Randomized Controlled Clinical Trial of a Combination of Somatostatin Analog and Dexamethasone Plus Zoledronate *vs.* Zoledronate in Patients with Androgen Ablation-refractory Prostate Cancer

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Abstract. *Background: As previously shown, the combination of standard androgen ablation therapy with somatostatin analog and dexamethasone in metastatic androgen ablation-refractory (stage D_3) prostate cancer (PrCa) patients has a favorable profile of side-effects, durable objective antitumor activity (up to 60% partial response rate) and palliative effects. Bisphosphonates interfere with bone remodeling at the sites of PrCa bone metastases and have been postulated to have indirect and/or direct anti-PrCa activity. Materials and Methods: A randomized controlled clinical trial was conducted to compare a combination of somatostatin analog (octreotide 20 mg i.m. every 28 days) and oral dexamethasone (4 mg daily for 1 month, gradually reduced to 1 mg daily by the fourth month, with a 1 mg daily maintenance dose thereafter) plus zoledronate (4 mg i.v. every 4 weeks) vs. zoledronate only. All patients in both arms remained in basic androgen blockade throughout the study. Results: Thirty-eight stage D_3 patients (mean age 72.8±6.8 years) were randomized to either treatment arm of the study. The trial was stopped after a pre-specified interim analysis met the criteria for early closure, i.e. significant difference in outcomes between the two treatment arms. Partial responses (PR, ≥50% PSA decline) were observed in 13 out of 20 patients with combination therapy vs. none with zoledronate. The combination therapy arm had significantly better outcome with respect to median progression-free survival (7.0 vs. 1.0 months, $p<0.0001$), median overall survival (OS) (12.0 vs. 9.0 months, $p=0.0027$), median PrCa-specific OS (defined as time from onset of therapy until PrCa-related death) (16 vs. 9.0 months, $p=0.0005$) and median duration of bone pain improvement (>14 vs. 4 months $p=0.00001$ by log-rank tests). Conclusion: For androgen ablation-refractory metastatic PrCa patients under androgen ablation, the combination of dexamethasone, somatostatin analog and zoledronate offered superior objective and palliative clinical responses than zoledronate alone.*

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Metastatic prostate cancer (PrCa) is almost always responsive to androgen ablation therapy, but eventually becomes hormone-refactory (stage D_3), with a median overall survival of <12 months, even with the administration of salvage cytotoxic chemotherapy (1-3). Interestingly, disease progression from hormonal therapy occurs predominantly in bone metastases, even in those cases when androgen ablation still offers sustained control of the disease at the primary site or non-skeletal metastases (4-10). The reason why bones are preferential sites for development of PrCa metastases and for establishment of drug-resistance is primarily attributed to cytokines and growth factors (collectively termed "survival factors") which are released by normal cellular constituents of the bone microenvironment and protect metastatic PrCa against the induction of apoptosis by antitumor agents, such as androgen ablation or cytotoxic chemotherapy (11-16). Insulin-like growth factor-1 (IGF-1) is a major survival factor for bone metastatic PrCa cells, which are exposed not only to high levels of liver-derived IGF-1 from the circulation, but also to increased local IGF-1 bioavailability in the microenvironment of bone metastases (11, 16, 17). In order to abrogate the protective

effect of IGF-1 or other "survival factors" on cancer cells, a combination regimen, which aimed at suppressing the bioavailability of IGF-1 and the activity of its downstream molecular effects, was developed. This regimen involves administration of somatostatin analog (SM-A), which reduces the growth hormone (GH)-dependent IGF-1 production, mainly in the liver (18) and oral dexamethasone (Dex), which suppresses the GH-independent increase of IGF-1 bioavailability in the bone metastases (19, 20), in combination with luteinizing hormone-releasing hormone analog (LHRH-A). This combination therapy was first studied in a case series of stage D_3 PrCa patients and led to encouraging preliminary results (21, 22), which were later confirmed in phase II studies (23) and in randomized clinical trials in comparison to salvage chemotherapy (24).

Zoledronate and other bisphosphonates interfere with the bone remodeling process in the sites of metastatic bone disease, preventing bone loss and reducing the rates of skeletal events associated with prolonged androgen ablation therapy (25, 26), while pre-clinical data also suggest direct antitumor effects in PrCa (27-29). Herein, considering these properties, and since zoledronate is the most potent clinically available bisphosphonate, we compared the objective clinical and palliative responses of stage D_3 PrCa patients receiving either surgical (orchiectomy) or pharmacological (LHRH-A) androgen ablation therapy to either zoledronate or the combination of zoledronate with SM-A and Dex.

Materials and Methods

Study population. Thirty-eight patients with androgen ablation-refractory (stage D_3) metastatic PrCa were prospectively evaluated for enrollment in our clinical trial after providing written informed consent. All enrolled patients were maintained in basic androgen blockade, either due to prior orchiectomy or continuation of LHRH-A therapy, for the duration of participation and follow-up in this study). All the patients had bony lesions at the time of original diagnosis of metastatic disease (stage D_2), and subsequently received combined androgen blockade (CAB; LHRH-A or orchiectomy plus anti-androgen: flutamide 750 mg three times daily or bicalutamide 50 mg daily). Following progression to stage D_3, all the patients, underwent anti-androgen withdrawal manipulation, while remaining on basic androgen ablation therapy. Furthermore, before enrolling on this trial, all the patients had failed salvage chemotherapy, while some had received radionuclide therapy with strontium-89 (four and two patients in the combination *vs.* zoledronate group, respectively) or rhenium-186 HEDP (three patients in the zoledronate) (Table I). Finally, estrogen-based hormonal therapy was administered to two patients in the combination group, respectively. At study entry, and for at least 3 months prior to randomization and the initiation of treatment, all patients had shown steadily increasing PSA values (confirmed by monthly measurements showing a >10 ng/mL net increase over the PSA nadir during hormonal therapy) and progressive deterioration of performance status. All the patients had diffuse bony lesions (>6 foci in bone scan) at the time of study entry.

Study design. The patients were randomly assigned to receive either zoledronate (4 mg *i.v.* every 4 weeks) or a combination of zoledronate with SM-A (octreotide 20 mg *i.m.* every 28 days) and oral Dex (4 mg daily for the first month of treatment, gradually reduced to 1 mg daily by the fourth month, with a 1 mg daily maintenance dose thereafter). All the participating patients were followed-up on an out-patient basis, according to the principles of the Declaration of Helsinki (30) and a protocol reviewed and approved by the local Ethics committee.

The concomitant presence of another malignancy and life expectancy of <3 months were criteria for exclusion. No patients were excluded from this study on the basis of cardiopulmonary, renal, gastrointestinal dysfunction or diabetes. No patients had evidence of measurable soft tissue metastases (except for lymph nodes), as assessed by computerized tomography (CT) scan. All the patients were followed-up with clinical and biochemical work-up at monthly intervals, while the repeat imaging work-ups (CT and/or bone scans) were performed every 6-12 months.

In view of the effects of SM-A on pancreatic function (31) and of DEX on blood glucose (21, 22), all patients in the combination therapy arm received instructions to appropriately modify their diet with regard to the intake of lipids (especially the day before and on the day of SM-A injections, to minimize gastrointestinal discomfort) and carbohydrates. Blood glucose levels were monitored bi-weekly during the first 3 months of therapy and monthly thereafter. For six patients, with known prior medical histories of diabetes, the oral anti-diabetic drug dose was increased, especially during the initial period of combination therapy, when DEX was administered at daily doses of 4, 3 and 2 mg during the first, second and third months of follow-up, respectively. Antacid therapy was prophylactically offered to two patients with known medical histories of chronic gastritis/peptic ulcer or gastro-esophageal reflux disease.

The clinical responses to treatment were defined, according to previously published criteria (22, 23), as either: i) progressive disease (PD) (progressive increase of PSA by ≥25% from baseline, for at least two consecutive measurements, and/or steady deterioration of pain score and performance status); ii) partial response (PR) (PSA decline by ≥50% over baseline value, for at least two consecutive assessments, accompanied by significant improvement in pain score and performance status); or iii) stable disease (SD) (PSA decline by <50% from baseline value, for at least two consecutive measurements, and accompanied by significant improvement in pain and performance status scores). Progression-free survival (PFS) was defined as the time between the onset of therapy and a steady rise in PSA levels by ≥50% over their nadir values, or the detection of new metastatic lesions by bone and CT scans, and/or documentation of steady deterioration in pain and performance status scores. In the absence of deterioration in clinical symptoms, a net increase (in two consecutive measurements) of PSA value by >10 ng/mL over the nadir PSA value was required to document disease progression. OS was calculated as the time between the onset of therapy and death or end of follow-up. PrCa-specific OS was defined as the time from the onset of therapy until PrCa-related death (with patient censoring at the end of follow-up, or non-prostate cancer-related death). For patients with decreases of PSA levels during therapy, the time to return to baseline PSA was defined as the time between the onset of treatment until a return of the serum PSA levels equal to or higher than the respective baseline PSA level.

Table I. *Clinical characteristics of patients at study entry (N = 38).*

	Zoledronate + Dex + SM-A (N=20)	Zoledronate (N=18)
Age (years) (mean±SD)	72.25±8.3	73.5±4.87
Prior hormonal therapies		
LHRH-A monotherapy	0 patients (0%)	0 patients (0%)
CAB (LHRH-A/orchiectomy plus anti-androgen)	20 patients (100%)	18 patients (100%)
LHRH-A plus anti-androgen	13 patients (65%)	11 patients (61.1%)
Orchiectomy plus anti-androgen	7 patients (35%)	7 patients (38.9%)
Anti-androgen WITHDRAWAL	20 patients; 5 patients with PSA decline for 3 months	18 patients; 1 patient with PSA decline for 3 months
Prior radiation therapy		
Strodium	4 patients (20%)	2 patients (11.1%)
Rnenium	0 patients (0%)	3 patients (16.67%)
PAIN SCORE (0-5 scale)	median = 5 (min = 1; max = 5)	median = 3 (min = 2; max = 4)

Evaluations of symptomatic improvement and quality of life were performed using the Eastern Cooperative Oncology Group (ECOG) - World Health Organization (WHO) performance status score (32), and a bone pain score (PS), which provides, on a 6-point scale (from 0 to 5), the composite expression of pain intensity and analgesic requirements (type and quantity of analgesics consumed) (*i.e.,* 0 = lack of bone pain without analgesics; 1 = occasional mild pain, not necessitating use of analgesics; 2 = constant moderate pain, necessitating use of non-opiate analgesics; 3 = constant pain (severe), necessitating constant consumption of common analgesics; 4 = severe constant pain, requiring use of opiate analgesics; and 5 = severe pain refractory even to opiate analgesics). Reduction of the ECOG or bone pain score lasting for >1 month was considered a palliative response.

Tumor markers, indices of bone and mineral metabolism and hormonal serum measurements were performed at monthly intervals/using commercially available standard kits for PSA (Abbott Laboratories, IMX kit), alkaline phosphatase (AP), testosterone (T; Testo-CT2 RIA kit, Schering-Plough Corp., S.p.A, Milan, Italy), dehydroepiandrosterone sulphate (DHEA-S; Immunotech RIA kit, Miami, FL, USA) and IGF-1 ELISA kits (R&D Systems Europe, Abingdon, UK).

Study end-points and statistical analyses. Patients were randomized (1:1) to zoledronate or the combination regimen. The primary end-point of the study was to compare the PFS between patients in the two treatment arms. PFS was calculated from the onset of treatment until disease progression or death (with patient censoring at end of follow-up or in cases of death from prostate cancer-unrelated causes). Secondary end-points included PrCa specific OS (defined as time from initiation of therapy until death from prostate cancer-related causes, with patient censoring at end of follow-up or in cases of death from prostate cancer-unrelated causes), overall survival and duration of bone pain palliation. This study was designed with a target accrual of 86 patients (with approximately 1:1 accrual ratio between the two arms) to detect a PFS for zoledronate-treated patients that was at least 60% shorter than in patients receiving the combination regimen (median PFS of less than 3 months *vs.* 7 months or more, respectively) with a statistical power of 80%, a two-sided critical alpha of 0.01 by log-rank test, a 36-month accrual period and a 12-month period of follow-up after the end of accrual. This sample size would also correspond to an 80% power of detection of a difference between the two arms, in terms of the main secondary end-point (*i.e.*, PrCa-specific OS) of 16 *vs.* 7 months for the combination therapy *vs.* zoledronate alone treatment arms. A formal pre-specified interim analysis was planned at the conclusion of 24 months of accrual (provided that >40% of the target patient accrual had been completed) with a significance level of $p<0.0005$ for early closure, on the basis of the difference in PFS between the two arms of the study.

To detect differences in biochemical measurements, bone pain and performance status scores among subgroups of patients enrolled in the trial, one-way analysis of variance was performed (with Friedman's test and Dunnett's C *post-hoc* tests, where appropriate). Survival analyses for calculation of median PFS and OS, as well as time to return to baseline bone pain, was performed with the Kaplan-Meier method and the log-rank test was used to detect differences in survival distributions between subgroups of patients. The log-rank tests were used to test the homogeneity of the survival functions across strata defined by baseline clinical/biochemical parameters, *e.g.,* PSA, AP, testosterone levels, *etc.* All statistical analyses were performed with the SPSS 11.1 statistical package.

Results

Patients' characteristics at study entry. The clinical characteristics of the 38 patients enrolled in our randomized controlled clinical trial are presented in Table I. All the patients had previously received and eventually failed combined androgen blockade (including LHRH-A administration plus anti-androgen in thirteen and eleven patients in the combination arm *vs.* the zoledronate arm, respectively, orchiectomy plus anti-androgen in seven and seven patients, in the two arms, respectively), anti-androgen withdrawal (with PSA declines observed in five and one patient in the combination *vs.* zoledronate arms, respectively) and salvage chemotherapy. Furthermore, some patients enrolled in our trial had previously

Table II. *Baseline biochemical parameters.*

	Zoledronate			Zoledronate + Dex + SM-A		
	Mean	SD	SE	Mean	SD	SE
PSA (ng/mL)	137.47	170.53	40.19	536.05	1367.21	305.72
AP (U/L)	197.44	32.72	7.712	308.8	415.42	92.89
IGF-1 (ng/mL)	191.72	18.85	5.68	198.6	64.38	28.79
Testosterone (ng/mL)	≤0.2			0.375	0.22	0.06
DHEA-S (_g/mL)	≤0.8			0.87	0.71	0.18

received radionuclide therapy with strontium-89 (four and two patients in the combination *vs.* zoledronate groups, respectively) or Rhenium-186 HEDP (three patients in the zoledronate group), as well as estrogen-based hormonal therapy (two patients in the combination arm). There were no statistically significant differences among the two arms in the baseline levels of PSA, AP, IGF-1 or DHEA-S ($p=0.176$, 0.158, 0.827 and 0.202, respectively, by the Mann-Whitney *U*-test), but patients assigned to the combination regimen had higher baseline levels of testosterone ($p=0.012$ by Mann-Whitney *U*-test), as well as worse bone pain scores ($p=0.011$ by Mann-Whitney *U*-test) than patients in the zoledronate + LHRH-A arm (Table II).

Objective clinical responses. A pre-specified interim analysis after 24 months of accrual revealed that, in the combination therapy arm, 13 out of 20 patients had PR, one of 20 patients had stable SD, and six patients had PD. In contrast, all 18 patients in the zoledronate arm had PD.

Progression-free survival (PFS) and prostate-cancer specific overall survival (OS). At interim analysis, the median PFS in the combination therapy arm and the zoledronate arm was 7 months (95% C.I.: 3.65-10.35 months) and 1 month (95% C.I.: 0.32-1.68 months), respectively ($p<0.0001$ by log-rank test) (Figure 1a). Because the results of the interim analysis for the primary end-point (PFS) in the two arms of the study, satisfied the pre-specified threshold of statistical significance for early closure ($p<0.005$), the trial was prematurely terminated.

The median OS in the combination therapy arm and the zoledronate arm was 12 months (95% C.I.: 8.08-15.92 months) and 9 months (95% C.I.: 4.87-13.13 months), respectively (log-rank test, $p=0.0027$) (Figure 2). During the follow-up period, four patients (all assigned to the combination therapy arm) died, from causes unrelated to PrCa or the combination regimen, while they were experiencing objective clinical responses to treatment. Therefore, the median PrCa-specific OS of the two arms were compared: 14 months (95% C.I.: 8.56-19.44 months) for the combination therapy arm *vs.* 9 months (95% C.I.: 4.87-13.13 months) for the zoledronate arm ($p= 0.0005$, log-rank test) (Figure 3).

To obtain insight into potential clinical/biochemical parameters that could influence the response of PrCa patients to the combination therapy, exploratory log-rank tests were used to test the homogeneity of the survival functions across strata defined by baseline clinical/biochemical parameters, including age, serum PSA, AP, IGF-1, testosterone, DHEA-S, bone pain score (dichotomized as < or > of the median baseline value of the respective parameter), prior treatment with cytotoxic chemotherapy or prior radiation therapy. However, in univariate analyses, none of these parameters had a statistically significant association with PFS ($p>0.05$, for all comparisons).

Bone pain status scores during therapy. The bone pain scores improved, in all (20 out of 20) patients in the combination regimen arm and in 15 of 18 patients in the zoledronate arm of the study. Importantly, the treatment-associated improvement in bone pain scores was sustained for a far shorter period of time for patients in the zoledronate arm. Indeed, the median time-to-return to baseline bone pain, as an expression of duration of palliative response, was only 4 months (95% C.I. 3.08-4.92 months) in the zoledronate arm, while bone pain did not return to its baseline levels in any of the patients in the combination arm for the duration of their follow-up (median follow-up of >14 months) ($p<0.0001$, log-rank test) (Figure 4).

Performance status. The performance status scores of patients showed significant differences during therapy in the combination regimen arm ($p<0.001$, non-parametric related samples Friedman test), with improved performance status at the time of PSA nadir than baseline ($p<0.01$, Dunnett's C test). Interestingly, improvements in performance status of patients were observed even for those whom the combination regimen failed to produce major objective clinical responses.

Profile of side-effects. The mean fasting blood glucose levels of patients receiving the combination therapy remained within normal levels (80-120 ng/dl or 3.5-6.3 mmol) throughout the study, although three patients without prior

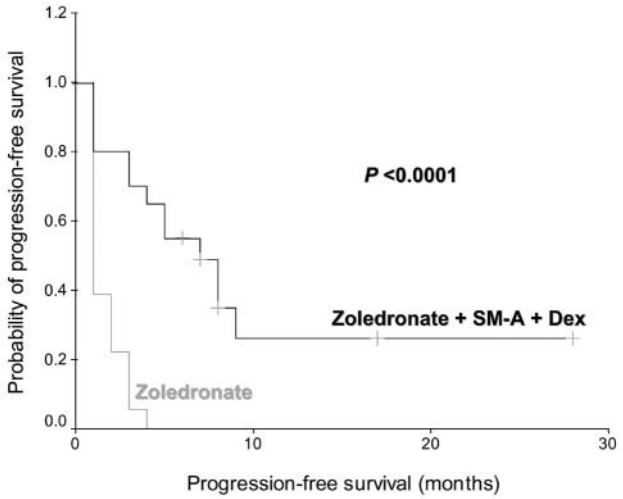

Figure 1. *Progression-free survival (PFS) curves (plotted with the Kaplan-Meier method) for androgen ablation-refractory metastatic PrCa patients receiving the SM-A+Dex+zoledronate combination regimen vs. zoledronate. The SM-A+Dex+zoledronate combination achieved longer PFS than zoledronate alone (median PFS of 7 vs. 3 months, respectively, p<0.0001, log-rank test).*

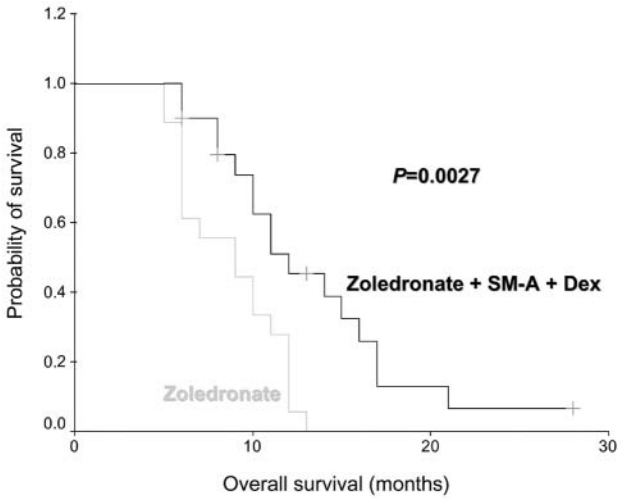

Figure 2. *Overall survival (OS) curves (plotted with the Kaplan-Meier method) for PrCa patients enrolled in our trial. The SM-A+Dex+zoledronate combination achieved longer OS than zoledronate alone (median PFS of 12 vs. 9 months, respectively, p=0.0027, log-rank test).*

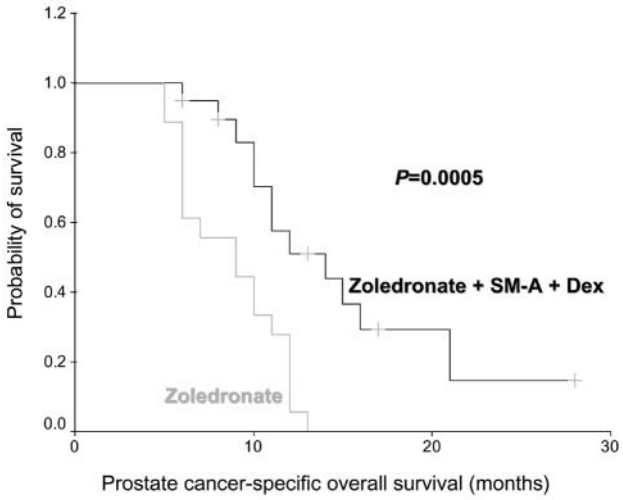

Figure 3. *Prostate cancer-specific overall survival (OS) Kaplan-Meier curves for PrCa patients enrolled in our trial. The SM-A+Dex+zoledronate combination achieved longer PrCa-specific OS than zoledronate alone (median PFS of 14 vs. 9 months, respectively, p=0.0005, log-rank test).*

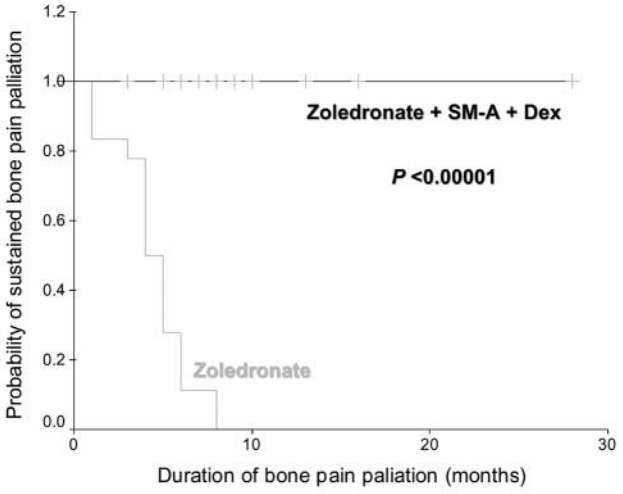

Figure 4. *Kaplan-Meier curves for the comparison of the duration of palliative response in PrCa patients enrolled in our trial. The SM-A+Dex+zoledronate combination achieved longer time to return to baseline bone pain than zoledronate alone (p<0.0001, log-rank test).*

history of diabetes developed transient hyperglycemia during the first 3 months of ASF treatment: in one patient, the maximum fasting blood glucose levels did not exceed 160 ng/dl (8.8 mmol/liter), and the hyperglycemia resolved after diet modification. In the other two cases, maximum fasting blood glucose levels did exceed 160 ng/dl, but also quickly returned to euglycemia after only temporary oral anti-diabetic therapy. Furthermore, three patients developed mild facial Cushingoid features, and five patients reported mild to moderate proximal muscle weakness. These side-effects rapidly subsided upon Dex reduction from 4 mg/day down to 1 mg/day. No major cardiovascular, renal, or liver-gastrointestinal toxicities were reported, while the mild gastrointestinal discomfort reported by one patient

was effectively controlled with oral administration of antacids and/or supplements of pancreatic enzymes.

Discussion

The microenvironment of the skeleton constitutes a favorable milieu for metastatic cancer cells to home, survive, proliferate and resist the anticancer activities of conventional therapies, such as cytotoxic chemotherapy or hormonal therapies (11-16, 22, 33). This probably explains not only why the bones are the most frequent targets for formation of metastases in PrCa, but also why these metastases are almost always the first, and frequently the only, foci where PrCa lesions become refractory to combined androgen blockade (6, 34, 35). Because our previous pre-clinical studies had documented that the signaling cascade of IGF-1/IGF-1R constitutes a key contributor to the protective effect of the bone microenvironment on PrCa cells (11, 33), we developed a combination regimen, which aimed at neutralizing this pathway and enhancing the anti-PrCa activity of conventional therapies (such as androgen ablation). This combination regimen contained SM-analogs, which suppress the growth hormone-dependent, mainly liver-derived, circulating levels of IGF-1 (18), and oral Dex, which suppresses the levels of bioavailable IGF-1 in the microenvironment of bone metastases (19, 20). This regimen, in combination with LHRH-A, was studied in a case series of stage D_3 PrCa patients and led to encouraging preliminary results (21, 22), which were later confirmed in phase II studies (23) and a randomized clinical trial, in comparison to salvage chemotherapy (etoposide & estramustine phosphate) (21-24). In particular, this combination therapy led to durable partial responses (in approximately 40-60% of patients) or disease stabilization (in another 20% of patients) (23). Importantly, it was not only associated with a very favorable profile of manageable side-effects (*e.g.*, hyperglycemia or mild gastrointestinal discomfort), but also led to extended median time to return to baseline tumor burden (as assessed by serum PSA levels), PFS survival, OS, PrCa-specific OS, as well as significant and durable improvement of bone pain and performance status (23, 24). Consistent with the original rationale for this regimen, statistically significant reductions in serum IGF-I levels were observed correlating with the time of best clinical response to the combination therapy.

Based on of the encouraging phase II experience with the SM-A+Dex– based therapy, we decided to further evaluate the clinical activity of combining zoledronate with this regimen, as compared to treatment with zoledronate alone, in stage D_3 patients with testicular androgen blockade. The rationale for this combination therapy was based on the ability of bisphosphonates, such as zoledronate, to modulate the regulation of local bone remodeling and the evidence, (reviewed in 11), that deregulation of this remodeling process is a key factor leading to local cytokine production and stromal interactions which confer resistance to PrCa cells, which are present in metastatic bone sites, against conventional therapies. Indeed, while PrCa has traditionally been associated with osteoblastic bone metastatic lesions, an emerging body of evidence has indicated that the development of PrCa bone metastases additionally requires osteoclastic activity (36, 37), suggesting that zoledronate (or other bisphosphonates) could have a favorable impact on the biological behavior of these metastases. In particular, we hypothesized that zoledronate (which principally targets osteoclast function) and the SM-A+Dex combination (with its direct and indirect effects on proliferation and/or anti-apoptotic responses of PrCa and/or osteoblasts) might comprehensively modulate multiple levels of local pathophysiological phenomena in the milieu of bone metastases and further improve the outcome of PrCa patients. This study was a randomized controlled clinical trial designed to test the hypothesis.

Consistent with previous experience with the SM-A+Dex combination, the zoledronate+SM-A+Dex arm of this study was associated with substantial rates of objective durable responses, palliative effects and favorable OS (in comparison to historical experience in this advanced stage of PrCa) (21-23). Patient accrual for this trial was terminated early, because a pre-specified interim analysis comparing the progression-free survival, overall survival and duration of bone pain palliation between the 2 arms of the study showed a significant difference, for all 3 parameters, in favor of the zoledronate +SM-A+Dex arm. Importantly, 65% of patients in this arm achieved >50% reduction in serum PSA (PR), while no such responses were observed in the zoledronate arm. Of note, while the two treatment arms did not differ in terms of baseline levels of PSA, AP, IGF-1 or DHEA-S, patients assigned to the combination regimen had higher baseline testosterone levels and worse bone pain scores than patients in the zoledronate arm, suggesting a more aggressive biological and clinical behavior of the disease among patients receiving the combination. Although the enrolled patients were randomly assigned to the treatment arms, imbalances between the treatment arms in terms of potential prognostic factors could have occurred by chance, possibly because of the premature termination of accrual. However, this difference is not confounding the results of our study, but instead reinforces the notion that the combination regimen, even when administered to patients with unfavorable clinical features, can offer more protracted objective and palliative responses than bisphosphonate therapy alone.

It should be emphasized that the results of the current trial are not inconsistent with the previous experience pertaining to the biological activity and clinical efficacy of bisphosphonates in PrCa. Saad *et al.* showed in a randomized controlled clinical trial that zoledronate reduced the rate of skeletal-related events in PrCa patients with bone metastases, decreased urinary

markers of bone resorption, and improved analgesic scores (25). However, no differences were reported with respect to time-to-disease progression, performance status, or quality-of-life scores among the zoledronate and placebo groups (25). Furthermore, in comparison to this latter study, our trial enrolled a more heavily-pretreated population of patients refractory to androgen ablation and chemotherapy, many of whom had also failed other therapeutic modalities (*e.g.*, radiotherapy). This raises the possibility that bisphosphonate therapy, such as zoledronate, may be potentially more active in the earlier stages of PrCa and that combination with other biologically active regimens, such as the SM-A+Dex-based regimen, may be required to offer disease control and palliative responses in the more advanced stages of PrCa. This hypothesis is not only supported by the results of our trials, but is also be consistent with pre-clinical data, which indicated marked increases in the numbers of osteoclasts at sites of early tumor invasion, but not at sites where tumor invasion was complete (36), therefore suggesting that the optimal timing for specific targeting of osteoclast function is the early stages of the establishment of bone metastases. Currently, there is no clearly determined approach to optimally define, which patients harbor bone metastases in sufficiently early stages to be responsive to bisphosphonates. However, we have recently shown (38, 39) that reverse-transcriptase polymerase chain reactions (RT-PCR) for both PSA and prostate-specific membrane antigen (PSMA) messenger RNA (mRNA) in peripheral blood and bone marrow biopsy samples can help identify patients with micrometastatic skeletal lesions of PrCa, even when conventional imaging techniques fail to identify the presence of these metastases. It is, therefore, possible that evidence of micrometastatic disease (according to RT-PCR analyses or other similar molecular assays), in the absence of metastases detectable by conventional imaging approaches, may represent a population of patients for which bisphosphonate therapy can be administered with the goal of preventing further establishment of macroscopic metastases lesions.

These findings generate an important framework for future efforts to improve the clinical activity of bisphosphonates or the SM-A+Dex combination regimen and provide a better range of therapeutic options for patients with advanced metastatic PrCa.

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