mountain View, Calif.). The mononuclear cell layer was removed, washed, and lysed in ice-cold RIPA buffer. Equivalent amounts of protein from each sample were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (Amersham Life Science, Little Chalfont, UK), as previously described [4]. Bcl-2 protein was detected with a monoclonal bcl-2 primary antibody and secondary horseradish peroxide-conjugated antibody (Dako, Copenhagen, Denmark). Actin was used as a control for total protein loading.

Results

Patient demographics

A total of 14 patients were entered into the protocol. The median age of the patients in the group was 66 years (Table 1). The total number of cycles received by all the patients was 23. Prior to enrollment in the study, all patients had demonstrated disease progression despite at least one prior chemotherapy regimen (median of two), and had an ECOG performance status ≤ 1. Patients had multiple primary tumor types (five with prostate cancer, one with ovarian cancer, one with gastric cancer, one with breast cancer, three with cervical cancer, one with small-cell lung cancer, and two with bladder cancer).

Hematologic toxicity

Hematologic toxicity grades 3 and 4 for the first cycle and all courses are shown in Tables 2 and 3. At a TAX dose of 80 mg/m^2, one patient experienced grade 3 neutropenia with fever requiring treatments to be held during the first cycle that was considered a DLT. All other episodes of hematologic toxicity were not dose-limiting, as defined in the Methods section, although one

Table 1 Patient demographics (values are number of patients, except age in years)

Entered study	14
Age (years)	
Median	66
Range	51–80
Sex	
Female	5
Male	9
Performance status	
0–1	14
2	0
Tumor origin	
Breast	1
Prostate	5
Lung	1
Gastric	1
Cervical	3
Ovary	1
Bladder	2
Prior chemotherapy	
Yes	14
No	0

Table 2 Hematologic toxicities by paclitaxel dose level (first cycle)

Dose level (mg/m^2)	Anemia		Neutropenia		Thrombocytopenia	
	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
70 ($n=3$)	0	0	1	0	0	0
80 ($n=11$)	0	0	4	1	0	0

Table 3 Hematologic toxicities by paclitaxel dose level (all courses)

Dose level (mg/m^2)	Anemia		Neutropenia		Thrombocytopenia	
	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
70 ($n=3$)	0	0	1	0	0	0
80 ($n=11$)	1	0	5	2	0	0

patient at the 80-mg/m^2 dose level had grade 4 neutropenia without fever that required a dose to be held during the first cycle. This patient had received two prior chemotherapy regimens before this study and recovered after 1 week. Another patient experienced grade 4 neutropenia without fever during the second cycle that lasted more than 2 weeks. One patient also had grade 3 anemia requiring blood transfusion. Thrombocytopenia was not observed in any patient during this study.

Non-hematologic toxicity

Non-hematologic toxicity grades 3 and 4 for the first cycle and all courses are shown in Tables 4 and 5. One patient had grade 4 hypertriglyceridemia in the first cycle at a TAX dose of 80 mg/m^2 that was defined as a DLT. All other non-hematologic toxicities were not dose limiting. One patient at a TAX dose of 70 mg/m^2 experienced grade 3 muscle weakness and fatigue during the second cycle, which required removal of the patient from the study. Three additional patients experienced grade 3 fatigue and myalgias at a dose of 80 mg/m^2 during the second cycle. A patient experienced grade 3 depression during the second cycle, and another patient had grade 3 hypertriglyceridemia during the second cycle.

Recommended phase II dose

The recommended phase II dose (RPTD) was 80 mg/m^2. No patient at the 70-mg/m^2 dose level had a DLT in the first cycle and the regimen was escalated to 80 mg/m^2. Although none of the first three patients enrolled at 80 mg/m^2 had DLT in the first cycle of therapy, we did not dose-escalate further because of multiple non-hematologic toxicities noted in the second cycle of therapy. Instead, we extended the second cohort to 11 patients. Out of the 11 patients at 80 mg/m^2 TAX, two had DLT in the first cycle (fever/neutropenia and hypertriglyceridemia), and five had grade 3 or 4 non-hematologic toxicity in the second cycle of therapy (three with fatigue and myalgia, one with depression, and one with hypertriglyceridemia).

Clinical responses and effect on PBMC bcl-2

One patient chose not to start treatment after registration leaving 13 patients evaluable for response. Two of these 13 patients only completed 4–5 weeks of a first cycle due to toxicity. During follow-up evaluation at 8 weeks, two patients had a biochemical partial response, five patients had stable disease, and six patients

Table 4 Non-hematologic toxicities by paclitaxel dose level (first cycle)

Dose level (mg/m^2)	Depression		Hypertriglyceridemia		Muscle weakness		Fatigue		Myalgia	
	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
70 ($n=3$)	0	0	0	0	0	0	0	0	0	0
80 ($n=11$)	0	0	0	1	0	0	0	0	0	0

Table 5 Non-hematologic toxicities by paclitaxel dose level (all courses)

Dose level (mg/m^2)	Depression		Hypertriglyceridemia		Muscle weakness		Fatigue		Myalgia	
	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
70 ($n=3$)	0	0	0	0	1	0	1	0	1	0
80 ($n=11$)	1	0	1	1	0	0	3	0	3	0

Table 6 Tumor responses

Response	Week 8	Week 16
Progressive disease	6	1
Stable disease	5	2
Partial response	2	1

had progressive disease. Of seven patients who had measurable disease, three had stable disease and four progressed. One of the patients with a biochemical partial response had advanced prostate cancer and an 82% decrease in PSA at 8 weeks, and an 87% decrease at 16 weeks. The other patient had advanced ovarian cancer and a 55% decrease of CA-125 at 4 weeks and 99% decrease at 8 weeks. Of the seven remaining patients with stable disease or a partial response at 8 weeks, three were removed from study due to toxicity prior to 16 weeks. Of the four patients assessed for response at 16 weeks, one continued to have a biochemical PR, one progressed, and two had stable disease. These results are summarized in Table 6.

To determine the feasibility of measurement of bcl-2 in PBMCs after therapy with this regimen for the future development of a pharmacodynamic marker of bcl-2 modulation, we determined the expression of bcl-2 in PBMCs before and during treatment with CRA/IFN/TAX. Bcl-2 protein was adequately assessed at baseline and following therapy in only 10 of 13 patients because of inadequate samples or processing in some patients. As shown in Fig. 1, bands representing bcl-2 protein expression are shown for baseline, day 2 and/or day 4 of the first cycle of therapy. Actin is shown to control for the amount of total protein loaded. Although the sample size was too small to draw conclusions, bcl-2 expression appeared to decrease on either day 2 or day 4 relative to actin in patients 4, 6, and 10. Patient 4 had progression of transitional cell cancer. Patient 6 had transitional cell cancer with stable disease, but came off of study because of toxicity. Patient 10 had prostate cancer with disease progression.

Discussion

This phase I study was conducted to determine the dosing, safety, and feasibility of determining PBMC bcl-2 in the regimen of weekly CRA/IFN/TAX for use in phase II trials in patients with prostate cancer or other advanced malignancies. This phase I study demonstrated the safety and clinical activity of the combination of weekly TAX combined with CRA/IFN, which builds on our prior study with CRA/IFN and TAX administered every 3 weeks [4]. Additionally, this study demonstrated the feasibility of determination of PBMC bcl-2 levels for future validation as a marker of tumor response.

CRA/IFN and weekly TAX was well tolerated, but induced grade 3 fatigue by the second cycle of therapy in 3 of 11 patients at the recommended phase II dose. In our prior study with an every-3-week schedule, grade 3 fatigue occurred in only 1 of 16 patients at the recommended phase II dose level [4]. Although safe at this dose, long-term therapy with IFN has been known to induce fatigue in a significant number of patients treated with multiple malignancies, and this effect is dose dependent [4, 6, 16]. In our prior studies, patients who experienced fatigue after treatment with CRA/IFN, were able to continue with a decreased dose of IFN per protocol [4]. In fact, a current trial in the Eastern Cooperative Oncology Group using CRA/IFN and TAX in patients with HRPC allows for a 50% dose reduction in IFN for grade 3 or 4 fatigue (Eastern Cooperative Oncology Group trial 3899). Taken together, the current study, prior studies, and ongoing studies support the recommended phase II dosing in this

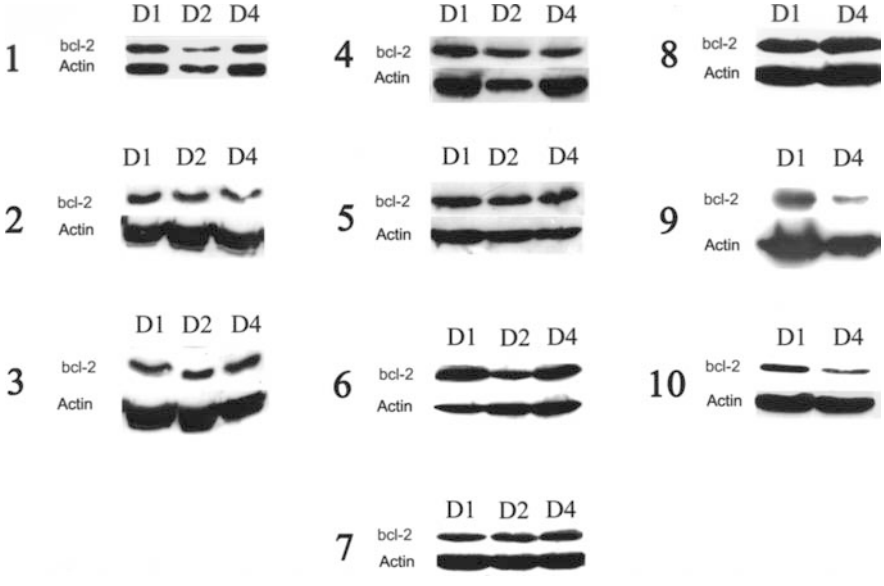

Fig. 1 Effect of CRA/IFN/TAX on patient PBMCs. Results from immunoblots are shown for ten patients. Protein was isolated from PBMCs prior to therapy and on day 2 and/or day 4 of the first cycle of therapy. Bcl-2 protein was detected with a monoclonal bcl-2 primary antibody and secondary horseradish peroxide-conjugated antibody (Dako, Copenhagen, Denmark). Actin is shown as a control for total protein loading

trial with a dose reduction of IFN in patients with grade 3/4 fatigue.

The regimen of CRA/IFN and weekly TAX is active in patients with multiple prior therapies. The rationale for conducting a study with CRA/IFN and weekly TAX after our prior study with TAX every 3 weeks was based on data suggesting the schedule-dependence of TAX in HRPC [15, 18]. Roth et al. studied TAX administered every 3 weeks in patients with HRPC and found a response in only one patient [15]. In contrast, Trevadi et al. studied the regimen of weekly TAX in patients with HRPC and observed a biochemical response rate of 40% [18]. Although only a phase I study, we did see evidence of antitumor activity indicating that further study is warranted to determine the response rate of the regimen, and if the combination has greater activity than monotherapy with TAX in the salvage setting.

The validation of easily obtainable clinical markers of relevant mechanisms of drug resistance is important to enhance drug development. Bcl-2 gene expression is common in prostate cancer and can increase cell viability and drug resistance through the abrogation of normal mechanisms of apoptosis [14]. Furthermore, an association has been demonstrated between resistance to androgen ablation and bcl-2 expression in prostate cancer [10]. Bcl-2 protein may also cause chemotherapy resistance in addition to resistance to androgen ablation. Tu et al. transfected Dunning-G rat prostate cancer cells with a bcl-2 expression vector and found that the transfectants were more resistant to cytotoxic effects compared to cells transfected with the empty vector [19]. We assessed PBMC bcl-2 in this study with the weekly regimen of CRA/IFN and TAX. Given the small sample size, and phase I nature of this study, it is premature to draw conclusions about the effect of bcl-2 and clinical benefit. Our prior efforts have demonstrated the feasibility of measuring bcl-2 in PBMCs both in a CRA/IFN/TAX regimen administered every 3 weeks, and with the use of bcl-2 antisense in patients with prostate cancer [4]. The effect on bcl-2 in the current study was minimal in three of the ten patients assessed only on days 1, 2, and/or 4. Future studies would be needed to determine if the assessment of PBMC bcl-2 at these time-points predicts clinical response. Studies of PBMC bcl-2 at different time-points, and as a ratio to proapoptotic proteins such as bax, may also be important.

In summary, this is the first study in which the safety and clinical activity of the combination of weekly TAX combined with CRA/IFN has been demonstrated in prostate cancer and patients with other advanced cancer. This study was planned as an effort to develop this regimen in phase II studies for prostate cancer, given the schedule-dependent activity of TAX in this disease, but further study would be reasonable in multiple advanced malignancies. Additionally, the assessment of PBMC bcl-2 is feasible in this weekly chemotherapy schedule, supporting further study.

References

1. Dahiya R, Park HD, Cusick J, Vessella RL, Fournier G, Narayan P (1994) Inhibition of tumorigenic potential and prostate-specific antigen expression in LNCaP human prostate cancer cell line by 13-cis-retinoic acid. Int J Cancer 59:126
2. DiPaola RS, Aisner J (1999) Overcoming bcl-2 and p53 mediated resistance in prostate cancer. Semin Oncol 26:112
3. DiPaola RS, Weiss RE, Cummings KB, Kong FM, Jirtle R, Anscher M, Gallo J, Goodin S, Thompson S, Rasheed Z, Aisner J, Todd M (1997) Effect of 13-cis retinoic acid and alpha interferon on transforming growth factor beta1 in patients with rising prostate specific antigen. Clin Cancer Res 3:1999
4. DiPaola RS, Rafi M Vyas V, Gupta E, Toppmeyer D, Rubin E, Patel G, Goodin S, Medina P, Zamek R, Zhang C, White E, Hait WN (1999) Phase I clinical and pharmacologic study of 13-cis retinoic acid, alpha interferon and paclitaxel in patients with prostate cancer and other advanced malignancies. J Clin Oncol 17:2213
5. Hu ZB, Minden MD, McCulloch EA (1996) Regulation of the synthesis of bcl-2 protein by growth factors. Leukemia 10:1925
6. Kirkwood JM, Bender C, Agarwala S, Tarhini A, Shipe-Spotloe J, Smelko B, Donnely S, Stover L (2002) Mechanisms and management of toxicities associated with high-dose interferon alfa-2b therapy. J Clin Oncol Sep 1; 3703 20(17):3703–18
7. Lippman SM, Kavanagh JJ, Paredes-Espinoza M, Delgadillo-Madrueno F, Paredes-Casillas P, Hong WK, Holdener E, Krakoff IH (1992) 13-cis-Retinoic acid plus interferon-2alpha: highly active systemic therapy for squamous cell carcinoma of the cervix. J Natl Cancer Inst 84:4
8. Lippman SM, Parkinson DR, Itri LM, Weber RS, Schantz SP, Ota DM, Schuterman MA, Krakoff IH, Gutterman JU, Hong WK (1992) 13-cis-Retinoic acid and interferon-2a: effective combination therapy for advanced squamous cell carcinoma of the skin. J Natl Cancer Inst 84:235
9. Majewski S, Szmurlo A, Marczak M, Jablonska S, Bollag W (1994) Synergistic effect of retinoids and interferon alpha on tumor-induced angiogenesis: anti-angiogenic effect on HPV-harboring tumor cell lines. Int J Cancer 57:81
10. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LW, Hsieh JT, Campbell ML (1992) Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. Cancer Res 52:6940
11. Morris MJ, Tong WP, Cordon-Cardo C, Drobniak M, Kelly WK, Slovan SF, Terry KL, Siedlecki K, Swanson P, Rafi M, DiPaola RS, Rosen N, Scher HI (2002) Phase I trial of BCL-2 antisense oligonucleotide (G3139) administered by continuous intravenous infusion in patients with advanced cancer. Clin Cancer Res 8:679
12. Rafi MM, Rosen RT, Vassil A, Ho C, Zhang H, Ghai G, Lambert G, Hait WN, DiPaola RS (2000) Modulation of bcl-2 and cytotoxicity by licochalcone-A, a novel estrogenic flavonoid. Anticancer Res 20:2653
13. Rafi MM, Vastano BC, Zhu N, Ho CT, Ghai G, Rosen RT, Gallo MA, DiPaola RS (2002) Novel polyphenol molecule isolated from licorice root (*Glycrrhiza glabra*) induces apoptosis, G2/M cell cycle arrest, and Bcl-2 phosphorylation in tumor cell lines. J Agric Food Chem 13:677
14. Reed JC (2002) Apoptosis-based therapies. Nat Rev Drug Discov 1:111
15. Roth BJ, Yeap BY, Wilding G, Kasimis B, McLeod D, Loehrer PJ (1993) Taxol in advanced, hormone refractory carcinoma of the prostate. A phase II trial of the Eastern Cooperative Oncology Group. Cancer 72:2457
16. Santhanam S, Decatris M, O'Byrne K (2002) Potential of interferon-alpha in solid tumours: part 2. Biodrugs 16:349
17. Sullivan GF, Amenta PS Villanueva JD, Alvarez CJ, Yang JM, Hait WN (1999) The expression of drug resistance gene products during the progression of human prostate cancer. Clin Cancer Res 4:1393

18. Trivedi C, Redman B, Flaherty LE, Kucuk O, Du W, Heilbrun LK, Hussain M (2000) Weekly 1-hour infusion of paclitaxel. Clinical feasibility and efficacy in patients with hormone refractory prostate carcinoma. Cancer 89:431

19. Tu S, McConnell K, Marin MC, Campbell ML, Fernandez A, von Eschenbach AC, McDonnell TJ (1995) Combination adriamycin and suramin induces apoptosis in bcl-2 expressing prostate carcinoma cells. Cancer Lett 93:147