A New Sustained-Release, 3-Month Leuprolide Acetate Formulation Achieves and Maintains Castrate Concentrations of Testosterone in Patients With Prostate Cancer

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ABSTRACT

Purpose: This clinical trial investigated the effectiveness, pharmacokinetic properties, and safety profile of leuprolide acetate 22.5-mg depot, a new 3-month leuprolide depot formulation, as androgen deprivation therapy for patients with prostate cancer.

Methods: A Phase III, open-label, multicenter study design for patients with prostate cancer, with patient inclusion assessed by the investigative site as patient's appropriate for androgen deprivation therapy. Patients received 2 separate intramuscular injections of leuprolide acetate 22.5-mg depot for a 3-month depot interval of therapeutic effect. Plasma testosterone concentrations were determined throughout the study. The primary efficacy analysis was the percentage of patients who achieve and maintain castrate testosterone levels (≤50 ng/mL) from days 28−168. Secondary end points included luteinizing hormone, follicle-stimulating hormone, prostate-specific antigen, and safety assessments. A pharmacokinetic study was also conducted in a subset of 30 patients.

Findings: All 163 patients enrolled in the study received at least 1 dose of study drug; 162 of them were fully evaluable and 151 completed the study. Castrate levels of testosterone were achieved and maintained from days 28−168 in 96.8% (95% CI, 92.5%−98.7%) of patients. Five patients presented with sporadic testosterone levels >50 ng/dL. By day 28, of the 161 patients, 150 (99.4%) had achieved castrate levels, and 127 (78.9%) had achieved testosterone concentrations ≤20 ng/dL. At study end, 149 of 151 patients (98.7%) patients achieved castrate testosterone levels, with 142 of 151 (94.0%) having testosterone levels ≤20 ng/dL. At study end, mean luteinizing hormone and follicle-stimulating hormone concentrations had decreased from baseline to below the lower limit of quantitation and below baseline levels, respectively, whereas mean serum prostate-specific antigen was reduced by 94.7% from baseline. Most patients (>96%) had no change in their World Health Organization/Eastern Cooperative Oncology Group score, whereby 84.0% of patients had a baseline score of 0. Bone pain, urinary pain, and urinary symptoms were infrequent and remained so throughout the study. After administration, leuprolide concentrations increased rapidly. The peak was followed by a decline up to day 28, maintaining sustained drug levels until the following dose on day 84. The most common related treatment-emergent adverse events, detected in >5% of patients, were hot flushes, fatigue, and injection site pain reported by 77.3%, 9.8%, and 9.2% of patients, respectively.

Implications: Leuprolide acetate 22.5-mg depot was effective in achieving and maintaining testosterone suppression. Safety and tolerability profiles were consistent with established profiles of androgen deprivation therapy. Clinical Trials.gov identifier: NCT01415960. (*Clin Ther*. xxxx;xxx:xxx) © 2019 Published by Elsevier Inc.

Keywords: Androgen deprivation therapy, Efficacy, Leuprolide depot, Prostate cancer, Testosterone.

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INTRODUCTION

Prostate cancer has a worldwide incidence of 1.1 million cases and represents a leading cause of cancer specific mortality globally.[1] Androgen deprivation therapy (ADT) has been the mainstay treatment for advanced prostate cancer. Current options for androgen deprivation are surgical castration (bilateral orchiectomy) or administration of luteinizing hormone (LH)−releasing hormone (LHRH) agonists[2] or antagonists.[3]

LHRH analogues, which are synthetic analogues of LHRH, have become the standard of care in the achievement of androgen suppressive therapy, including the potential use for intermittent ADT application, with the consideration for testosterone recovery, as well as the avoidance of the physical and psychological morbidity associated with surgical castration.[4,5] On initiation of LHRH ADT, anterior pituitary LHRH receptors are stimulated (agonized), thereby inducing a transient supraphysiologic increase in LH and follicle-stimulating hormone (FSH), leading to the testosterone surge, which may last from 3 to 7 days.[6] However, on long-term LHRH agonist administration, there is resultant desensitization of these receptors, resulting in down-regulation and a subsequent suppression of serum LH, FSH, and testosterone.[7,8]

In addition to LHRH agonists providing the mainstay of treatment for advanced prostate cancer, they may also serve an adjuvant role in patients with newly diagnosed prostate cancer. Indeed, in patients with locally advanced or high-risk localized disease, the addition of neoadjuvant and adjuvant hormone therapy is now considered the standard of care for these men treated with radiation therapy.[9,10]

Leuprolide acetate is a synthetic nonapeptide LHRH analogue with increased duration of action and increased affinity for the pituitary receptor, the targeted receptor for endocrine suppression of androgen production.[11−14] Various depot formulations of leuprolide have been developed to avoid issues associated with patient convenience and to enhance patient adherence.[15−20] A leuprolide acetate 3.75-mg depot was previously developed as a monthly sustained-release formulation of leuprolide. Its tolerability and efficacy were proven during a Phase III clinical trial in patients with prostate cancer.[21]

Leuprolide acetate 22.5-mg depot is a unique 3-month, sustained-release formulation of microencapsulated leuprolide acetate. Leuprolide acetate 22.5-mg depot presents a double control sustained-release delivery system. This innovative approach consists of microspheres formed by a biodegradable polymer of polylactic acid and small quantities of triethyl citrate (TEC), obtained by a coacervation process. TEC, acting as a hydrophobic second barrier, controls the diffusion rate of leuprolide acetate entrapped inside a polymeric matrix throughout the microsphere. This formulation differs from the 1-month formulation in the polymer composition, which permits a prolonged leuprolide release. Leuprolide acetate 22.5-mg depot has been approved in 26 European countries and more recently in the United States.

This Phase III, open-label, multicenter clinical study was performed to investigate the efficacy, pharmacokinetic properties, and safety profile of leuprolide acetate 22.5-mg depot in suppressing testosterone levels in patients with prostate cancer. The primary efficacy end point of the study was the proportion of castrated patients (ie, testosterone levels $\leq$50 ng/dL) over the total number of the intent-to-treat (ITT) patients at specific time points. During US Food and Drug Administration (FDA) evaluation, reviewers requested efficacy results to be presented according to their current standards for LHRH analogues approval (Kaplan−Meier analysis), which ensure not only achievement but also maintenance of testosterone suppression throughout the study. Therefore, efficacy study results are presented based on Kaplan−Meier analysis.

PATIENTS AND METHODS

Study Design

The efficacy of leuprolide acetate 22.5-mg depot was evaluated in an open-label, multicenter, multiple-dose clinical trial. Patients with prostate cancer were administered leuprolide acetate 22.5-mg depot in 2 discrete doses separated during a 3-month interval. The patients received the first intramuscular injection of study drug initially at baseline (day 0) and the second dose at day 84. The duration of the study was 6 months. Plasma testosterone determinations were obtained in all patients at specific time points throughout the study as listed below. The

pharmacokinetic profile of leuprolide was investigated during the six 6 in a subset of 30 patients, involving different US investigative sites.

Patients

Patients with biopsy-proven carcinoma of the prostate, who in the judgment of the trial investigators could benefit from ADT, were enrolled in the study at 25 US investigational sites. Study eligible patients were >18 years old, had a World Health Organization (WHO)/Eastern Cooperative Oncology group (ECOG) score of 0, 1, or 2; had eugonadal testosterone levels at screening >150 ng/dL; had adequate renal and hepatic function, a life expectancy of at least 1 year, and no known hypersensitivity to the active ingredient or excipients of the study formulation. Patients were excluded if they had brain metastases, spinal cord compression, severe urinary tract obstruction, severe pain from extensive osseous deposits, previous cancer systemic therapy, coexistent malignant tumor, or serious concomitant diseases or if they had undergone previous ADT (within 12 months before the screening visit and no ADT administrations that involved >6 months), previous treatment with antiandrogens or androgen receptor blockers (unless a 3-month washout was achieved), or previous orchiectomy, adrenalectomy, or hypophysectomy. Adequate washouts for the following therapies and medications were required for inclusion: previous prostate surgery, external beam radiotherapy, brachytherapy, thermotherapy, cryotherapy, any investigational drug use (5 half-lives of its physiologic action or 3 months, whichever was longer), use of 5α-reductase inhibitors (3 months), or use of over-the-counter or alternative medical therapies with an estrogenic or antiandrogenic effect (3 months). The study protocol was approved by US Copernicus Institutional Review Board for all centers, except for 1 site that was approved by its local institutional review board (Greenville Hospital System). All patients provided their written informed consent before enrollment.

Investigational Product

Leuprolide acetate 22.5-mg depot formulation consists of polylactic polymer microspheres, incorporating TEC as release modulator agent, which entrap 22.5 mg leuprolide acetate. For an easier reconstitution and administration of the study product, a device (Mixject, Medimop Medical Projects Ltd, West Pharmaceutical Services, Exton, Pennsylvania), was used to connect the prefilled syringe that contained the diluent (ie, 2 mL of 0.8% mannitol) with the vial that contained the study product, allowing a direct drug reconstitution and subsequent intramuscular injection into the gluteal area, anterior thigh, or deltoid.

Study Procedures

The screening visit was performed within 14 days of the first study drug administration. Patients who met all eligibility criteria were offered study enrollment.

Blood samples for testosterone, LH, and FSH were collected on day 0 (baseline) and day 84 (before dosing and 1 and 4 h after dosing) and on days 2, 14, 28, 56, 86, 112, and 168. Blood samples for testosterone (8 mL) were collected in chilled lithium-heparinized tubes and then centrifuged at 1600 *g* at 4 °C for 15 min to obtain plasma. Blood samples for LH and FSH (6 mL) were collected into polyethylene tubes, kept at room temperature for 30 min, and then centrifuged at 2500 *g* for 10 min to obtain serum. All samples were stored at –20 °C until analyzed. On the basis of a plasma volume of 250 μL, the determination of testosterone in plasma samples were performed by LC-MS/MS after liquid–liquid extraction. Testosterone-d3 was used as the internal standard. Interassay and intra-assay accuracy and precision were within 10.7%, and the lower limit of quantitation (LLOQ) was set to 10 ng/dL. A Gemini C18 (Phenomenex, Torrance, California) and an AB Sciex API 3000 (SCIEX, Concord, Ontario, Canada) were used as HPLC and mass spectrometers, respectively. LH and FSH levels were determined by chemiluminescence immunoassay with an LLOQ of 2.0 and 3.6 mIU/mL for LH and FSH, respectively.

Blood samples for prostate-specific antigen (PSA) were collected on day 0 (baseline) and day 84 (before dosing), and on days 14, 28, 56, 112, and 168. Blood samples (9 mL) were collected into polyethylene tubes, kept at room temperature for 30 min to separate serum, centrifuged at 2500 *g* for 10 min, and stored at –20 °C until analyzed. PSA levels were determined by chemiluminescence immunoassay, with an LLOQ of 0.36 ng/mL.

Patients eligible for the pharmacokinetic substudy provided blood samples for leuprolide on day 0 (baseline) and day 84 (before dosing and 1 and 4 h after dosing) and on days 2, 14, 28, 56, 86, 112, and 168. Patients not in the pharmacokinetic substudy provided leuprolide samples only on days 28, 56, 84 (before dosing), and 112. Blood samples were collected in chilled polypropylene tubes that contained 25 μM PPack EDTA (4.5 mM) and aprotinin (500 kIU/mL of blood) and then centrifuged at 1600 *g* at 4 °C for 15 min. Samples were stored at −70 °C until analyzed. Analysis was performed using an enzymatic immunoassay. All analyses were performed by using validated methods at a central laboratory (Nuvisan GmbH, Neu-Ulm, Germany).

Bone pain, urinary pain, and urinary symptoms were assessed. The intensity of pain was assessed using a Likert 10-point scale (with 1 indicating no pain/symptoms and 10 indicating worst pain/symptoms possible). The WHO/ECOG performance status was assessed at screening and on days 0 (baseline) 14, 28, 56, 84, 112, and 168.

The safety profile of the drug was assessed by the incidence of treatment-emergent adverse events (TEAEs); occurrence of hot flushes assessed on days 0 (baseline), 2, 14, 28, 56, 84, 86, 112, and 168; clinical laboratory parameters (including hematology, blood chemistry, and urinalysis) at screening and on days 28, 84, 112, and 168; ECGs assessment at screening and on days 0 (only if ≥ 8 days after screening), 28, 84, 112, and 168; and vital sign measurements at screening and on days 0, 28, 56, 84, 112, and 168. Local tolerability (eg, burning, pain, redness, swelling, and bruising) was assessed after administration of each dose. Patients were provided with a diary to record any signs or symptoms of adverse events (AEs) after each injection. At each visit, the patients were questioned about AEs and concomitant medications. Final assessment and evaluation took place approximately 3 months after the second dose (day 168).

Statistical Analysis

Efficacy analyses were performed using the ITT population. Safety analyses were performed on all enrolled patients. Pharmacokinetic analysis was performed on a subset of 30 patients.

Efficacy was evaluated according to the current FDA-recommended method of Kaplan−Meier analysis and the current FDA standards for gonadotropin-releasing hormone analogue approval, which indicate that the lower bound of the 95% CI for the point estimate of the percentage of patients who achieve and maintained castrate testosterone levels during the treatment period should not be >90%.

The percentage of patients with testosterone suppression (≤50 ng/dL) from days 28−168 was calculated by the Kaplan−Meier method for right-censored observations.[22] Patients in whom testosterone suppression failed were considered failures on the first day of a testosterone measurement >50 ng/dL. Patients who prematurely discontinued without T concentration > 50 ng/dL and those who were successfully suppressed through day 168 were censored at their last measured testosterone value. On FDA request, a more restricted analysis was performed treating missing testosterone values as failures.

Summary statistics were presented as sample size, mean (SD), SD, and median for continuous variables and group frequencies and percentages for categories of categorical variables. In general, percentages were calculated using the total nonmissing responses by time point. Pharmacokinetic and pharmacodynamic parameters were measured or calculated for C_{max} and T_{max}, considering 2 intervals: days 0−84 after first administration and days 84−168 after second administration. The statistical analysis was performed using SAS software, version 9.2 for Windows (SAS Institute, Cary, North Carolina). The pharmacokinetic analysis was performed using WinNonlin software, version 6.1 (Certara USA Inc, Princeton, New Jersey) and SAS software, version 9.2. AEs and local adverse reactions were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 14.1.

RESULTS

All 163 study patients enrolled received at least 1 dose of the study medication and thus constituted the safety population. One patient was not included in the ITT population (n = 162) because of consent withdrawal within a few days after the first study drug administration; thus, no efficacy assessment was available. The number of patients who completed the study was 151. Twelve patients

terminated the study prematurely for reasons not related to treatment (Figure 1).

Baseline enrollee demographic characteristics as well as prostate cancer stage of the ITT population are presented in Table I. All patients included in the ITT had testosterone levels >150 ng/dL at screening.

The trial assessed 2 intramuscular leuprolide acetate 22.5-mg depot injections separated by a 3-month interval. Castrate levels of testosterone (≤50 ng/dL) were achieved and maintained from days 28−168 in 96.8% (95% CI, 92.5%−98.7%). Five patients had a testosterone level above castration. One patient did not achieve castration at day 28; nevertheless, once castration was achieved, it was maintained. The other 4 patients did not maintain castration. Three of them presented an isolated testosterone escape (i.e. T levels > 50 ng/dL).

A more restricted analysis was performed that included not only patients with noncastrate testosterone levels but also patients with missing testosterone values between days 28 and 168 as failures. Four patients had a missing testosterone value, 1 of them at day 28, resulting overall in achievement and maintenance of castration of 94.3% (95% CI, 89.4%−97.0%).

After the first injection on day 0, mean (SD) testosterone concentrations rapidly increased from baseline levels (ie, 400.4 [167.4] ng/dL) reaching a C_{max} of 626.0 (274.0) ng/dL at day 2 (median T_{max}). Thereafter, testosterone levels rapidly decreased, and 160 of the 161 patients (99.4%) achieved castration by day 28; remarkably, 35 patients achieved onset of castration by day 14. In addition, at day 28, 127 of the 161 patients (78.9%) achieved a more stringent criterion of testosterone ≤20 ng/dL. Mean testosterone concentrations were maintained at <20 ng/dL from day 28 onward. On day 168, of 151 patients, 149 (98.7%) had castrate testosterone levels; specifically, 142 (94.0%) had testosterone levels ≤20 ng/dL (Figure 2).

After the first administration on day 0, leuprolide concentrations increased rapidly (Figure 3) from nondetectable to 46.1 and 33.6 ng/mL at 1 and 4 h, respectively. The peak was followed by a decrease during several days, maintaining sustained drug levels until the following dose on day 84. The profile of leuprolide concentrations after the second administration on day 84 was similar to that following the first dose on day 0. Hence, the leuprolide peak was followed by a decrease in

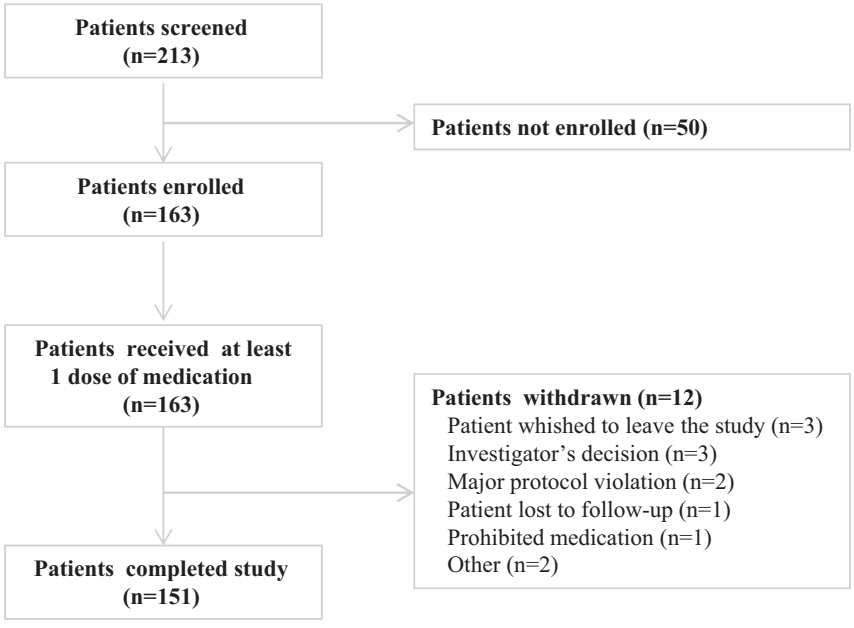

Figure 1. Disposition of patients.

Table I. Demographic characteristics, baseline characteristics, and TNM disease stage of patients included in the intent-to-treat study population.

Characteristic	Finding (n = 162)
Age, y	
Mean (SD)	71.0 (9.0)
Range	47–91
Weight, kg	
Mean (SD)	88.9 (18.6)
Range	51.7–158.8
Race, %	
White	62.3
Black	30.2
Hispanic	4.9
Asian	1.2
Other	1.2
TNM stage, %	
T stages	
T1	43.2
T2/T3/T4	56.2
NA	0.6
Stage N	
N0	91.4
N1	5.6
N2	1.9
NA	1.2
Stage M	
M0	88.3
M1	11.1
NA	0.6

NA = not available.

leuprolide concentrations, and leuprolide plateau levels were maintained up to study end (day 168). The leuprolide C_{max} for days 0–84 (dose 1) and days 84–168 (dose 2) were similar and corresponded to mean (SD) values of 46.8 (18.0) ng/mL and 48.3 (18.6), ng/mL, respectively. Both C_{max} values were observed approximately 2 h after each administration. Furthermore, in a post hoc analysis, testosterone concentrations were classified within 3 ranges (<20, 20–50, and ≥50 ng/dL) and correlated with the corresponding leuprolide plasma concentrations (Figure 4). Mean leuprolide concentrations were comparable among the 3 testosterone ranges.

After the first administration of leuprolide acetate 22.5-mg depot, there was an initial increase above baseline levels in mean serum LH concentrations at 1 and 4 h after dosing followed by a decrease below baseline level on day 14. From day 28 to study end, mean serum LH concentrations were below the LLOQ at all time points. No increases were detected just after second administration. With respect to FSH, there was a transient increase from baseline in mean serum FSH concentrations just after first dose (1 and 4 h after dosing). At day 14, mean serum FSH concentrations were below the LLOQ, and these levels were maintained up to day 56, where a slight but negligible increase was observed. Mean serum FSH concentrations remained well below baseline values from day 56 until the end of the study.

At study end, PSA levels decreased in all patients, with the exception of 1. At baseline, 108 of 162 patients (66.7%) had serum PSA concentrations that were ≥4 ng/mL. At 12 and 24 weeks of treatment, 87.6% and 89.1% of patients with elevated PSA levels at baseline achieved a PSA <4 ng/mL. Furthermore, at study end, mean serum PSA was reduced by 94.7% from baseline (Figure 5).

The WHO/ECOG performance status scores at study entry indicated that 84.0% of the patients in the ITT population were "fully active and able to carry on all pre-disease performances without restriction" (score of 0), 13.6% were "restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature" (score of 1), and 2.5% were "ambulatory and capable of all self-care but unable to carry out any work, up and about >50% of waking hours" (score of 2). Results of the WHO/ECOG assessments found that the activity status of the patients was consistent throughout the study. At study completion (day 168), the percentage of patients reporting scores of 0, 1 and 2 were 82.9%, 14.5%, and 2.6%, respectively. Almost all patients (>95%) had no change in their WHO/ECOG performance status from baseline.

Bone pain, urinary pain, and urinary symptoms were infrequent and remained so throughout the study; mean scores were 1.02–1.42 for all assessments (Table II).

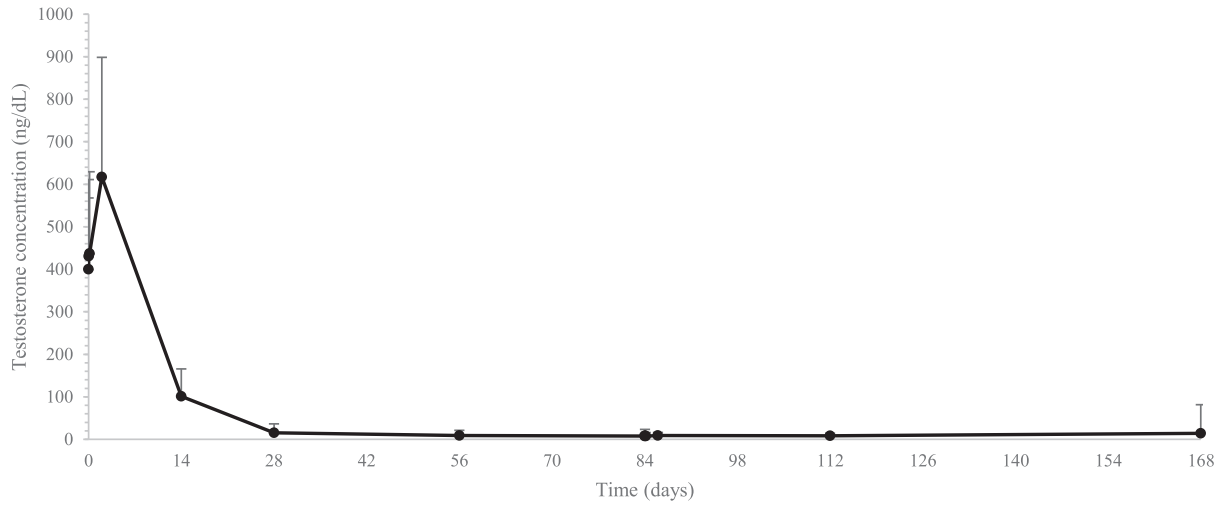

Figure 2. Mean testosterone concentrations in the intent-to-treat population. Error bars indicate SDs.

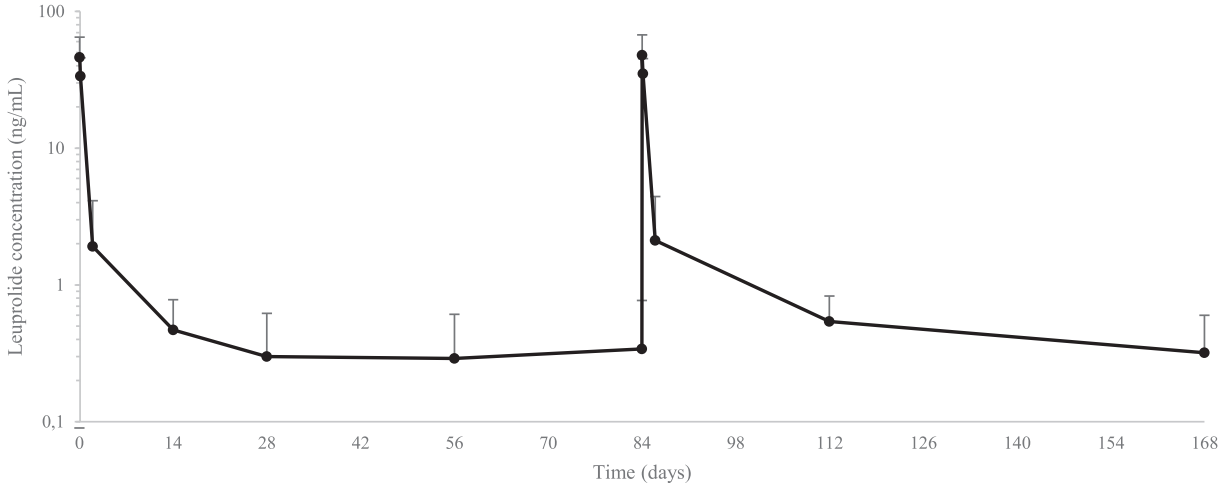

Figure 3. Mean leuprolide concentrations in the pharmacokinetic population. Error bars indicate SDs.

No TEAE led to intervention, including withdrawal or dose reduction of study drug, or significant additional concomitant therapy. A total of 141 patients of the safety population of 163 (86.5%) experienced TEAEs considered to be related to the study drug. The most frequently related TEAEs were hot flushes, fatigue, and injection site pain reported by 77.3%, 9.8%, and 9.2% of patients, respectively (Table III). Other related TEAEs were reported by <5% of patients. Apart from injection site pain, other local related TEAEs reported in >1% of patients are listed in Table IV.

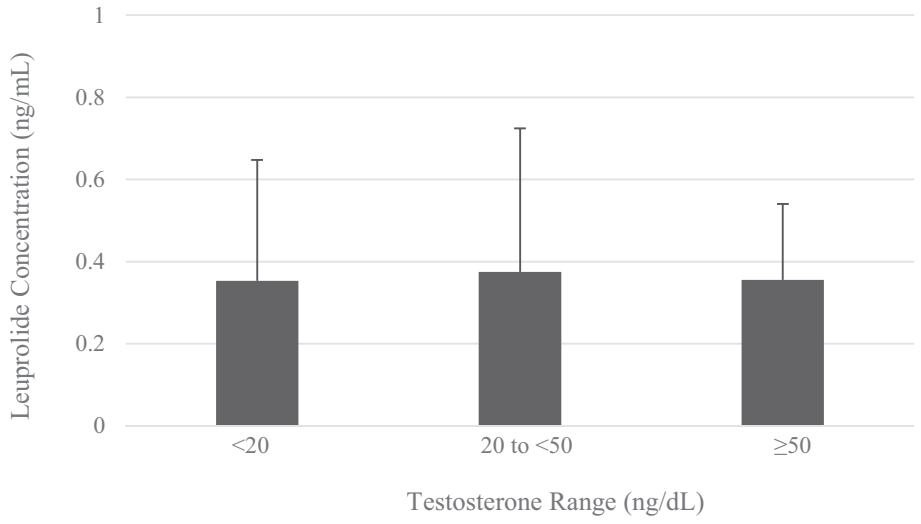

Figure 4. Correlation between mean leuprolide and testosterone concentrations when the latter is classified in 3 different concentration levels. Error bars indicate SDs.

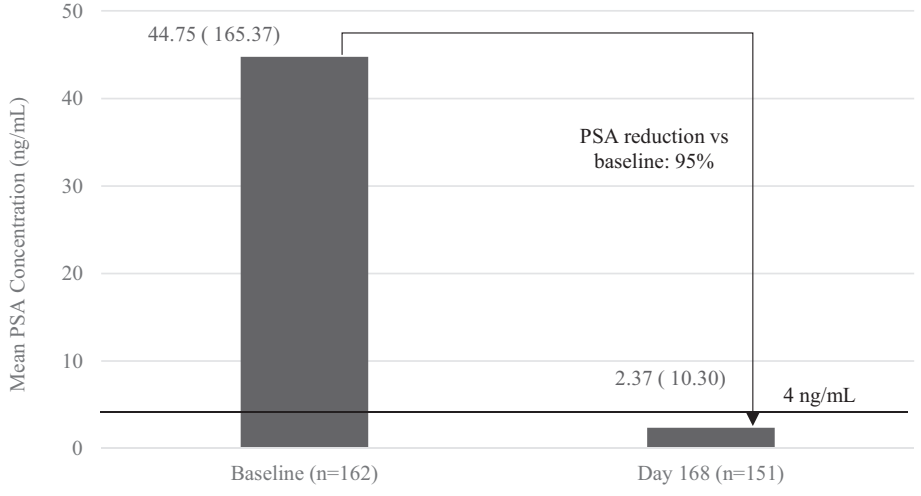

Figure 5. Mean (SD) prostate-specific antigen (PSA) concentrations in the intent-to-treat population at baseline and at study end and percentage of decrease from baseline.

In most patients, related TEAEs were of mild or moderate intensity. There were 14 serious AEs reported by 12 patients (7.4%) after administration of leuprolide acetate 22.5-mg depot. All serious AEs except 1 were deemed by the investigator as not related to the study treatment. The only serious AE assessed by the investigator as unlikely to be related to the study treatment occurred in 1 patient with peripheral arterial disease and consisted of myocardial infarction that required hospitalization.

Table II. Summary of bone pain, urinary pain, and urinary symptoms scores during treatment with leuprolide acetate 22.5-mg depot in the intent-to-treat population.*

Assessment Time	Bone Pain	Urinary Pain	Urinary symptoms
Day 0 (baseline)	1.1	1.0	1.4
Day 2	1.1	1.1	1.4
Day 14	1.2	1.1	1.4
Day 28	1.1	1.0	1.4
Day 56	1.2	1.1	1.4
Day 84	1.1	1.1	1.3
Day 86	1.1	1.0	1.3
Day 112	1.3	1.1	1.4
Day 168 (final visit)	1.1	1.1	1.3

* Scores are based on a 10-point scale, ranging from 1 for no pain or symptoms to 10 for worst pain or symptoms.

No serious TEAEs led to withdrawal of a patient from the study. No deaths occurred during the study. There were no clinically significant trends observed in blood pressure or heart rate measurements, ECG parameters, or liver, renal, and all other laboratory values throughout the study. No clinically relevant changes from baseline or shifts in values occurred during the study.

Table III. Total number of patients with treatment-emergent adverse events related to study drug (in ≥5% of patients) classified by the Medical Dictionary for Regulatory Activities in body system and preferred term in the safety population.

System Organ Class and Preferred Term*	No. (%) of patients (N = 163)
Vascular disorders	
Hot flush	126 (77.3)
General disorders and administration site conditions	
Fatigue	16 (9.8)
Injection site pain	15 (9.2)

* Some patients had symptoms in >1 category.

DISCUSSION

This study reports the efficacy of a new 3-month, sustained-release leuprolide acetate 22.5-mg depot formulation in achieving and maintaining castrate testosterone levels from days 28 through 168 after patients with prostate cancer received 2 quarterly sequential doses of study drug. Testosterone castrate levels (<50 ng/dL) were achieved and maintained in 96.8% (95% CI, 92.5–98.7) of the ITT population from days 28–168. The study successfully achieved its primary end point. This newly developed 3-month, sustained-release formulation of microencapsulated leuprolide is effective in achieving and maintaining castrate testosterone levels. Moreover, when applying a more stringent criteria, imputing missing testosterone values as failures, the efficacy of the study drug remains >94% [(94.3%; 95% CI, 89.4–97.0). Remarkably, for the 3 missing values, preceding and succeeding testosterone concentrations were below the LLOQ.

Onset of castrate testosterone levels occurred within 28 days after the first leuprolide acetate 22.5-mg depot injection in 99.4% of evaluable patients. The onset and extent of testosterone suppression were similar to those observed for other current leuprolide depot formulations.[15,20] By the end of the study (day 168), patients receiving leuprolide acetate 22.5-mg depot had reached a mean (SD) testosterone level of 13.8 (67.6) ng/dL, which is similar to the testosterone level achieved with other approved LHRH depot formulations.[23,24] Although the optimal testosterone level to effect castration has yet to be determined,[25] current testing methods have found that the mean value of testosterone after surgical castration is 15 ng/dL.[26] This finding led to a discussion of the current ideal definition of castration to achieve, possibly a lower level of testosterone suppression, sometimes defined as <20 ng/dL.[27] This lower threshold of testosterone suppression was achieved in the study by 78.9% of the patients on day 28 and in 94.0% by the end of the investigation.

The leuprolide pharmacokinetic profile after both administrations confirmed a reduction of the initial peptide release compared with that of other marketed

Table IV. Total number of patients with local treatment-emergent adverse events related to study drug (in ≥1% of patients) classified by the Medical Dictionary for Regulatory Activities in body system, preferred term, and lower-level term in the safety population.

System Organ Class and Preferred Term*	No. (%) of patients	No. (%) of injections
Injection site pain	15 (9.2)	17 (5.3)
Injection site pain	8 (4.9)	8 (2.5)
Injection site burning	7 (4.3)	7 (2.2)
Injection site erythema	5 (3.1)	6 (1.9)
Injection site induration	4 (2.5)	4 (1.3)

* Some patients had symptoms in >1 category.

3-month leuprolide formulations.[15,20] According to the pharmacokinetic analysis, the release of leuprolide acetate 22.5-mg depot can be described by an immediate release phase followed by a mixed diffusion-erosion release phase. This unique pharmacokinetic profile results from a double-control sustained peptide release system. The combination of polymer and TEC barriers modulates the initial release, ensuring constant levels of leuprolide during the 3-month period. Compared with other pharmacokinetic studies on leuprolide acetate 22.5 mg 3-month formulations,[15,20] in which the initial release of the pharmacologic drug leads to leuprolide concentrations up to 127 ng/mL at 4 h, with this new formulation only a value of 33.6 ng/mL is attained in the same period. In addition, as the pharmacokinetic results suggested, despite the lowest initial release, there was no accumulation of leuprolide after repeated study drug administration.

Similar to other LHRH agonists, initial treatment with leuprolide acetate 22.5-mg depot caused a surge in FSH and LH release with the corresponding increase in testosterone levels. This testosterone surge has been clinically associated with worsening of bone pain, urinary obstruction, or other symptoms during the first treatment week, causing the so-called flare phenomenon.[28,29] Patients at higher risk of clinical flare are those with an advanced stage disease, especially those with widespread bony metastasis.[29] In the present study, there was little if any increase in the means of bone pain, urinary pain, and urinary symptoms score in the 2 days after administration, suggesting that there were no reported flare symptoms. However, specific trial exclusion criteria were set to avoid enrolment of patients who could potentially have clinical flare because previous treatment with antiandrogens was not allowed.

The levels of PSA, a clinical prostate tumor progression marker, were decreased in almost all patients in the study. More than 89% of patients with PSA levels ≥4 ng/mL at baseline had their PSA values < 4 ng/mL at the end of the study, reinforcing the effectiveness of the treatment.

Regarding WHO/ECOG performance status, no significant change was observed during the 6-month treatment. Patient assessment of bone pain, urinary pain, and urinary symptoms were relatively few at study entrance and remained low during the study period. Overall, these results support an adequate functional status and good symptom control.

Once castration was achieved, testosterone elevations occurred in 4 patients (2.5%). Three patients presented a single testosterone elevation: 1 of them at day 86 and the remaining 2 at the last study visit. The last patient presented various testosterone elevations. Remarkably, none of these 4 patients had an LH increase associated with the transient testosterone elevation. Three patients had no exacerbation of clinical symptoms accompanied by a >80% PSA reduction from baseline. The other patient presented with lower urinary tract symptoms and extremely high PSA levels at baseline. Nevertheless, this patient experienced a 68% PSA reduction at the last study visit.

Testosterone escapes are well-documented biochemical results associated with the administration of LHRH agonists and can occur at any time during ADT.[30,31] The mechanism of action

of testosterone escapes related to LHRH treatment failure is a transient activation of LH production with the consequent testosterone increase.[30] To reactivate this axis, LHRH could stimulate the anterior pituitary gland to release LH and FSH. LH stimulates the Leydig cells of the testes to secrete testosterone.[32] Therefore, any testosterone elevation not associated with an increase in LH levels might not be considered as treatment failure; for example, other endogenous testosterone sources can be considered. Considering that all transient testosterone elevations of the study after castration have no associated LH elevation, it can be inferred that they share the same extrahypothalamic–pituitary–gonadal axis origins.

Furthermore, in the post hoc analysis shown in Figure 3, the patient testosterone range above the castration limit identified leuprolide concentrations almost identical to the patients with testosterone values < 20 ng/dL. This finding suggests that testosterone elevations observed during the study are not related to leuprolide concentration but perhaps to other explanations (eg, adrenal production, pituitary receptor mutations). Moreover, it has been considered that this type of transient testosterone elevations are not clinically meaningful and have not had a clinically significant effect on patient outcomes.[33]

Historically, when reviewing options of ADT for prostate cancer, surgical castration (orchiectomy) is considered to be the gold standard against which pharmacologic treatments are compared.[32] The gold standard surgical castration (orchiectomy) has an efficacy of 100% regarding suppression of testicular testosterone (testosterone produced by the hypothalamic–pituitary–gonadal axis), but it does not have a complete efficacy of suppressing the serum testosterone level below the castration limit. This phenomenon occurs presumably because of potential additional sources of testosterone, which are not produced by the hypothalamic–pituitary–gonadal axis and thus may result in failure to achieve testosterone levels consistently <50 ng/dL. Recently, several studies[34,35] reveal that apart from the pituitary–gonadal axis, pituitary-adrenal and pituitary-intratumoral androgen axes may have a critical role during ADT. These studies[34,35] found that in patients treated with ADT the pituitary-adrenal axis mediated by adrenocorticotropic hormone has a central role in the regulation of androgen synthesis, suggesting that the sporadic lack of testosterone suppression is not related to hypothalamic–pituitary–gonadal axis inhibition failure. In particular, the androgen precursor dehydroepiandrosterone was found most commonly in prostate cancer tissues and played a significant role in the synthesis of testosterone and dihydrotestosterone in prostate cancer tissues after ADT. Aligned with these findings, the additional therapies for castrate-resistant prostate cancer are based on pituitary-adrenal axis testosterone production inhibition together with androgen receptor inhibition.[36] A probable source of the few testosterone elevations in these 4 patients is the hypothalamic–pituitary–adrenal axis where leuprolide acetate has no known action.

Regarding the drug safety profile for this study, the most common related TEAEs were hot flushes and fatigue. These class effects are related to the pharmacologic consequence of testosterone deprivation attributable to suppression of the hypophysis–gonadal axis. The main underlying mechanism for hot flushes are low testosterone levels that interrupt the negative feedback mechanism in hypothalamic noradrenaline production and reset the hypothalamic thermoregulatory center, resulting in vasomotor hot flushing.[37] Because hot flushes are associated with ADT, they do not seem to be a dose-dependent effect. Therefore, no differences in tolerability between leuprolide acetate 22.5-mg depot and other leuprolide depot marketed formulations[23] were expected. Variability in the incidence of hot flushes associated with ADT has been reported in the range of 44%–80%.[37–39] Although the incidence of hot flushes in the present study was within the published ranges, patients were directly asked about the presence of this event. Inclusion of routine assessment of hot flushes at different time points throughout the study could result in a higher incidence of hot flushes reporting compared with the spontaneous reporting method used in other studies. Fatigue, the second most common related TEAE reported in the study, is a major concern for quality of life, especially in men with less aggressive disease.[37] Fatigue could be explained by increased fat mass along with the loss of lean muscle mass in combination with pain and depression in patients undergoing ADT. The incidence of fatigue reported

in the present study is in line with data reported in similar studies conducted with other LHRH depot marketed formulations.[40]

Local related TEAEs are detailed in Table IV as percentage of patients and injections. Overall, 20 patients (12.3%) reported a local related TEAE, corresponding to 32 of the 320 total injections administered (10%). Injection site pain and injection site burning were the most frequent related local TEAE, when these are classified by the MedDRA lower-level term, representing only 4.9% and 4.3% of patients or 2.5% and 2.2% of total injections, respectively. In contrast, a previous study[40] of a 3-month LHRH formulation found burning/stinging (21.7% of injections) and pain (6% of patients; 3.5% of injections) as the most common treatment-related injection site reactions. The better local safety profile of leuprolide acetate 22.5-mg depot may be explained by the lack of *N*-methyl pyrrolidone, which is present in other leuprolide depot formulations currently on the market.

Overall, the results of the present study reveal a safety profile consistent with the leuprolide safety profile established in previous clinical studies and reported for pharmacologic ADT used in the treatment of prostate cancer.[21]

CONCLUSIONS

The results of this study indicate that the new 3-month leuprolide acetate 22.5-mg depot formulation is effective in achieving and maintaining testosterone concentration below castration levels in patients with prostate cancer and is well tolerated.

CONFLICTS OF INTEREST

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Dr. Shore, as the principal investigator of this trial, was involved in study design, interpreted the data, and reviewed the drafts of the manuscript. Dr. Parente was responsible of the project conceptualization and was involved in the clinical trial study design. Moreover, he was the project leader for the development of leuprolide acetate formulation. Ms. Sanahuja and Dr. Guerrero managed the project at the sponsors site; in addition, they contributed, together with Dr. Gambús and Dr. Parente, to analysing the data, writing the

manuscript, and creating figures. All authors were involved in the decision to submit the article for publication; accordingly, they read and approved the final version of the manuscript. The sponsor was not involved in the collection and interpretation of study data. Study collection and writing the clinical study report were performed by a contract research organization.

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