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ORIGINAL ARTICLE

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Natesto™, a novel testosterone nasal gel, normalizes androgen levels in hypogonadal men

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SUMMARY

Advantages of testosterone nasal gel include ease of administration, low dose, and no risk of secondary transference. The efficacy and safety of testosterone nasal gel was evaluated in hypogonadal males. The ninety-day, randomized, open-label, dose-ranging study, included potential dose titration and sequential safety extensions to 1 year. At 39 US outpatient sites, 306 men (mean age 54.4 years) with two fasting morning total serum testosterone levels <300 ng/dL were randomized (n = 228, b.i.d. dosing; n = 78, t.i.d. dosing). Natesto™ Testosterone Nasal Gel was self-administered, using a multiple-dose dispenser, as two or three daily doses (5.5 mg per nostril, 11.0 mg single dose). Total daily doses were 22 mg or 33 mg. The primary endpoint was the Percentage of patients with Day-90 serum total testosterone average concentration (C_{avg}) value within the eugonadal range (≥300 ng/dL, ≤1050 ng/dL). At Day 90, 200/273 subjects (73%; 95% CI 68, 79) in the intent-to-treat (ITT) population and 180/237 subjects (76%; 71, 81) in the per-protocol (PP) population were in the normal range. Also, in the normal range were 68% (61, 74) of ITT subjects and 70% (63, 77) of PP subjects in the titration arm, as well as, 90% (83, 97) of ITT subjects and 91% (84, 98) of PP subjects in the fixed-dose arm. Natesto™ 11 mg b.i.d. or 11 mg t.i.d. restores normal serum total testosterone levels in most hypogonadal men. Erectile function, mood, body composition, and bone mineral density improved from baseline. Treatment was well tolerated; adverse event rates were low. Adverse event discontinuation rates were 2.1% (b.i.d.) and 3.7% (t.i.d.). This study lacked a placebo or an active comparator control which limited the ability to adequately assess some measures.

INTRODUCTION

Male hypogonadism is a clinical syndrome resulting from failure of the testis to produce physiologic levels of testosterone as a result of disruption at one or more levels of the hypothalamic-pituitary-testicular axis (Bhasin *et al.*, 2010). Both primary and secondary testicular failure result in low testosterone levels, clinical symptoms, and impaired spermatogenesis (Wu *et al.*, 2010). Testosterone deficiency has many detrimental effects, including impaired sexual function, reduced energy levels, mood disturbances, and reduced quality of life. It also contributes to the development of osteoporosis, increased fat mass, and muscular atrophy (Basaria, 2010; Bhasin *et al.*, 2010).

Extensive evidence supports testosterone replacement therapy (TRT) for men with hypogonadism (Wang *et al.*, 2000; Steidle *et al.*, 2003; Tracz *et al.*, 2006; Reyes-Vallejo *et al.*, 2007; Emmelot-Vonk *et al.*, 2008; Chiang *et al.*, 2009; Bhasin *et al.*, 2010). TRT can be administered by injections (intramuscular or subcutaneous implantation), transdermally (gels, axillary solution, and patches) and transmucosally (buccal system) (Basaria, 2010; Bhasin *et al.*, 2010). Despite the overall effectiveness of TRT, different types of testosterone products are associated with various adverse reactions. Testosterone esters have highly variable pharmacokinetics and are associated with injection site pain and polycythemia. Skin adhesion problems, skin or mucosal irritation, or unintentional testosterone transference to women and children are examples of undesired properties of existing TRT preparations (Steidle *et al.*, 2003; Korbonits *et al.*, 2004; Wang *et al.*, 2004; Merhi & Santoro, 2007; Basaria, 2010; Cabrera & Rogol, 2013). These limitations and adverse reactions have encouraged the investigation of other modes of delivery for TRT including the intranasal route of administration.

The nasal mucosa offers high permeability and high bioavailability, as the drug is not subject to first-pass metabolism (Mattern *et al.*, 2008; Banks *et al.*, 2009). A previous study showed that a single dose nasal gel in eight hypogonadal men showed rapid absorption with T_{max} at ca. 60 min (Mattern *et al.*, 2008). Multiple daily doses (2 or 3) were required to achieve the appropriate levels of circulating testosterone. The half-life ranged from

10 to 100 min 100 min. As such, testosterone levels returned nearly to baseline between doses (Mattern *et al.*, 2008; Natesto Prescribing Information 2014). Administration via the intranasal route is simple safe and rapid, requiring only a few seconds per day (Pires *et al.*, 2009).

Acerus Pharmaceuticals Corp. (Mississauga, ON Canada) developed Natesto™ Testosterone Nasal Gel as a hormone replacement therapy for males with hypogonadism. Here, we describe the randomized, open-label, dose-ranging study to evaluate the efficacy and safety of testosterone nasal gel in the treatment of male hypogonadism. The primary endpoint is based on pharmacokinetic analyses which demonstrate the ability to achieve testosterone levels in the normal range.

MATERIALS AND METHODS

Study design

Thirty-nine US outpatient sites were recruited into this phase 3, randomized, open-label study sponsored by Acerus Pharmaceuticals SRL. The study design (Fig. 1) consisted of three parts: Part 1:a 3- to 7-week Screening Period that included (i) 2-week washout for subjects previously on topical TRT or 4-week washout for subjects previously on injectable testosterone enanthate and cypionate (washout was confirmed by measurement of morning total testosterone) and (ii) measurement of baseline parameters for various safety and secondary efficacy; Part 2: a 2-arm 90-day pharmacokinetic efficacy treatment period during which subjects received study drug either in a fixed-dose arm (t.i.d., 5.5 mg/nostril, 11 mg/dose, 33 mg/day) or in a titration arm, starting at twice daily (b.i.d., 22 mg/day) with potential dose adjustment to t.i.d (33 mg/day) on Day 45 based on morning serum total testosterone levels; Part 3: two sequential Safety Extension Periods of 90 and 180 days during which subjects continued on study drug at their Day 90 dose level. Maximum subject participation was 406 days.

Protocol and informed consent forms were approved by each center's Institutional Review Board before study initiation. The study was conducted in accordance with the Declaration of Helsinki and all applicable laws and regulations of the United States, and in compliance with Good Clinical Practice Guidelines. Each subject signed an informed consent form before initiation of study procedures.

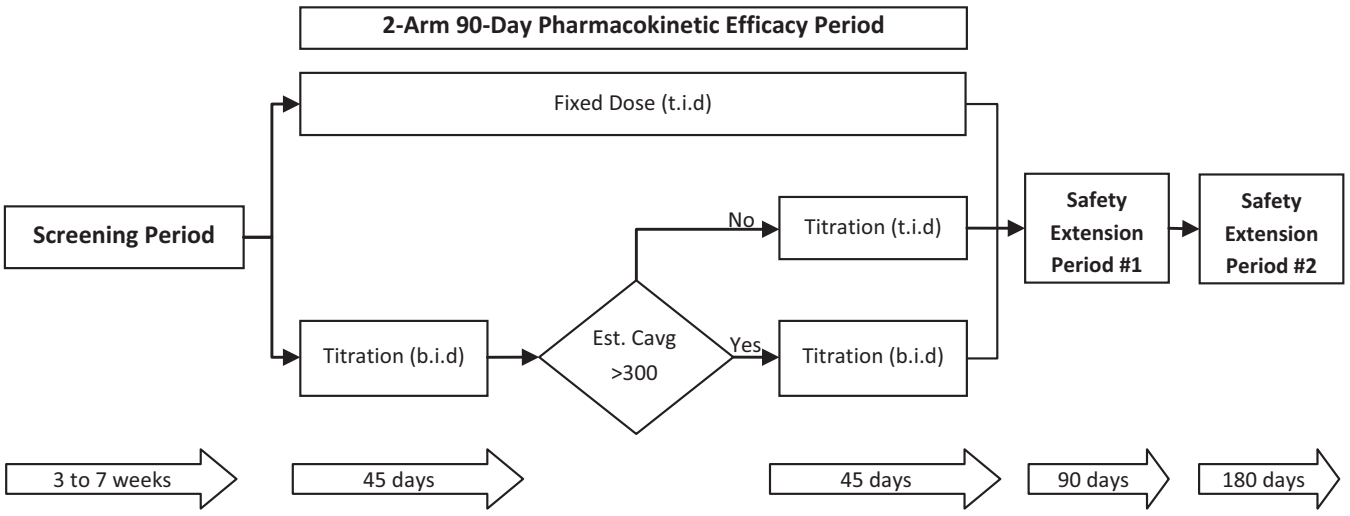
Study drug

Natesto™ Testosterone Nasal Gel, manufactured by Haupt Pharma (Regensburg, Germany), is a 4.5% testosterone gel. The drug is administered from a non-pressurized, manual pump dispenser equipped with a specialized nasal applicator which administers 125 μL (5.5 mg testosterone) of the thixotropic gel directly onto the mucosa of the nasal vestibule of each nostril (total dose 11 mg). Light massaging of the nostrils spreads the gel inside the lower nasal cavity, from where it is absorbed into the bloodstream (Natesto™ (testosterone) Nasal Gel Prescribing Information, 2014).

Eligibility criteria

Men aged 18 to 80 years were eligible if they had two fasting morning (0900 h ± 30 min) screening serum testosterone levels <300 ng/dL; a body mass index between 18.5 and 35 kg/m^2, inclusive; and a hemoglobin level ≥13.0 g/dL. Eligible subjects required an otorhinolaryngological (ENT) examination, including nasal endoscopy, without clinically significant abnormal findings, a normal prostate based on digital rectal examination, and a serum prostate specific antigen (PSA) level <4.0 ng/mL. Exclusion criteria included nasal disorders, inflammation (e.g., Sjogren's syndrome), nasal or sinus surgery, recent nasal fracture, deviated septum, as well as significant intercurrent disease or laboratory abnormalities. Respiratory conditions resulting in exclusion included active allergies, mucosal inflammatory disorders, sinus disease, nasal disorders or surgery, history of asthma and ongoing asthma treatments, and history of sleep apnea. Patients were also ineligible if they were receiving drugs that interfered with the metabolism of testosterone. Also excluded were shift workers, individuals who used any type of intranasal medication, smoked >10 cigarettes (or equivalent) per day, regularly consumed more than 4 units of alcohol daily, or had a history or current evidence of substance abuse. Detailed exclusion criteria appear as Supplemental Data S1.

Figure 1 Study design.



Study procedures

Randomization occurred on Day 1 via the ClinTrak™ Interactive Voice Response System (Medpace, Inc.; Cincinnati, OH), with subjects randomly assigned in a 3:1 ratio to the titration or the fixed-dose arm, respectively (Fig. 2). Following randomization, all subjects were instructed on the use of the medication dispenser, and then received their first dose in the clinic at 2100 h. Subjects assigned to the titration arm were instructed to take 1 actuation (5.5 mg) per nostril of study medication at 0700 h and 2100 h (b.i.d., total dose 22 mg/day), while subjects assigned to the fixed-dose arm took 5.5 mg per nostril at 0700 h, 1300 h, and 2100 h (t.i.d, total dose 33 mg/day). Subjects in the titration arm having an estimated serum total testosterone average concentration (C_{avg}) of <300 ng/dL (measured at Day 30) were up-titrated to t.i.d. on Day 45. Subjects continued treatment through the 90-day Treatment Period and, as applicable, through both Safety Extension Periods out to 1 year (Supplemental Data S1).

Efficacy and pharmacokinetic/safety assessments

The primary efficacy variable was the percentage of subjects with a serum total testosterone C_{avg} within the eugonadal range (≥300 ng/dL, ≤1050 ng/dL) on Day 90. Efficacy was assessed for subjects in the titration and fixed-dose arms. Secondary efficacy measures included change from baseline to Day 90 in sexual function measured by International Index of Erectile Function (IIEF) (Rosen *et al.*, 2002) and mood states measured by Positive and Negative Affect Schedule scores (PANAS) (Watson *et al.*,1988), and Day-180 and 360 changes from baseline in body composition and bone mineral density (BMD).

Secondary pharmacokinetic endpoints were based on the Day-90 dose regimen (b.i.d. or t.i.d.) and included:

- Number and percentage of subjects with a serum total testosterone C_{max} in pre-specified categories: ≤1500 ng/dL, ≥1800 and ≤2500 ng/dL, and >2500 ng/dL
- Complete pharmacokinetic profile (C_{avg}, C_{min}, C_{max}, and T_{max}) of serum total testosterone
- Serum DHT and estradiol pharmacokinetic parameters
- DHT_{avg}/T_{avg} ratio

Safety variables and clinical safety assessments at Day 90, Day 180, and Day 360 included:

- The incidence, severity and causation of treatment-emergent adverse events (TEAEs), including local tolerance, cardiovascular and androgen-specific adverse events (AEs).
- Clinical laboratory measurements, including chemistry profile, liver function tests, fasting lipid profile, hematology, urinalysis, PSA, endocrine profile, 12-lead electrocardiogram (ECG) parameters, vital signs, physical examination parameters and ENT examination.

ANALYTICAL METHODS

Bioanalytical measurements for serum hormones (total testosterone, DHT and Estradiol) were performed using a validated method using an API 4000 LC-MS/MS system at Analytical Biochemical Laboratory (ABL, Assen, Netherlands). The analytical range of the assay was 0.500–50.0 ng/mL for testosterone, 0.100–10.0 ng/mL for DHT and 5.00–100 pg/mL for estradiol. Free testosterone concentrations were calculated using the measured concentrations for total testosterone, albumin and SHBG. Clinical laboratory measurements for safety were performed by Medpace Reference Laboratories (Cincinnati, OH).

Subjects' experience with intranasal administration

Subjects receiving study medication for ≥90 days were invited to answer questions about their experience. To the question "Did you have any issues with the intranasal administration of testosterone nasal gel?" subjects could answer "Yes," "No," or "Neutral." To the question "How many days did it take for you to become confident in applying the testosterone nasal gel?" subjects could answer "Less than 1 day," "1–2 days," or "3+ days." Subjects having experience with other forms of TRT were also asked: "Did not having to touch the gel serve as benefit to you?" and could answer "Yes," "No," or "Neutral." Subjects with prior TRT experience were also asked: "If TBS-1 (the nasal gel study drug) was on the market, and it cost you the exact same out of pocket, would you stay on it or go back to your old one?" and could answer "Stay on TBS-1," "Go back to previous medication".

Statistical analysis

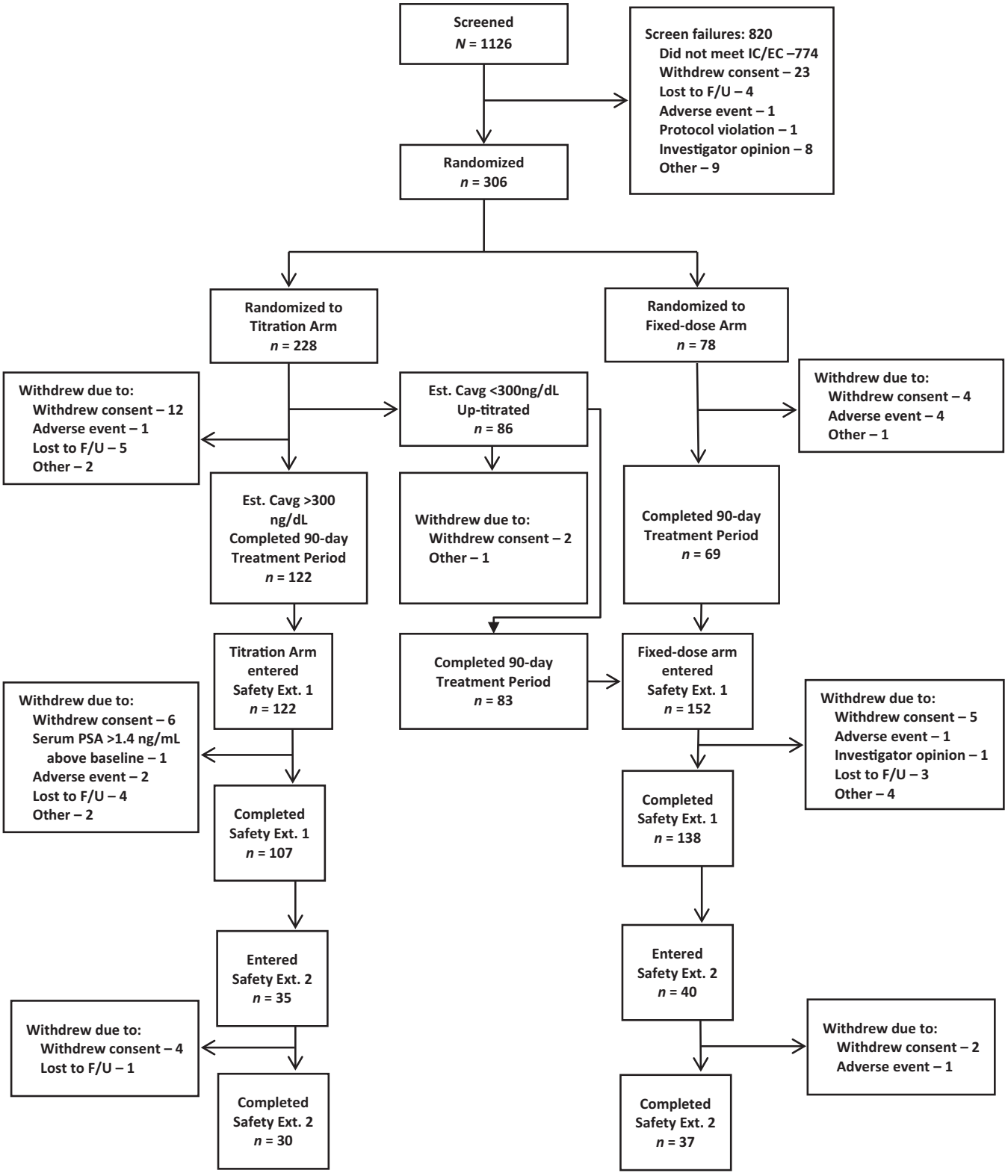
Data analysis populations were the intent-to-treat (ITT) population (subjects who received study drug and had at least 1 valid post-baseline efficacy measurement), the per-protocol population (PP; ITT subjects who completed the 90-day Treatment Period with no major protocol deviations), and the safety population (any subject who received study drug and had safety measurements). The primary efficacy analysis, which included subjects with valid 24-h serum total testosterone C_{avg}, was performed for ITT and PP populations in the fixed-dose and titration arms. Secondary pharmacokinetic analyses included grouping based on Day 90 dose regimen (b.i.d. or t.i.d.) for the ITT population. Total testosterone C_{max} was evaluated in the ITT and PP populations (Additional statistical methods are presented in the online Supplement).

According to the randomization scheme, 228 and 78 subjects were assigned to the titration arm and the fixed dose arm. The ITT population comprised 226 subjects in the titration arm and 77 in the fixed-dose arm. The PP population included 170 subjects in the titration arm and 67 in the fixed-dose arm. On Day 45, 85 titration-arm subjects were up-titrated to the t.i.d. dose. The safety populations are defined by 142 and 164 subjects, on b.i.d. and t.i.d, respectively. Demographic and baseline characteristics (Table 1) were similar between treatment arms and dose-level groupings. During the Treatment Period and Safety Extension Periods 1 and 2, respectively, the mean overall exposure to study medication was 86.1, 175.7, and 348.2 days. Exposure was similar across groups.

Supplemental section

The Supplemental Section includes more detailed information about study procedures, statistical analysis, concomitant medications, Day-90 serum total testosterone C_{max} values (Table S1), serum DHT and estradiol pharmacokinetic values (Table S2), and IIEF and PANAS scores (Tables S3 and S4). Table S5 presents an overview of TEAEs (Table 2).

Figure 2 Disposition of subjects. Titration Arm: includes subjects who were randomly assigned to b.i.d. dosing of study medication on Day 1 and could be up-titrated to t.i.d. dosing on Day 45. Fixed Dose Arm: includes subjects who were randomly assigned to t.i.d. dosing on Day 1 and remained on t.i.d. dosing throughout the study. Abbreviations: EC, exclusion criteria; Ext., extension; IC, inclusion criteria; F/U, follow-up; PSA, prostate-specific antigen.



RESULTS

Figure 3 shows a plot of 24-h serum total testosterone concentration-time curves at Day 90 by treatment regimen. The mean total testosterone C_{avg} increased from 200.8 ng/dL at baseline into the normal range in all groups after 90 days of treatment (Table 3). Mean total testosterone C_{avg} were 375 and 421 ng/dL

Table 1 Demographic and baseline characteristics in the randomized population

Characteristics	Titration arm[a] (*n* = 228)	Fixed-Dose Arm[b] (*n* = 78)	Total (*N* = 306)
Mean (SD) age, years	54.4 (10.9)	54.4 (11.5)	54.4 (11.0)
Not Hispanic or Latino, *n* (%)	196 (86.0)	69 (88.5)	265 (86.6)
Race, *n* (%)			
Asian	13 (5.7)	3 (3.8)	16 (5.2)
Black or African American	14 (6.1)	4 (5.1)	18 (5.9)
White	201 (88.2)	70 (89.7)	271 (88.6)
Other	0	1 (1.3)	1 (0.3)
Mean (SD) weight, kg	93.2 (14.6)	93.7 (13.4)	93.3 (14.3)
Mean (SD) height, cm	177.1 (7.0)	176.9 (6.6)	177.1 (6.9)
Mean (SD) BMI, kg/m^2	29.6 (3.7)	29.9 (3.2)	29.7 (3.6)
Hypogonadism etiology, *n* (%)			
Primary	167 (73.2)	52 (66.7)	219 (71.6)
Secondary	61 (26.8)	26 (33.3)	87 (28.4)
Mean (SD) hypogonadism duration, y	4.5 (4.0)	5.0 (5.7)	4.6 (4.5)
Testosterone therapy at Screening, *n* (%)			
None	168 (73.7)	56 (71.8)	224 (73.2)
Injection	29 (12.7)	10 (12.8)	39 (12.7)
Oral	1 (0.4)	2 (2.6)	3 (1.0)
Topical	31 (13.6)	10 (12.8)	41 (13.4)
Buccal	0	0	0
Previous testosterone treatment, subjects, *n* (%)			
None currently or naive	98 (43.0)	32 (41.0)	130 (42.5)
Injection	30 (13.2)	11 (14.1)	41 (13.4)
Oral	5 (2.2)	1 (1.3)	6 (2.0)
Topical	38 (16.7)	13 (16.7)	51 (16.7)
Buccal	4 (1.8)	1 (1.3)	5 (1.6)
Mean (SD) Screening values			
Fasting serum total testosterone, ng/dL	197.6 (67.9)	210.3 (51.5)	200.8 (64.3)
DHT, ng/dL	18.8 (8.9)	20.5 (9.0)	19.2 (9.0)
Estradiol, pg/mL	17.8 (6.9)	19.6 (7.5)	18.2 (7.1)

BMI, body mass index; C_{avg}, average concentration. [a]Subjects were randomly assigned to b.i.d. dosing of study medication on Day 1 and could be up-titrated to t.i.d. dosing on Day 45. [b]Subjects were randomly assigned to t.i.d. dosing on Day 1 and remained on t.i.d. dosing throughout the study.

for b.i.d and t.i.d. regimens, respectively. Among subjects whose C_{avg} value was in the normal range, the mean values were 415 ng/dL for the b.i.d. and 428 ng/dL for the t.i.d. regimens. Geometric mean total testosterone C_{max} values did not exceed the upper limit of normal (ULN) for the b.i.d. and t.i.d. regimens at Day 90.

The percentages of ITT subjects (95% CI in parentheses) whose total testosterone C_{avg} were in the normal range was 73% (68, 79) in the total population, 68% (61, 74) in the titration arm, and 90% (83, 97) in the fixed-dose arm. Percentages of PP subjects whose C_{avg} was within the normal range were 76% (71, 81) for the total population, 70% (63, 77) for the titration arm, and 91% (84, 98) for the fixed-dose arm. For ITT subjects in the titration arm, 71% (62, 79) on b.i.d. dosing and 63% (53, 74) on t.i.d. had C_{avg} values in the normal range.

In addition, 88.6% of the ITT population had mean testosterone C_{max} at Day 90 below 1500 ng/dL (Table S1). Nine (3.3%) subjects had C_{max} between 1800 and 2500 ng/dL. One subject showed a C_{max} >2500 ng/dL (3570 ng/dL); this subject, presumably did not discontinue concomitant finasteride treatment prior to the study as evidenced by increased testosterone AUC and an unusually low DHT/T ratio as a result of inhibition of 5a-reductase which blocks conversion of testosterone to DHT. No safety concerns were identified for this subject.

At screening, the overall mean DHT concentration (ITT) was 19.2 ng/dL. At Day 90, mean DHT C_{avg} values increased to 33.2–40.1 ng/dL across all treatment groups and were in the range of normal (25.5–97.8 ng/dL). Geometric mean DHT C_{max} values did not exceed the ULN. Day-90 mean DHT/T ratio ranged from 0.089 to 0.094 ng/dL across groups, mimicking physiological levels (Watson *et al.*, 1988) (Table S2). Likewise, the overall mean estradiol concentration at screening was 18.2 pg/mL. For all groups at Day 90, mean estradiol C_{avg} values remained within the normal range of healthy males (3–81 pg/mL), and geometric mean estradiol C_{max} values did not exceed the ULN (Table S2).

Improvement from baseline in mean erectile function ($p < 0.0001$ for mean total IIEF with b.i.d. and t.i.d. dosing) and in mood scores ($p < 0.0001$ for mean positive affect, $p < 0.01$ for negative affect) achieved statistical significance by Day 90 (Tables S3 and S4). Positive changes from baseline in body composition (increase in lean body mass from baseline, $p = 0.0384$ at Day 180) were observed in ITT population.

Safety results

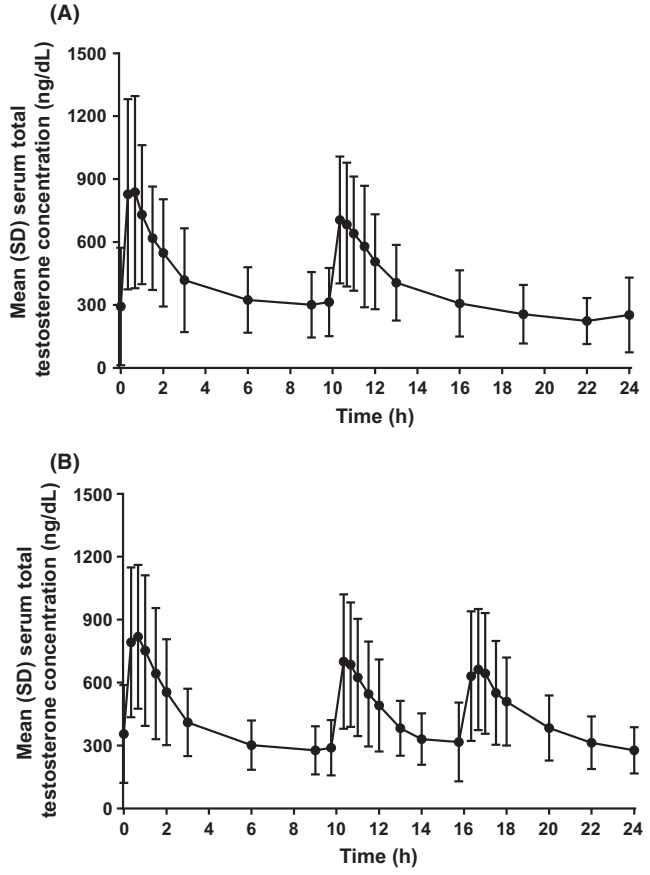
Proportions of subjects with ≥1 TEAE and ≥1 possibly drug-related TEAE were, respectively, 63.4% and 30.3% for the b.i.d. regimen and 64.6% and 40.9% for the t.i.d. regimen (Table S5). Most TEAEs and drug-related TEAEs were mild in severity; 37.6% of subjects had TEAEs and 26.1% had drug-related TEAEs of mild severity. Overall, 4.6% of subjects had ≥1 severe TEAE and 1 subject (0.3%) had a severe drug-related TEAE (myalgia, which did not require study drug discontinuation or dose adjustment and remitted after initiating concomitant medication). Eight (2.6%)

Table 2 Primary efficacy measure: percentage of testosterone nasal gel-treated subjects with serum total testosterone C_{avg} in the normal range at Day 90 of the treatment period by treatment and by population[a]

Time point, population	Variable	Titration arm[b] (*n* = 226)	Fixed-dose arm[c] (*n* = 77)	b.i.d.[d] (*n* = 141)	t.i.d.[e] (*n* = 162)	Total (*N* = 303)
Day 90, ITT	*n* with C_{avg} data	204	69	122	151	273
	n in normal range	138	62	86	114	200
	% in normal range (95% CI)	68 (61, 74)	90 (83, 97)	71 (62, 79)	76 (69, 82)	73 (68, 79)
Day 90, PP	*n* with C_{avg} data	170	67	102	135	237
	n in normal range	119	61	76	104	180
	% in normal range (95% CI)	70 (63, 77)	91 (84, 98)	75 (66, 83)	77 (70, 84)	76 (71, 81)

b.i.d., twice daily; C_{avg}, average concentration; CI, confidence interval; ITT, intent-to-treat; PP, per-protocol; t.i.d., three times daily. [a]Criterion for success was ≥75% of subjects with a serum total testosterone C_{avg} value in the normal range, with the lower 95% CI ≥65%. [b]Subjects were randomly assigned to b.i.d. dosing of study medication on Day 1 and could be up-titrated to t.i.d. dosing on Day 45. [c]Subjects were randomly assigned to t.i.d. dosing on Day 1 and remained on t.i.d. dosing throughout the study. [d]Subjects were randomly assigned to b.i.d. dosing on Day 1 and remained on b.i.d. dosing throughout the study. [e]Subjects were assigned to t.i.d. dosing, either on Day 1 or Day 30.

Figure 3 Plot of 24-h total testosterone concentration-time curves by treatment regimen and time point at Day 90 in the intent-to-treat population. Data are shown for the b.i.d. dosing (n = 141) (A), and the t.i.d. dosing (n = 77) (B).



subjects had a serious AE (SAE), including abdominal mass, pneumonia, angina pectoris, gastroesophageal reflux disease, Rocky Mountain spotted fever and acute coronary syndrome, hip fracture, ligament rupture, and internal injuries because of a motorcycle accident. One subject died during the Treatment Period from internal injuries sustained in a motorcycle accident. Observed SAEs were not considered related to study medication, as assessed by the primary investigator.

Nine subjects discontinued the study because of a TEAE: 3 (2.1%) from the b.i.d. and 6 (3.7%) from the t.i.d. groups. Seven (2.3%) subjects discontinued due to a drug-related TEAE: 3 (2.1%) receiving b.i.d. and 4 (2.4%) receiving t.i.d. therapy. Additional TEAE findings are presented as Supplemental Data.

Table 4 shows commonly reported androgenic, local tolerance, and other TEAEs. Increased hematocrit (57 and 56%) was reported in 1 subject (0.7%) in the b.i.d. and in 1 subject (0.6%) in the t.i.d. group, respectively; these events were reported during Safety Extension 1. The mean baseline hematocrit value was 44.8%. A slight decrease (to 44.1%) at Day 90 was attributed to blood withdrawal for the pharmacokinetic analysis. Mean values were 45.6% and 45.3%, respectively, at Day 180 and Day 360. These increases were consistent with TRT. During study drug treatment, hematocrit values did not exceed the ULN in the majority of subjects. Eight subjects (2.6%) had hematocrit values ≥54%. All abnormal hemoglobin, hematocrit, and red blood cell (RBC) values were borderline and not clinically significant. No subjects discontinued the study because of hematology abnormalities.

Mean baseline PSA values were 0.97 ng/mL in the b.i.d. and 1.17 ng/mL in the t.i.d. groups. Mean increases from these baseline values at Day 180 and Day 360, respectively, were 0.01 and 0.06 ng/mL in the b.i.d. and 0.09 and 0.21 ng/mL in the t.i.d. groups. Events of increased PSA (either greater than by 1.4 μg/L or higher than 4.0 μg/L) were reported in 10 (6.1%) subjects in the t.i.d. group (Treatment Period, n = 6; Safety Extension 1, n = 4; Safety Extension 2, n = 0). No events of PSA increase were reported in the b.i.d. group at any point.

No clinically meaningful changes in vital sign measurements, physical examination findings, or ECG results, including changes in the QT interval duration, were detected. There were no clinically significant changes for any treatment group in the mean values of chemistry, hematology, or urinalysis parameters, or in liver function or endocrine profiles. No notable changes in lipid profiles were observed. Overall mean concentration changes from baseline at Day 360 were −2.4% for high-density lipoprotein cholesterol, +0.8% for low-density lipoprotein cholesterol, and +0.2% for total cholesterol. For triglycerides, there was a median increase of 3.3%.

At Day 90, 2.6% of subjects had abnormal ENT findings and 11.0% had ENT symptoms, the most common in both cases was "other, not specified." At Day 180 and Day 360, respectively, 3.7% and 0% of subjects had abnormal ENT findings, and 7.4% and 6.1% showed ENT symptoms.

Ninety-nine (32.4%) subjects (b.i.d., n = 33; t.i.d., n = 66) with at least 90 days exposure drug exposure completed a patient survey: 25/99 subjects reported minor issues with testosterone nasal gel administration, the most common ($N > 2$) were taste/smell

Table 3 Pharmacokinetics of serum total testosterone by treatment regimen at Day 90 of the treatment period in the intent-to-treat population

Group	Statistic	AUC_{0-24} (ng × h/dL)	C_{avg} (ng/dL)	C_{min} (ng/dL)	C_{max} (ng/dL)	T_{max} (hr)
b.i.d.[a],	*n*	122	122	122	122	122
n = 141	Mean (SD)	9007.5 (3092.5)	375.3 (128.9)	186.3 (92.6)	1045.7 (467.1)	1.4 (2.5)
	Geom. mean	8590.2	357.9	166.8	958.0	0.7
	Median	8412.2	350.5	164.0	987.5	0.7
t.i.d.[b],	*n*	151	151	151	151	151
n = 162	Mean (SD)	9285.3 (2684.9)	386.9 (111.9)	200.9 (72.7)	934.9 (381.2)	1.0 (1.0)
	Geom. mean	8918.6	371.6	187.6	861.7	0.7
	Median	9068.0	377.8	192.0	884.0	0.7

AUC_{0-24}, area under the curve from 0 to 24 h; b.i.d., twice-daily; C_{avg}, average concentration; C_{max}, maximum concentration; C_{min}, minimum concentration; Geom., geometric; t.i.d., three times daily; T_{max}, time to maximum concentration. [a]Subjects were randomly assigned to b.i.d. dosing on Day 1 and remained on b.i.d. dosing throughout the study. [b]Subjects were assigned to t.i.d. dosing, either on Day 1 or Day 30.

Table 4 Summary of treatment-emergent adverse events and serious adverse events by study period and treatment

Category, Event	Incidence of TEAEs and SAEs, *n* (%)							
	Treatment period		Safety extension 1		Safety extension 2		All study periods	
	b.i.d.[a] (*n* = 143) [b]	t.i.d.[c] (*n* = 163)	b.i.d.[a] (*n* = 120)	t.i.d.[c] (*n* = 152)	b.i.d.[a] (*n* = 34)	t.i.d.[c] (*n* = 40)	b.i.d.[a] (*n* = 142) [b]	t.i.d.[c] (*n* = 164)
Commonly reported androgenic TEAEs by SOC and preferred term[d]								
Investigations	3 (2.1)	6 (3.7)	5 (4.2)	7 (4.6)	2 (5.9)	4 (10.0)	8 (5.6)	30 (18.3)
PSA increased	0	6 (3.7)	0	4 (2.6)	0	0	0	10 (6.1)
Commonly reported local tolerance TEAEs by SOC and preferred term[d]								
Nervous system	13 (9.1)	15 (9.2)	1 (0.8)	10 (6.6)	4 (11.8)	1 (2.5)	16 (11.3)	26 (15.9)
Parosmia	7 (4.9)	5 (3.1)	0	3 (2.0)	1 (2.9)	1 (2.5)	7 (4.9)	9 (5.5)
Dysgeusia	1 (0.7)	3 (1.8)	1 (0.8)	2 (1.3)	0	0	2 (1.4)	5 (3.0)
Respiratory, thoracic, and mediastinal disorders	33 (23.1)	41 (25.2)	18 (15.0)	19 (12.5)	6 (17.6)	8 (20.0)	27 (19.0)	55 (33.5)
Rhinorrhea	8 (5.6)	11 (6.7)	3 (2.5)	2 (1.3)	1 (2.9)	0	10 (7.0)	14 (8.5)
Epistaxis	6 (4.2)	8 (4.9)	3 (2.5)	4 (2.6)	1 (2.9)	3 (7.5)	9 (6.3)	11 (6.7)
Nasal discomfort	6 (4.2)	5 (3.1)	5 (4.2)	2 (1.3)	1 (2.9)	0	10 (7.0)	8 (4.9)
Nasal dryness	6 (4.2)	3 (1.8)	2 (1.7)	3 (2.0)	0	1 (2.5)	7 (4.9)	6 (3.7)
Nasal congestion	5 (3.5)	5 (3.1)	1 (0.8)	0	1 (2.9)	2 (5.0)	5 (3.5)	7 (4.3)
Nasal mucosal disorder	4 (2.8)	0	2 (1.7)	1 (0.7)	0	0	6 (4.2)	1 (0.6)
Upper-airway cough syndrome	1 (0.7)	3 (1.8)	0	0	0	0	1 (0.7)	3 (1.8)
Rhinalgia	1 (0.7)	2 (1.2)	1 (0.8)	0	1 (2.9)	0	2 (1.4)	2 (1.2)
Skin and subcutaneous tissue disorders	9 (6.3)	10 (6.1)	5 (4.2)	7 (4.6)	2 (5.9)	2 (5.0)	14 (9.9)	17 (10.4)
Scab	3 (2.1)	5 (3.1)	3 (2.5)	2 (1.3)	2 (5.9)	2 (5.0)	8 (5.6)	8 (4.9)
Skin fissures	0	1 (0.6)	0	0	2 (5.9)	0	2 (1.4)	1 (0.6)
Other commonly reported TEAEs by SOC and preferred term[d]								
Infections and infestations	15 (10.5)	24 (14.7)	11 (9.2)	18 (11.8)	8 (23.5)	10 (25.0)	33 (23.2)	44 (26.8)
Nasopharyngitis	4 (2.8)	7 (4.3)	2 (1.7)	5 (3.3)	3 (8.8)	5 (12.5)	9 (6.3)	16 (9.8)
Upper respiratory tract infection	1 (0.7)	3 (1.8)	3 (2.5)	3 (2.0)	2 (5.9)	1 (2.5)	6 (4.2)	7 (4.3)
Urinary tract infection	1 (0.7)	0	1 (0.8)	0	0	2 (5.0)	2 (1.4)	2 (1.2)
Bronchitis	2 (1.4)	3 (1.8)	1 (0.8)	3 (2.0)	0	1 (2.5)	3 (2.1)	5 (3.0)
Tooth abscess	0	4 (2.5)	0	0	0	0	0	4 (2.4)
Investigations	3 (2.1)	21 (12.9)	5 (4.2)	7 (4.6)	2 (5.9)	4 (10.0)	8 (5.6)	30 (18.3)
Blood CPK increased	1 (0.7)	4 (2.5)	1 (0.8)	0	0	0	2 (1.4)	4 (2.4)
Musculoskeletal and connective tissue disorders	12 (8.4)	13 (8.0)	4 (3.3)	7 (4.6)	2 (5.9)	2 (5.0)	17 (12.0)	21 (12.8)
Back pain	4 (2.8)	3 (1.8)	0	2 (1.3)	0	0	4 (2.8)	5 (3.0)
Pain in extremity	2 (1.4)	1 (0.6)	0	3 (2.0)	1 (2.9)	1 (2.5)	3 (2.1)	5 (3.0)
Arthralgia	1 (0.7)	3 (1.8)	2 (1.7)	1 (0.7)	0	0	3 (2.1)	4 (2.4)
Nervous system disorders	13 (9.1)	15 (9.2)	1 (0.8)	10 (6.6)	4 (11.8)	1 (2.5)	16 (11.3)	26 (15.9)
Headache	2 (1.4)	3 (1.8)	0	3 (2.0)	2 (5.9)	0	4 (2.8)	6 (3.7)
SAEs by SOC and preferred term								
Cardiac disorders								
Angina pectoris					1 (2.9)		1 (0.7)	
Acute coronary syndrome		1 (0.6)						1 (0.6)
Injury, poisoning, and procedural complications								
Broken Hip	1 (0.7)						1 (0.7)	
Fall and ligament fracture	1 (0.7)						1 (0.7)	
Motorcycle accident and internal injuries due to motorcycle accident		1 (0.6)						1 (0.6)
Infections and infestations								
Rocky mountain spotted fever				1 (0.7)				1 (0.7)
Gastrointestinal disorders								
Vomiting						1 (2.5)		1 (0.7)
Gastroesophageal reflux disease						1 (2.5)		1 (0.7)
Abdominal mass		1 (0.6)						1 (0.6)

CPK, creatine phosphokinase; PSA, prostate-specific antigen; SOC, system organ class; TEAE, treatment-emergent adverse event. SAE, serious adverse event. [a]Subjects were randomly assigned to b.i.d. dosing on Day 1 and remained on b.i.d. dosing throughout the study. [b]One subject was assigned to t.i.d. regimen on Day 45, but did not up-titrate until after Day 90. This subject is included in the b.i.d. group up until Day 90 and in the t.i.d. group afterwards. [c]Subjects were assigned to t.i.d. dosing, either on Day 1 or Day 30. [d]Incidence ≥2% in either treatment group.

(7), cold/runny nose (7) soreness/irritation (5), gel running down throat (3) and crustiness (2); 83.8% reported feeling confident about administering the product within 2 days of initiating therapy; and 48.0% of subjects, previously on other testosterone therapy ($n = 50$), remarked that not touching the gel was beneficial. Lastly, 58/99 subjects who were receiving other TRT medication prior to study initiation, were asked whether they would return to their previous TRT product or switch to Natesto if the products were of same out-of-pocket cost: 74% (b.i.d) and 68% (t.i.d) stated that they would choose Natesto vs. going back to their previous TRT product. Responses were statistically similar between b.i.d. and t.i.d. groups.

DISCUSSION

TRT products are a class of well-studied and well-understood agents, which are currently approved based on pharmacokinetic measurements, namely the percentage of subjects with testosterone C_{avg} in the normal range. Safety is determined on a larger

set of data including limiting testosterone C_{max} levels, metabolite profiles, PSA induction, adverse events and tolerability.

In the case of testosterone nasal gel, 90% of hypogonadal subjects in the fixed-dose arm and 68% of subjects in the titration arm (ITT) were in the eugonadal range (300–1050 ng/dL) after 90 days of treatment with testosterone nasal gel. Subject's mean total testosterone C_{avg} level after 90 days was 415 ng/dL when taking the b.i.d. dose and 428 ng/dL when receiving the t.i.d. dose. These levels are consistent with the mean C_{avg} (418 ng/dL) reported for a large, population-based epidemiological survey of healthy adult males aged 30–79 years (Litman *et al.*, 2006). After considering the protocol violations, the PP percentage of subjects achieving normal serum testosterone is 91% in the fixed-dose arm and 71% in the titration arm. Protocol violations included failure to up-titrate subjects upon direction of physician or at patient request (b.i.d. being adequate for symptoms) despite estimated C_{avg} values <300 ng/dL. Notably, the percentage of PP subjects in the normal range on the b.i.d. dose of the titration arm was 75% (95% CI, 66–83%).

Each individual dose of nasal gel provides a reproducible short-acting peak that returns near to baseline by the time of the next dose. While there are up to three peaks per profile, C_{max} values were consistently below 1500 mg/d and only 3.3% of subjects had values of 1800–2500 ng/dL. While one subject showed a C_{max} >2500 ng/dL (3570 ng/dL); this subject would appear to have violated the protocol by continuing finasteride treatment. No safety concerns were identified for this subject.

The peaks-and-troughs PK profile did not appear to have a negative impact on symptomatology. There were statistically significant improvements because of treatment in mean values for the erectile function and mood, and positive trends in improvement for body composition and BMD when compared to pretreatment baseline values.

DHT, a testosterone metabolite, is a very potent androgen implicated in benign prostatic hypertrophy, prostate cancer (Andriole *et al.*, 2004) and more recently implicated as a cardiovascular risk factor (Borst *et al.*, 2014). Treatment with testosterone nasal gel results in mean DHT C_{avg} values (33.2–40.1 ng/dL) and DHT/T ratios (<0.1) which remain in the normal physiological range (Litman *et al.*, 2006). These values are generally at the lower end of the range when compared to other approved TRT products, whose mean values range from 77 to 451 ng/dL for DHT C_{avg} and from 0.1 to 0.7 for the DHT/T ratio (Axiron Prescribing Information 2010, Aveed Prescribing Information 2013). Consistent with these observations, there were no subjects on the b.i.d. dose and only 6.1% on the t.i.d. dose that showed increased PSA levels. Mean PSA changes over 180 and 360 days of treatment, respectively, are 0.01 and 0.06 ng/mL for b.i.d. and 0.09 and 0.21 ng/mL for t.i.d. doses. These changes were below the safety limit for PSA increase of 0.3 ng/mL in young men, 0.44 ng/mL in older men (1) and the PSA change was lower than changes reported for other marketed TRT products, whose increases range from 0.13 to 1.5 ng/dL (Axiron Prescribing Information, 2010; Aveed Prescribing Information, 2013). Changes in estradiol levels because of treatment were also unremarkable.

The incidence of TEAEs was similar in subjects who received b.i.d. and t.i.d. treatment, and most events were of mild severity. The frequency of TEAEs leading to discontinuation was low (b.i.d., 2.1%; t.i.d., 3.7%). The most commonly reported TEAEs related to local nasal tolerance, but the incidence was low with both dosing regimens. Frequencies of the most commonly reported events were: nasopharyngitis, 6.3% and 9.8%; rhinorrhea, 7.0 and 8.5%; and epistaxis, 6.3 and 6.7%, for b.i.d and t.i.d treatments, respectively.

A post-study survey was administered to gage how subjects responded to using this novel delivery route. Survey respondents, regardless of whether they were receiving b.i.d. or t.i.d. dosing, reported that they were confident about administering the product within 2 days of starting treatment. Most found that not having to touch the gel was beneficial and nearly 70% subjects previously treated with another TRT said that they would be willing to switch to Natesto, suggesting that the multiple daily dosing was not a major inconvenience.

Some limitations of this study should be noted. Pharmacokinetic analysis is the key primary endpoint to determine efficacy on testosterone therapies. The study was not blinded and did not include an active comparator or a placebo control limiting the usefulness of a variety of measures including secondary efficacy endpoints (IIEF, PANAS and BMD) which were analyzed against baseline values and between the b.i.d. and t.i.d. regimens and are only indicators. Subjects with nasal disorders were excluded from the study (exclusion criteria). Single dose pharmacokinetics of nasal testosterone administration were determined in subjects with active seasonal rhinitis (unpublished results) and treated with oxymetazoline; the results showed a relative decrease in testosterone absorption. It is recommended that patients consult their doctor when nasal inflammation occurs.

CONCLUSION

The intranasal route is increasingly used for systemic drug delivery because it allows for lower dose levels because of efficient absorption and avoidance of first-pass metabolism (Mattern *et al.*, 2008; Banks *et al.*, 2009). This route benefits subjects because application is rapid, non-invasive, convenient, and avoids secondary transference observed with other topical products.

This randomized, open-label, dose-ranging study showed that Natesto™ Testosterone Nasal Gel restores testosterone levels in most hypogonadal men. Statistically significant improvements from baseline were observed in erectile function and mood. Natesto was generally well-tolerated with only a low incidence of local reaction. Furthermore, DHT, DHT/T, and PSA levels produced by the nasal gel are among the lowest levels observed with a commercial formulation of testosterone and suggest an excellent safety profile. Furthermore, nasal therapy was well accepted by most survey respondents. Natesto™ is currently approved for use in the United States.

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DISCLOSURES

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

Table S1. Number and percentage of subjects with serum total testosterone C_{max} values in selected ranges at Day 90 of the treatment period by treatment and population[a].

Table S2. Pharmacokinetics of serum total Dihydrotestosterone and serum Estradiol by treatment at Day 90 of the treatment period in the intent-to-treat population.

Table S3. Mean change from baseline in international index of erectile function total scores at Day 90 of the treatment period in the intent-to-treat population.

Table S4. Mean change from baseline in positive and negative affect schedule scores at Day 90 of the treatment period in the intent-to-treat population.

Table S5. Overview of treatment-emergent adverse events by study period and treatment.

