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Variation in Chromogranin A Serum Levels During Intermittent Versus Continuous Androgen DeprivationTherapy for Prostate Adenocarcinoma

Alessandro Sciarra,[1] Salvatore Monti,[2] Vincenzo Gentile,[1] Gianna Mariotti,[1] Antonio Cardi,[1] Giuseppe Voria,[1] Rossana Lucera,[1] and Franco Di Silverio[1]*

[1]*Department of Urology U. Bracci, University La Sapienza, Rome, Italy*
[2]*Endocrinology Department, University La Sapienza, Rome, Italy*

OBJECTIVES. It has been hypothesized that continuous androgen-suppression therapy produces hyperactivation of neuroendocrine (NE) cells and an increase in chromogranin A (CgA) in prostate carcinoma (PC). The aim of this study was to verify whether the intermittent administration of androgen deprivation (IAD) reduces the risk of CgA increase in PC cases treated with complete androgen deprivation (CAD).

MATERIALS AND METHODS. We analyzed changes in serum CgA levels in patients with PC who successfully responded to the first 24 months of IAD versus continuous CAD therapy. Two different populations were analyzed: Type 1 = pT3pN0M0 prostate cancers with biochemical (PSA) progression after RRP; Type 2 = metastatic PC directly submitted to CAD. Cases in Type 1 and Type 2 population were randomly assigned to IAD versus continuous CAD therapy. Forty cases each in Type 1 and Type 2 population were included in the analysis. At 1, 3, 6, 12, 18, 24 months of IAD versus continuous therapy, serum levels of CgA compared to PSA levels were analyzed.

RESULTS. In population Type 1 and Type 2, in the group of cases continuously treated with CAD (Group 2), there was a significant trend to increase for CgA levels from baseline to 24 months of therapy. On the contrary, no significant variations were found in cases treated with IAD (Group 1). Either in population Type 1 or Type 2, at 12- and 24-month follow-up, mean and median serum levels of CgA were significantly ($P < 0.005$) lower in Group 1 than in Group 2.

CONCLUSIONS. The present study represents the first evidence in the literature that the intermittent administration of CAD therapy significantly reduces the increase in serum CgA levels during CAD therapy. *Prostate 55: 168–179, 2003.* © 2003 Wiley-Liss, Inc.

KEY WORDS: **prostate adenocarcinoma; neuroendocrine; chromogranin A; intermittent androgen deprivation**

Sciarra Alessandro (Assistant Professor in Urology, Department of Urology U. Bracci, University La Sapienza, Rome, Italy); Monti Salvatore (Endocrinologist, Endocrinology Department, University La Sapienza, Rome, Italy); Gentile Vincenzo (Associated Professor in Urology, Department of Urology U. Bracci, University La Sapienza, Rome, Italy); Mariotti Gianna (Urologist, Department of Urology U. Bracci, University La Sapienza, Rome, Italy); Cardi Antonio (Resident in Urology, Department of Urology U. Bracci, University La Sapienza, Rome, Italy); Voria Giuseppe (Resident in Urology, Department of Urology U. Bracci, University La Sapienza, Rome, Italy); Lucera Rossana (Resident in Urology, Department of Urology U. Bracci, University La Sapienza, Rome, Italy); and Di Silverio Franco (Chief Professor in Urology, Department of Urology U. Bracci, University La Sapienza, Rome, Italy).

*Correspondence to: Franco Di Silverio, Department of Urology ''U. Bracci,'' University La Sapienza, V. Policlinico, 00161 Rome, Italy. E-mail: sciarrajr@hotmail.com

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INTRODUCTION

In recent years, increasing attention has been focalized on neuroendocrine (NE) differentiation of prostate adenocarcinoma and, in particular, on its possibile clinical significance [1–3].

Serum levels of NE markers, particularly Chromogranin A (CgA), could reflect the NE activity of prostate carcinoma and could be used during follow-up evaluation. CgA appears to be the best marker of NE activity in the prostate [4,5].

It has been stressed that prostate carcinomas with NE differentiation tend to be more aggressive and resistant to hormonal therapy [4–8].

Moreover, some studies have shown that the number of NE tumor cells [9] and CgA serum levels increase during hormonal therapy [10–12] for prostate cancer. Ahlgren et al. [12] studied the extent of NE differentiation in prostate cancers submitted to radical prostatectomy (RRP) after 3 months' hormonal treatment. Both the number of CgA positive cells and the proportion of NE positive tumors were significantly greater ($P < 0.003$) in the neoadjuvant treated group than in an untreated control group.

The hypothesis we considered is: if CgA expression reflects NE activity in the prostate, continuous androgen suppression therapy would seem to produce a hyperactivation of NE cells in the prostate. This may be one of the mechanisms used by prostate cancer to progress to an androgen independent tumor during hormone therapy. We suggest that different types of hormone therapy may influence CgA levels and NE differentiation in prostate cancer differently.

Intermittent androgen deprivation (IAD) is proposed in prostate cancer patients to delay the time to tumor progression due to castration therapy resistance [13].

IAD therapy has been proposed not only as monotherapy in patients with advanced prostate cancer but as Tunn [14,15], Bruchowsky, and Di Silverio [16–18] have suggested, also as an attractive option for the treatment of men with prostate cancer who have failed radical prostatectomy or radiation therapy as demonstrated by a progressive increase in prostate specific antigen (PSA) levels.

Therefore, the aim of this study is to verify whether the intermittent administration of androgen deprivation reduces the risk of serum CgA increase in prostate cancer cases treated with hormone therapy.

In this study, we attempted to analyze the direct effect of two different modalities of androgen deprivation therapy on CgA levels. We did not analyze the effect of the response to therapy on CgA levels and the possible association of CgA levels to tumor progression during therapy, and for this reason, we considered only cases that successfully responded to hormone therapies.

MATERIALS AND METHODS

We prospectively analyzed changes in serum CgA levels in patients with prostate adenocarcinoma who successfully responded to the first 24 months of IAD therapy versus continuous hormone-therapy. In both groups, complete androgen deprivation (CAD) using LHRH agonists (triptorelin 3.75 mg) in combination with an antiandrogen (flutamide 250 mg every 8 hr) was performed.

Experimental Design

The study objectives called for a design that would detect a statistically significant difference between measures (months 24 versus baseline) of 25% at $P \leq 0.05$ with a power 90% (Type II or beta error of 0.1). The effect size seemed to be clinically reasonable. Using standard power analysis methods, a sample size of 20 subjects in each group of therapy (IAD versus continuous CAD) was estimated.

Subject Selection

Two different populations were analyzed: Type 1 = pT3pN0M0 prostate adenocarcinomas with biochemical (PSA) progression after RRP; Type 2 = metastatic prostate carcinomas (M1)(TNM 1997) [19] directly submitted to CAD. In each population, cases were randomly assigned to IAD versus continuous therapy.

We describe the study design and the Materials and Methods for each of the two populations considered.

Population type 1. A clinical protocol of IAD in men with biochemical failure after RRP was started in January 1994 [17].

Inclusion into this study was based on the following criteria:

- No preoperative hormone or radiation therapy.
- RRP with regional lymphadenectomy at our institution.
- Histologically proven adenocarcinoma of the prostate.
- Pathological stage pT3pN0 (TNM 1997) [19].
- Only biochemical failure after surgery (no later than 12 months after RRP).

Between January 1994 and December 1998, 306 patients with clinically localized prostate cancer underwent RRP and bilateral pelvic lymphadenectomy at our institution; a total of 62 patients fulfilled the inclusion criteria and accepted to be randomly assigned to IAD versus continuous CAD therapy. None of these

cases presented a history of other disorders or therapies or conditions known to interfere with CgA levels (NE malignancies, previous or concomitant other neoplastic history, adrenal "incidentalomas," endocrine diseases, other endocrine malipulation therapies, uncontrolled blood hypertension).

Fifthyeight out of the 62 cases (93.5%) responded to their first 12 weeks of CAD and they achieved the baseline for randomization (Fig. 1).

In the present study, we analyzed the first 20 consecutive cases for each group of randomization (20 cases in IAD versus 20 cases in continous CAD) that successfully responded to the first 24 months of treatment.

Population type 2. A clinical protocol of IAD in metastatic prostate cancer was started in January 1994 (unpublished data).

Between January 1994 and December 1998, 148 prostate cancer cases with distant metastases (M1; TNM 1997) [19] were detected and followed up in our Department. This group was drawn from a larger population referred for therapy of histologically confirmed prostate cancers.

Inclusion into this protocol was based on the following criteria:

- Histological diagnosis of prostate adenocarcinoma obtained at prostate biopsy.
- No previous surgery on the prostate gland, radiation, or hormone therapies.
- Distant metastases evidenced with a positive total body bone scan.

A total of 125 cases satisfied the inclusion criteria and accepted to be randomly assigned to IAD versus continuous CAD therapy. All cases were consecutively obtained from the list of our department. None of these cases showed a history of other disorders or therapies or conditions known to interfere with CgA levels (NE malignancies, previous or concomitant other neoplastic history, adrenal "incidentalomas," endocrine diseases, other endocrine malipulation therapies, uncontrolled blood hypertension).

Seventy-three out of the 125 cases (58.4%) responded to their first 12 weeks of CAD and they achieved the baseline for randomization (Fig. 2).

In the present study, we analyzed the first 20 consecutive cases for each group of randomization (20 cases in IAD and 20 cases in continuous CAD) that successfully responded to the first 24 months of treatment.

Patients selected for the study were randomly assigned to continuous CAD or IAD therapy. In alla

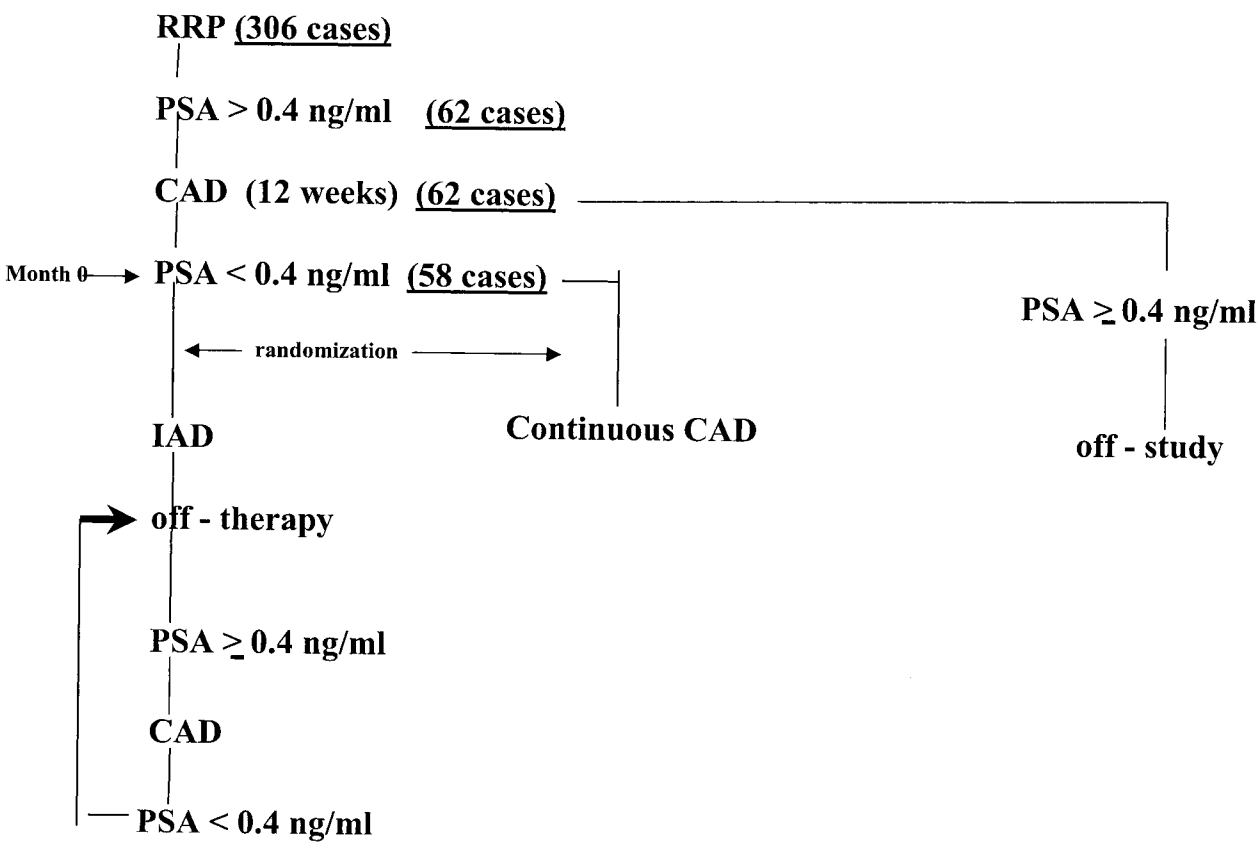

Fig. 1. Study design in population Type I.

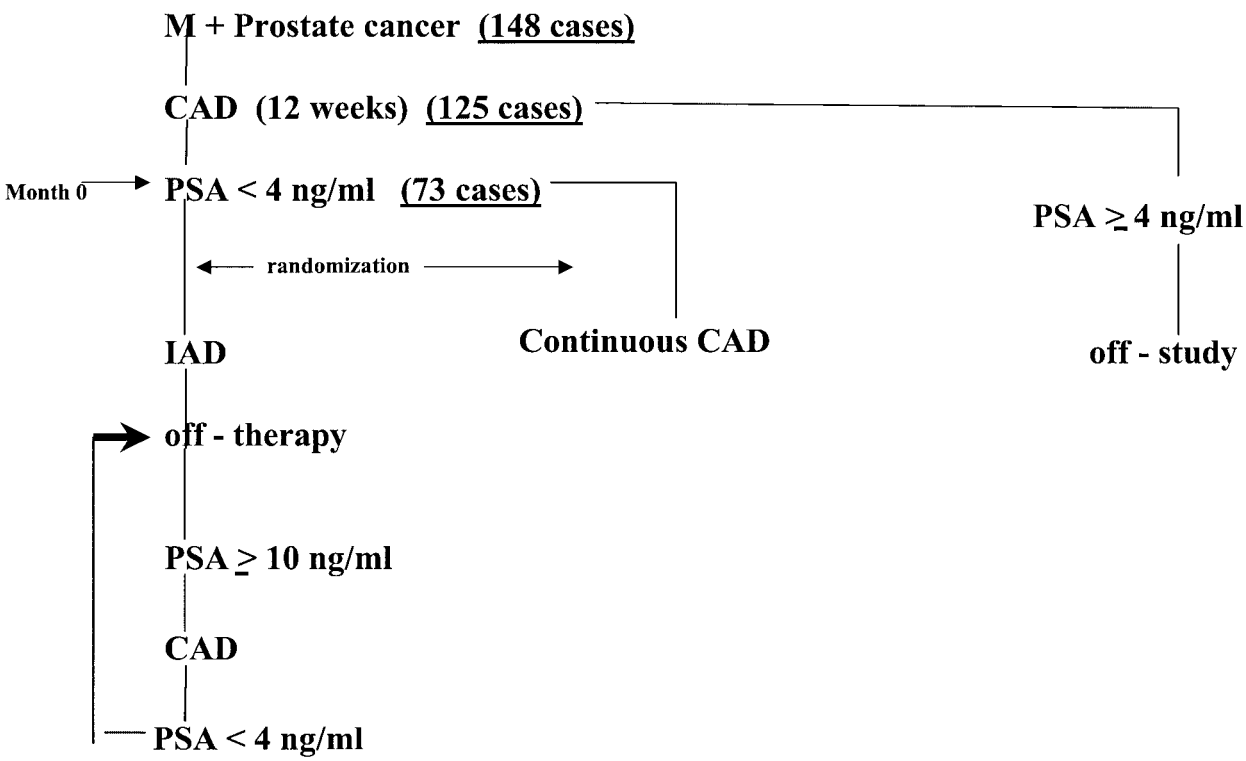

Fig. 2. Study design in population Type 2.

cases, signed informed consent was obtained prior to the study.

Experimental Protocol

Population type 1—study design. Signed informed consent was obtained in all cases prior to the study. After RRP, when serum PSA levels exceeded 0.2 ng/ml, PSA determinations were repeated at 2-weekly intervals. In this protocol, patients were admitted to the study once PSA progressed over 0.4 ng/ml. PSA progression was defined as three or more consecutive elevated PSA levels (over 0.4 ng/ml).

The study design is illustrated in Figure 1. In all cases, the initial treatment period with CAD was limited to 12 weeks. After this period, an acceptable nadir was considered to be a serum PSA level of less than 0.4 ng/ml, stable or decreasing. Patients were then randomly assigned to continuous versus intermittent CAD therapy.

In the IAD group (Group 1), CAD was then withheld until serum PSA increased to a value of $\geq$0.4 ng/ml, (the ''off'' treatment phase). The subsequent ''on'' treatment phases lasted for the time needed to reach the nadir PSA level again with a stable or decreasing value.

In Group 1, during the first cycle of treatment, serum PSA levels were measured every 4 weeks during the ''on'' treatment period and weekly during the ''off'' treatment period. From the second cycle, PSA levels were measured every 4 weeks both during the ''off'' and ''on'' treatment periods. IAD treatment was considered to have failed when the patient was no longer able to cycle ''off'' treatment or in case of clinical progression. These patients were eligible for further systemic or local palliative treatments as considered appropriate by the treating physician.

In Group 2, PSA levels were measured every 4 weeks for the first year of treatment and then every 8 weeks for the following period. In Group 2, treatment was considered to have failed when PSA increased to over 0.4 ng/ml despite continuous CAD or in the case of clinical progression.

In both Group 1 and Group 2, a biopsy of the urethrovesical anastomosis, an abdominal-pelvic magnetic resonance, and a total body bone scan were performed in all cases at 12-monthly intervals during follow-up or upon detection of therapy failure to determine clinical progression of the disease.

At baseline (PSA progression after RRP), month 0 (after 12 weeks of CAD), and after randomization at 1, 3, 6, 12, 18, 24-month interval of therapy, serum levels of CgA were analyzed. Moreover, in the IAD treated group, blood samples for the determination of CgA and testosterone were also obtained at the end of either the ''off'' or the ''on'' phase of each cycle of therapy to analyze variations between ''on'' and ''off'' phases.

Population type 2—study design. In all 40 patients, the initial treatment period with CAD was limited to 12 weeks. After this period, an acceptable nadir was considered to be a serum PSA level of less than 4 ng/ml, stable or decreasing. Patients were then randomly assigned to intermittent (Group 1) versus continuous CAD (Group 2) therapy.

In the IAD group (Group 1), CAD was then withheld until serum PSA increased to a value of $\geq$10 ng/ml (the ''off'' treatment phase). The subsequent ''on'' treatment phases lasted for the time needed to again reach the nadir PSA level of less than 4 ng/ml with a stable or decreasing value. During the first treatment cycle, PSA levels were measured every 4 weeks in the ''on'' treatment period and weekly in the ''off'' treatment period. Starting from the second cycle, PSA levels were measured every 4 weeks both in the ''off'' and ''on'' treatment periods.

In Group 2, patients continuously treated with CAD were followed and PSA levels were measured every 4 weeks for the first year of treatment and, therefore, every 8 weeks for the following period.

In both Group 1 and Group 2, a total body bone scan was performed in all cases at baseline and at 6-month intervals during follow-up or upon the detection of PSA progression in order to determine clinical progression of the disease. A total body magnetic resonance was performed at baseline and at 1-year intervals.

In Group 1, treatment was considered to have failed when the patient was no longer able to cycle ''off'' treatment or in the case of clinical progression (new distant metastases). In Group 2, treatment was considered to have failed when PSA progressed during therapy or in the case of clinical progression (new distant metastases). PSA progression was defined as two consecutive (at weekly intervals) elevated PSA levels over 4 ng/ml.

At baseline (before starting CAD therapy), month 0 (after 12 weeks of CAD), and after randomization at 1, 3, 6, 12, 18, 24-month intervals of therapy, serum levels of CgA were analyzed. Moreover, in the IAD treated group, blood samples for the determination of CgA were also obtained at the end of either the ''off'' or the ''on'' phase of each cycle of therapy to analyze variations between ''on'' and ''off'' phases.

Serum CGA

CgA was measured in serum samples by RIA using a commercial kit (CIS bio International, Cedex, France). The detection limit of this kit was 1.5 ng/ml. The inter-assay and intra-assay coefficient of variation of CgA assay was 5.8% and 3.8%, respectively. Normal reference value for CgA assay was $<$80 ng/ml.

In each case, the same serum sample was also used to determine PSA levels (Hybritech Inc., San Diego, California). Testosterone levels were measured using commercially available RIA diagnostic kits (Testo CT2 Kit, Schering-Plough). All samples were evaluated centrally in the laboratory of our University. Each blood sample was collected in the early morning after an overnight fast. Serum samples were immediately frozen and stored at -20°C until analysis.

Statistical Methods

Statistical analyses were carried out using the statistical package of SPSS 10.0.7.

Descriptive statistics were used to characterize the different parameters (mean, median, range). Differences between mean values were assessed using Student's *t*-test.

Between-Therapy Group differences (IAD versus continuous CAD) were tested using repeated measures analysis of variance (r-MANOVA). To this extent, the Machuly sphericity test was also performed to test the spherical form of the common covariance matrix and to make valid the F-test and associated *P* values for the within-subject factors. The degree of association among the different variables was determined using the Pearson's r correlation test, validated by the Spearman's rank and Kendal tau correlation tests.

RESULTS

PopulationType I

Clinical and pathological characteristics of the 40 patients are described in Table I. All 40 cases exhibited a primary postoperative decrease in serum PSA to below the detection limit after RRP (0.2 ng/ml). These 40 patients were admitted to the study once PSA levels progressed over 0.4 ng/ml. All patients showed an early PSA progression, no later than 9 months from RRP. In all cases, a total body bone scan, abdominal–pelvic magnetic resonance, and a biopsy taken at the urethrovesical anastomosis upon the detection of PSA progression proved negative. All 40 cases successfully responded to the first 24 months of therapy with no clinical evidence of local recurrence or disease progression.

We limited our analysis to PSA and CgA variations during these first 24 months: following this period, patients continued therapy. No significant difference in the baseline characteristics and in serum PSA and CgA levels between Group 1 (IAD) and Group 2 (continuous CAD) were found ($P > 0.05$) (Table I). Moreover, at randomization after the first 12 weeks of CAD that all 40 cases performed (time 0), no significant differences in serum PSA and CgA levels between Group 1 and Group 2 were found ($P > 0.05$) (Table I).

Cycling characteristics of the 20 patients included in Group 1 and mean and median PSA and CgA

TABLE I. Clinical and Pathological Characteristics of the 40 Cases Included as PopulationType I

	Group 1 (IAD)	Group 2 (continuous CAD)	*P* value
N. cases	20	20	
Age (yrs)	64.9 ± 3.4 (65); 56–70	65.4 ± 3.1 (66); 58–70	0.691
Pathological stage at RRP			
T3a N0 (n°)	12	12	
T3b N0 (n°)	8	8	
Gleason score at RRP			
6 (3 + 3)	3	3	
7 (3 + 4)	6	4	
7 (4 + 3)	9	11	
8 (5 + 3)	2	2	
Surgical margins			
Negative	20	20	
Pre-RRP PSA (ng/ml)	11.80 ± 2.56 (11.0); 8.0–16.50	11.12 ± 2.46 (12.5); 8.0–16.0	0.689
PSA after RRP (ng/ml)	0.12 ± 0.02 (0.15); 0.10–0.15	0.10 ± 0.02 (0.15); 0.05–0.15	0.095
Time to PSA progression after RRP (months)	5.20 ± 1.20 (5); 3–9	4.80 ± 1.90 (5); 3–9	0.431
PSA at baseline (ng/ml) (progression after RRP)	1.20 ± 0.40 (1.20); 0.70–1.70	1.30 ± 0.36 (1.30); 0.80–1.90	0.411
PSA at randomization (ng/ml) (after 12 weeks of CAD) (time 0)	0.14 ± 0.04 (0.13); 0.10–0.20	0.16 ± 0.04 (0.15); 0.10–0.20	0.122
CgA at baseline (ng/ml) (progression after RRP)	36.60 ± 6.82 (37.50); 27–50	38.10 ± 6.88 (38.40); 26–52	0.493
CgA at randomization (ng/ml) (after 12 weeks of CAD) (time 0)	45.80 ± 6.93 (47.30); 35.0–58.0	42.90 ± 6.03 (45.20); 28.0–50.0	0.166

Statistical significance of differences between Group 1 and Group 2 is reported (*P* values).

variations during the first 4 cycles of IAD are shown in Table II; all these cases completed at least 4 treatment cycles. In all Group 1 cases, during each ''on'' phase of therapy, the serum testosterone dropped into the castration range <1.5 nmol/L. On the contrary, at the end of each ''off'' phase, serum testosterone was higher than 8 nmol/L (range 9.30–19.50 nmol/L) in all cases.

During the first 24 months of follow-up, in none of the 20 patients in Group 1 did serum PSA fail to decrease during the ''on'' treatment period and all bone scans, magnetic resonance results were normal (with no evidence of metastases or local recurrence).

In Group 2, all 20 patients responded to CAD therapy. In all the 20 cases included in Group 2 at each interval of the 24-month follow-up, PSA serum levels were less than 0.4 ng/ml and none of these cases showed clinical progression.

Serum CgA analysis. CgA variation during 24 months of IAD versus continuous CAD therapy and differences between groups are shown in Figure 3. The results of the repeated measures of analysis of variance ($F = 12.190$; $P = 0.001$; power = 0.925) support the hypothesis that a statistically significant difference exists between IAD and continuous CAD therapy on the overall CgA measurements.

In particular, the linear regression analysis showed that, in Group 2, there was a significant trend for CgA levels to increase from baseline to 24 months of therapy ($\alpha = 38.42$; $\beta = 1.75$; $P = 0.0001$). On the contrary, no significant variations were found in Group 1 ($\alpha = 40.23$; $\beta = -0.22$; $P = 0.425$).

At the 12-month follow-up after randomization, mean and median serum levels of CgA were significantly ($P = 0.004$) lower in Group 1 (40.20 ± 8.66; median 40.25 ng/ml) than in Group 2 (49.15 ± 10.03; median 48.20 ng/ml). This difference increased at the 24 months' follow-up (Group 1: 40.0 ± 8.40 median 41.25; Group 2: 57.50 ± 9.38 median 56.40 ng/ml; $P = 0.0001$).

Furthermore, the median increase in serum CgA levels from baseline to the 12- and 24-month interval of therapy was lower in Group 1 (baseline-12 months: 8.1%, range from −9% to +20%; baseline-24 months: 10.8%, range from −10% to +24%) than in Group 2 (baseline-12 months: 26.3%, range 14–60%; baseline-24 months: 47.4%, range 28–93%) ($P < 0.001$).

However, either at baseline or during therapy, all mean and median CgA levels remained into the normal reference values for our assay.

In Group 1, no significant ($P > 0.05$) difference in CgA serum levels between ''on'' and ''off'' therapy phases was found from cycle 2 to cycle 4 (Table II).

TABLE II. Cyclic Characteristics of the 20 Cases Included as Group I in PopulationType I

	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Median length of cycle (weeks)	20	24	28	32
Time ''off'' therapy (weeks)	7.10 ± 1.70 (8); 5–10	13.80 ± 1.80 (14); 12–16	18.10 ± 1.70 (18); 16–20	22.60 ± 1.0 (22); 20–24
Time to PSA nadir during ''on'' therapy (weeks)	10.0 ± 1.80 (8); 4–12	9.50 ± 1.20 (10); 8–12	10.20 ± 1.80 (10); 8–12	10.0 ± 1.60 (10); 8–12
Nadir PSA during ''on'' therapy (ng/ml)	0.14 ± 0.04 (0.13); 0.10–0.20	0.17 ± 0.07 (0.20); 0.10–0.30	0.12 ± 0.03 (0.20); 0.10–0.20	0.18 ± 0.07 (0.20); 0.10–0.30
Highest PSA level during ''off'' therapy (ng/ml)	1.26 ± 0.20 (1.40); 0.80–1.80	1.38 ± 0.22 (1.40); 1.0–1.80	1.22 ± 0.20 (1.20); 0.80–1.50	1.32 ± 0.24 (1.20); 0.80–1.80
Testosterone levels during ''on'' therapy (nmol/L)	0.78 ± 0.20 (0.80); 0.60–1.40	0.70 ± 0.15 (0.65); 0.60–1.20	0.60 ± 0.18 (0.60); 0.40–1.20	0.67 ± 0.20 (0.65); 0.40–1.20
Testosterone levels during ''off'' therapy (nmol/L)	12.28 ± 2.04 (12.40); 9.30–16.50	14.48 ± 2.03 (14.80); 9.30–18.70	15.40 ± 2.87 (15.90); 10.0–19.50	15.56 ± 1.96 (15.80); 9.50–19.0
CgA during ''on'' therapy (ng/ml)	45.80 ± 6.93 (47.30); 35.0–58.0	40.16 ± 5.38 (39.0); 30.40–53.70	40.22 ± 5.68 (40.45); 27.30–56.80	41.25 ± 5.16 (41.30); 29.30–52.70
CgA during''off'' therapy (ng/ml)	40.74 ± 5.12 (41.40); 27.20–48.60	38.76 ± 6.14 (38.40); 26.50–50.40	39.16 ± 5.63 (39.20); 27.40–48.20	39.65 ± 5.84 (40.30); 28.80–48.30
Statistical significance of differences in CgA levels between ''on'' and ''off'' phase (*P* value)	0.012	0.448	0.557	0.356

Testosterone and CgA levels were determined at the end of each ''off'' and ''one'' phase.

Fig. 3. Analysis of variance repeated measures analysis of variance (r-MANOVA). Population Type I: median Chromogranin A (CgA) variations during 24 months of intermittent administration of androgen deprivation (IAD) (Group I) versus continuous complete androgen deprivation (CAD) therapy (Group 2). Normal reference values for our assay are indicated.

In both Group 1 and Group 2, at each interval of follow-up, a no significant association between CgA and PSA levels during therapy was found($r < 0.200$; $P > 0.05$); CgA level increase was independent of PSA variations.

If we classify patients on the basis of Gleason score (Gleason score < 7 versus Gleason score ≥ 7), at 12 and 24-months' follow-up, a significant difference in serum CgA levels between Group 1 and Group 2 was found either in cases with a Gleason score ≥ 7 tumors (12 months; $P = 0.0001$; 24 months: $P = 0.0001$) or in Gleason score < 7 (12 months: $P = 0.0001$; 24 months: $P = 0.0001$) (Table III).

PopulationType 2

Clinical characteristics of the 40 patients are described in Table IV. All 40 cases had fewer than three metastatic sites on bone scan.

Forty cases successfully responding to the first 24 months of IAD versus continuous CAD therapy are analyzed. We limited our analysis to PSA and CgA variation during the first 24 months: following this period, patients continued therapy.

No significant differences in the baseline characteristics and serum PSA and CgA levels between Group 1 and Group 2 were found ($P > 0.05$) (Table IV). Moreover, at randomization after the first 12 weeks of CAD that all 40 cases underwent (time 0), no significant differences in serum PSA and CgA levels between Group 1 and Group 2 were found ($P > 0.05$) (Table IV).

TABLE III. PopulationType I: Serum CgA Levels According to Gleason Score in Group I and Group 2 Cases

		Baseline	12 months	24 months
Group 1	Gleason score < 7	29.80 ± 2.28 (30.40)	30.40 ± 5.55 (30.20)	31.20 ± 6.87 (30.15)
Group 1	Gleason score ≥ 7	38.87 ± 6.29 (40.20)	40.47 ± 6.88 (41.40)	42.93 ± 6.75 (43.50)
Group 2	Gleason score < 7	31.20 ± 2.28 (32.20)	43.80 ± 5.02 (45.40)	53.24 ± 4.35 (52.30)
Group 2	Gleason score ≥ 7	40.40 ± 6.33 (40.10)	50.93 ± 10.76 (50.30)	59.43 ± 10.39 (57.40)
Group 1 versus Group 2	Gleason score < 7: (P value)	0.060	0.0001	0.0001
Group 1 versus Group 2	Gleason score ≥ 7: (P value)	0.448	0.0001	0.0001

Statistical significance of differences between Group 1 and Group 2 is reported (P value).

Cycling characteristics of the 20 patients included in Group 1 and mean and median PSA and CgA variations during the first three cycles of IAD are shown in Table V; all these cases completed at least three treatment cycles. During the first 24 months of follow-up, in none of the 20 cases in Group 1 did serum PSA fail to decrease during the ''on'' treatment period and no variation at all bone scans and magnetic resonances were found.

In Group 2, all 20 cases responded to CAD therapy. In all cases included in Group 2, at each interval of the 24 months' follow-up, PSA serum levels were less than 4 ng/ml and none of these cases showed further clinical progression (new metastatic sites).

Serum CgA analysis. At 12 and 24 months of therapy, mean or median CgA levels were higher than normal reference values for our assay only in Group 2.

CgA variations during 24 months of IAD versus continuous CAD therapy and differences between groups are shown in Figure 4. The results of repeated measures analysis of variance ($F = 3.242$; $P = 0.080$; power = 0.419) demonstrate that, in the population Type 2, a statistically significant difference between IAD and continuous CAD therapy on the overall CgA measurements is not reached.

However, the linear regression analysis showed that in Group 2, there was a significant trend for CgA levels to increase from baseline to 24 months of therapy ($\alpha = 72.36$; $\beta = 3.5$; $P = 0.0001$) whereas no variations were found in Group 1 ($\alpha = 83.18$; $\beta = -0.88$; $P = 0.347$). At 12 months' follow-up after randomization, mean and median serum levels of CgA were significantly ($P = 0.021$) lower in Group 1 (78.35 ± 14.21; median 76.30 ng/ml) than in Group 2 (91.95 ± 20.95; median 93.20 ng/ml). This difference increased at the 24-month follow-up (Group 1: 77.70 ± 19.13, median 74.30 ng/ml; Group 2: 101.95 ± 26.16, median 102.40 ng/ml; $P = 0.002$).

Also, the increase in serum CgA levels from baseline to the 12- and 24-month follow-up was lower in Group 1 (baseline-12 months: 5.5%, range from -2% to $+14\%$; baseline-24 months: 2.8%, range from -1% to $+14\%$) than in Group 2 (baseline-12 months: 31%, range 12–

TABLE IV. Clinical Characteristics of the 40 Cases Included as PopulationType 2

	Group 1 (IAD)	Group 2 (continuous CAD)	P value
N. cases	20	20	
Age (yrs)	72.4 ± 3.5 (72); 67–77	72.8 ± 2.7 (73); 68–77	0.760
Clinical stage			
T3 N0 M1	20	20	
Gleason score			
6 (3 + 3)	4	4	
7 (3 + 4)	5	4	
7 (4 + 3)	8	10	
8 (5 + 3)	3	2	
Pre-treatment PSA (ng/ml) (at baseline)	42.25 ± 13.20 (39.50); 22.0–65.0	41.35 ± 12.76 (38.40); 24.0–63.0	0.828
PSA at randomzation after 12 weeks CAD (ng/ml) (time 0)	2.45 ± 0.78 (2.50); 1.0–3.80	2.44 ± 0.70 (2.40); 1.30–3.80	0.966
CgA at baseline (ng/ml) (pre-treatment)	73.45 ± 16.77 (72.50); 50.60–110.80	73.40 ± 13.86 (71.30); 54.50–100.30	0.992
CgA at randomization (ng/ml) (after 12 weeks of CAD) (time 0)	89.20 ± 17.0 (86.40); 60.80–130.60	82.80 ± 15.99 (85.20); 58.70–120.20	0.228

Statistical significance of differences between Group 1 and Group 2 is reported (P values).

TABLE V. Cyclic Characteristics of the 20 Cases Included as Group I in Population Type 2

	Cycle 1	Cycle 2	Cycle 3
Median length of cycle (weeks)	24	24	32
Time ''off'' therapy (weeks)	10.90 ± 1.0 (12); 8–12	12.80 ± 2.40 (12); 8–16	14.90 ± 2.50 (16); 8–20
Time to PSA nadir during ''on'' therapy (weeks)	10.20 ± 1.60 (12); 8–12	12.70 ± 2.10 (12); 8–16	16.50 ± 2.80 (16); 8–20
Nadir PSA during ''on'' therapy (ng/ml)	2.40 ± 1.0 (2.50); 1.0–3.80	2.0 ± 0.80 (2.0); 1.0–3.80	2.60 ± 1.0 (2.50); 1.0–3.70
Highest PSA level during ''off'' therapy (ng/ml)	16.20 ± 3.50 (16.50); 11.0–20.10	17.10 ± 3.30 (16.40); 11.10–22.30	15.80 ± 2.80 (15.45); 11.10–20.10
CgA during ''on'' therapy (ng/ml)	89.20 ± 17.0 (86.40); 60.80–130.60	80.35 ± 13.15 (81.60); 60.30–110.50	82.65 ± 16.13 (80.60); 60.40–122.10
CgA during ''off'' therapy (ng/ml)	77.35 ± 12.14 (78.20); 57.60–102.30	76.74 ± 13.24 (76.50); 56.50–100.90	73.40 ± 15.24 (72.60); 53.10–99.70
Statistical significance of differences in CgA levels between ''on'' and ''off'' phase (P value)	0.015	0.343	0.062

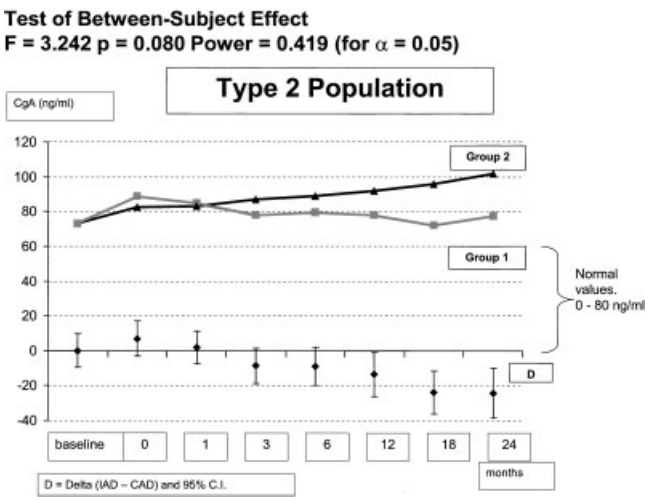

Fig. 4. Analysis of variance (r-MANOVA). Population Type 2: median CgA variations during 24 months of IAD (Group I) versus continuous CAD therapy (Group 2). Normal reference values for our assay are indicated.

46%; baseline-24 months: 43.7%, range 20–64%) ($P < 0.001$).

In Group 1, CgA levels were lower in the ''off'' phases of each cycle, but the difference did not reach significance in cycle 2 and cycle 3 (Table V).

In both Group 1 and Group 2, at each interval of follow-up, a no significant association between CgA and PSA levels during therapy was found ($r < 0.200$; $P > 0.05$): CgA level increase was independent of PSA variations.

If we classify patients on the basis of Gleason score, (Gleason score < 7 versus ≥ 7), at 12 and 24 months' follow-up, a significant difference in serum CgA levels between Group 1 and Group 2 was found either in cases with Gleason score ≥ 7 (12 months: $P = 0.0001$; 24 months: $P = 0.0001$) or Gleason score < 7 (12 months: $P = 0.0001$; 24 months: $P = 0.0001$) (Table VI). The highest increase in serum CgA levels during therapy was found in Group 2—Gleason score ≥ 7 cases (baseline-24 months: 43%) (Table VI).

DISCUSSION

To our knowledge, this represents the first study reported in the literature indicating that the intermittent and continuous administration of androgen deprivation therapy each produce different effects on serum CgA levels.

Jongsma et al. [9] demonstrated that proliferation of prostatic cancer cell lines under the condition of androgen depletion can be modulated by neuropeptides which are known to be produced by NE cells, and the androgen suppression can lead to an induction of NE differentiation in prostate tissue.

TABLE VI. PopulationType 2: Serum CgA Levels According to Gleason Score in Group I and Group 2 Cases

		Baseline	12 months	24 months
Group 1	Gleason score < 7	57.0 ± 4.63 (57.0)	60.50 ± 4.04 (60.20)	59.0 ± 4.78 (60.35)
Group 1	Gleason score ≥ 7	84.42 ± 12.01 (83.20)	90.17 ± 14.80 (88.0)	90.17 ± 13.92 (87.20)
Group 2	Gleason score < 7	58.86 ± 3.29 (60.15)	67.14 ± 1.35 (68.40)	72.14 ± 7.58 (70.0)
Group 2	Gleason score ≥ 7	81.31 ± 10.42 (84.60)	105.31 ± 11.69 (100.20)	118.0 ± 16.04 (120.30)
Group 1 versus Group 2	Gleason score < 7 (P value)	0.151	0.0001	0.0001
Group 1 versus Group 2	Gleason score ≥ 7 (P value)	0.387	0.0001	0.0001

Statistical significance of differences between Group 1 and Group 2 is reported (P value).

Some studies showed that the number of NE tumor cells [9] and CgA serum levels increase with escape of human prostate tumor from hormonal therapy [10,11,12,21].

In a previous study, we analyzed [1] serum concentration and prostate tissue gene expression (RT-PCR) of CgA and PSA in prostate cancer patients submitted to RRP. Patients were stratified on the basis of neoadjuvant CAD therapy (1–3 or 6 months). We found that in prostate cancer treated with CAD for three months or longer, both serum and tissue mRNA levels of CgA were significantly ($P < 0.01$) higher than in the untreated group.

The present study investigates whether the intermittent administration of androgen-deprivation therapy reduces the risk of an increase in CgA levels and hyperactivation of NE prostate cells.

We confirmed the effect of CAD therapy on serum CgA levels and we reported significant differences using IAD therapy.

IAD is proposed in prostate cancer patients to delay the time to tumor progression due to castration therapy resistence [5]. Using IAD therapy, it is possible that the cyclic replacement of androgens during the ''off'' therapy phases reduces the opportunity of a NE selection and serum CgA increase produced by CAD therapy.

Several studies have evidenced that NE differentiation and CgA are significantly expressed in advanced and in poorly differentiated prostate cancers [2,3]. In the present study, we tried to consider the influence of stage and histological grading on CgA level modifications during therapy. In fact, we analyzed two different populations, the first with locally advanced prostate cancer submitted to RRP who showed only biochemical progression after surgery, and the second with metastatic prostate cancer. Higher levels of serum CgA either at baseline or during therapy were found in population Type 2 (metastatic prostate cancer). In particular, as in previous reports (20), in population Type 1 (non metastatic prostate cancer after RRP), at baseline but also after 24 months of therapy, CgA levels remained into the normal reference value for our assay. On the contrary, in population Type 2, at baseline CgA levels were close to or higher than the normal reference value and they significantly increased after 24 months of continuous CAD. However, we underline that, at present, there are not enough data to define a normal range for CgA related to the presence of a prostate adenocarcinoma; all existing ranges refer to the presence of pure NE malignancies or diseases. Interestingly, when analyzing only patients with poorly differentiated tumors, focal NE differentiation represents a more significant factor [22,23]. In this study, poorly differentiated tumors showed higher levels of CgA and at the 24-month interval, the highest difference in serum CgA levels between IAD and continuous therapy was found in cases with Gleason score ≥ 7 ($P < 0.0001$). However, in both populations, most of our cases presented a Gleason score ≥ 7 and the limited number of patients considered reduces the significance of these results according to Gleason score.

In both populations, we considered very favorable cases that successfully responded to hormone therapy. In this way, we try to analyze the direct effect of two different modalities of androgen deprivation therapy on CgA serum levels regardless of the response of the tumor to therapy. It was not our aim to analyze CgA variations in relation to the clinical response of prostate cancer to IAD versus continuous therapy.

In population Type 1, we decided to use a low nadir of PSA to modulated therapy, such as 0.4 ng/ml, as described in the protocol [17]. This has been considered the cut-off for a significant detectable PSA level indicative of residual disease after RRP [24]. Kurek et al. [15] and Tunn [14] used a PSA nadir of 3 ng/ml in patients with PSA progression after RRP treated with IAD.

Also, if the PSA nadir used to modulate therapy was low (population Type 1) and the total period of examination was limited to 24 months, in both populations, the number of cycles of IAD therapy (four cycles in population Type 1 and three cycles in population Type 2) and the median length of each ''on'' and ''off'' phase were comparable with previous studies [14,15] and significant to analyze differences with a contin-

uous administration of CAD therapy. The fact that the duration of IAD cycles and ''off'' phases increased over time confirms that our cases successfully responded to therapy. A similar increase was not reported in all previous experiences on IAD, but less homogeneous populations and not only responding to therapy cases were analyzed [25,26].

Moreover, in comparison with other previous studies on IAD, we decided to use a limited initial period of induction with CAD (only 12 weeks). Other authors started with 24 weeks of CAD before modulating the ''off'' and ''on'' phases of treatment [14,25–27]. A prolonged initial period of induction with CAD therapy in the IAD group could produce a significant increase in CgA serum levels in this initial phase, reducing the possibility of comparing variations of CgA with continuous therapy during the following phases of IAD treatment.

As expected, a lower percentage of cases in population Type 2 than in population Type 1 responded to their first 12 weeks of induction with CAD and achieved baseline for randomization.

The fact that we selected responding populations for our analysis is a positive aspect that increases the significance of our results. In this way, we were able to demonstrate that, even in a favorable group of patients, regardless of the response of the tumor to hormone therapy, continuous administration of CAD, despite stable low PSA levels, produces a significant increase in CgA levels. On the contrary, the intermittent administration of CAD produces no significant increase in serum CgA levels.

Using the analysis of variance, the difference between IAD and continuous CAD therapy on the overall CgA measurements, reached statistically significance only in population Type 1 ($P = 0.001$) but not in population Type 2 (metastatic tumors) ($P = 0.080$). Therefore, a clear evidence for a therapy main effect on CgA levels was obtained only in population Type 1. However, either in population Type 1 or Type 2, a significant trend for CgA levels to increase during continuous CAD but not during IAD (linear regression model) was found.

This difference between IAD and continuous therapy is significant even after only 12 months of therapy. As previously reported [1,12], the effect of androgen deprivation on CgA levels is rapidly obtained and sustained with time. The advantage of a cyclic suspension of therapy is also appreciable over a short-term period.

We also verified that during the first cycle of IAD, the difference in serum CgA levels between ''on'' and ''off'' therapy phases was significant whereas, in the following cycles, no further significant differences were found. Therefore, IAD therapy progressively reduced the effect of CAD on serum CgA levels also during the ''on'' phases of the same therapy.

We underline that the elevations in CgA levels reported in our study are modest if 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 the presence of NE cells with a focal distribution in the common prostatic adenocarcinoma [1]. Therefore, in prostate adenocarcinoma cases, it is not possible to expect the same levels and some variations in CgA than those found in pure NE tumors. However, in our cases continuously treated with CAD, after 24 months of treatment, we found a higher than 40% median increase in serum CgA levels that, in our opinion, it is not only statistically significant but also clinically relevant.

It is important to note that the CgA serum level increase was independent of PSA variations. As reported in previous studies [1,25], no significant correlation was found between PSA and CgA serum levels: CgA may represent a useful index regardless of the role of PSA.

A possible limit of our study is the determination of only serum expression of CgA. Some authors showed that serum levels of CgA could reflect the NE activity of prostate carcinoma and they reported a significant correlation between serum and tissue expression of CgA in prostate cancer [1,4,16]. In the present study, in only a subgroup of 20 cases from population Type 1, prostate tissue samples were previously obtained at RRP and analyzed by RT-PCR for CgA and PSA mRNA expression. This subgroup was not well distributed between cases treated with IAD and continuous therapy because it was determined before randomization of patients. So, for these reasons, we did not include these data in our results. However, the expression of CgA mRNA has been found in all these tissue samples; and a significant association between tissue expression and serum levels of CgA ($r = 0.469$; $P = 0.039$) was confirmed.

CONCLUSIONS

On the basis of our results, we can affirm that the modality of the administration of CAD therapy significantly influences serum CgA levels in prostate cancer patients. In particular, the present study represents the first evidence in the literature that intermittent administration of CAD therapy significantly reduces the increase in serum CgA levels during androgen deprivation.

On the contrary, following prior evidence that supports a significant relationship between serum CgA levels, tissue expression of CgA and NE activity in prostate cancer, we can only hypothesize that IAD

therapy may reduce the risk of NE hyperactivation in prostate cancer during androgen deprivation therapy. After these results, the critical aspect is the demonstration that this increase in CgA levels and the activation of NE cells are associated with the development of progression towards the hormone-independent clinical phenotype of prostate cancer. The fact that continuous hormone-therapy produces a significant increase in CgA serum levels even in patients who successfully respond to therapy may indicate that: (1) serum CgA level increase is not an index for prostate tumor progression but is only the result of the direct effect of hormone-therapy on NE cells; (2) a serum CgA level increase despite stable PSA levels may precede tumor progression.

Any definitive conclusion on the independent value of CgA in patients submitted to CAD and whether IAD enhances progression-free or overall survival of prostate cancer patients, for example, by reducing the hyperactivation or the selection of NE prostate cells during hormone therapy, has to be ascertained in larger prospective randomized and long-term clinical trials.

It will be interesting to verify whether our cases having a significant increase of CgA during therapy will present earlier clinical progression than cases with stable CgA values.

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