

Review Articles

SOMATOSTATIN ANALOGUES AND ESTROGENS IN THE TREATMENT OF ANDROGEN ABLATION REFRACTORY PROSTATE ADENOCARCINOMA

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ABSTRACT

Purpose: Prostate cancer progression to androgen ablation refractory stage D3 corresponds to cancer cell escape from androgen withdrawal induced apoptosis. Of note, salvage chemotherapy can extend the median survival of approximately 10 months in patients with stage D3. Therefore, novel therapeutic strategies that target the molecular basis of androgen resistance are required.

Materials and Methods: The MEDLINE and Current Content databases were used to find studies of the use of estrogens and somatostatin analogues for D3 prostate adenocarcinoma. We also analyzed the rationale and clinical results of our combination therapy using lanreotide and ethinylestradiol.

Results: Negative experiences have been reported with somatostatin analogues as monotherapy. On the other hand, the median progression-free survival reported in our experience using lanreotide acetate plus ethinylestradiol clearly surpassed the 10-month survival historically described in stage D3 cases.

Conclusions: The use of somatostatin analogues in combination therapy for D3 prostate cancer sustains the novel concept in cancer treatment in which therapies may target not only cancer cells, but also the microenvironment in combination, which can confer protection from apoptosis.

KEY WORDS: prostate, prostatic neoplasms, estrogens, somatostatin, adenocarcinoma

Prostate cancer progression to androgen ablation refractory stage D3 corresponds to cancer cell escape from androgen withdrawal induced apoptosis.¹ In this development enhancement of growth factor stimulation has an essential role in the up-regulation of survival signals and constitutive proliferation. Of note, salvage chemotherapy can extend the median survival of approximately 10 months in stage D3 cases.^{2,3} Therefore, novel therapeutic strategies that target the molecular basis of androgen resistance are required.

We have previously proposed a combination therapy of ethinylestradiol and somatostatin analogue to reintroduce objective clinical responses in patients with metastatic androgen ablation refractory prostate cancer.⁴ In this study we reviewed the literature on the use of estrogens and somatostatin analogues for prostate adenocarcinoma and analyzed the rationale and clinical results of our combination therapy.

MATERIALS AND METHODS

The MEDLINE and Current Contents databases were used to identify studies of the use of estrogens and somatostatin analogues for D3 prostate adenocarcinoma. Research was mainly focused on studies performed in the last 20 years.

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ESTROGENS

Estrogen therapy continues to have an active role in the secondary treatment of prostate cancer, producing biochemical prostate specific antigen (PSA) responses in a quarter to two-thirds of patients.⁵ Smith et al reported that low dose diethylstilbestrol (DES) (1 mg daily) produced biochemical responses in 43% of men in whom primary androgen deprivation therapy failed.⁶ Other trials have shown response rates of 62% to 79% with a mean time to progression of 6 to 7.5 months.^{7–9} A review of high dose intravenous fosfestrol treatment as secondary treatment in 139 men with progressive metastatic disease showed biochemical responses in 37% and a palliative benefit in 53%.¹⁰

The mechanism of action of estrogens in prostate cancer consists in different targets. Estrogens effect negative feedback inhibition of the luteinizing hormone releasing hormone (LH-RH) axis, producing biochemical castration within 3 to 9 weeks. As shown in hormone insensitive prostate cancer cell lines, they can also exert a direct cytotoxic effect at the prostate cell level, leading to mitotic arrest and apoptosis.^{5,11} Cunha et al suggested that estrogens may influence prostate carcinogenesis via paracrine mechanisms mediated by the stromal microenvironment.¹² At the human bone level Moverare et al noted that effects of estrogen receptor (ER) α activation may be also mediated by insulin-like growth factor I (IGF-I) expression.¹³ Moreover, in prostate cancer cell lines Grande et al found that estrogens down-regulate endothelin-1, a mediator of the osteo-

blastic response of bone to metastatic prostate cancer and also a modulator of several effects in prostate tissue, including cell growth, inhibition of apoptosis and angiogenesis.¹⁴

DES is the prototypical estrogen for prostate adenocarcinoma treatment. European Organisation for the Research and Treatment of Cancer trial 30805 demonstrated the equivalent efficacy of 1 mg DES daily to orchiectomy¹⁵ and 1 trial showed superior results over antiandrogen monotherapy.¹⁶ However, use has been limited primarily by cardiovascular toxicity. Earlier European Organisation for the Research and Treatment of Cancer trials demonstrated an 11% rate of cardiovascular mortality in 185 men on 3 mg DES daily.¹⁷ In different countries the use of DES as estrogen therapy for prostate cancer has been discontinued and the synthetic estrogen ethinylestradiol is used instead.¹⁸ As with DES, different doses are reported for ethinylestradiol.^{18,19} A dose of 0.05 to 1 mg ethinylestradiol daily has proved to be able to lower plasma testosterone permanently below the castrate level.¹⁸

Interest in estrogen therapy has been also rekindled by recent trials suggesting that parenteral administration of estrogens may avoid much cardiovascular toxicity.²⁰ Most recently Ockrim et al reported encouraging biochemical results in 20 patients with locally advanced or metastatic cancer treated with transdermal estradiol patches.²¹

In conclusion, the last International Consultation on Prostate Cancer recommended that estrogens should remain a mainstay of secondary therapy.⁵ Efforts should be made to secure more reliable availability of the preparation and avoid the cardiovascular toxicity of DES.

SOMATOSTATIN ANALOGUES

Native somatostatin is characterized as an inhibitory peptide with exocrine, endocrine and autocrine activity.²² The general inhibitory function of somatostatin is wide ranging and it affects a number of organ systems. The effect of somatostatin on various organ systems is thought to be mediated via specific somatostatin receptors (SSTRs). To date 5 subtypes (SSTRs 1 to 5) have been identified and cloned in human tissue.²³ While all 5 subtypes display an affinity similar to that of somatostatin, there are major differences in the binding of currently available somatostatin analogues^{24,25} to various SSTR subtypes. The highly SSTR2 affine octapeptide somatostatin analogues such as octreotide remain the drugs of choice for application in a majority of pure neuroendocrine (NE) tumors since such tumors most often predominantly express SSTR2.²⁶ However, other somatostatin derivatives such as lanreotide, which have good affinity for SSTR5 in addition to that for SSTR2, may advantageously identify SSTR5 expressing tumors. Not only octreotide and lanreotide, but also other somatostatin analogues such as vapreotide have been analyzed in human prostate cancer.²⁷ Octreotide, lanreotide and vapreotide bind with high affinity to SSTR2 and to a lesser extent to SSTR5, have low affinity for SSTR3 and do not bind SSTR1 and SSTR4.²⁵ More recently the new somatostatin analogue SOM-230 has been introduced.²⁸ SOM-230 binds with high affinity SSTR1, SSTR2, SSTR3 and SSTR5, and with low affinity SSTR4. In rats, monkeys and dogs SOM-230 potently and dose dependently decreases IGF-I.²⁹ Clinical trials are needed to investigate the pharmacokinetic, toxicity and antiproliferative action at different tumor levels of this new compound.

Investigations concerning the exact intracellular mechanisms effected by different SSTRs with regard to cellular proliferation and apoptosis induction are ongoing. In humans SSTR2 and SSTR5 have been involved in the control of growth hormone (GH) release and SSTR5 appears to be important in the control of insulin release.³⁰ Important data have been presented on SSTR activated signal transduction

pathways that are responsible for growth inhibition and apoptosis induction. SSTR3 and to a lesser extent SSTR2 can induce apoptosis, and SSTR1, SSTR4 and SSTR5 have an inhibitory effect on the cell cycle, while SSTR1 might be involved in angiogenesis.³⁰ Recently SSTR subtype expression was characterized in various neoplastic tissues.²⁶ There appears to be a predominance of only 1 or 2 SSTR subtypes in most tumors investigated.

The occurrence and localization of SSTRs in the prostate has been a matter of debate with conflicting results. In patients with prostate cancer undergoing radical prostatectomy Halmos et al observed on reverse transcriptase-polymerase chain reaction that SSTR1 and SSTR5 are expressed in prostate homogenates.³¹ However, reverse transcriptase-polymerase chain reaction tends to overestimate the real contribution of various messengers for receptors and it also detects mRNA from immune cells, nerves and vessels. In benign and malignant human prostatic tissues Reubi et al noted on somatostatin radioligand binding that octreotide only binds to the stromal structures of prostate tissue and not to prostate cancer cells, suggesting high SSTR2 expression only in fibromuscular cells.³² On the contrary, radioligand SST-28 binding was found in prostate cancer cells, suggesting preferential expression of SSTR1, which was also confirmed by *in situ* hybridization studies.³² Reubi et al evaluated approximately 200 tumors for SSTR subtype protein expression using a specific binding assay.²⁶ They noted that prostate carcinomas predominantly expressed SSTR1, although some specimens showed unspecific binding with no displacement by any somatostatin selective compound. This might in part be explained by the finding that somatostatin analogues could produce dimerization with other G-protein coupled receptors, leading to increased binding affinity and a change in SSTR subtypes.^{25,33} The group also reported that, on the contrary, SSTR2 is expressed in nonneoplastic human prostatic stroma.^{26,32} Other, more recent immunohistochemical and *in situ* hybridization studies have permitted the identification of specific somatostatin subtype proteins in human prostatic tissue. Hansson et al confirmed that messenger RNA for SSTR2 is preferentially located in the stromal compartment, while SSTR4 is confined to the epithelial prostatic cells and, furthermore, is up-regulated in prostate cancer compared to benign prostatic hyperplasia (BPH).³⁴ By the immunohistochemical analysis of radical prostatectomy specimens Dizzei et al identified SSTR1 in cancerous and NE prostatic cells, whereas SSTR2 was found in the stroma and in peritumor blood vessels.³⁵ SSTR3 was demonstrated to be present on the cell membrane of BPH and malignant areas. Strong SSTR4 immunoreactivity was found in tumor cells compared with less intensity in adjacent BPH areas. On the contrary, SSTR5 was not detectable.

Long acting somatostatin analogues have been developed that are specifically designed for antitumor activity. Schally synthesized more than 300 analogues using solid phase methods, resulting in octapeptide super analogues, which are more potent and have a longer duration of action than native somatostatin and octreotide.³⁶

Several clinical trials have demonstrated impressive efficacy of somatostatin analogues for various hypersecretory disorders resistant to standard therapy.²³ They have also proved useful for the management of symptoms caused by neuroendocrine diseases. The primary effect of somatostatin analogues is not a direct cytotoxic effect of neuroendocrine cells, but rather inhibition of the release of various peptides hormones secreted by neuroendocrine cells.²³ The observation that somatostatin analogues inhibit the release of various neuroendocrine products has stimulated interest in their use as antiproliferative and pro-apoptotic agents.

Antiproliferative and pro-apoptotic actions of somatostatin analogues have been demonstrated in various tumor models, including those of the breast, prostate, colon and pancre-

as.³⁶⁻³⁸ However, SSSTRs were not expressed in all experimental tumor models, also suggesting indirect mechanisms of somatostatin analogue mediated tumor control. They could be accomplished via the inhibition of GH or tumor growth factors such as IGF-I.³⁰ Other potential mechanisms are through the inhibition of tumor angiogenesis or immune modulation. Several experimental studies suggest that somatostatin analogues inhibit angiogenesis *in vitro* and *in vivo*.³⁹ Moreover, somatostatin analogues have a wide therapeutic index and they are apparently free of major side effects.³⁶ Most reported side effects are gastrointestinal in nature, including minor nausea, diarrhea and constipation.

Clinical trials of and experience with somatostatin analogues as monotherapy for prostate cancer have shown negative results (see table).^{4,40-47} Octreotide was used to treat patients with advanced hormone refractory prostate cancer in a study of Logothetis et al.⁴³ The dose of octreotide applied in 24 cases was 0.1 mg subcutaneously every 8 hours for 6 weeks. No patients had objective evidence of tumor regression and in 10 serum prostate acid phosphatase increased at an accelerated rate after 1 to 2 months of treatment. However, 6 patients underwent salvage chemotherapy after octreotide therapy, of whom 5 achieved objective tumor regression. Therefore, the group concluded that octreotide monotherapy might stimulate prostatic tumor growth but it may also sensitize tumor cells to subsequent chemotherapy.

A total of 30 patients with hormone refractory prostate cancer were treated with a slow release formulation of lanreotide (30 mg intramuscularly once weekly) by Maulard et al.⁴⁴ Toxicity related to treatment was minor, and performance status and bone pain improved in 40% and 35% of patients, respectively, but a PSA decrease of at least 50% was reported in only 20%.

SOMATOSTATIN ANALOGUES IN COMBINATION THERAPY: RATIONALE AND FIRST EXPERIENCE

The mechanism of action of somatostatin analogues may suggest the use of these drugs not as monotherapy, but rather as combination therapy for tumors such as prostate cancer. Also, in breast cancer favorable results have been obtained by the use of somatostatin analogues in combination therapy. A total of 22 postmenopausal patients with metastatic breast cancer were randomized to 40 mg tamoxifen daily or a combination of 40 mg tamoxifen plus 0.2 mg octreotide 3 times daily subcutaneously.⁴⁸ An objective response was found in 36% of the patients treated with tamoxifen alone and in 55% of those treated with combination therapy.

The management of metastatic neoplasms has traditionally relied on therapeutic modalities, which almost exclusively aim at directly inducing cancer cell death. However, the *in vivo* response of malignant cells to anticancer therapies is directly influenced by the local microenvironment in

which they reside (or metastasize).⁴⁹ Microenvironment factors may attenuate the antitumor activity of several cytotoxic agents on neoplastic cells. In particular, organ sites frequently involved in metastatic advanced disease appear to confer on neoplastic cells protection from anticancer drug induced apoptosis. This protection may be mediated by several mechanisms, including growth factors, cytokines released by the normal cellular and constituents of the host-tissue microenvironment.⁵⁰ Also, stromal factors may confer increased survival on cancer cells. In prostate cancer cell lines Ozen et al observed that fibroblast growth factor expression is increased.⁵¹ They reported the unexpected dependence of prostate cancer cells on fibroblast growth factor receptor signal transduction to arrest in the G2 phase of the cell cycle before cell death. As previously presented,^{26,32,52} SSSTR2 is predominantly expressed in prostatic stroma. Therefore, SSSTR2 may also be involved in cancer cell control through the stromal influence.

Additional emphasis should be placed on the design of novel treatments that can neutralize the protection that the microenvironment offers to tumors cells. An example of the role of the microenvironment in protecting tumor cells from anticancer therapies is in the setting of hormone refractory prostate cancer. For years it has been a widely accepted notion that resistance to hormonal therapy is an outcome exclusively determined at the genetic level and involving mutations that neutralize pro-apoptotic intracellular pathways and/or activate anti-apoptotic ones.⁴⁹ It is now well documented that this resistance can also be conferred by epigenetic mechanisms.⁵² These mechanisms result from the interaction of tumor cells with the local microenvironment at local or metastatic sites. Mediators of this interaction are also neuropeptides secreted by NE cells in prostate tissue and IGF-I. The locally bioavailability of these peptides and growth factors on prostate cancer cells activates anti-apoptotic mechanisms more than proliferative direct effects. They represent real survival pathways involved in prostate cancer progression and androgen deprivation therapy resistance. The development of survival factor mediated resistance to anticancer therapies is a major hurdle preventing long lasting clinical responses to conventional or investigational therapies.⁴⁹ This realization led to the novel concept of antisurvival (ASF) therapy for prostate cancer as a component of anticancer treatments and to the concept of combination therapy for hormone refractory disease. This approach is novel because, instead of attempting directly to induce cancer cell apoptosis, it aims at neutralizing the protective effect conferred on cancer cells by survival factors. This neutralization alone may not induce apoptosis but it can enhance the sensitivity or reverse the resistance of tumors cells to other anticancer strategies with direct cytotoxic effects.^{49,50,52}

On this basis, Koutsilieris et al first proposed combination therapy with dexamethasone and long acting somatostatin

Clinical experiences with somatostatin analogues for hormone refractory prostate cancer

Treatment	Dose	No. Pts	Results	References
Octreotide	100 µg × 3/Day subcutaneously	7	Pain decrease	Carteni et al ⁴⁰
Octreotide	600-1,350 µg/Day subcutaneously	10	Disease progression after 21 days	Dupont et al ⁴¹
Octreotide	400-1,000 µg/Day subcutaneously	5	Temporary halt in PSA increase	Verhelst et al ⁴²
Octreotide	100 mg × 4/Day subcutaneously	22	Prostate tumor growth stimulation	Logothetis et al ⁴³
Lanreotide	30 mg × 1/Wk intramuscularly	30	20% Partial response (PSA decrease), 40% improved performance status	Maulard et al ⁴⁴
Lanreotide	4-24 mg/Day subcutaneously	25	No modifications	Figg et al ⁴⁵
Octreotide	Not clarified	14	Symptom-free responses	Vainas et al ⁴⁶
Lanreotide + dexamethasone	30 mg/14 Days intramuscularly + 4 mg/day orally	11	90% objective (PSA decrease) + symptomatic response, 7-mo progression-free survival	Koutsilieris et al ⁴⁷
Lanreotide acetate + ethinylestradiol	73.9 mg × 1/4 Wks intramuscularly + 1 mg/day orally	10	90% Objective (PSA decrease) + symptomatic response, 18.5-mo progression-free survival	Di Silverio and Sciarra ⁴

analogue in patients with stage D3 prostate cancer, ie patients with metastatic prostate cancer that had become refractory to combined androgen blockade.⁴⁷ In this setting GH independent and GH dependent production of IGF-I has been implicated in the development of an epigenetic form of cancer cells resistance to pro-apoptotic therapies. Among its diverse pharmacological effects dexamethasone acts to down-regulate the GH independent local bioavailability of IGF-I, whereas somatostatin analogue suppresses the level of GH dependent IGF-I. In particular, dexamethasone decreases the hydrolysis of IGF binding proteins (BPs), thereby, increasing the local concentration of IGF-BPs.⁵⁰ Koutsilieris et al reported that the increased IGFBP concentration decreases the bioavailability of IGFs to prostate cancer cells and osteoblasts.⁵⁰ On the other hand, using primary human fetal pituitary cell cultures it was demonstrated that GH secretion was suppressed equally by somatostatin analogues preferential for SSTR2 or SSTR5.⁵³ Patients with GH secreting pituitary adenomas who received somatostatin analogues showed a significant decrease in circulating, GH dependent IGF-I.⁵⁴

This paradigm of an ASF therapy, which was practically an antiIGF-I therapy, yielded objective responses and major improvement of bone pain and performance status in D3 cases. The treatment schedule includes administration of oral dexamethasone plus long acting somatostatin analogue (lanreotide or octreotide as intramuscular injections) in combination with androgen ablation therapy. In the initial cohort of patients receiving combination therapy median overall survival clearly surpassed 12 months and post-relapse performance status and bone pain were significantly improved compared to baseline status even months after relapse. The stimulating feature of this ASF approach is that its combination with LH-RH analogues can reintroduce clinical responsiveness to LH-RH analogues.

COMBINATION THERAPY WITH ETHINYLESTRADIOL AND SOMATOSTATIN ANALOGUE: RATIONALE AND CLINICAL EXPERIENCE

We have previously analyzed whether the combination of ethinylestradiol and lanreotide can offer objective responses and/or symptomatic improvements in patients with stage D3 prostate cancer.⁴ We followed the study design of Koutsilieris et al.⁴⁷ As did Koutsilieris et al, we evaluated patients with metastatic androgen ablation refractory prostate cancer. However, in contrast to Koutsilieris et al, we discontinued LH-RH analogue and started combination therapy with ethinylestradiol and lanreotide acetate.

The rationale for our combination therapy was: 1) to inhibit the protective (anti-apoptotic) effect of the NE system on prostate adenocarcinoma cells (somatostatin analogue), 2) use a new mechanism to induce castration (estrogen) and 3) to add a direct cytotoxic effect on prostate cells (estrogen) (fig. 1). Some studies have shown that the number of NE tumor cells^{55,56} and serum chromogranin A (CgA) increase during hormonal therapy⁵⁵⁻⁵⁹ for prostate adenocarcinoma. As previously underlined, at the cellular level refractoriness to androgen ablation therapy occurs principally because prostate cancer cells can be rescued from androgen ablation induced apoptosis. It has been shown that Bcl2 proto-oncogene, which is an anti-apoptotic factor, is preferentially expressed in foci of prostate adenocarcinoma cells in the vicinity of NE differentiation.^{60,61} In hormone refractory (D3) prostate cancer NE cells may protect prostate adenocarcinoma cells from anti-cancer therapies through the neutralization of pro-apoptotic intracellular pathways.

The rationale for somatostatin analogue therapy for D3 prostate tumors is not only to induce directly cancer cell apoptosis, but also neutralize the protective effect conferred on cancer cells by survival factors derived by NE prostate cells and the microenvironment. The somatostatin analogues octreotide and lanreotide are highly SSTR2 affine.^{25,26} Considering the predominant localization of SSTR2 in the stromal compartment of the prostate and peritumor blood vessels³⁵ and not on NE prostate cells, the effects of these analogues might be indirect by stromal control. However, somatostatin analogues seem to interact at tissue level, also through a receptor binding independent mechanism.²³

The antigonadotropic effect of estrogens has been exploited therapeutically. Experimental and clinical evidence suggests that estrogen therapy may be superior to castration in terms of efficacy for advanced prostate cancer.^{6,9,15,18,19} Moreover, after analyzing prostatectomy specimens of untreated and treated (combined androgen blockade [CAB]) patients with prostate cancer Kruithof-Dekker et al reported that androgen deprivation leads to up-regulation of estrogen receptor expression in prostate cancer tissue.⁶² Torlakovic et al underlined that in particular ER β was expressed in 93% of prostate adenocarcinomas and it was positively associated with primary Gleason score and grade.⁶³ PC-3, an aggressive prostate cancer cell line with invasive properties in nude mice, expressed higher levels of ER β than the LNCaP, non-metastasizing cell line, whereas no difference for ER α expression could be observed.⁶³ These findings suggest that ER β may have a role in the process of prostate adenocarcinoma

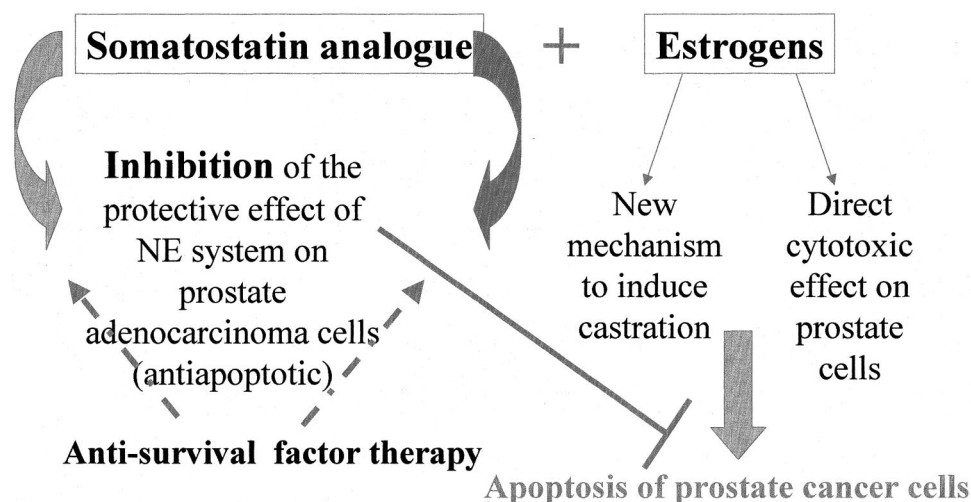


FIG. 1. Rationale for our combination therapy with somatostatin analogue and estrogen

dedifferentiation. However, another experience did not show statistically significant differences in ER β expression between androgen dependent and hormone refractory prostate carcinomas.⁶⁴ It has been supposed that the beneficial effect of estrogens is based not only on the decrease in the androgen concentration, but also on a simultaneous, direct cytotoxic effect^{3,5,11} on prostate cancer cells. In hormone prostate cancer cell lines Robertson et al found that estrogen cytotoxicity at the prostate tissue level was ER independent.¹¹ Grande et al found that estrogens down-regulate endothelin-1, a mediator of the osteoblastic response and pain responses of bone to metastatic prostate cancer and a modulator of cell growth, apoptosis and angiogenesis, also at the prostate cancer level.¹⁴ These data support our rationale, that is to discontinue LH-RH analogue and substitute estrogen therapy for it. An important question is whether the responses achieved in our study were most likely indirect evidence of a potential survival benefit offered by combination therapy rather than a response to lanreotide only or ethinylestradiol only.

As previously shown, in advanced hormone refractory prostate cancer negative experiences have been reported with the use of somatostatin analogues as monotherapy.⁴⁰⁻⁴⁶ On the other hand, the median progression-free survival reported in our study clearly surpassed the 10-month survival historically described in patients with stage D3 even when estrogen therapy or salvage chemotherapy is administered.^{3,12} However, additional studies are required to elucidate fully the precise in vivo mechanism of action for the combination of estrogens with somatostatin analogues.

In our first experience we prospectively evaluated 10 consecutive patients with stage D3 disease who received combination therapy consisting of 1) oral ethinylestradiol (1 mg daily) and 2) lanreotide (73.9 mg lanreotide acetate intramuscularly every 4 weeks) (fig. 2).⁴ In this first experience 90% of cases (95% CI 55.5 to 99.8) had an objective complete (PSA less than 4 ng/ml) or partial (at least a 50% PSA decrease from baseline) clinical response to combination therapy, corresponding to a statistically significant (compared to baseline refractoriness) rate of re-introduction of responsiveness to the combination with lanreotide and ethinylestradiol (McNemar's paired chi-square test $p < 0.01$). In all cases PSA responses were accompanied by a concomitant, statistically significant decrease in the bone pain score

($p \leq 0.0001$) as well as by significant improvement in the Eastern Cooperative Oncology Group (ECOG) performance status score ($p < 0.0001$). The symptomatic improvement in pain and performance status appeared to be temporally associated with changes in objective response markers and it is suggested that the main mechanism of action of this combination therapy affects mechanisms regulating the growth and/or survival of metastatic cells rather than involving a nonspecific anti-inflammatory or analgesic effect.⁴⁷ The rate and time to achieve the symptomatic and objective responses that we described are comparable to those in the study of Koutsilieris et al.⁴⁷ However, with our combination therapy we achieved a longer duration of objective responses. In particular, the median duration of the bone pain response, ECOG response and progression-free survival was 17.5 (95% CI 12 to 19), 18 (95% CI 12 to 19) and 18.5 (95% CI 14 to 21) months in our study, and 13 (95% CI 12 to 14), 19 (95% CI 13 to 25) and 7 (95% CI 3 to 10) months in the study of Koutsilieris et al.⁴⁷

Comparison of serum CgA at baseline, during followup, at maximal response and at relapse from therapy revealed a significant change in CgA during the course of combination therapy (Friedman's nonparametric ANOVA $p < 0.0001$). We observed a significant decrease in serum CgA during the administration of combination therapy (median maximum decrease 38.4%, 95% CI 33.2 to 50.3, range 28.6% to 64.9%) compared with baseline CgA.

In our patients time to CgA nadir was lower than time to PSA nadir. Therefore, it seems that the CgA response preceded the PSA response. In our study baseline CgA was similar to that reported in other experiences with metastatic prostate cancer.^{59,60} The significant decrease in circulating CgA documented in this cohort of patients suggests that a reduction in NE activity on prostate cancer cells may be a mechanism accounting for at least part of the encouraging responses that were observed. A possible mechanism for such a response was suggested by Hansson³⁴ and Gonzalez-Barcena²⁷ et al, namely that lower IGF-I induced by somatostatin analogue therapy may decrease NE prostatic activity and secretion. Again, this may support the indirect activity of somatostatin analogue therapy on prostate cancer.

Interestingly patient serum CgA was not significantly increased at relapse, suggesting that NE activity may not be involved at relapse from this combination therapy. The modifications in CgA reported in our study are lower compared with those observed in pathologically confirmed NE tumors, such as small cell carcinoma of the lung. However, we must remember that NE differentiation of prostate adenocarcinoma consists of NE cells with focal distribution in the common prostatic adenocarcinoma.^{59,60} A limit of our analysis may be the determination of only serum CgA expression. However, none of our patients presented with a history of other disorders known to interfere with CgA levels. Some groups have reported a significant correlation between serum and tissue expression of CgA in prostate cancer.^{59,60} Moreover, in 8 cases we had the opportunity to analyze CgA expression at the prostate tissue level by immunohistochemistry. Prostate tissue specimens were obtained by transrectal ultrasound guided prostate biopsy, and formalin fixed and paraffin embedded prostate specimens were sectioned to 5 μ m prior to analysis. Diffuse immunohistochemical staining for CgA was found in biopsies obtained in D3 cases at baseline from our therapy. On the contrary, limited and focal CgA staining was noted in cases with an objective clinical response to our combination therapy (fig. 3).

No major treatment related side effects were reported during combination therapy. In all cases gynecomastia and mild breast pain developed. It is true that none of our patients had a history of severe cardiovascular diseases at baseline but the dose of ethinylestradiol (1 mg) and the duration of followup

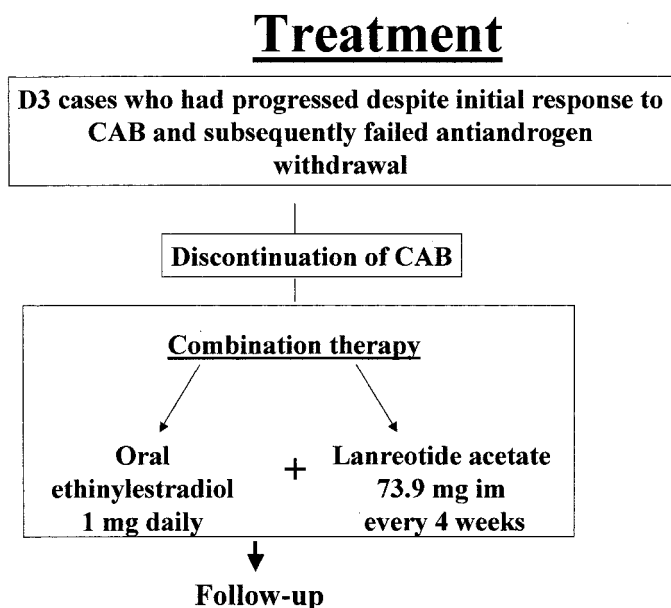


FIG. 2. Study design for our trial of lanreotide acetate and ethinylestradiol combination therapy in D3 prostate cancer cases. *im*, intramuscularly.

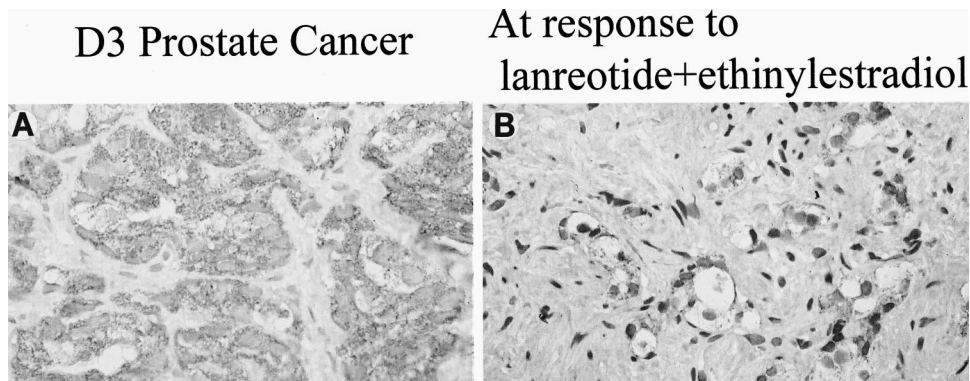


FIG. 3. Immunohistochemical staining for CgA in D3 cases at baseline (A) and at objective clinical response to combination therapy with ethinylestradiol plus lanreotide acetate (B). Formalin fixed, paraffin embedded prostate specimens were obtained and sectioned to 5 μm prior to analysis. Dark stained cells were indicative of diffuse CgA staining (A), and focal and limited CgA staining (B). Reduced from ×400.

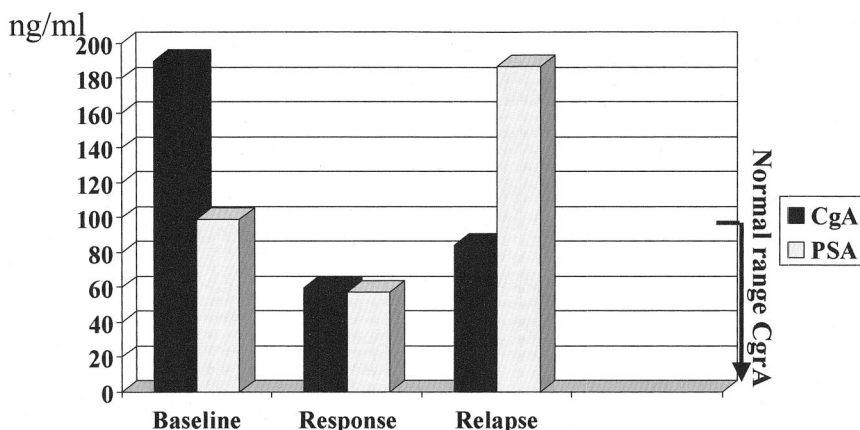


FIG. 4. Mean serum CgA and serum PSA at baseline, at response and at relapse from combination therapy. Combination treatment was associated with significant mean CgA decrease ($p < 0.0001$). In contrast, combination therapy relapse was not associated with significant increase in CgA vs those during response to therapy ($p > 0.05$).

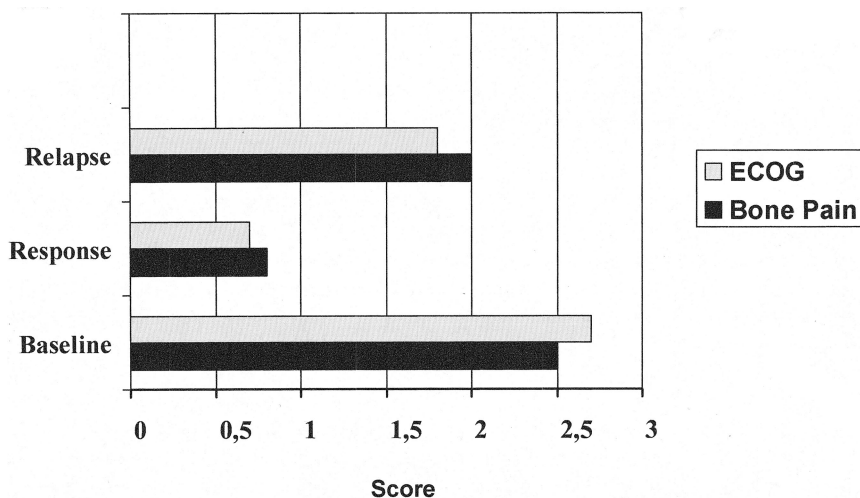
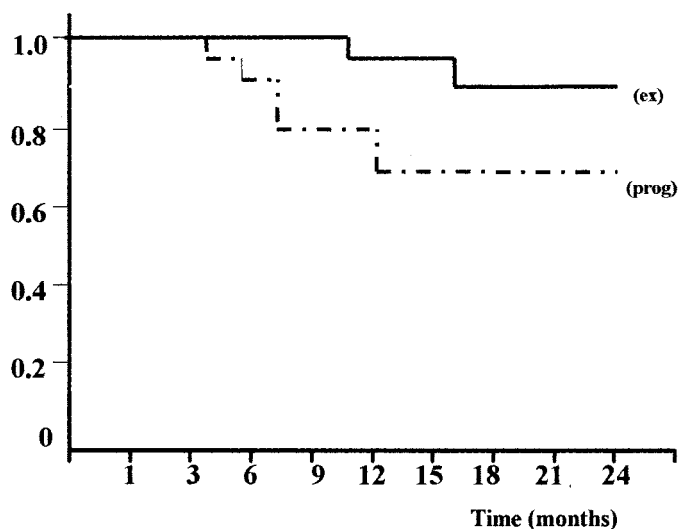


FIG. 5. Mean bone pain and ECOG scores at baseline, at response and at relapse from combination therapy. Combination treatment was associated with significant improvement in ECOG and bone pain ($p < 0.0001$). Symptomatic improvement in pain and performance status appeared to be temporally associated with changes in objective response markers.

(no longer than 24 months) may also have contributed to differences vs other experiences with estrogen therapy.^{17,20}

As of January 2004, 20 patients with D3 disease have been included in our analysis and received combination therapy with ethinylestradiol and lanreotide (unpublished data). Criteria for inclusion and the study protocol were similar to those previously described.⁴

Figures 4 to 6 show clinical results achieved in this population of 20 D3 prostate adenocarcinoma cases. Results continue to be encouraging and supportive of the rationale for our combination therapy. In particular, in January 2004 19 of the 20 cases (95%) showed an objective (complete in 5 or 25% and partial in 14 or 70%) clinical response to combination therapy, as demonstrated by at least a 50% PSA de-



Number of patients at risk						
Group	N	3 months	6 months	12 months	18 months	24 months
ex	20	20	17	10	6	1
prog	20	20	16	10	6	1

FIG. 6. Kaplan-Meier projections (cumulative proportion of patients) for cancer specific (*ex*) and progression-free (*prog*) survival in 20 D3 cases receiving ethinylestradiol plus lanreotide therapy.

crease from baseline. In only 1 case was the biochemical response accompanied by a decrease in the number of bone metastases on bone scan. Two of the 20 patients (10%) died of prostate cancer at 10 and 16 months, respectively, and 6 (30%) had clinical progression with PSA increasing to more than 50% of the PSA nadir at a mean of 7.8 months (median 7, range 4 to 12) during followup. The other 14 patients (70%) were still without disease progression at a median of 16.5 months (mean 13.9, range 4 to 24) of followup during combination therapy. Figure 6 shows Kaplan-Meier projections of cancer specific and progression-free survival. Again, no major treatment related side effects were reported during combination therapy and none of our patients discontinued the treatment due to side effects related to combination therapy.

CONCLUSIONS

It should be emphasized that any conclusion regarding the usefulness of this combination therapy in comparison to other proposed treatment strategies for stage D3 prostate cancer can only be drawn in randomized, controlled clinical trials. Our clinical trial and that of Koutsilieris et al⁴⁷ were not designed to address directly the question of whether ASF treatment can prolong the survival of these patients in comparison to other strategies. The results of our study indicate that such randomized trials are warranted because the combination of ethi-

nylestradiol and lanreotide had a favourable toxicity profile, offered objective and symptomatic responses in patients with limited treatment options and refractoriness to conventional hormonal therapy strategies and, in particular, offered a median overall survival that was superior to the 10-month median survival in patients with hormone refractory disease. This combination therapy also sustains the novel concept in cancer treatment, in which therapies may target not only cancer cells, but also its microenvironment in combination, which can confer protection from apoptosis.

REFERENCES

- Landstrom, M., Damber, J. E. and Bergh, A.: Prostatic tumor regrowth after initially successful castration therapy may be related to a decreased apoptotic cell death rate. *Cancer Res*, **54**: 4281, 1994
- Koutsilieris, M., Laroche, B., Thabet, M. and Fradet, Y.: The assessment of disease aggressivity in stage D2 prostate cancer patients. *Anticancer Res*, **10**: 333, 1990
- Hudes, G. R., Greenberg, R., Krigel, R. L., Fox, S., Scher, R., Litwin, S. et al: Phase II study of estramustine and vinblastine, two microtubule inhibitors, in hormone-refractory prostate cancer. *J Clin Oncol*, **10**: 1754, 1992
- Di Silverio, F. and Sciarra, A.: Combination therapy of ethinylestradiol and somatostatin analogue reintroduces objective clinical responses and decreases chromogranin A in patients with androgen ablation refractory prostate cancer. *J Urol*, **170**: 1812, 2003
- Carroll, P. R., Altwein, J., Brawley, O., Cockett, A., Cooperberg, M., Hirao, Y. et al: Management of disseminated prostate cancer. In: 3rd International Consultation on Prostate Cancer-Paris. Edited by L. Denis, G. Bartsch, S. Khoury, M. Murai and A. Partin. United Kingdom: Plymbridge Distributors, Ltd., pp. 251-284, 2003
- Smith, D. C., Redman, B. G., Flaherty, L. E., Li, L., Strawderman, M., Pienta, K. J. et al: A phase II trial of oral diethylstilbestrol as a second-line hormonal agent in advanced prostate cancer. *Urology*, **52**: 257, 1998
- Farrugia, D., Ansell, W., Singh, M., Philp, T., Chinegwundoh, F. and Oliver, R. T.: Stilboestrol plus adrenal suppression as salvage treatment for patients failing treatment with luteinizing hormone-releasing hormone analogues and orchiectomy. *BJU Int*, **85**: 1069, 2000
- Orlando, M., Chacon, M., Salum, G. and Chacon, D. R.: Low-dose continuous oral faspesol is highly active in hormone-refractory prostate cancer. *Ann Oncol*, **11**: 177, 2000
- Rosenbaum, E., Wygoda, M., Gips, M. et al: Diethylstilbestrol is an active agent in prostate cancer patients after failure to complete androgen blockade. *Proc Am Soc Clin Oncol*, **19**: 1372A, 2000
- Droz, J. P., Kattan, J., Bonnay, M., Chraïbi, Y., Bekradda, M. and Culine, S.: High-dose continuous-infusion fosfestrol in hormone-resistant prostate cancer. *Cancer*, **71**: 1123, 1993
- Robertson, C. N., Roberson, K. M., Padilla, G. M., O'Brien, E. T., Cook, J. M. and Fine, R. L.: Induction of apoptosis by diethylstilbestrol in hormone-insensitive prostate cancer cells. *J Natl Cancer Inst*, **88**: 908, 1996
- Cunha, G. R., Hayward, S. W., Wang, Y. Z. and Riche, W. A.: Role of the stromal microenvironment in carcinogenesis of the prostate. *Int J Cancer*, **107**: 1, 2003
- Moverare, S., Venken, K., Eriksson, A. L., Andersson, N., Skrtic, S., Wergedal, J. et al: Differential effects on bone of estrogen receptor alpha and androgen receptor activation in orchiectomized adult male. *Proc Natl Acad Sci USA*, **100**: 13573, 2003
- Grande, M., Carlstrom, K., Stege, R., Poussette, A. and Faxen, M.: Estrogens affect endothelin-1 mRNA expression in LNCaP human prostate carcinoma cells. *Eur Urol*, **41**: 568, 2002
- Robinson, M. R., Smith, P. H., Richards, B., Newling, D. W., de Pauw, M. and Sylvester, R.: The final analysis of the EORTC Genito-Urinary Tract Cancer Co-Operative Group phase III clinical trial (protocol 30805) comparing orchidectomy, orchidectomy plus cyproterone acetate and low dose stilboestrol in the management of metastatic carcinoma of the prostate. *Eur Urol*, **28**: 273, 1995
- Chang, A., Yeap, B., Davis, T., Blum, R., Hahn, R., Khanna, O. et al: Double-blind, randomized study of primary hormonal treat-

- ment of stage D2 prostate carcinoma: flutamide versus diethylstilbestrol. *J Clin Oncol*, **14**: 2250, 1996
17. de Voogt, H. J., Smith, P. H., Pavone-Macaluso, M., de Pauw, M., Suci, S. and Members of the European Organization for Research or Treatment of Cancer Urological Group: Cardiovascular side effects of diethylstilbestrol, cyproterone acetate, medroxyprogesterone acetate and estramustine phosphate used for the treatment of advanced prostatic cancer: results from European Organization for Research on Treatment of Cancer trials 30761 and 30762. *J Urol*, **135**: 303, 1986
 18. Langeveld, J. W., Lycklama, A., Nijeholt, A. A. and Jonas, U.: Oestrogen in the treatment of prostatic carcinoma. What is the safe and effective dose of ethinyloestradiol? *Br J Urol*, **63**: 76, 1989
 19. Shearer, R. J., Hendry, W. F., Somerville, I. F. and Fergusson, J. D.: Plasma testosterone: an accurate monitor of hormone treatment in prostatic cancer. *BJU*, **45**: 668, 1973
 20. Hedlund, P. O. and Henriksson, P.: Parenteral estrogen versus total androgen ablation in the treatment of advanced prostate carcinoma: effects on overall survival and cardiovascular mortality. The Scandinavian Prostatic Cancer Group (SPCG)-5 Trial Study. *Urology*, **55**: 328, 2000
 21. Ockrim, J. L., Lalani, E.-N., Laniado, M. E., Carter, S. S. and Abel, P. D.: Transdermal estrogen therapy for advanced prostate cancer-forward to the past? *J Urol*, suppl., **167**: 175, abstract 702, 2002
 22. Newman, J. B., Lluís, F. and Townsend, C. M., Jr.: Somatostatin. Edited by J. C. Thompson, G. H. Greeley, Jr., P. L. Rayford and C. M. Townsend, Jr.: In: *Gastrointestinal Endocrinology*. New York: McGraw-Hill Book Co. pp. 286-299, 1987
 23. Hejna, M., Schmidinger, M. and Raderer, M.: The clinical role of somatostatin analogues as antineoplastic agents: much ado about nothing? *Ann Oncol*, **13**: 653, 2002
 24. Pollak, M. N. and Schally, A. V.: Mechanisms of antineoplastic action of somatostatin analogs. *Proc Soc Exp Biol Med*, **217**: 143, 1998
 25. Lamberts, S. W., van der Lely, A. J. and Hofland, L. J.: New somatostatin analogs: will they fulfill old promises? *Eur J Endocrinol*, **146**: 701, 2002
 26. Reubi, J. C., Wasar, B., Schaer, J. C. and Laissue, J. A.: Somatostatin receptor sst1-sst5 expression in normal and neoplastic human tissues using receptor autoradiography with subtype-selective ligands. *Eur J Nucl Med*, **28**: 836, 2001
 27. Gonzalez-Barcena, D., Schally, A. V., Vadillo-Buenfil, M., Cortez-Morales, A., Hernandez, L. V., Cardenas-Cornejo, I. et al: Response of patients with advanced prostatic cancer to administration of somatostatin analog RC-160 (vapreotide) at the time of relapse. *Prostate*, **56**: 183, 2003
 28. Bruns, C., Lewis, I., Briner, U., Meno-Tetang, G. and Weckbecker, G.: SOM230: a new somatostatin peptidomimetic with broad somatotropin release inhibiting factor SRIF receptor binding and a unique antisecretory profile. *Eur J Endocrinol*, **146**: 707, 2002
 29. Weckbecker, G., Briner, U., Lewis, I. and Bruns, C.: SOM230: a new somatostatin peptidomimetic with potent inhibitory effects on the growth hormone/insulin-like growth factor-I axis in rats, primates, and dogs. *Endocrinology*, **143**: 4123, 2002
 30. Lamberts, S. W., de Herder, W. W. and Hofland, L. J.: Somatostatin analogs in the diagnosis and treatment of cancer. *Trends Endocrinol Metab*, **13**: 451, 2002
 31. Halmos, G., Schally, A. V., Sun, B., Davis, R., Bostwick, D. G. and Plonowski, A.: High expression of somatostatin receptors and messenger ribonucleic acid for its receptor subtypes in organ-confined and locally advanced human prostatic cancers. *J Clin Endocrinol Metab*, **85**: 2564, 2000
 32. Reubi, J. C., Waser, B., Schaer, J. C. and Markwalder, R.: Somatostatin receptors in human prostate and prostate cancer. *J Clin Endocrinol Metab*, **80**: 2806, 1995
 33. Rocheville, M., Lange, D. C., Kumar, U., Sai, R., Patel, R. C. and Patel, Y. C.: Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *J Biol Chem*, **275**: 7862, 2000
 34. Hansson, J., Bjartell, A., Gadaleanu, V., Dizeyi, N. and Abrahamsson, P. A.: Expression of somatostatin receptor subtypes 2 and 4 in human benign prostatic hyperplasia and prostatic cancer. *Prostate*, **53**: 50, 2002
 35. Dizeyi, N., Konrad, L., Bjartell, A., Wu, H., Gadaleanu, V., Hansson, J. et al: Localization and mRNA expression of somatostatin receptor subtypes in human prostatic tissue and prostate cancer cell lines. *Urol Oncol*, **7**: 91, 2002
 36. Schally, A. V.: Oncological applications of somatostatin analogues. *Cancer Res*, **48**: 6977, 1988
 37. Smith, J. P. and Solomon, T. E.: Effects of gastrin, proglumide, and somatostatin on growth of human colon cancer. *Gastroenterology*, **95**: 1541, 1988
 38. Murphy, W. A., Lance, V. A., Moreau, S., Moreau, J. P. and Coy, D. H.: Inhibition of rat prostate tumor growth by an octapeptide analog of somatostatin. *Life Sci*, **40**: 2515, 1987
 39. Woltering, E. A., Watson, J. C., Alperin-Lea, R. C., Sharma, C., Keenan, E., Kurozawa, D. et al: Somatostatin analogs: angiogenesis inhibitors with novel mechanisms of action. *Invest New Drugs*, **15**: 77, 1997
 40. Carteni, G., Biglietto, M., Tucci, A. and Pacilio, G.: Sandostatin, a long-acting somatostatin analogue in the treatment of advanced metastatic prostate cancer. *Eur J Cancer*, **26**: 161A, 1990
 41. Dupont, A., Boucher, H., Cusan, L., Lacourciere, Y., Emond, J. and Labrie, F.: Octreotide and bromocriptine in patients with stage D2 prostate cancer who relapsed during treatment with flutamide and castration. *Eur J Cancer*, **26**: 770, 1990
 42. Verhelst, J., De Longueville, M., Ongena, P., Denis, L. and Mahler, C.: Octreotide in advanced prostatic cancer relapsing under hormonal treatment. *Acta Urol Belg*, **62**: 83, 1994
 43. Logothetis, C. J., Hossain, E. A. and Smith, T. L.: SMS 201-995 in the treatment of refractory prostatic cancer. *Anticancer Res*, **14**: 2731, 1994
 44. Maulard, C., Richaud, P., Droz, J. P., Jessueld, D., Dufour-Esquerre, F. and Housset, M.: Phase I-II study of the somatostatin analogue lanreotide in hormone-refractory prostate cancer. *Cancer Chemother Pharmacol*, **36**: 259, 1995
 45. Figg, W. D., Thibault, A., Cooper, M. R., Reid, R., Headlee, D., Dawson, N. et al: A phase I study of the somatostatin analogue somatuline in patients with metastatic hormone-refractory prostate cancer. *Cancer*, **75**: 2159, 1995
 46. Vainas, G., Pasaitou, V., Galaktidou, G., Maris, K., Christodoulou, K., Constantinidis, C. et al: The role of somatostatin analogues in complete antiandrogen treatment in patients with prostatic carcinoma. *J Exp Clin Cancer Res*, **16**: 119, 1997
 47. Koutsilieris, M., Mitsiades, C., Dimopoulos, T., Iannidis, A., Ntounis, A. and Lambou, T.: A combination therapy of dexamethasone and somatostatin analog reintroduces objective clinical responses to LH-RH analog in androgen ablation-refractory prostate cancer patients. *J Clin Endocrinol Metab*, **86**: 5729, 2001
 48. Bontenbal, M., Foekens, J. A., Lamberts, S. W., de Jong, F. H., van Putten, W. L., Braun, H. J. et al: Feasibility, endocrine and anti-tumor effects of a triple endocrine therapy with tamoxifen, a somatostatin analogue and an anti-prolactin in postmenopausal metastatic breast cancer: a randomized study with long-term follow-up. *Br J Cancer*, **77**: 115, 1998
 49. Koutsilieris, M., Mitsiades, C., Dimopoulos, T., Vacalicos, J., Lambou, T., Tsintavis, A. et al: Combination of dexamethasone and a somatostatin analogue in the treatment of advanced prostate cancer. *Expert Opin Investig Drugs*, **11**: 283, 2002
 50. Koutsilieris, M., Mitsiades, C. and Sourla, A.: Insulin-like growth factors I and urokinase-type plasminogen activator bioregulation system as a survival mechanism of prostate cancer cells in osteoblastic metastases: development of anti-survival factor therapy for hormone-refractory prostate cancer. *Mol Med*, **6**: 251, 2000
 51. Ozen, M., Giri, D., Ropiquet, F., Mansukhani, A. and Ittmann, M.: Role of fibroblast growth factor receptor signaling in prostatic cancer cell survival. *J Natl Cancer Inst*, **93**: 1783, 2001
 52. Reyes-Moreno, C., Sourla, A., Choki, I., Doillon, C. and Koutsilieris, M.: Osteoblast-derived survival factors protect PC-3 human prostate cancer cells from adriamycin apoptosis. *Urology*, **52**: 341, 1998
 53. Shimon, I., Taylor, J. E., Dong, J. Z., Bitonte, R. A., Kim, S., Morgan, B. et al: Somatostatin receptor subtype specificity in human fetal pituitary cultures. Differential role of SSTR2 and SSTR5 for growth hormone, thyroid-stimulating hormone, and prolactin regulation. *J Clin Invest*, **99**: 789, 1997
 54. Davies, P. H., Stewart, S. E., Lancranjan, L., Sheppard, M. C. and Stewart, P. M.: Long-term therapy with long-acting octreotide (Sandostatin-LAR) for the management of acromegaly.

- Clin Endocrinol, **48**: 311, 1998
55. Angelsen, A., Syversen, U., Stridsberg, M., Haugen, O. A., Mjølnerod, O. K. R. and Waldum, H. L.: Use of neuroendocrine serum markers in the follow-up of patients with cancer of the prostate. *Prostate*, **31**: 110, 1997
56. Abrahamsson, P. A.: Neuroendocrine differentiation and hormone-refractory prostate cancer. *Prostate*, suppl., **6**: 3, 1996
57. Monti, S., Sciarra, A., Falasca, P. and Di Silverio, F.: Serum concentrations and prostatic gene expression of chromogranin A and PSA in patients affected by prostate cancer and benign prostatic hyperplasia. *J Endocrinol Invest*, suppl., **23**: 53, 2000
58. Sciarra, A., Monti, S., Gentile, V., Mariotti, G., Cardi, A., Voria, G. et al: Variation in chromogranin A serum levels during intermittent versus continuous androgen deprivation therapy for prostate adenocarcinoma. *Prostate*, **55**: 168, 2003
59. Sciarra, A., Mariotti, G., Gentile, V., Voria, G., Pastore, A., Monti, S. et al: Neuroendocrine differentiation in human prostate tissue: is it detectable and treatable? *BJU Int*, **91**: 438, 2003
60. Jongsma, J., Oomen, M. H., Noordzij, M. A., Romijn, J. C., van Der Kwast, T. H., Schroder, F. H. et al: Androgen-independent growth is induced by neuropeptides in human prostate cancer cell lines. *Prostate*, **42**: 34, 2000
61. Segal, G., Cohen, F., Haffejee, Z. and Savage, N.: BCl2 proto-oncogene expression in prostate cancer and its relationship to the prostatic neuroendocrine cell. *Arch Pathol Lab Med*, **118**: 616, 1994
62. Kruithof-Dekker, I. G., Tetu, B., Janssen, P. J. and Van der Kwast, T. H.: Elevated estrogen receptor expression in human prostate stromal cells by androgen ablation therapy. *J Urol*, **156**: 1194, 1996
63. Torlakovic, E., Lilleby, W., Torlakovic, G., Fossa, S. D. and Chibbar, R.: Prostate carcinoma expression of estrogen receptor-beta as detected by PPG5/10 antibody has positive association with primary Gleason grade and score. *Hum Pathol*, **33**: 646, 2002
64. Linja, M. J., Savinainen, K. J., Tammela, T. L., Isola, J. J. and Visakorpi, T.: Expression of ERalpha and ERbeta in prostate cancer. *Prostate*, **55**: 180, 2003