

Leflurozole in Male Obesity-Associated Hypogonadotropic Hypogonadism: Ph 2b Double-Blind Randomised Controlled Trial

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Abstract

Objective

Assessment of the efficacy and safety/tolerability of the aromatase inhibitor leflutrozolet to normalise testosterone in Obesity-associated Hypogonadotropic Hypogonadism (OHH).

Design

Placebo-controlled, double-blind, RCT, in 70 sites in Europe/USA.

Methods

Patient inclusion criteria: men with BMI of 30–50kg/m², morning total testosterone (TT) <10.41nmol/L, and two androgen deficiency symptoms (at least one of sexual dysfunction). Patients randomised to weekly leflutrozolet (0.1/0.3/1.0mg) or placebo for 24 weeks. Primary endpoint: normalisation of TT levels in ≥75% of patients after 24 weeks. Secondary endpoints (included): time to TT normalisation and change in LH/FSH. Safety was assessed through adverse events and laboratory monitoring.

Results and Conclusions

Of 2103 screened, 271 were randomised, 81 discontinued. Demographic characteristics were similar across groups. Mean BMI was 38.1kg/m², and TT 7.97nmol/L. The primary endpoint was achieved in all leflutrozolet-treated groups by 24 weeks with a dose-tiered response; mean TT 15.89; 17.78; 20.35nmol/L, for leflutrozolet 0.1mg, 0.3mg, 1.0mg groups respectively, versus 8.04nmol/L for placebo. LH/FSH significantly increased in leflutrozolet vs placebo groups. No improvement in body composition or sexual dysfunction were observed. Semen volume/total motile sperm count improved with

1 leflutrozoled vs placebo. Treatment-emergent adverse events, more common in
2 leflutrozoled-treated groups included, raised haematocrit, hypertension, increased PSA,
3 and headache. Some reduction in lumbar bone density was observed with leflutrozoled
4 (mean -1.24%, -1.30%, -2.09%), and 0.66% for 0.1mg, 0.3mg, 1.0mg, and placebo,
5 respectively, without change at the hip. This RCT of leflutrozoled in OHH demonstrated
6 normalisation of TT in obese men. FSH/LH and semen parameter changes support that
7 leflutrozoled may preserve/improve testicular function.

8

Significance Statement

Lifestyle changes of weight loss and physical exercise are the first line of management of obesity-associated male hypogonadotropic hypogonadism, but in practice may be difficult to achieve and sustain. Testosterone replacement therapy is not suitable for men who wish to maintain fertility. Alternatives, such as Aromatase Inhibition and Selective Oestrogen Receptor Modulators (SERMs) that block the oestrogen suppressing effects on the Hypothalamic-Pituitary Gonadotropin (HPG) axis are of interest, but placebo-controlled data in the obese male population are limited. This article presents the efficacy and safety results from a 6-month, double-blind placebo controlled, dose-ranging study of an oral aromatase inhibitor, leflutroazole, in male obesity-associated hypogonadotropic hypogonadism (OHH). Leflutroazole was developed specifically for aromatase inhibition in non-oncological disorders, with relatively low aromatase suppression. Of 2103 screened, 271 eligible subjects were randomised (the majority of screen failures being due to testosterone ineligibility) to 3 different doses of leflutroazole and placebo, for 24 weeks treatment and 12 weeks follow up. All primary and secondary endpoints of testosterone normalisation were met, with rapid onset of effect and increases in gonadotropins. Despite testosterone normalisation, no benefit was observed on exploratory endpoints of sexual dysfunction, cardio-metabolic or body composition after 24 weeks. Patients with OHH experienced improvement in sperm parameters in a predefined sub-set analysis. The potential benefit as a treatment option for men with sub-fertility associated with OHH, needs to be considered in the context of the small but statistically significant reduction in bone mineral density.

1 Introduction

2 Hypogonadotropic Hypogonadism (HH) is a condition in which the testes fail to produce
3 physiological concentrations of testosterone and sperm due to impaired central
4 (pituitary) regulation. Key symptoms of hypogonadism include reduced sexual desire,
5 erectile dysfunction, and fatigue (1, 2, 3, 4, 5). Other features include loss of muscle
6 mass, insulin resistance, and subfertility (4, 6). In addition to pituitary structural
7 abnormalities, HH can be caused by obesity (2, 7).

8
9 Aromatase is highly expressed in adipose tissue, where it converts testosterone to
10 oestradiol (8). This results in high oestradiol levels, that feed back to the hypothalamic–
11 pituitary–gonadal (HPG) axis, suppressing gonadotropin secretion, testicular
12 testosterone production, and spermatogenesis (9, 10).

13
14 Treatment options for men with obesity-associated hypogonadotropic hypogonadism
15 (OHH) remains an unmet need. Obesity is the single most important modifiable factor
16 resulting in functional testosterone deficiency (11), creating a vicious cycle with
17 testosterone deficiency causing increased adipogenesis and visceral fat increase.
18 Management is currently focussed on lifestyle changes, particularly achieve weight
19 reduction and increase in physical exercise. However significant weight loss of >15%
20 body weight is required to increase free testosterone and may be difficult to sustain (12,
21 13). Treatment with testosterone is challenging, requiring monitoring and dose
22 adjustment to manage potential risks (e.g. erythrocytosis) of supra-physiological
23 testosterone levels (2) and may lead to Infertility (14, 15). Short term therapy with

1 respect to cardiovascular concerns appears safe, but there is paucity of long-term data
2 (16). Approaches to stimulating the HPG axis using Selective Oestrogen Reuptake
3 Modulators (SERMs) and Aromatase Inhibitors have shown evidence of increasing
4 testosterone and gonadotropins, improving body composition and sexual dysfunction,
5 without adverse effects on fertility. However, randomised placebo-controlled trial data
6 on efficacy on symptoms, signs and safety in the obese population, particularly in long
7 term studies, are limited. (17, 18).

8
9 Aromatase inhibition can potentially restore endogenous testosterone levels to the
10 physiological range by suppressing testosterone to oestradiol conversion (19, 20, 21,
11 22, 23). Reducing oestradiol levels, and thus decreasing negative feedback on
12 hypothalamic–pituitary function, FSH/LH secretion is expected to increase, stimulating
13 testosterone production and restoring spermatogenesis (24, 25).

14
15 Leflurozole (BGS649) is an aromatase inhibitor being investigated as a treatment for
16 OHH. Leflurozole was specifically developed to be dosed at lower levels and with less
17 suppressive action than aromatase inhibitors approved for management of cancer. The
18 long half-life of leflurozole offers convenient once-weekly dosing, and its oral
19 formulation provides advantages over testosterone injections, or topical formulations,
20 which carry the risk of transference (2, 14). Given that leflurozole is expected to restore
21 physiological function of the HPG axis, supra-physiological levels of testosterone and
22 LH/FSH suppression may be less likely versus testosterone replacement therapy. This

1 may be beneficial to men with OHH wishing to maintain fertility, or as treatment for
2 OHH-associated sub-fertility (26).

3
4 There is limited randomised controlled trial (RCT) data regarding aromatase inhibitors in
5 men with HH. Appropriate dosing of aromatase inhibitors is important, as evidence
6 suggests adverse effects are dose-dependent (26); suppression of oestradiol may
7 impact bone mineral density (BMD) (20, 24), and free testosterone can rise to supra-
8 physiological levels with incorrect doses, because of low and unchanging sex hormone-
9 binding globulin (SHBG) levels (22).

10
11 Here, we report the results of a 24-week, Phase IIb, dose-ranging study designed to
12 assess the efficacy and safety/tolerability of leflutroazole for the treatment of men with
13 OHH.

14 15 **Materials and methods**

16 17 ***Dose selection***

18 Leflutroazole dose selection for this study was guided by a Phase II, dose-finding study
19 (21). Based on that study, three weekly doses (0.1mg, 0.3mg, and 1.0mg) were
20 selected for dose-ranging, targeting achieving total testosterone (TT) levels at low,
21 middle and high aspects of the normal physiological range. The rationale was based on
22 observations that testosterone levels at the higher end of the physiological range may
23 be associated with better clinical outcomes (1).

Ethical conduct of the study

The study was conducted in accordance with Good Clinical Practice, the International Council on Harmonization of Technical Requirements for Pharmaceuticals for Human Use guidelines, applicable regulations, guidelines governing clinical study conduct, and the ethical principles based on the Declaration of Helsinki. Written informed consent was obtained from each patient.

Study design

This was a multicentre, double-blind, randomised, placebo-controlled, parallel-group trial (Clinical Trials.Gov NCT02730169). The study was conducted at 70 sites (United States, United Kingdom, Italy, and Spain), between June 2016 and February 2018. The study had three stages: a screening period (up to 28 days); a treatment period (24 weeks), where eligible patients were randomised 1:1:1:1 to the three leflutroazole doses or identical placebo (manufactured by Almac Clinical Services), and a 12-week follow-up period (**Figure 1**).

Randomisation numbers were generated using the following procedure to ensure that treatment assignment was unbiased and concealed from subjects and investigator staff.

A subject randomisation list was produced by ICON Biostatistics using a validated system that automates the random assignment of subject numbers to randomisation numbers. These randomisation numbers were linked to the different treatment regimens, which in turn are linked to medication numbers. A separate medication list

was produced using a validated system that automated the random assignment of medication numbers to packs containing the investigational drug. Subjects, investigational staff, persons performing the assessments and data analysts (with the exception of unblinded IDMC staff/members) were blinded to treatment allocation until after all data were entered and data base locked.

The study was conducted in adult (18–65 years) males, with a body mass index (BMI) of 30–50kg/m². Eligible participants had below-normal TT levels (morning testosterone levels taken before 11:00 am <10.41nmol/L [$<300\text{ng/dL}$] on at least two separate occasions, at least 3 days apart), LH levels below the upper limit of normal (ULN) and oestradiol levels within or above the normal range of approved assay. Additionally, participants had ≥ 2 symptoms of androgen deficiency, for ≥ 2 months prior to first screening visit, with at least one of these being of sexual dysfunction (list of symptoms and signs of androgen deficiency and exclusion criteria can be found in **Supplementary material**)

Independent data monitoring committee

Safety data were evaluated throughout the study by an independent data monitoring committee who met approximately every 12 weeks during the study.

Endpoints

The primary endpoint was normalisation of morning TT levels (10.41–34.70nmol/L [$300\text{--}1000\text{ng/dL}$]) in $\geq 75\%$ of men after 24 weeks of treatment. Secondary endpoints

included: time to normalisation of TT levels; change in LH and FSH levels. See **Supplementary material** for exploratory endpoints.

Safety was assessed by recording adverse events and laboratory safety monitoring, including measurement of haematocrit, PSA, and vital sign assessment.

Assessments

Sex hormones

Sex hormones were measured at screening, baseline, Day 8, Week 4, and every 4 weeks up to Week 24, and in follow-up at Week 36.

Testosterone

Serum TT (liquid chromatography and tandem mass spectrometry (LC/MS)) and SHBG radioimmunoassay levels were assessed using a CDC-accredited assay for screening and in study assessment. Constants for binding of testosterone to albumin and/or SHBG were used to calculate free and bioavailable (bound to albumin) testosterone (28). Testosterone measurements were performed before 11:00 am to reduce effects of circadian rhythm on plasma levels. Testosterone analyses were carried out by ARUP Laboratories, Salt Lake City, Utah. FSH and LH were measured by an immunoenzymatic method. Oestradiol was measured by radioimmunoassay (lower limit of quantitation 34.7pmol/L), with an ultrasensitive LC/MS assay used to quantitate values that fell below the lower limit of the radioimmunoassay (LC/MS with having lower limit of quantitation of 0.7pmol/L).

Semen parameters

Sperm count, semen volume, concentration, motility and morphology assessments were performed according to WHO guidelines (2) at baseline, Week 12, and Week 20 during the randomisation period.

Bone mineral density

BMD was assessed at baseline and Week 24. All available DEXA scans from clinical sites underwent a centralised, blinded over-read (ICON Medical Imaging Services) to assess acceptability of scans for analysis.

Body Composition

Fat and fat-free mass was measured by bio-impedance.

Patient-reported outcomes

Change in total and domain scores on PRO measures of sexual function (international Index of Erectile Function, PROMIS SexSF); fatigue (PROMIS Fatigue SF); and Quality of Life (SF-36) were assessed at baseline, Week 4, 8, 12, 24, and 36, with the exception of SF-36 which was not assessed at Weeks 4 and 8 (**Supplementary material**).

Assessment of safety

Treatment-emergent adverse events (TEAEs) were classified by system organ class and preferred term according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 19.1 or higher. See **Supplementary material** for protocol-defined stopping criteria.

Statistical methods

The intention-to-treat (ITT) population included all patients who were randomised, received ≥ 1 dose of study medication, and provided a baseline and ≥ 1 post-baseline testosterone value. The safety population included all patients who received ≥ 1 dose of the study medication. The Statistical Analysis Plan and Population allocation was determined prior to unblinding the study.

Demographic and baseline characteristics were summarised using the ITT population. Primary, secondary and exploratory efficacy endpoints were analysed using the ITT population, using last observation carried forward imputation for missing data. Safety variables were analysed using the safety population.

Continuous variables were summarised using descriptive statistics. For binary outcomes, the proportion of patients with a response was summarised and compared between treatment regimens using Fisher's exact test. A Last Observation Carried Forward approach was used for missing data for the primary endpoint. A sensitivity analysis of the primary endpoint was performed using a non-responder imputation. In

1 this approach, subjects with missing testosterone values were deemed as not achieving
2 testosterone normalisation.

3
4 For all continuous outcomes, where appropriate, a mixed model for repeated measure
5 analysis was performed, with change from baseline as the outcome and treatment, visit,
6 treatment by visit interaction, baseline value, and baseline by visit interaction as
7 covariates. Values below the limit of quantitation for an assay were considered as zero
8 for analysis.

9
10 The study was powered at 90% to detect a difference in the proportion of patients with
11 normalisation of testosterone at Week 24 greater than the pre-specified performance
12 goal of 75% at the 2.5% significance level, for which 67 patients were required to be
13 randomised to each dose arm. All statistical testing was two-sided conducted at the
14 significance (alpha) level of 0.05.

15 16 CONSORT Statement

17 We used the CONSORT checklist when writing our report (Schulz KF, Altman DG,
18 Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for
19 reporting parallel group randomised trials).

20 Author Responsibilities

21 HJ was lead investigator involved in the design and conduct of the study and data
22 interpretation. JP was involved in the design of the study and statistical analysis plan

and data interpretation. WM led the operational elements of trial execution and data management for the study. AD was a study investigator and provided input to the data interpretation. HR was a study investigator and provided input to the data interpretation.

Results

Patients

Between May 2016 and February 2018, 2,103 patients were screened, and 271 patients were randomised to either leflutrozone (n=200) or placebo (n=71) (**Figure 2**). The main reason for screen failure (60.5%) was TT being above the eligibility criterion. Among randomised patients, 190 (70.1%) completed the treatment period, the last subject last visit being May 2018. Overall, the mean (SD) duration of follow up was 174.4 (51.8) days.

Baseline demographic and clinical characteristics

Demographic characteristics were generally similar across treatment groups, apart from numerically higher Black or African Americans in the leflutrozone-treated groups versus placebo (**Table 1**). Mean (SD) TT at baseline was 7.97 (2.28) nmol/L, with 31.7% of patients having levels <6.94nmol/L (**Table 1**). Baseline levels of free and bioavailable testosterone, and other hormones are shown in **Table 1** and **Supplementary material**.

Primary and secondary endpoints

Total testosterone

Of the 271 randomised subjects 24-week data were available for the ITT analysis on 52/67 at 0.1mg, 40/65 at 0.3mg, 39/67 at 1.0mg and 51/71 on placebo. The primary endpoint of achieving TT normalisation (10.41–34.70nmol/L) in $\geq 75\%$ of men in the ITT population by Week 24 was met in all leflutroazole treatment groups. Morning TT normalisation was achieved in $>90\%$ of patients in the two highest leflutroazole treatment groups: 88.1% (0.1mg), 95.4% (0.3mg), and 94.0% (1.0mg), versus 9.9% for placebo ($P<0.0001$ for all vs placebo; **Figure 3**). Some patients showed normalised TT levels at baseline, prior to initiation of leflutroazole treatment: 10.4% (0.1mg), 18.5% (0.3mg), 16.4% (1.0mg), and 9.9% (placebo). This aligns with other studies and reflects the variability of TT levels. The sensitivity Analysis in which those subjects with no TT results available at week 24 were assessed as non-responders also demonstrated significantly higher rate of % subjects with normalisation of testosterone compared to placebo ($p<0.001$ for 0.1 mg, 0.3 mg and 1.0 mg, data in Supplementary material).

Mean (SD) TT at Week 24 demonstrated a dose-tiered response, being 15.89 (3.73), 17.78 (5.56), 20.35 (6.66), and 8.04nmol/L (2.36), for the leflutroazole 0.1mg, 0.3mg, 1.0mg, and placebo groups, respectively (**Figure 4**). There was no statistically significant difference in TT between the leflutroazole treatment groups at baseline or at any time during the study. Testosterone levels increased rapidly with leflutroazole treatment, with normal testosterone levels achieved in 80.6% (0.1mg), 85.5% (0.3mg), and 89.1% (1.0mg) of patients by Day 8.

One (2.6%) patient in the leflutrozone 1.0mg group had overshoot testosterone at Week 24; (maximum testosterone level 41.29nmol/L).

LH and FSH

LH (normal range males: 0.57–12.57mIU/mL) and FSH (normal range males: 0.95–11.95mIU/mL) were significantly increased in the leflutrozone groups versus placebo (**Figure 5**). At Week 24, mean LH increased by 2.1 (0.1mg), 3.6 (0.3mg), and 5.2mIU/mL (1.0mg) in the leflutrozone groups, versus placebo (-0.04mIU/mL). FSH increased by 4.84, 6.18, and 8.28mIU/mL, respectively, versus placebo (0.04mIU/mL). A dose-dependent leflutrozone response was observed for the LH and FSH increases at Week 24: $P<0.001$ for leflutrozone 1.0mg versus 0.1mg or versus 0.3 mg (both); $P=0.007$ (LH) and $P=0.022$ (FSH) for leflutrozone 0.1mg versus 0.3mg.

Exploratory endpoints

Bioavailable and free testosterone changes

Free testosterone and bioavailable testosterone increased in the leflutrozone groups versus placebo. At Week 24, mean bioavailable testosterone increased by 5.73, 6.67, 9.14, and 0.10nmol/L in the leflutrozone 0.1mg, 0.3mg, 1.0mg, and placebo groups, while free testosterone increased by 2.08, 2.46, 3.29, and 0.03nmol/L, respectively.

At Week 24, change from baseline in bioavailable testosterone and free testosterone was statistically significant, with p-values all <0.001 for each leflutrozone treatment group

versus placebo. At Week 24, free and bioavailable testosterone increased in a dose-dependent manner in the leflutroazole groups (**Supplementary material**).

Semen parameters

A subset of 139 patients provided semen samples for the exploratory analysis. At Week 20, there was a significant improvement (least squares [LS] mean difference [95% confidence interval (CI)]) in semen volume (1.4 [0.4, 2.3] mL, $P=0.006$), spermatozoa ($270.1 [65.0, 475.2] \times 10^6/\text{ejaculate}$, $P=0.011$), and total motile sperm count ($127.7 \times 10^6/\text{ejaculate}$, $P=0.030$) in the leflutroazole 1.0mg group versus placebo, with similar trends at lower doses (**Table 2**). An improvement in sperm count translated into improvements in severity of oligospermia, with 11/16 (68.8%) of those with baseline oligospermia normalising by Week 20, including 4/8 (50%) with baseline severe oligospermia (<5 million per mL) (**Table 3**).

Inhibin A and B

Inhibin B increased from baseline in all leflutroazole groups without dose effect, with increases by Week 24 of 14.6 ($P=0.006$), 9.9 ($P=0.77$) and 12.3ng/L ($P=0.026$) over placebo, in the 0.1mg, 0.3mg and 1.0mg groups, respectively. There were no significant changes in inhibin A in the leflutroazole versus placebo arms.

Oestradiol levels

Mean oestradiol levels decreased from baseline to Week 24 in the leflutroazole groups (-40.6pmol/L [0.1mg], -45.1pmol/L [0.3mg], and -51.4pmol/L [1.0mg]), versus a small

6.6pmol/L increase with placebo (**Figure 5**). Differences between the leflutroazole groups and placebo were significant for mean oestradiol levels (all $P<0.001$) and the TT/oestradiol ratio ($P=0.002$ for the leflutroazole 0.1mg group and $P<0.001$ for 0.3mg and 1.0mg groups). Significant differences between the level of change in the leflutroazole groups indicated a dose-dependent response for both mean oestradiol levels and the TT/oestradiol ratio.

Patient-reported outcomes

At Week 24, there were no significant improvements in sexual function, as measured by IIEF and PROMIS SexFS. At Week 24, PROMIS SexFS sexual function domains showed a statistically significant decrease in the domain of 'Interest in sexual activity' in the 0.3mg and 1.0mg groups, but did not meet the clinically meaningful threshold. There were no significant changes in fatigue (PROMIS Fatigue SF), overall health (PGI-S), or HRQoL (SF-36).

Cardiometabolic and body composition outcomes

No statistically significant changes from baseline were shown in triglycerides, HbA1c, glucose, or CRP during the study ($P>0.05$; data not shown). A slight increase in cholesterol was observed in the leflutroazole 1.0mg group (least squares (LS) mean differences (95% CI) versus placebo of 0.237 (0.012, 0.462) mmol/L, $P=0.039$ at week 24, with no significant change for lower doses (**Supplementary material**). At Week 24, statistically significant differences were observed in mean high-density lipoprotein (HDL) cholesterol. The LS mean differences (95% CI) in HDL cholesterol were -0.082 (-0.137,

-0.028), $P=0.003$ and -0.072 (-0.126, -0.019) mmol/L, $P=0.008$ in the leflutroazole 0.3mg and 1.0mg groups versus placebo, with no change in the 0.1mg group.

Body composition measurements by bioimpedance showed no statistically significant differences at week 24 for change from baseline in Total Body Fat (kg) LS mean of 2.12 (-3.00, 7.34); 3.91 (-1.63, 9.45), -0.89 (-6.06, 4.29) and 3.06 (-1.90, 8.03) in the leflutroazole 0.1mg, 0.3mg, 1.0mg groups and placebo groups. The changes in Visceral Adipose Tissue (kg) were -0.145 (-1.08, 0.79); 0.681 (-0.349, 1.711); -0.48 (-1.42, 0.46) and 0.18 (-0.72, 1.07) in the leflutroazole 0.1mg, 0.3mg, 1.0mg groups and placebo groups. Whole body skeletal muscle mass change (kg) at week 24 was -2.05 (-5.16, 1.07); 1.62 (-1.76, 5.00); 0.59 (-2.62, 3.79); and -2.83 (-5.92, 0.26). in the leflutroazole 0.1 mg, 0.3mg, 1.0mg groups and placebo groups

Numerical decreases in insulin and HOMA-IR were observed in the leflutroazole groups versus placebo (**Supplementary material**), but none met statistical significance. There was no improvement in fasting glucose or glycosylated HbA1c.

Safety endpoints

Overall, 164 (60.5%) patients reported 429 TEAEs during the study, with the leflutroazole 1.0mg group having a higher frequency (70.1%; **Table 4**) versus other treatment groups or placebo. The most common TEAEs reported in the leflutroazole 1.0mg group versus placebo were raised haematocrit >54% (7.5% vs 0%), hypertension defined as >130/80mmHg (7.5% vs 0%), a PSA increase of >3.5nmol/L above screening level (6.0% vs 2.8%), and headache (6.0% vs 2.8%). None of the increased PSA events

1 were due to prostate cancer. There was a higher incidence of hypertension in the
2 leflutroazole groups versus placebo, and a significant increase in systolic blood pressure
3 (SBP) and diastolic blood pressure (DBP) that was limited to the 1.0mg leflutroazole
4 group: SBP LS mean difference, 4.1mmHg, $P=0.030$; DBP, 3.1mmHg, $P=0.028$). A
5 small but statistically significant increase in creatinine was observed in leflutroazole
6 groups versus placebo at Week 24 (5.70 μ mol/L [0.1mg], 6.10 μ mol/L [0.3mg], and
7 8.27 μ mol/L [1.0mg]). This elevation was evident by Day 8 of treatment, did not progress
8 and was not associated with increased blood urea nitrogen. These adverse events
9 generally resolved on cessation of study drug treatment.

10
11 A higher number of serious TEAEs occurred in the leflutroazole 1.0mg group versus
12 placebo (12 vs 9), while the other leflutroazole groups were similar to each other and
13 lower than placebo (**Table 4**). Two serious TEAEs in the same patient (1.5%) in the
14 leflutroazole 0.1mg group were considered treatment-related (atypical noncardiac chest
15 pain and hypertension).

16
17 More patients in the leflutroazole groups discontinued study treatment versus placebo,
18 with a dose-dependent frequency of 9.0% (0.1mg), 12.1% (0.3mg), and 19.4% (1.0mg)
19 versus 2.8% (placebo) (**Table 4**). The most common reason for discontinuation was
20 raised haematocrit or increased PSA (1.5%, 4.5%, and 13.4% in the leflutroazole 0.1mg,
21 0.3mg, and 1.0mg groups). These parameters resolved upon study drug cessation. No
22 deaths were reported during this study.

Bone mineral density

BMD at baseline across the treatment groups was 1.25 (SD 0.14) g/cm² total hip, 1.01 (SD 0.16) g/cm² femoral neck, and 1.23 (SD 0.20) g/cm² lumbar spine. There were no significant changes from baseline for total hip DEXA scan density at Week 24 for any leflutroazole dose. A small but statistically significant reduction from baseline in DEXA scan density in the lumbar spine was observed at Week 24 for all the leflutroazole groups versus placebo. Mean percentage change (SD) from baseline was -1.24% (2.616), -1.30% (3.792), -2.09% (3.255), and 0.66% (3.037) in the leflutroazole 0.1mg, 0.3mg, 1.0mg, and placebo treatment groups, respectively (**Supplementary material**). Based on T-score assessments, the reductions in BMD did not translate into development of osteoporosis or new osteopenia in the leflutroazole arms over the 24 weeks.

Discussion

This placebo-controlled RCT assessed the efficacy and safety of aromatase inhibition in OHH, and confirmed that excess oestrogen suppresses the HPG axis in men with obesity. This study demonstrated that leflutroazole rapidly normalised TT in patients with OHH, with normalisation in >80% of subjects by Day 8, and with the effect maintained throughout the study period. Leflutroazole treatment groups showed a dose-dependent increase from baseline in mean TT to levels across the normal physiological range. These results were supported by dose-dependent responses in free and bioavailable testosterone. Only one patient, in the leflutroazole 1.0mg group, had a single episode of overshoot of testosterone above the physiological range over the 24 weeks. This

1 No improvements were observed for the symptoms and signs of hypogonadism in this
2 study. The 6-month duration of the study may not be long enough to show
3 improvements in cardiometabolic and body composition effects which have been shown
4 to take 12–18 months to develop upon testosterone normalisation (32).

5
6 The reduction in sexual desire with the highest doses of leflutroazole versus placebo,
7 albeit not meeting the clinically meaningful threshold, may be an effect of lowering
8 oestradiol, which plays a role in erectile function and libido (33). Additionally, the erectile
9 function of obese men may also be affected by other factors including psychological
10 factors or suprapubic fat pads (34, 35).

11 The efficacy profile appears similar to that described in a recent metanalysis of SERMs
12 in androgen deficient obese men (36), where consistent increases in testosterone, LH
13 and FSH and improved semen quality, have been observed with both clomiphene
14 citrate and enclomiphene citrate, however effects on sexual dysfunction when tested in
15 short-term placebo-controlled trials have not been clearly demonstrated (18).

16
17 Overall, leflutroazole was well-tolerated in this patient population. The increase in
18 adverse effects behind the higher incidence of TEAEs/serious TEAEs with the highest
19 leflutroazole dose (1.0mg), was mostly due increased haematocrit, increased PSA, and
20 hypertension. In patients with HH, an increase in haematocrit may be considered an
21 expected effect of increased testosterone normalising the mild anaemia of HH (37).

22 There were no treatment emergent thromboembolic events. All AEs resolved after drug
23 cessation, and within the 12-week follow-up period of the study. There was evidence of

1 an effect on blood pressure of leflutroazole, particularly at the highest 1.0mg dose, with a
2 small, but statistically significant change from baseline in SBP and DBP. Hypertension
3 is a recognised adverse drug reaction for testosterone-elevating treatments (37).
4 However, it should be noted too that there were numerically higher Black and African
5 American patients in the leflutroazole-treated groups versus placebo. This may have
6 influenced these results, given that African Americans are more likely to develop severe
7 hypertension than White patients (38). Further formal blood pressure studies will need
8 to characterise this, and to better elucidate the risk-benefit and dose choice for future
9 development. Hypertension was managed effectively with standard antihypertensive
10 medications.

11
12 Concerns have been raised about the potential increased cardiovascular risk of
13 testosterone replacement therapy (39, 40). However, RCTs have not demonstrated
14 evidence for increased cardiovascular risk where testosterone is within the physiological
15 range (41), and the maintenance of the normal feedback loop and lack of supra-
16 physiological levels with leflutroazole treatment is a potential benefit of this drug. The
17 reversibility of effects of haematocrit and blood pressure, and responsiveness to
18 antihypertensives is reassuring, along with a potential therapeutic window, with the
19 lowest doses demonstrating limited effects on blood pressure and haematocrit.

20
21 Small, but statistically significant reductions in BMD were observed in the most
22 oestrogen-sensitive site of the lumbar spine, but without evidence of drug-associated
23 osteopenia/osteoporosis developing. Obesity is associated with raised BMD, possibly

1 due to biomechanical loading (42), as was demonstrated in this study population at
2 baseline. This may reduce the risk of development of osteopenia and osteoporosis. Six
3 months is considered too short to determine BMD effects; thus, the extension study
4 MBGS206 was specifically designed to investigate BMD over a more appropriate period
5 of 48 weeks.

6 7 8 ***Limitations of the study***

9 The two morning testosterone screening samples were not taken in fasted state which
10 could have led to variation in the measurement. Androgen deficiency was defined by
11 low levels of total testosterone in the presence of typical symptoms. Due to the known
12 effects of obesity on reduction in SHBG, free testosterone may have been a better
13 determinant of hypogonadism. Free testosterone was low in only 41% of patients at
14 baseline which may have reduced the population of subjects with true deficiency. A
15 single morning TT value was used as the primary endpoint of testosterone
16 normalisation when levels are higher due to circadian rhythm; using this value may have
17 overestimated the response rate. However, even using a higher threshold of 12.15–
18 40.60nmol/L (350–1170ng/dL) inclusive to account for circadian rhythm at Week 24, the
19 number of patients with TT in this higher range was 54 (80.6%), 60 (92.3%), and 61
20 (91.0%) for the leflutroazole 0.1mg, 0.3mg, and 1.0mg groups, respectively, versus 5
21 (7.0%) with placebo ($P<0.001$ for all leflutroazole groups compared to placebo). An
22 immunoassay was used for estradiol measurements in the study but had inadequate

sensitivity for the low levels that occurred in some patients on leflutrolole, meaning that a second more sensitive LCMS was used for some of the samples.

Not all patients provided semen samples and centralised testing was not used for the semen analyses, which may have increased variability of results. In addition, as none of the placebo patients had oligospermia at baseline, there was no comparator for the improvement in oligospermia observed in patients receiving leflutrolole. A key limitation of the study was it was not powered for the exploratory endpoints, therefore definitive conclusions cannot be made on the effect of leflutrolole on clinical outcome measures of PROs, cardiometabolic or body composition endpoints. It was also of inadequate duration to detect changes in cardiometabolic and body composition parameters that may take months or years to develop. In addition, although eligibility criteria required ≥ 1 symptom of sexual dysfunction with the screening checklist, not all patients subsequently reported abnormalities of sexual function in the specific PROs at baseline International Index of Erectile Function (IIEF) or PROMIS Sex Short Form, which may affect the ability of these instruments to detect an effect.

Conclusions

Leflutrolole offers convenient weekly dosing that has the potential to rapidly normalise testosterone without supra-physiological peaks in circulating testosterone levels.. A clear dose-dependent effect of 0.1mg, 0.3mg and 1.0mg on final testosterone levels within the normal range indicates potential for a treat-to-target approach for patients with OHH. Effects on free and bioavailable testosterone were consistent with those of

1 TT, supporting the conclusion that the effect was not secondary to changes in SHBG. It
2 is possible that the lack of improvement by 24 weeks in symptoms of erectile
3 dysfunction, body composition and cardio-vascular parameters may reflect a longer time
4 for improvement in these parameters in this population with obesity and its co-
5 morbidities. However, in the context of reduction in lumbar spine BMD, elevation in
6 blood pressure and increase in cholesterol at the highest doses, leads to the conclusion
7 that aromatase inhibition by leflutroazole using the lower doses of <1.0 mg weekly may
8 be preferred doses to continue to explore in chronic dosing.

9 The dose-dependent increases in FSH and LH associated with what appear to be
10 meaningful changes in sperm parameters, including those with oligospermia at study
11 entry, warrant further investigation of leflutroazole in men with OHH who wish to preserve
12 fertility or for men with sub-fertility related to secondary gonadotropin deficiency. The
13 doses of 0.1mg and 0.3mg showed efficacy on fertility parameters, with a more
14 favourable adverse event profile than the highest dose of 1.0 mg, informative for further
15 investigations planned to explore the appropriate therapeutic dose, which will be further
16 informed by the outcomes of the extension study.

Declaration of interest, Funding and Acknowledgements

Declaration of interest

THJ is president of the Androgen Society; has received consultancy fees, meeting/travel support and participated in advisory boards for Mereo Biopharma; has participated in advisory boards, educational lectures, webinars, and has received research grants from Besins Healthcare; has participated in advisory boards and educational lectures, and has received research grants from Bayer. WM has received stock options and is an employee of Mereo Biopharma. JP has received stock options and is an employee of Mereo Biopharma. AD and HR have nothing to declare.

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Compliance with ethical standards

The study was conducted in accordance with the 1964 Declaration of Helsinki. The
 ethical review board for each of the study sites (boards listed in Appendix) approved the study
 and study amendments. All patients provided written informed consent before taking
 part. This study does not contain any experiments with animals.

Data availability

Data analyses that support the findings of this study are available from the
 corresponding author upon reasonable request; however, primary data will not be made
 publicly available while leflutroazole is still in development.

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ACCEPTED MANUSCRIPT

1 **Tables**

2 Table 1. Baseline patient demographics and sex hormone concentrations (ITT
3 population)

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8 Figure 1: Study design

9 Figure 2: Patient disposition

10 Figure 3: Proportion of patients achieving normalisation of total testosterone (LOCF, ITT
11 population)

12 Figure 4. Total testosterone over time (ITT population)

13 Figure 5: Observed date at baseline and Week 24 for (a) LH, (b) FSH, (c) oestradiol and
14 (d) total testosterone/oestradiol ratio (ITT population)

Figure 1.**Figure 1 Study design****Figure 1 Footnote**

A minimum of 25 patients per treatment arm were to be invited to participate in a 6-month extension study (Protocol MBGS206), starting at Visit 8 (Week 24 EOT). This transfer was handled by the Interactive Response Technology and the blinding was to be maintained. Patients participating in the 6-month extension had their last study visit at Visit 8 (Week 24 EOT).

If one of the leflutrolole study arms at Visit 3 (Week 4) fulfilled discontinuation criteria as determined per Data Monitoring Committee, the dosing for the ineffective or unsafe arm(s) was to be stopped and no further randomisation to that arm was to be performed.

Abbreviations: D, day; EOT, end of treatment; PK, pharmacokinetic; W, week.

Figure 2:**Figure 2 Title: Patient disposition****Figure 2: Footnote**

Abbreviations: ITT, intention to treat

Figure 3.

Figure 3 Title: Proportion of patients achieving normalisation of total testosterone (LOCF, ITT population)

Figure 3 Footnote

Normalised testosterone level was defined as 10.41–34.70 nmol/L (300–1000 ng/dL) inclusive.

Percentages were based on number of non-missing values as denominator.

P values were obtained using Fisher's exact test. Baseline was defined as the last non-missing value collected before the first study treatment administration, including unscheduled assessments. *** $P < 0.001$ vs placebo.

Abbreviations: ITT, intention to treat; LOCF, last observation carried forward.

Figure 4.

Figure 4 Title: Total testosterone over time (ITT population)

Figure 4 Footnote:

Data are mean \pm 95% Confidence Intervals.

Abbreviations: ITT, intention to treat.

Figure 5:

Figure 5 Title: Observed data at baseline and Week 24 for (A) LH, (B) FSH, (C) oestradiol and (D) total testosterone/oestradiol ratio (ITT population)

Figure 5 Footnote

Data are mean \pm standard deviation. *** $P < 0.001$ vs placebo.

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Table 1. Baseline patient demographics and sex hormone concentrations (ITT population)

	Leflutrozolet 0.1 mg (n=67)	Leflutrozolet 0.3 mg (n=65)	Leflutrozolet 1.0 mg (n=67)	Placebo (n=71)
Age, years	51.4 (8.4)	51.6 (7.3)	49.6 (9.7)	50.9 (9.2)
Race, n (%)				
White	53 (79.1)	48 (73.8)	49 (73.1)	61 (85.9)
Black or African American	12 (17.9)	13 (20.0)	15 (22.4)	7 (9.9)
Asian	0	2 (3.1)	2 (3.0)	0
Other	2 (3.1)	2 (3.1)	1 (1.5)	3 (4.2)
BMI, kg/m ²	37.9 (5.4)	38.2 (5.1)	38.0 (5.6)	38.2 (5.1)
Total testosterone, nmol/L	7.9 (2.2)	8.3 (2.2)	7.85 (2.55)	7.86 (2.27)
Free testosterone, nmol/L	1.66 (0.46)	1.80 (0.46)	1.7 (0.7)	1.7 (0.5)
Bioavailable testosterone, nmol/L	4.5 (1.3)	4.8 (1.3)	4.5 (1.5)	4.5 (1.3)
Oestradiol, pmol/L	85.4 (36.8)	82.6 (28.1)	78.4 (28.5)	88.5 (33.3)
Total testosterone/oestradiol ratio	107.8 (57.7)	111.2 (45.3)	113.9 (57.9)	98.9 (36.8)
SHBG, nmol/L Normal Range (11-80nmol/L)	24.4 (8.5)	23.0 (8.4)	24.4 (8.9)	23.6 (9.8)
Haemoglobin (g/dl)	14.5 (1.9)	14.4 (1.1)	14.3 (1.1)	14/4 (1.1)
Haematocrit (ratio)	0.46 (0.04)	0.46 (0.03)	0.45 (0.03)	0.46 (0.03)
Symptoms of Sexual Dysfunction N (%)				

Reduced sexual desire (libido) and sexual activity	63 (94.0%)	55 (83.3%)	60 (89.6%)	63 (88.7%)
Decreased spontaneous erections	59 (88.1%)	58 (87.9%)	53 (79.1%)	62 (87.3%)
Reduced intensity of orgasm	38 (58.2%)	40 (60.6%)	48 (71.6%)	49 (69.0%)
History of Sleep Apnoea N (%)	6 (9%)	12 (18.2%)	7 (10.4%)	14 (19.7%)

Data are mean (SD) unless otherwise stated.

Abbreviations: BMI, body mass index; ITT, intention-to-treat; SD, standard deviation. SHBG, sex hormone-binding globulin.

Table 2. Change from baseline in semen parameters at Week 20 (ITT population)

	Leflurozole 0.1 mg (n=40)	Leflurozole 0.3 mg (n=32)	Leflurozole 1.0 mg (n=38)	Placebo (n=29)
Volume of semen, mL				
Patients included in model, n	31	23	24	20
LS mean difference (95% CI) vs placebo	0.7 (-0.2, 1.6)	0.8 (-0.2, 1.9)	1.4 (0.4, 2.3)**	
Spermatozoa, 10 ⁶ /ejaculate				
Patients included in model, n	30	23	23	18
LS mean difference (95% CI) vs placebo	130.4 (-65.3, 326.1)	90.2 (-126.5, 306.8)	270.1 (65.0, 475.2)*	
Sperm concentration, 10 ⁹ /L				
Patients included in model, n	29	20	22	20
LS mean difference (95% CI) vs placebo	51.0 (6.5, 95.5)*	17.6 (-35.1, 70.3)	58.5 (11.8, 105.1)*	
Sperm motility, %				
Patients included in model, n	31	23	24	20
LS mean difference (95% CI) vs placebo	2.7 (-8.2, 13.5)	-0.1 (-12.3, 12.1)	-1.2 (-12.6, 10.2)	
Total motile sperm count, 10 ⁶ /ejaculate				
Patients included in model, n	29	19	22	20
LS mean difference (95% CI) vs placebo	86.267 (-24.5, 197.1)	54.2 (-77.3, 185.7)	127.7 (12.6, 242.8)*	
Sperm morphology, %				
Patients included in model, n	31	23	22	19
LS mean difference (95% CI) vs placebo	-1.9 (-8.4, 4.7)	-2.6 (-9.8, 4.6)	-4.1 (-11.2, 3.0)	

N=number of patients in the ITT population.

Analysis was based on a linear MMRM with change from baseline as the outcome including treatment, visit, treatment by visit interaction, baseline value and baseline by visit interaction as covariates.

Baseline was defined as the last non-missing value collected up to 48 hours post the first study treatment administration, including unscheduled visits. For both motility and morphology, the percentage normal was reported.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Abbreviations: CI, confidence interval; ITT, intention to treat; LS, least square; MMRM, mixed model for repeated measures; SE, standard error.

Table 3. Shift in sperm parameters at Week 20 (ITT population)

Baseline category	Post-baseline category			
	Normal	Mild OS	Moderate OS	Severe OS
	n	n	n	n
Placebo (n=29)				
Normal	11	1	2	1
Mild OS	-	-	-	-
Moderate OS	-	-	-	-
Severe OS	-	-	-	1
Leflurozole (n=199; all doses combined)				
Normal	29	1	1	-
Mild OS	4	-	-	-
Moderate OS	3	1	-	1
Severe OS	3	-	1	4

Baseline was defined as the last non-missing value collected up to 48 hours post the date of first dose of study medication. Missing baseline assessments were excluded from this analysis.

Mild OS: 10–15 million/mL; moderate OS: 5–10 million/mL; severe OS: <5 million/mL.

Abbreviations: OS, oligospermia; ITT, intention to treat.

Table 4. Summary of TEAEs (safety population)

	Leflutrozone 0.1 mg (n=67)	Leflutrozone 0.3 mg (n=66)	Leflutrozone 1.0 mg (n=67)	Placebo (n=71)
Patients with at least 1 TEAE	37 (55.2)	39 (59.1)	47 (70.1)	41 (57.7)
TEAEs	4 (6.0)	6 (9.1)	6 (9.0)	2 (2.8)
Haematocrit increased	1 (1.5)	4 (6.1)	5 (7.5)	0
Polycythaemia	3 (4.5)	2 (3.0)	0	1 (1.4)
Oedema peripheral	0	0	0	1 (1.4)
Sleep apnoea syndrome	0	0	1 (1.5)	0
Treatment-related TEAEs	17 (25.4)	14 (21.2)	23 (34.3)	13 (18.3)
Serious TEAEs	2 (3.0)	2 (3.0)	5 (7.5)	5 (7.0)
Treatment-related serious TEAEs	1 (1.5)	0	0	0
TEAEs leading to death	0	0	0	0
TEAEs leading to permanent study discontinuation	6 (9.0)	8 (12.1)	13 (19.4)	2 (2.8)
Common TEAEs (≥4.5% in any group)				
Upper respiratory tract infection	3 (4.5)	3 (4.5)	2 (3.0)	5 (7.0)
Haematocrit increased	1 (1.5)	4 (6.1)	5 (7.5)	0
Prostate specific antigen increased	2 (3.0)	1 (1.5)	4 (6.0)	2 (2.8)
Pain in extremity	1 (1.5)	0	3 (4.5)	0
Back pain	2 (3.0)	3 (4.5)	2 (3.0)	1 (1.4)
Arthralgia	3 (4.5)	3 (4.5)	1 (1.5)	1 (1.4)
Headache	7 (10.4)	3 (4.5)	4 (6.0)	2 (2.8)
Dizziness	3 (4.5)	0	2 (3.0)	0
Fatigue	1 (1.5)	4 (6.1)	1 (1.5)	2 (2.8)
Cough	1 (1.5)	0	3 (4.5)	1 (1.4)

Hypertension	3 (4.5)	2 (3.0)	5 (7.5)	0
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Data are number of patients (%), with percentages based on the total number of patients in the safety population. TEAE was defined as an event occurring or worsening on or after the first dose of study medication.

TEAESIs include cardiovascular events (acute myocardial infarction, brain stroke, transient ischemic attack, unstable angina, congestive heart failure), prostate cancer, lower extremity oedema \geq Grade 3, polycythaemia as measured by a haematocrit $>54\%$, fragility fracture, development of sleep apnoea, development of osteoporosis or low mineral density as per DEXA measurement (T-score ≤ -2.5 for men ≥ 50 years or Z-score ≤ -2 for men < 50 years of age), and breast cancer. Classification of AEs were based on MedDRA Version 20.1.

Abbreviations: AE, adverse event; DEXA, dual energy X-ray absorptiometry; TEAE, treatment-emergent adverse events; TEAESI, treatment-emergent adverse events of special interest.

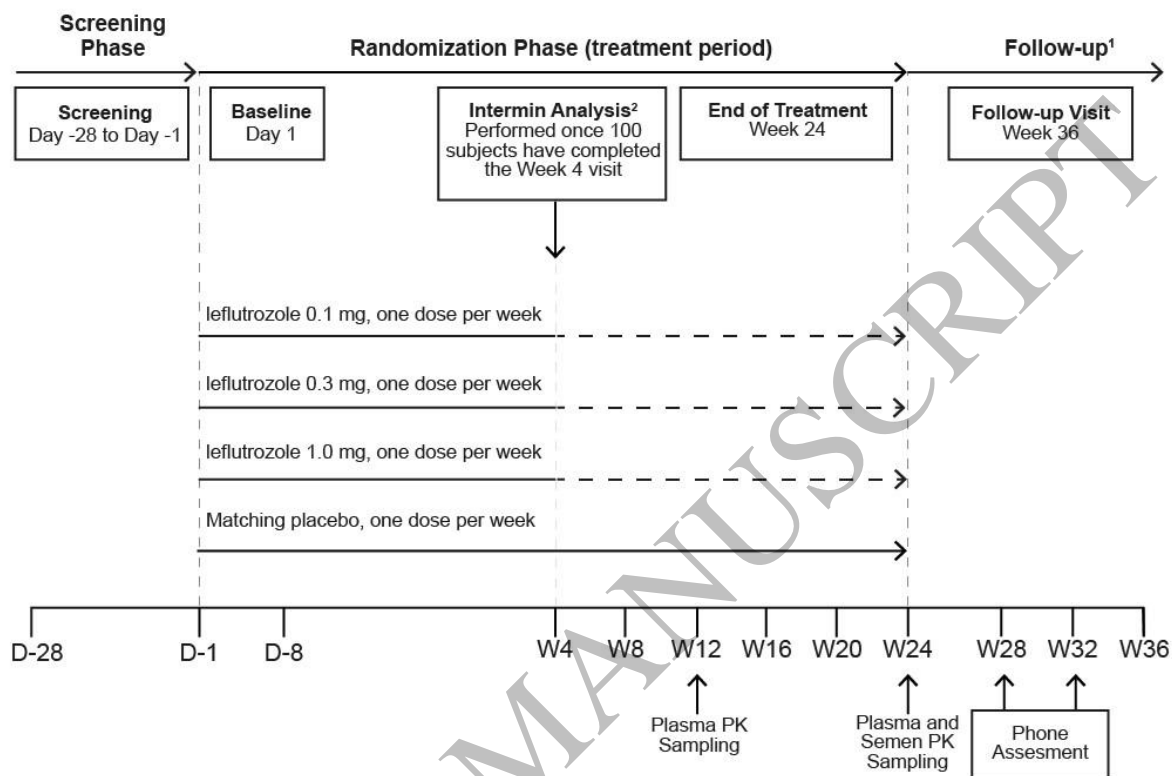


Figure 1
47x31 mm (x DPI)

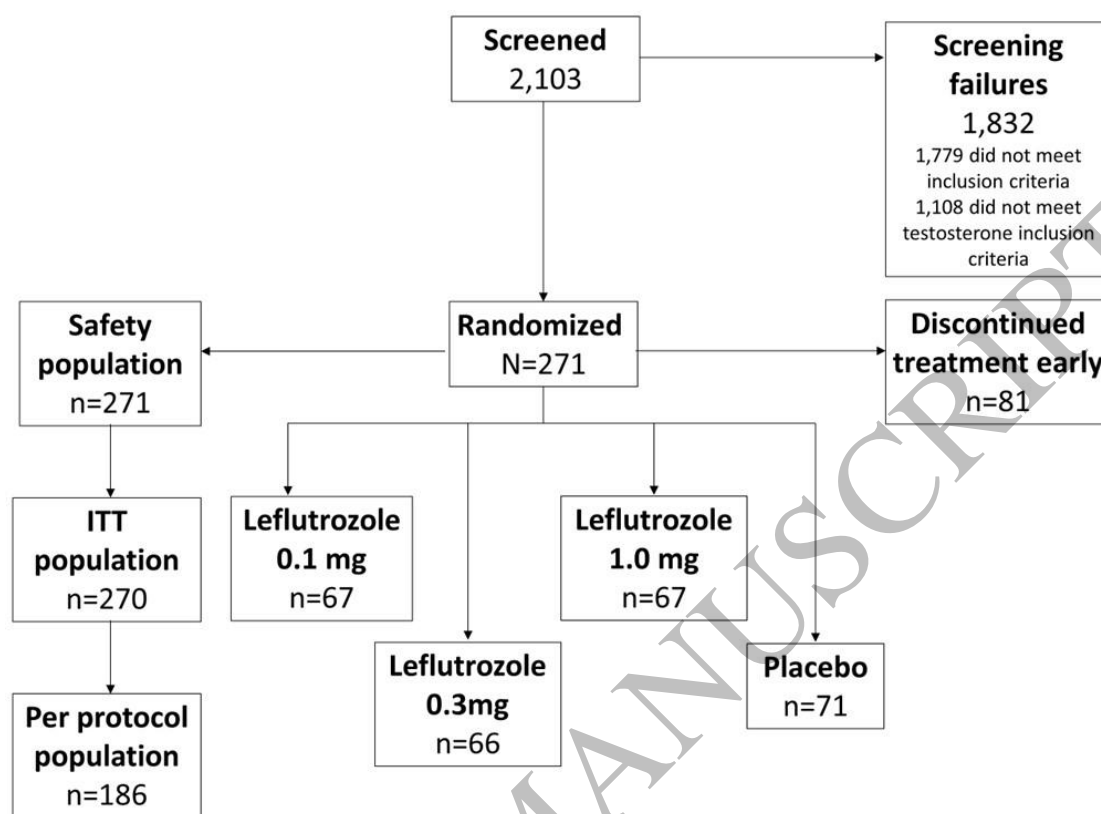


Figure 2
43x31 mm (x DPI)

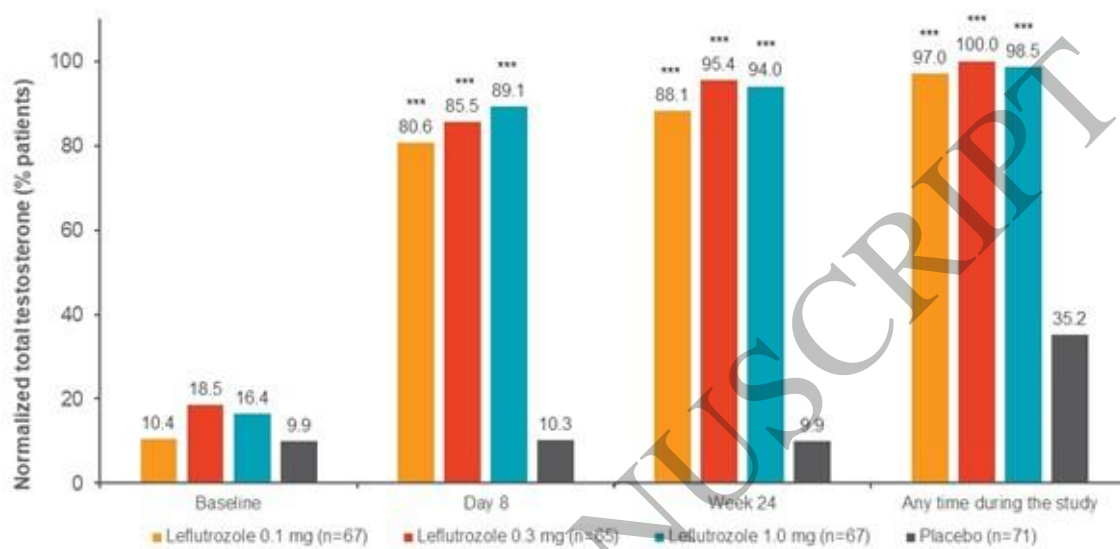


Figure 3
30x17 mm (x DPI)

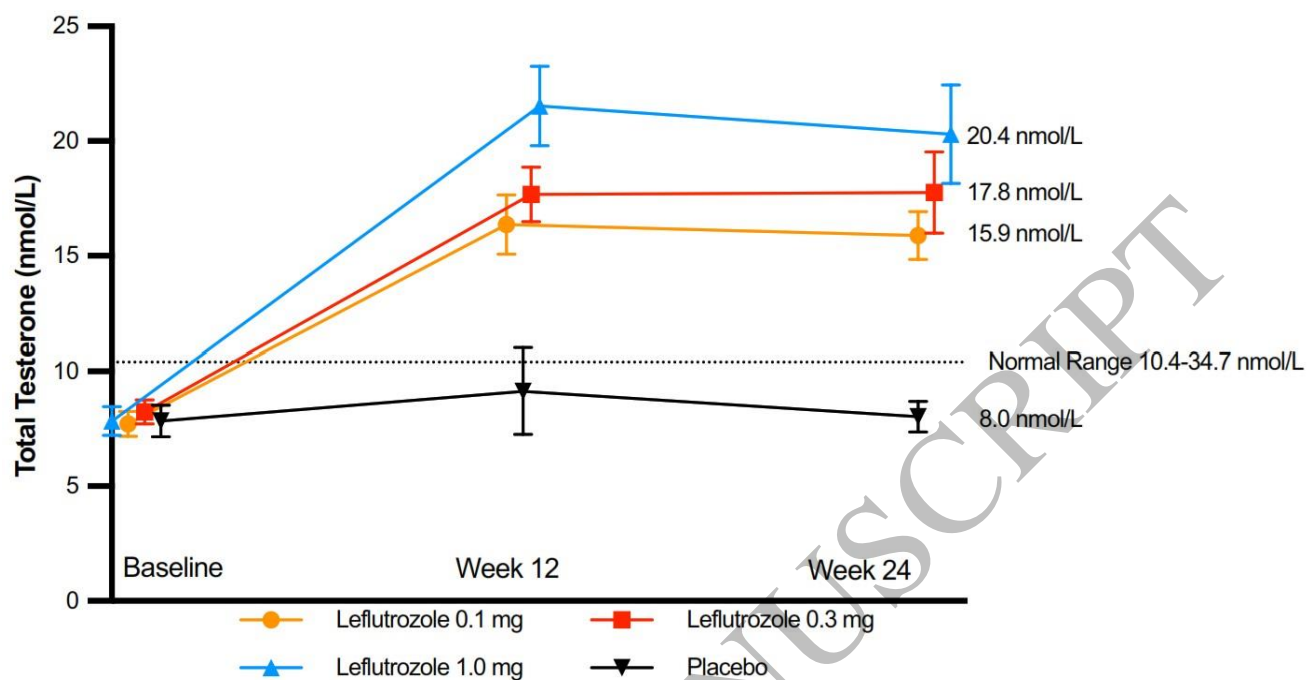


Figure 4
60x31 mm (x DPI)

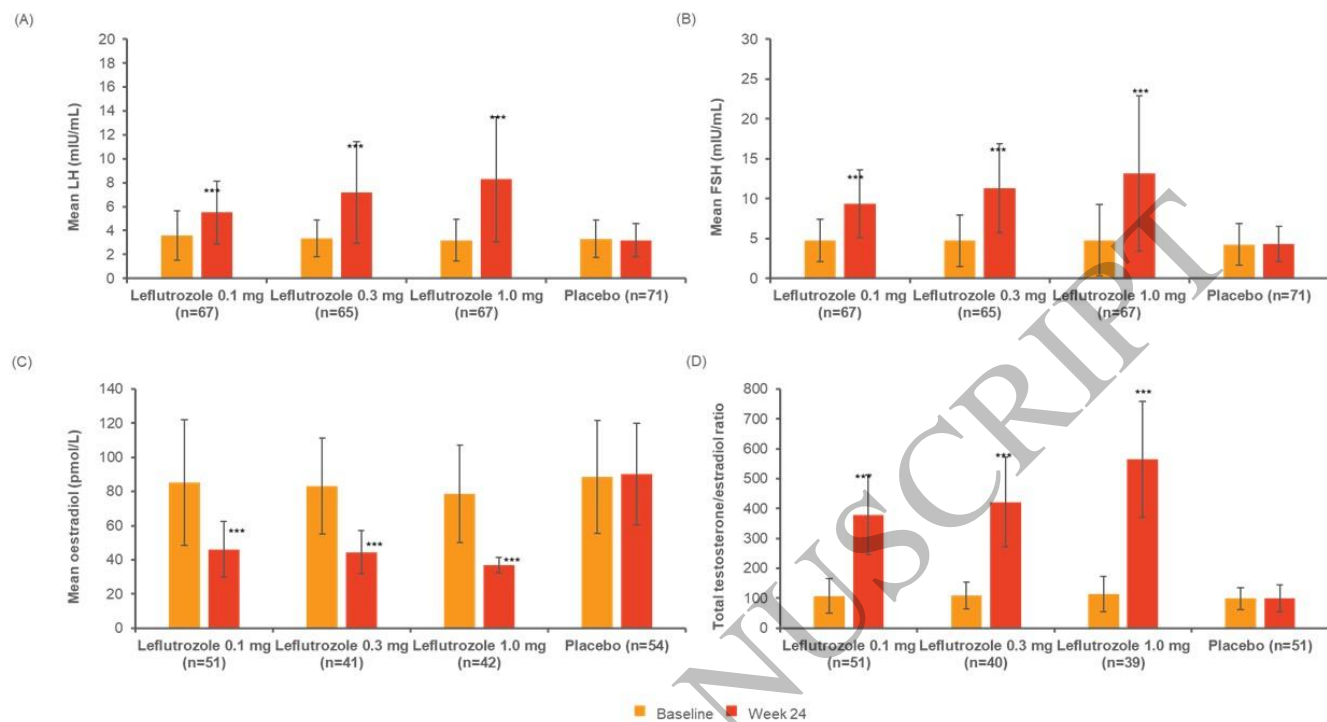


Figure 5
53x29 mm (x DPI)