25 January 2018
EMA/88321/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

EnCyzix

International non-proprietary name: enclomifene

Procedure No. EMEA/H/C/004198/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	EnCyzix
Applicant:	Renable Pharma Limited 20-22 Bedford Row WC1R 4JS UNITED KINGDOM
Active substance:	ENCLOMIFENE CITRATE
International Non-proprietary Name/Common Name:	Enclomifene
Pharmaco-therapeutic group (ATC Code):	Not assigned
Therapeutic indication(s):	Treatment of hypogonadotropic hypogonadism (secondary hypogonadism) in adult men aged ⩽60 years with a body mass index (BMI) ⩾25 kg/m2 which has been confirmed by clinical features and biochemical tests in patients which have not responded to diet and exercise
Pharmaceutical form(s):	Capsule, hard
Strength(s):	8.5 mg and 17 mg
Route(s) of administration:	Oral use
Packaging:	bottle (HDPE)
Package size(s):	30 capsules

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List of abbreviations

ACTH	Adrenocorticotropic Hormone
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AUC	Area Under the Curve
BCS	Biopharmaceutics Classification System
b.i.d	*bis in die* (twice a day)
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
Cavg	Average concentration
CEP	Certificate of Suitability of the EP
COSY	Correlation Spectroscopy
Cmax	Maximum concentration
DEPT	Distortionless enhancement by polarization transfer
DHT	Dihydrotestosterone
dL	Deciliter (100 mL)
E2	Estradiol-17β
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
FDA	Food and Drug Administration
FBG	Fasting Blood (Serum or Plasma) Glucose
FSH	Follicle-Stimulating Hormone
FTG	Fasting Triglycerides
GC	Gas Chromatography
GCP	Good Clinical Practice
GnRH	Gonadotropin Releasing Hormone
GHRH	Growth Hormone Releasing Hormone
Hb/Hgb	Haemoglobin
HbA1c	Haemoglobin A1c in serum as a percent
Hct	Haematocrit
HDL-C	High Density Lipoprotein Cholesterol
hGH	Human Growth Hormone (GH)
HH	Hypogonadotropic Hypogonadism
HDPE	High Density Polyethylene
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High performance liquid chromatography
HSQC	Heteronuclear Single-Quantum Correlation
ICH	International Conference on Harmonization
IGF-1	Insulin-like Growth Factor-1
IR	Infrared
ITT	Intention to Treat
KF	Karl Fischer titration
kg	Kilogram(s)

LDL-C	Low Density Lipoprotein Cholesterol
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
mg	Milligram(s)
mL	Milliliter
ng	Nanogram
NMR	Nuclear Magnetic Resonance
NMT	Not more than
Ph. Eur.	European Pharmacopoeia
pg	Picogram
OHA	Oral Hypoglycemic
PK	Pharmacokinetic
PRL	Prolactin
PP	Per Protocol
PRO	Patient Reported Outcome
PSA	Prostate Specific Antigen
QC	Quality control
RBC	Red Blood Cell
rpm	revolutions per minute
SAE	Serious Adverse Event
SHBG	Sex Hormone Binding Globulin
SmPC	Summary of Product Characteristics
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TGA-DSC	Thermo-Gravimetric Analysis- Differential Scanning Calorimetry
TSH	Thyroid Stimulating Hormone
TT	Total Testosterone in serum or plasma
USP	United States Pharmacopoeia
WBC	White Blood Cell
XRPD	X-Ray Powder Diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Renable Pharma Limited submitted on 12 September 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for EnCyzix, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 March 2015.

The applicant applied for the following indication:

Treatment of hypogonadotrophic hypogonadism (secondary hypogonadism) in adult men with a body mass index (BMI) ≥25 kg/m2 wishing to preserve testicular function and spermatogenesis

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that enclomifene was considered to be a known active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision (P/0204/2015) on the granting of a (product-specific) waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Scientific Advice

The applicant did not seek scientific advice at the CHMP.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Nithyanandan Nagercoil Co-Rapporteur: Joseph Emmerich

- The application was received by the EMA on 12 September 2016.
- The procedure started on 29 September 2016.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 16 December 2016. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 19 December 2016. The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on 6 January 2017.
- During the meeting on 26 January 2017, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 9 August 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 18 September 2017.
- During the PRAC meeting on 28 September 2017, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP.
- During the CHMP meeting on 12 October 2017, the CHMP agreed on a list of outstanding issues to be sent to the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 14 November 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 29 November 2017.
- During the CHMP meeting on 14 December 2017, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 25 January 2018, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to EnCyzix.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

EnCyzix is intended for the treatment of hypogonadotrophic hypogonadism (secondary hypogonadism) in adult men with a body mass index (BMI) ≥ 25 kg/m2 wishing to preserve testicular function and spermatogenesis.

There are two types of hypogonadism; primary (testicular failure), and secondary (hypothalamic/pituitary dysfunction) (Basaria, 2013).

- Primary hypogonadism, often caused by trauma, chemotherapy, mumps, genetic conditions and advanced age. Primary hypogonadism is associated with low testosterone (T) and elevated luteinising hormone (LH).
- Secondary hypogonadism tends to be associated with obesity, but can also be transiently induced by illness, stress and medication. Secondary hypogonadism is associated with low T with low/normal LH (Tajar, 2010), and low follicle stimulating hormone (FSH).

2.1.2. Epidemiology

The prevalence of hypogonadism (including both primary and secondary), in the EU has been reported to be between 2.1 and 12.8% (Haring et al, 2010; Tajar et al, 2012). In one of these studies, conducted in Germany, the reported incidence was 11.7 cases per 1000 person-years (Haring et al, 2010). In a US study, the incidence was reported as 12.3 per 1000 person-years (Araujo et al, 2004).

2.1.3. Aetiology and pathogenesis

Primary hypogonadism can be caused by trauma, medication, infection or genetic conditions; it can also be a condition of advanced age. Primary hypogonadism is associated with low testosterone and elevated LH. Hypogonadotrophic hypogonadism (secondary hypogonadism), can be caused by a number of factors including disorders of both the hypothalamus and the pituitary. This causes a reduced secretion of gonadotrophins and a subsequent decrease in the production of testosterone by the testes.

In males, obesity, stress and certain medications are the greatest contributors to this disorder, with obesity being the single greatest cause, due to the negative feedback effects of oestrogen, which is produced by aromatase in fat tissue.

The European Male Aging Study (EMAS), a large international epidemiological assessment of hypogonadism, concluded that secondary hypogonadism is overwhelmingly associated with obesity (BMI >30) and the risk of developing secondary hypogonadism significantly increases if men become overweight (BMI 25-30) (Tajar et al, 2010).

2.1.4. Clinical presentation and diagnosis

Testosterone and dihydrotestosterone (DHT) are endogenous androgens, responsible for the normal growth and development of the male sex organs and for maintenance of secondary sex characteristics. These effects include the growth and maturation of prostate, seminal vesicles, penis, and scrotum; the development of male hair distribution, such as facial, pubic, chest, and axillary hair; laryngeal enlargement, vocal cord thickening, alterations in body musculature, and fat distribution. Testosterone and DHT are necessary for the normal development of secondary sex characteristics. Androgens also promote retention of nitrogen, sodium, potassium, phosphorus, and decreased urinary excretion of calcium. They increase protein anabolism and decrease its catabolism.

Symptoms associated with male hypogonadism include impotence and decreased sexual desire, fatigue and loss of energy, mood depression, increase in visceral fat, regression of secondary sex characteristics and osteoporosis.

2.1.5. Management

Available treatment for hypogonadism consists largely of testosterone replacement therapy with several authorised treatments available throughout the EU. These are available in different dosage forms, including injections, oral medication, trans-dermal patches and gels. The injectable treatments are associated with peaks and troughs in serum testosterone levels, which can cause unwanted changes in libido, mood and energy levels. Oral preparations may be associated with hepatotoxicity and undesirable effects on serum lipid profile and carbohydrate metabolism.

A specific disadvantage of testosterone replacement therapy of secondary hypogonadism is that the direct increase in serum testosterone has a negative feedback effect on the Hypothalamic-Pituitary-Gonadal (HPG) axis. This, ultimately, causes a suppression of the HPG axis, so reducing the endogenous production of LH and FSH. Decreased stimulation of the testes ensues, resulting in endogenous testosterone production and spermatogenesis both being reduced.

About the product

Enclomifene is the trans isomer of clomifene which is a mixture of two geometric isomers; cis (zuclomifene or Z isomer) and trans (enclomifene or E isomer). Clomifene is approved in the EU for the treatment of ovulatory failure in women.

Enclomifene citrate is a selective oestrogen receptor modulator (SERM) which acts by blocking the oestrogenic suppression of the HPG axis. As a result, the pituitary secretes more LH and FSH, and this stimulates the testes to produce more testosterone. Men with secondary hypogonadism have functional but under-stimulated testes. In the secondary hypogonadal male, enclomifene citrate restores LH release that, in turn, stimulates endogenous testosterone production by the testes leading to the restoration of normal physiological levels of testosterone.

The initially claimed indication for EnCyzix (also mentioned in this report as Androxal) is "*for the treatment of hypogonadotrophic hypogonadism (secondary hypogonadism) in adult men with a body mass index (BMI) ≥ 25 kg/m2 wishing to preserve testicular function and spermatogenesis*".

During the evaluation, the applicant amended the proposed indication to "*for the treatment of hypogonadotropic hypogonadism (secondary hypogonadism) in adult men aged ≤ 60 years with a body mass index (BMI) ≥ 25 kg/m2 which has been confirmed by clinical features and biochemical tests in patients which have not responded to diet and exercise*".

The recommended starting dose with EnCyzix is 8.5 mg once daily after which morning serum testosterone concentrations should be measured to ensure that the desired levels (300-1040 ng/dL) of testosterone are achieved.

If the serum testosterone concentration is below 450 ng/dL after 4 to 6 weeks of treatment, the daily dose should be increased to 17 mg.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as hard capsules containing 8.5 mg or 17 mg of enclomifene (as citrate) as active substance.

Other ingredients in the capsule content are microcrystalline cellulose and magnesium stearate. Ingredients of the capsule shell are: gelatin, titanium dioxide (E171) and black iron oxide (E172). Ingredients of the printing ink are: shellac, propylene glycol and black iron oxide (E172).

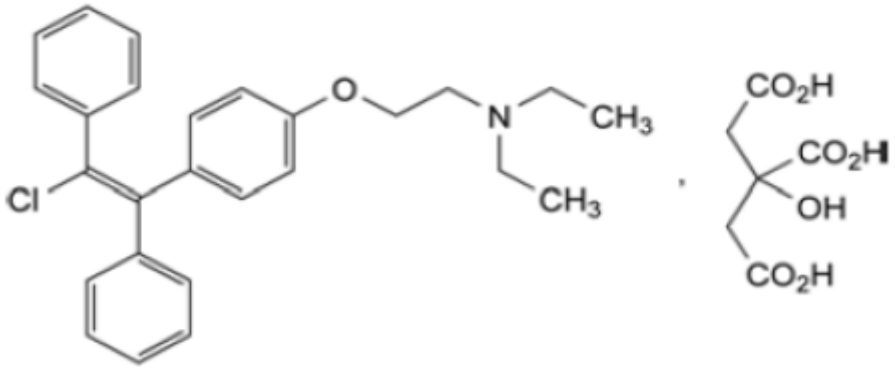
The proposed packaging consists of high density polypropylene (HDPE) bottle fitted with a polypropylene child resistant closure and an aluminium induction seal.

2.2.2. Active Substance

General information

The chemical name of enclomifene citrate is 2-[4-[(*E*)-2-chloro-1,2-di(phenyl)vinyl]phenoxy]ethyl-diethyl-amine, citrate corresponding to the molecular formula $C_{26}H_{28}ClNO$, $C_6H_8O_7$. It has a molecular mass of 598.1 g/mol and the following structure:

Figure 1: Structural formula of enclomifene citrate.



The chemical structure of enclomifene citrate was confirmed by a combination of elemental analysis, NMR spectroscopy (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC), mass spectrometry and IR spectroscopy. In addition, an X-Ray Powder Diffraction (XRPD) and a Thermo-Gravimetric Analysis-Differential Scanning Calorimetry (TGA-DSC) analyses were performed, showing that enclomifene citrate manufactured according to the described manufacturing process systematically leads to the desired polymorphic Form I (anhydrate) and there is no change in the polymorphic form during stability. A polymorph screening study was performed at the request of the CHMP.

The active substance is a white to pale yellow slightly hygroscopic powder, sparingly soluble in methanol, ethanol and heptane; and slightly soluble in ethyl acetate, tetrahydrofuran and acetone. Enclomifene citrate is a BCS Class III (low permeability and high solubility) substance.

Enclomifene has a non - chiral molecular structure. Enclomifene is the (*E*)-isomer of clomifene. Clomifene citrate is described in Ph. Eur. and USP monographs, and is a mixture of (*E*)- and (*Z*)-isomers of 2-[4-(2-chloro-1,2diphenylethenyl)phenoxy]-*N*,*N*-diethylethanamine dihydrogen citrate. The *Z*-isomer is therefore an isomeric impurity in the active substance and its content is routinely controlled by HPLC.

Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory. A single manufacturer carries out the manufacture of the active substance.

Enclomifene citrate is synthesized in two main chemical transformation steps followed by crystallisation steps, purification and salt formation, using a single well defined starting material with an acceptable specification. Three synthesis intermediates are isolated. During the procedure, at the request of CHMP, extensive additional information was provided on the starting material synthesis, potential mutagenic impurities (including their purging) and the control strategy, in order to ensure the satisfactory quality of the active substance throughout its lifecycle.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, the starting material and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. At the request of the CHMP the list of all impurities with a structural alert assessed by (Q)SAR was provided and described along with the control strategy. Ames tests have shown that the starting material and the first synthesis intermediate are non-mutagenic. Potentially mutagenic impurities were evaluated and to clearly assess the potential mutagenicity of two of these impurities, Ames tests were launched. As a quality recommendation, any positive results from these Ames tests should be reported to EMA. Potential and actual impurities were appropriately discussed with regards to their origin and characterised.

During development, the active substance manufacturer used to manufacture non-clinical and clinical batches and primary finished product stability batches was changed, and a new manufacturer was proposed for the EU registration and future commercial batches. The differences in the active substance manufactured by different manufacturers were evaluated and appropriately justified. The limits for all specified impurities are at or even below the ICH qualification threshold of 0.15%, and any unspecified impurities are limited to not

more than the ICH identification threshold of 0.10% (for a maximum drug substance dose of ≤ 2g/day) for both active substance manufacturers.

Batch results from active substance batches of the current and previous manufacturer have been compared, and it was found that the drug substance quality of both active substance manufacturers is comparable.

The active substance is packaged in an appropriate primary and secondary packaging configuration. The primary packaging complies with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The active substance specification includes tests for: appearance, identification (IR), identification of citrate (wet chemical analysis), water content (KF), sulfated ash (Ph. Eur.), heavy metals (USP), related substances (HPLC), assay (HPLC), residual solvents (GC) and particle size distribution (Ph. Eur.).

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. The equivalency of USP method for testing of heavy metals and Ph. Eur. 2.4.8 Method D has been appropriately demonstrated. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data (n=3 commercial scale) of the active substance produced by the manufacturer proposed for marketing are provided. The results are within the specifications and consistent from batch to batch.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. The equivalency of USP method for testing of heavy metals and Ph. Eur. 2.4.8 Method D has been appropriately demonstrated. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Stability

Stability data from 3 pilot scale batches of active substance from the proposed manufacturer stored in smaller versions of the intended commercial package for up to 24 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. Additional supporting data from 3 pilot scale batches from the manufacturer used to manufacture non-clinical and clinical batches and primary finished product stability batches was provided.

The parameters tested are the same as for release. The analytical methods used were the same as for release and were stability indicating all tested parameters were within the specifications.

Photostability testing following the ICH guideline Q1B was performed on one batch. The active substance was shown to be photosensitive.

Results under stressed conditions: heat, heat-humidity, acidic, alkaline and oxidative were also provided on one pilot batch. The heat and heat-humidity samples were stable, samples exposed to acidic conditions showed minor degradation and samples exposed to alkaline conditions showed insignificant degradation. Exposure to oxidative conditions resulted in significant degradation.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 24 months with no special storage conditions, in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The finished product is presented as immediate release size 3 hard capsules containing 8.5 mg or 17 mg of enclomifene (as citrate) as active substance. The hard gelatin capsules are light grey and opaque. The strengths differ in the number of black printed lines on the base of the capsule, one in the 8.5 mg strength and two in the 17 mg strength, and the actual strength numbers 8.5 or 17 imprinted in black above the lines.

As mentioned earlier in the report, enclomifene citrate is a BCS Class III (low permeability and high solubility) substance.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. Microcrystalline cellulose is used as filler, magnesium stearate as lubricant, the capsule shell is made of gelatin, titanium dioxide (E171) is used as a colourant/opacifier, black iron oxide (E172) is used as a colourant. Shellac, propylene glycol and black iron oxide (E172) are used in the printing ink. There are no novel excipients used in the finished product formulation. The list of excipients is included in paragraph 2.1.1 of this report. The active substance is compatible with the excipients (microcrystalline cellulose and magnesium stearate) and the gelatin capsule shells as demonstrated by the stability data (see stability section).

The formulation was established early in development and it is a simple powder formulation, consisting only of the active substance, a filler, and a lubricant, which are all encapsulated into a gelatin capsule. The formulation remained largely unchanged throughout development, the only changes being the change of capsule colour and imprints; and the change of the active substance and finished product manufacturers.

At the request of the CHMP, extensive data was provided to support the demonstration that the clinical batches are representative of the commercial scale product to be marketed (i. e. to support changes during manufacturing process development and change in manufacturers).

The dissolution study was performed in three different dissolution media covering the physiological pH range (pH 1.0: 0.1N HCl, pH 4.5: acetate buffer, pH 6.8: phosphate buffer) and with $f2$ calculation where possible, demonstrating the similarity between clinical batches from the two finished product manufacturers. The dissolution profiles were deemed similar (ref. Note for Guidance on the Investigation of Bioavailability and Bioequivalence).

In addition, the excipients (microcrystalline cellulose and magnesium stearate) employed for clinical batches and the commercial batches have similar characteristics or grades. The excipients characteristics which may have an impact on the bioavailability were assessed and additional controls (particle size for microcrystalline cellulose and specific surface area for magnesium stearate) have been proposed and are satisfactory. The similarity of the equipment used at the clinical development site and at the commercial site has been demonstrated.

The dissolution method was modified during the procedure at the request of the CHMP and its discriminatory power was considered adequate.

The proposed primary packaging is a high density polypropylene (HDPE) bottle fitted with a polypropylene child resistant closure and an aluminium induction sea. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of eight main steps: pre-blending, sifting, two blending steps, dry granulation, final blending, encapsulation and packaging. The process is considered to be a standard manufacturing process. The critical steps are appropriately controlled by the routine in-process control tests.

Validation of the manufacturing process will be conducted prior to launch on three consecutive production-scale batches per strength, in line with the provided validation master plans, which is considered acceptable. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance, identification (HPLC), assay (HPLC), uniformity of dosage units (Ph. Eur., HPLC), dissolution (HPLC), water content (KF), impurities (HPLC) and microbial limits (Ph. Eur.).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for two pilot scale batches per strength, manufactured by the proposed finished product manufacturer with active substance obtained from the current active substance manufacturer, confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification. Additional supportive batch analyses results on clinical trial and primary stability batches have been provided.

The finished product is released on the market based on the pre-defined acceptable specifications, through traditional final product release testing.

Stability of the product

Stability data from three pilot scale batches of each strength of the finished product stored for up to 24 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of the medicinal product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing.

The analytical procedures used are stability indicating.

All values remained well below the specification limit.

In addition, one batch per strength was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The samples were tested for appearance, assay, dissolution and related substances prior to and after light exposure. Based on the results, a statement ‘Store in the original package in order to protect from light’ was proposed for the product information.

An in-use stability study was performed on two finished product batches (one per strength) packaged in the container closure system intended for marketing. All results for in-use stability were well within specification limits.

Based on available stability data, the proposed shelf-life of 24 months and 'Store in the original package in order to protect from light' storage condition are acceptable.

Adventitious agents

Gelatine obtained from bovine sources is used in the product. Valid TSE CEP from the suppliers of the gelatine used in the manufacture is provided.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During the assessment, extensive additional data was requested by the CHMP and provided by the Applicant. These included data on the proposed regulatory starting material, discussion on potential genotoxic impurities, active substance polymorphism, finished product QC dissolution method and a discussion of equivalence of the finished product manufacturing process used during the development and the one proposed for commercialization.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk of the product.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on TSE safety.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. Potentially mutagenic impurities from active substance synthesis were evaluated and to clearly assess the potential mutagenicity of two of these impurities, Ames tests were launched with results being expected to be reported to EMA.

2. The Applicant is recommended to provide the results of partial revalidation of the revised QC dissolution method.

2.3. Non-clinical aspects

2.3.1. Introduction

The drug substance is enclomifene citrate (chemically referred to as (E)-2-[4-(2-chloro-1,2-diphenylethenyl) phenoxy]-N,N-diethylethanamine), which is the citrate salt and the trans isomer of clomifene.

Information generated from the clinical use of clomifene over several decades for the mixture of the isomers has been supplemented by the applicant specifically for the single enclomifene isomer, in terms of pharmacology, pharmacokinetics and toxicology.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro

A series of non-GLP studies were conducted by the applicant to determine the affinity of different clomifene isomers and their metabolites for human oestrogen receptors, and the agonist and/or antagonist potency at these receptors.

Study AB18747: This study confirmed that the test compounds were ligands for ERα and ERβ, and active at biological concentrations.

Study AB23728: Enclomifene was a ligand for ERα and ERβ, but with approximately 8x less affinity for the latter. 4-OH-Enclomifene was the strongest ligand tested in this experiment and demonstrated comparable affinity for both ERα and ERβ receptors; at biological and serum concentrations above 0.5 nM, it would be anticipated to stimulate activity. DDE-enclomifene bound ERα more strongly than ERβ by a factor of approximately 13. NO-En was the weakest ligand tested in this experiment for ERα and demonstrated 3x less affinity for ERβ. 4-OH-DE-Zu was a relatively weak ligand for ERα and weaker for ERβ.

Study AB23894: The order of antagonist activity for the ERβ/Coactivator for the ligands assessed here was 4-OH En >> En > 4-OH-Zu >NO-En > 4-OH DEZu > DDE-En > Zu. The 17-OH-CDB-4124 was not a ligand for either ER. The order of antagonist activity for the ERα/Coactivator for the ligands assessed here was 4-OH En > En > 4-OH-Zu >NO-En > 4-OH DE-Zu > DDE-En > Zu.

Study AB24026: In this study it was demonstrated that 4-OH-En would be anticipated to stimulate activity at the receptors at biological and serum concentrations circa. >0.5 nM and 4-OH-Zu would be anticipated to stimulate activity at circa. >12 nM. The binding was consistent with a single binding site on each recombinant ER molecule.

Study AB25938: In this study 4-OH –DE-Enclomifene and DE-enclomifene were ligands for ERα and ERβ. They were both strong binders of ERα. DE-enclomifene had approximately 4x higher affinity for ERα than ERβ. Both test compounds would be anticipated to stimulate activity at ERα at biological and serum concentrations at >0.5 nM. 4-OH-DE-En would also be anticipated to stimulate activity at ERβ at this concentration and DE-En would be expected to fully stimulate ERβ α τ $\geq$ 1.5 nM. The binding was consistent with a single binding site on each recombinant ER molecule.

Study AB25977: 4-OH-DE Enclomifene was shown to be an antagonist ligand for ERα association with its Coactivator at a low concentration and for ERß association with its Coactivator at an approximately 2x higher concentration. De-ethyl-En clomifene was an antagonist ligand for ERα association with its Coactivator and for ERß association with its Coactivator at approximately 2x higher concentration.

A summary of these results are presented in **Table 1**.

Table 1. Binding constants and antagonist potency. Enclomifene, Zuclomifene and key metabolites of enclomifene

	Ligand Binding Affinity Ki nM.		**Coactivator Assay Antagonist potency IC_{50} nM.**	
Compound	ERα	ERß	ERα	ERß
Enclomifene	0.47	>12	16	59
Zuclomifene	ND	ND	610	630
4-OH enclomifene	0.096	0.12	2.21	0.53
N-desethyl-enclomifene	0.093	0.23	22	40
4-OH N-desethyl enclomifene	0.059	0.094	0.77	0.30

ND No data

Source: ab18747, ab23728, ab23894, ab24026, ab25938, ab25977

In vivo

In vivo primary pharmacodynamics studies measured the effect of enclomifene on testosterone, luteinising hormone (LH) and follicle stimulating hormone (FSH) levels in the rat and baboon.

Study R-004-01: The effect of oral administration clomifene and its isomers, enclomifene and zuclomifene, for 6 days on total serum testosterone and body weight was investigated in Sprague-Dawley rats.

During the time course of the trial, there were clear and significant losses in total serum testosterone between baseline and day 6 for all the clomifene groups at every dose level in the trial. In general zuclomifene drastically reduced total serum testosterone in male rats in a dose-dependent fashion. Enclomifene often reduced serum testosterone but not dose dependently.

The administration of zuclomifene was associated with body weight loss up to 7 days after the treatment period for rats receiving the high dose. In contrast, enclomifene did not result in body weight loss that was dose dependent.

Study R-006-01: The effects of oral administration of clomifene and its isomers, enclomifene and zuclomifene for 12 days was evaluated in baboons.

A significant increase in mean total serum testosterone was observed in the enclomifene group but not for the clomifene or zuclomifene groups during the treatment period. Enclomifene produced higher levels of testosterone compared to clomifene and zuclomifene on day 6 ($p = 0.03$ and $p = 0.00002$ respectively) and compared to zuclomifene on day 12 ($p = 0.047$). No changes in mean serum LH/FSH were observed with any of the groups.

Secondary pharmacodynamic studies

Study 11829: The objective of this study was to evaluate the uterotropic effects following once-daily oral administration of clomifene isomers for 30 days to ovariectomised (OVX) C57BL/6 mice.

The uterotrophic response of the mouse was principally characterised by minimally visible effects (dilation of uterine glands and increase in mean uterine weights) for both isomers. There were no deaths, clinical signs or adverse effects on body weight in any dose group. There were statistically significant increases in absolute and relative uterine weights in the control (sham), oestradiol benzoate, tamoxifen, enclomifene and zuclomifene-treated groups as compared to the OVX controls.

The highest increases in uterine weights (absolute and relative) were noted in the oestradiol benzoate group (332% and 367%) followed by control (sham; 174% and 208%), tamoxifen (115% and 136%), enclomifene (82% and 104%) and zuclomifene-treated groups (61% and 91%). The increases in uterine weights were attributed to the oestrogenic effects of the compounds. The uterus of oestradiol benzoate and tamoxifen-treated mice showed several characteristic oestrogenic changes.

Safety pharmacology programme

Study 1155013: In this study the effects of Androxal on the hERG related potassium current were assessed using whole-cell patch clamp electrophysiology methods in human embryonic kidney (HEK293) cells.

Perfusion of Androxal I0nM- 300nM did not inhibit hERG channel mediated potassium currents to any significant degree. Based on these data, an IC50 could not be calculated.

No further safety pharmacology studies were submitted with enclomifene; however evaluations were undertaken as part of the rat 6 month and dog 9 month toxicology studies. The applicant states that in the rat study no treatment-related changes in motor activity, arousal, neuromuscular or pulmonary function or sensorimotor activity were observed. Furthermore, in the dog study all ECG traces were quantitatively normal and no morphologic abnormalities were seen. There were no changes in haematological parameters.

Pharmacodynamic drug interactions

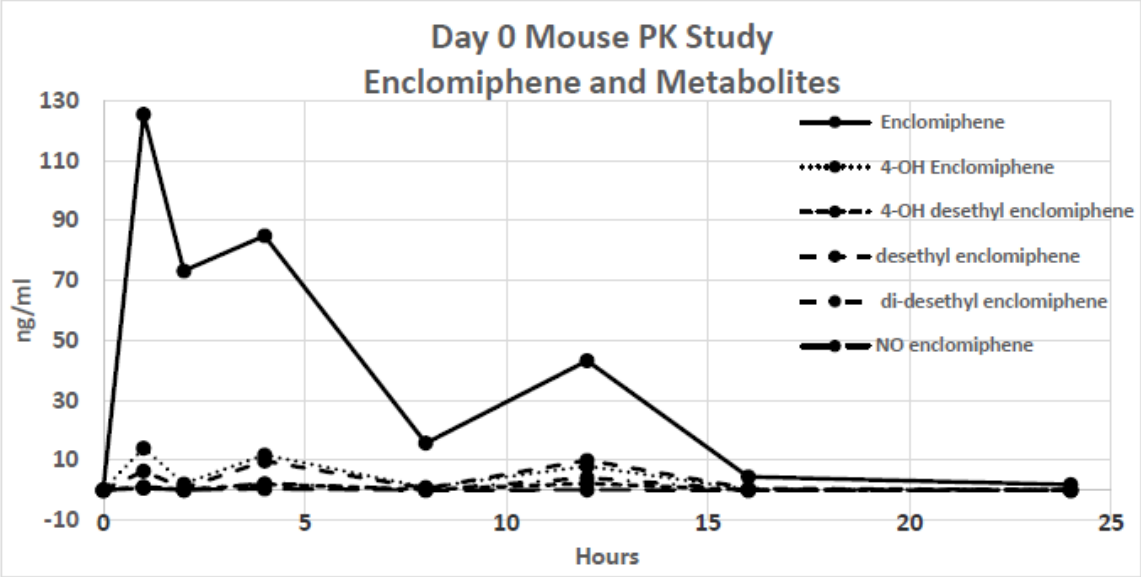
No studies of pharmacodynamics drug interactions were submitted.

2.3.3. Pharmacokinetics

Absorption

Results from **Study 18518-14 (non-GLP)**, in which 40 mg/kg of the test item was orally administered to male mice in order to determine acute PK parameters for enclomifene and its metabolites are presented in **Figure 2.**

Figure 2. Mouse single dose PK study – Enclomiphene and Metabolites absorption/time profile



See also results from toxicokinetics analysis in the 6 and 9 months pivotal toxicology studies in rats and dogs in section 2.3.4.

Distribution

Study 8308484: Distribution of ^{14}C-Enclomifene and ^{14}C-Zuclomifene was widely distributed in tissues and organs of mice following a single oral dose administration and was selectively associated with melanin-containing tissues. Tissue levels of radioactivity were consistently higher than plasma levels but with no selectivity for reproductive organs. Elimination was essentially complete by 72 hours except for retention in melanin containing tissues; residual radioactivity was still present in the eye and uveal tract at 864 hours. In the pivotal dog 9 month toxicology study, ophthalmoscopy showed no abnormalities in controls or the low and mid-dose groups.

Metabolism

In vitro

Study XT124119: Twenty-nine potential metabolites were detected in this study conducted in rat hepatocytes; only seven were present at levels high enough for UV detection, the 22 additional components were detected by mass spectrometry only. Two of the metabolites corresponded to the isomerisation product zuclomifene and the hydroxylation metabolite 4-OH-enclomifene. The predominant routes of metabolism of

enclomifene citrate were identified as hydroxylation, N-deethylation, glucuronide or methyl conjugation and oxidative dechlorination.

Study 0337-171-01: The objective of this study was to generate a profile of the metabolites of enclomifene citrate in cryopreserved hepatocytes from Sprague-Dawley rat, Beagle dog, Cynomolgus monkey and human.

Five major metabolites were observed in the incubations: M3, M13, M14, M15 and M22. All were seen at high levels in the 3 µM incubations and all except M3 were seen at high levels in the 30 µM incubations. The major transformations were hydroxylation dealkylation and dehydrogenation.

The metabolites produced by combination of incubations from rat and dog or rat and monkey covered all produced by human hepatocytes incubated with 3 µM enclomifene citrate except for M3. This was produced by the dog and monkey hepatocytes incubated with 30 µM enclomifene citrate only.

Excretion

No dedicated excretion studies were submitted.

Pharmacokinetic drug interactions

Inhibition of Cytochrome P450 enzymes by enclomifene citrate in human microsomes (Study report XT125125): This study was designed to evaluate the ability of Androxal to inhibit, *in vitro*, the major CYP enzymes in human liver microsomes with the aim of ascertaining the potential of Androxal to inhibit the metabolism of concomitantly administered drugs.

Under the experimental conditions, Androxal was shown to be a direct inhibitor of CYP2B6, CYP2C8, CYP2C19, CYP2D6 and CYP3A4/5 with IC50 values ranging from 1.2 µM for CYP2D6 to 24 µM for CYP2C19. The rank order of IC50 values from lowest to highest was CYP2D6 < CYP2C8 < CYP3A4/5 (midazolam 1´-hydroxylation) < CYP3A4/5 (testosterone 6β-hydroxylation) $\approx$ CYP2B6 < CYP2C19.

In addition, there was evidence that Androxal was a direct inhibitor of CYP1A2 and CYP2C9 with a maximum of 31% and 39% inhibition of these activities; however, the IC50 values are reported as > 50 µM since less than 50% inhibition was observed.

Inhibition of CYP450 enzymes by 4-OH enclomifene in human hepatocytes (Study XT135112): This study was designed to evaluate the ability of 4-OH enclomiphene to inhibit, *in vitro*, the major CYP enzymes in human liver microsomes with the aim of ascertaining the potential of 4-hydroxyenclomiphene to inhibit the metabolism of concomitantly administered drugs.

Results suggest that 4-OH enclomiphene was a direct inhibitor of every CYP enzyme examined. The rank order of IC50 values from lowest to highest was CYP2C8 > CYP2D6 > CYP3A4/5 (midazolam 1′-hydroxylation) > CYP2C19 > CYP2B6 > CYP3A4/5 (testosterone 6β-hydroxylation) > CYP2C9 > CYP1A2.

Induction of Cytochrome P450 enzymes by enclomifene citrate in cultured human hepatocytes. (Study XT123158): The objective of this study was to investigate the effects of treating primary cultures of fresh human hepatocytes with Androxal on the expression of cytochrome P450 (CYP) enzymes.

Under the conditions of this study, 10 µM Androxal caused an increase in CYP2B6 and CYP3A4 mRNA levels and caused increases in CYP2B6 and 3A4/5 activity, but had little or no effect on CYP1A2 mRNA levels or activity. The enzyme most affected with Androxal treatment was CYP3A4 with increases near half or almost as effective as rifampin at increasing CYP3A4 mRNA and/or activity, followed by CYP2B6 with minor increases in activity and mRNA. Of note, treatment with 100 µM Androxal caused toxicity in all three human hepatocyte cultures evaluated, consequently mRNA or activity results were unable to be collected.

Inhibition of small molecule transporters by enclomifene and 4-OH enclomifene (Study report XT138042): This study was designed to evaluate Androxal as an inhibitor of human P gp, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2K transporters and a substrate of human P-gp and BCRP transporters in vitro.

Enclomifene was evaluated as an inhibitor of a wide range of transporters using concentrations up to 15 µM. In particular significant inhibition seen was of the bidirectional transport of digoxin across CaCo-2 (P-gp/MDR1) cells where the IC50 was 4.2 µM, indicating that Enclomifene itself may be a substrate for P-gp.

2.3.4. Toxicology

Single dose toxicity

No single dose toxicology studies with enclomifene were submitted. The oral LD50 of the isomeric mixture of E and Z clomifene is 1700 mg/Kg in mice and 5750 mg/Kg in rats.

Repeat dose toxicity

The repeat-dose toxicity studies are summarised in **Table 2**.

Table 2. Summary of major findings in repeat dose toxicology performed with enclomifene

Study ID	Duration/Dose (mg/kg)/ Route	Species/ No. of animals	Major findings
17053-12/ non GLP	12 week: 4, 40/oral Enclomifene citrate or zuclomiphene citrate	Mice/15/ group	• One low dose animal death (zuclomiphene) Clinical signs: edema, hunched posture, activity decrease, emaciation, piloerection, growth on anterior, lump on leg, alopecia on anterior, lesion on anterior, and swollen testes. Also white mass in chest cavity and black mass in the abdomen • Necropsy: small spleen (high dose enclomiphene); small epididymis (high dose zuclomiphene) • Significant weight gain (low dose enclomiphene) • Significant weight loss (zuclomiphene) • Higher testes weight (high dose enclomiphene) • Lower testes weight (low dose enclomiphene) • Weight reduction: testes, seminal vesicles, epididymides, femoral muscle, liver and kidneys (high-dose zuclomiphene); testes, seminal vesicles, epididymides, liver and kidneys (low-dose zuclomiphene)
1155-003/GLP		CD® [Crl:CD® (SD)] rat/35/ group	• Reduced body weight (-32%) and food consumption (-23%) (all treated groups) • No effect of treatment was seen in clinical findings, functional observational battery, pulmonary, ophthalmoscopic, and urinalysis

	6 months: 0.5/5/10/oral		evaluations. • Statistically significant lower cholesterol values (no dose response; all treatment groups) • Pharmacological hormonal changes • Dose proportional increases in Cmax, and AUC_{0-24} • Minimal to moderate smaller prostrate (mid and high-dose) • Reduction in prostate gland, pituitary gland and epididymides weight (all treatment groups) • Microscopic changes: prostate gland, seminal vesicles, coagulating glands, testes, preputial glands, and kidneys. • The prostate glands, seminal vesicles, and coagulating glands - minimal to severe atrophy (all dose levels) • Minimal Leydig cell atrophy/depletion - testes (all dose levels) • Preputial glands - subacute inflammation (0.5 and 10 mg/kg/day); minimal to mild abscess formation (males; 0.5 and 5 mg/kg/day) • kidneys - minimal to mild tubular dilatation and minimal tubular mineralization (all dose levels) • statistically significant reduced Erythrocyte count, haemoglobin, and hematocrit value (mid- high-dose) • redcued reticulocyte count (all dose groups) • mildly prolonged Prothrombin time and activated partial thromboplastin time (dose-dependent and statistically significant)
1155-016/GLP	25 days: 0.5 or 5.0 for 11 days then 0.025 thereafter/oral	CD® [Crl:CD® (SD)] rat/5/group	• Increased incidence of 'few/absent' faeces recordings (m+f) • Days 1-11: significant decrease in mean body weight/body weight gain. Females slightly more affected • Days 12-26, body weight gains exceeded that of controls (5.0 to 0.025 mg/kg group) • Weeks 0-2: reduced food consumption (all dose groups); recovering in weeks 2-4 for 5.0/0.025 group. • No macroscopic or organ weight changes
1155-004	9 months: 2/10/40 then 20/oral	Beagle dog/11 males/ group	• Top dose reduced to 20 mg/kg based on high mortality rate in first 7 weeks – 2 dogs died on days 20 and 43 (hepatopathy). • Lacrimation in all treatment groups • Bodyweight increases • No effects on ECG/haematology/coagulation/clinical chemistry/ • Increases in testosterones levels – variable • Mid- and High-dose ophthalmoscopic abnormalities (mild cataracts) • Possible test article-related organ weight changes were seen in the adrenal glands, liver, and thyroid/parathyroid glands. • NOAEL = 2mg/kg/day
10818-07	9 months: 2/4/10/6/8/oral	Beagle dog/5 males/ group	• Oral administration not linked to pathological effects in eyes of male beagle dogs

Genotoxicity

A summary of the genotoxicity studies submitted are presented in **Table 3**.

Table 3. Overview of in vitro and in vivo genotoxicity studies

Type of test / study ID / GLP status	Test system	Concentrations / Metabolising system	Results
Gene mutations in bacteria **ZN01-100** GLP	*S. typhimurium* (TA1535, TA1537, TA98, TA100) +/-S9	Enclomiphene citrate or Zuclomiphene citrate: 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 µg/plate	Negative
L5178Y Tk+/- Mouse Lymphoma Forward Mutation Assay **769285 (21984)** GLP	L5178Y cell line +/- S9	500, 1500, 5000 $\mu g.ml^{-1}$.	Negative
In Vivo Rat Bone Marrow Micronucleus Assay **769290 (22008)** GLP	CD-1 mice	250, 500, and 1000 mg/kg and administered via oral gavage	Negative

Carcinogenicity

The oncogenic potential of enclomifene was investigated in transgenic-mice over a 26-week period (**Table 4**) and in in rats following 104 weeks of daily oral administration (**Table 5**).

Table 4. 26 week transgenic mouse carcinogenicity study (AB37CE.7G8R.BTL)

Study ID /GLP	Dose/Route	Mean plasma concentration ng/ml	Species/No. of animals	Major findings
AB37CE.7G8R.BTL/GLP	Positive control urethane 1000mg/kg/d) 10, 50, 100 and 200 mg/kg/d enclomiphene	Males: 13.9/342/1100 ng/ml (10/50/100 mg/kg) females: 15.6/374/1347 ng/ml (10/50/100 mg/kg: week 27)	Tg.rasH2 mice (25/sex/group)	• All high dose animals died = early termination of the 200 mg/kg/day dose group • 12/25 deaths in 100 mg/kg males and all TK males at 100 mg/kg • Cause of deaths: Necrosis/inflammation of intestine (m) • Clinical signs: dose-proportional incidences of thin appearance, hunched posture, lethargic behaviour, ruffled fur, diarrhoea, rapid and shallow respiration and tremors. • Decrease body weight gain (statistically significant and dose-proportional; m+f) • Gross changes: spleen, ovaries, seminal vesicles, skin, testes, thymus and uterus • tumor and proliferative effects – sporadic; not dose-related or statsistically significant • no NOAEL

Table 5. 104 week rat carcinogenicity study (1155-017)

Study ID /GLP	Dose/Route	Mean plasma concentration ng/ml	Species/No. of animals	Major findings
1155-017/GLP	0, 0.0125, 0.025, 0.05 mg/kg enclomiphene	Below LLOQ	CD® [Crl:CD® (SD)] rat (60/sex/group)	• For 1st 18 months lower mean body weight for males: statistically significant; regained weight in last 6 months of study • lower mean body weight for females throughout study: statistically significant • dose-related reduction in food consumption • tumor and proliferative effects – not statistically significant incidences of neoplasm • dose-related increase in the incidence and severity of minimal to severe centrilobular hepatocellular vacuolation - males. • a slight increase in the incidence and severity of minimal to mild centrilobular hepatocellular hypertrophy – males • livers of males at 0.05 mg/kg/day had a slight increase in the incidence and severity of clear cell foci • **TK:** The results of all of the plasma samples from treated animals were below the lower limit of quantitation (<5.00 ng/mL). • no NOAEL

Reproduction Toxicity

The effect of enclomifene on fertility was investigated in male mice in which the test article was administered via oral gavage to male mice for 28 days prior to mating and continued to euthanasia. The major findings of this study are summarised in **Table 6.**

Table 6. Functional Effects of enclomifene on Male Fertility in Mice Via Oral Gavage (Study 1155-005)

Study ID /GLP	Dose/Route	Mean plasma concentration ng/ml	Species/No. of animals	Major findings
1155-005/GLP (Segment I)	28 days prior to mating: 0, 40, 100, 150[a]/200 mg/kg enclomiphene		Mice/CD-1 Crl:CD1 (ICR) 30 males/group (~ 5 weeks old) 30 untreated females/group (~ 8 weeks old)	• No adverse effects on reproductive parameters (mating, fertility, fecundity indices) in either males or females at 40 or 100 mg/kg/day. The high dose, 200/150 mg/kg/day, produced mortality in all males; fertility could not be assessed. At 100 mg/kg/day, changes were noted in sperm parameters (lower sperm concentration, decreased sperm motility, higher abnormal sperm) and in uterine parameters (increased resorptions and post-implantation loss); decreased sperm concentration was also observed at 40 mg/kg/day. Based on these data, the No-Observed-Adverse-Effect-Level (NOAEL) for fertility was 100 mg/kg/day.

[a]Due to mortality, the dose level was reduced on Day 11.

Toxicokinetic data

In the male rat pivotal study, pharmacokinetic parameters for enclomifene are summarised in **Table 7**.

On the basis of the results of this study, a no-observed-adverse-effect-level (NOAEL) was not achieved, because of weight loss or failure to gain weight at all doses.

Table 7. Mean pharmacokinetic parameters for enclomifene in study 1155-003

Dose (mg/kg/day)	Day	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)	r2	$t_{1/2}$ (hr)
0.5	1	5.82	1	28.2		
	14	22.1	4	85.7		
	90	10.8	1	46.1	0.9217	5.9
	180	9.31	2	48.1		
5	1	42.6	1	307	0.9978	3.2
	14	136	1	694	0.9548	2.9
	90	157	1	1,046	0.9239	3.7
	180	165	2	999	0.9779	4.5
10	1	133	1	726	0.9825	3.1
	14	270	1	1,931	0.9991	3.2
	90	259	4	2,496	0.9770	4.3
	180	319	1	2,105	0.9925	5.2

Following a request from the CHMP and in order to determine the exposure margin limits of enclomifene and its main metabolites, comparison of AUC and Cmax between the highest dose in 1155-003 and human data from study ZA-205 was performed (**Tables 8** and **9**).

Table 8. Cmax (ng/mL) and calculated overage for study 1155-003

Analyte	C_{max} in Rat 180 days 10 mg/kg/day	C_{max} in Human 25 mg daily 12 weeks	Overage
Enclomifene	319	5.79	55.1
4-OH enclomifene	47.9*	3.42	14
Desethyl enclomifene	23.9*	2.58	9. 3
4-OH desethyl enclomifene	9.90*	1.75	5.7

Table 9. AUC exposure levels (ng.hr/mL) and overages for study 1155-003

Analyte	AUC in Rat 180 days 10 mg/kg/day	AUC in Human 25 mg daily 12 weeks	Overage
Enclomifene	2105	76.4	27.6
4-OH enclomifene	315.8*	58.8	5.4
Desethyl enclomifene	155.8*	41.7	3.7
4-OH desethyl enclomifene	58.9*	33.4	1.8

Study 1155-004 was the pivotal non-rodent repeat dose toxicology study and its results are summarised in **Table 10.** The no-observed-adverse-effect-level (NOAEL) was considered to be 2 mg/kg/day, the lowest level evaluated due to presence of ocular findings.

Table 10. Mean pharmacokinetic parameters for enclomiphene in male dogs in pivotal 9 month study (1155-004)

Sampling	Dose	C_{max} (ng/mL)		T_{max} (ng/mL)		AUC_{0-24} (ng•hr/mL)		$t_{1/2}$ (hr)	
Day	(mg/kg/day)	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Day 1	2	7.50 ± 1.34	11	1.45 ± 0.93	11	16.0 ± 6.8	11	7.8	1
	10	48.9 ± 35.1	11	1.91 ± 1.45	11	159 ± 107	11	2.4 ± 0.7	7
	40	480 ± 333	11	2.27 ± 1.19	11	2,516 ± 1,530	11	6.6 ± 1.9	7
Week 7	40	1,128 ± 721	10	2.60 ± 0.97	10	7,106 ± 5,254	10	9.4 ± 3.3	10
Day 90	2	7.37 ± 1.19	7	1.86 ± 1.86	7	14.4 ± 6.1	7		0
	10	143 ± 63.5	7	1.71 ± 0.49	7	683 ± 199	7	17.0 ± 3.8	3
	20	472 ± 273	7	3.43 ± 2.23	7	2,469 ± 1,298	7	10.0 ± 2.8	5
Day 272	2	15.5 ± 7.4	7	2.86 ± 1.07	7	45.6 ± 19.9	7	3.1	1
	10	261 ± 136	7	2.29 ± 0.76	7	1,372 ± 601	7	15.4 ± 4.2	6
	20	477 ± 122	7	3.43 ± 2.23	7	3,946 ± 848	7	18.7 ± 9.1	6

Cmax overage compared with ZA-205 was low for enclomifene and there was no overage for the metabolites and there was no overage for enclomifene or any metabolite when compared with exposure in ZA-205 (data not shown).

Further pharmacokinetic data for enclomifene and its main metabolite 4-OH enclomifene in dogs were collected in study 10818-07and are summarised in **Tables 11** and **12**.

Table 11. Mean C_{max} concentrations of enclomifene and 4-OH enclomifene (study 10818-07)

		Enclomifene 2 mg/kg (Mean ±SD)	Enclomifene 4 mg/kg (Mean ±SD)	Enclomifene 6 mg/kg (Mean ±SD)	Enclomifene 8 mg/kg (Mean ±SD)	Enclomifene 10 mg/kg (Mean ±SD)
Day 0 ng/mL	Encl	7.24 ±2.86	14.9 ±11.5	16.7 ±12.0	33.5 ±23.3	30.2 ±9.20
	4-OH Encl	1.78 ±0.69	6.58 ±3.88	6.19 ±5.10	19.1 ±17.0	15.8 ±3.60
Day 90 ng/mL	Encl	22.5 ±13.8	39.3 ±24.8	33.1±18.0	50.1 ±34.6	103 ±57.0
	4-OH Encl	7.62 ±4.50	14.6 ±8.70	14.3 ±20.9	20.0 ±10.9	39.8 ±23.7
Day 270 ng/mL	Encl	24.4 ±21.8	49.5 ±27.5	116 ±76.0	110 ±131	95.4 ±62.6
	4-OH Encl	5.81 ±6.08	24.7 ±18.2	31.2 ±23.4	49.5 ±65.6	39.3 ±27.1

Table 12. Mean $AUC_{0\text{-}24hr}$ values for enclomifene and 4-OH enclomifene (study 10818-07)

		Enclomifene 2 mg/kg (Mean ±SD)	Enclomifene 4 mg/kg (Mean ±SD)	Enclomifene 6 mg/kg (Mean ±SD)	Enclomifene 8 mg/kg (Mean ±SD)	Enclomifene 10 mg/kg (Mean ±SD)
Day 0 ng.hr/mL	Encl	19.1 ±2.8	51.0 ±19.3	71.1 ±42.9	130 ±79	112 ±26
	4-OH Encl	6.07 ±1.3	28.2 ±11.5	32.1 ±22.7	100 ±91	77.8 ±31.1
Day 90 ng.hr/mL	Encl	117 ±73	241 ±116	197 ±60	324 ±200	495 ±274
	4-OH Encl	41.1 ±19.3	111 ±71	72.6 ±81.4	146 ±89	232 ±128
Day270 ng.hr/mL	Encl	123 ±51	268 ±102	434 ±197	620 ±579	507 ±209
	4-OH Encl	31.7 ±22.8	151 ±99	171 ±113	372 ±472	252 ±166

Source: Study 10818-07 Auxiliary Metabolite Report Table 15

Tables 13 and **14** summarise Cmax and AUV values and overages for all analytes at a dose of 10 mg/kg/day enclomifene citrate in study 10818-07.

Table 13. Cmax (ng/mL) and calculated overage for study 10818-07

Analyte	C_{max} in Dog 10 mg/kg/day 272 days	C_{max} in Human 25 mg/day 12 weeks	Overage
Enclomifene	95.4	5.79	16.5
4-OH enclomifene	39.3	3.42	11.5
Desethyl enclomifene	32.2	2.58	12.5
4-OH desethyl enclomifene	24.0	1.75	13.7

Table 14. AUC exposure levels (ng.hr/mL) and overages for study 101818-07

Analyte	AUC in Dog 10 mg/kg/day 272 days	AUC in Human 25 mg/day 12 weeks	Overage
Enclomifene	507	76.4	6.6
4-OH enclomifene	252	58.8	4.3
Desethyl enclomifene	184	41.7	4.4
4-OH desthyl enclomifene	161	33.4	4.8

Other toxicity studies

No further studies were submitted.

2.3.5. Ecotoxicity / environmental risk assessment

Table 15. Summary of main study results

Substance (INN / Invented Name):enclomiphene			
CAS-number (if available):			
PBT screening			
Parameter	***Test protocol***	*Result*	**Conclusion**
Bioaccumulation potential- log K_{ow} (log D_{ow})	OECD123	log D_{ow} = 2 pH4 log D_{ow} = 4.95 pH 7 log D_{ow} = 6.52 pH 9	Potential PBT substance
PBT-assessment			
Persistence (P)-DT50 ay 12°C or DT50 ay 20°C	OECD 308	$DT50_{water}$ = 6.4-11.0 days $DT50_{sediment}$ = 1582.9-4058.1 days $DT50_{system}$ = 654-1171 days	vP
	OECD 307	$DT50_{soil}$ = >1000 days	vP
Bioaccumulation-BCF	OECD 305	BCF_{SS} = 548 L/kg	Not B
Toxicity- NOEC	OECD 211	0.026 mg/L	Definitive conclusion will be provided upon completion of the fish life cycle study
PBT-statement :			
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.125	µg/L	> 0.01 threshold (Y)
Other concerns (e.g. chemical class)			(N)
Phase II Physical-chemical properties and fate			
Study type	**Test protocol**	**Results**	**Remarks**
Adsorption-Desorption	OECD 106	K_{oc} = 0.899 L/kg (log KOC = -0.0460) for the citrate salt and 4.40 x 10^4 L/kg (log KOC = 4.64)	[^{14}C]enclomiphene citrate solution can be classified as being 'immobile' in soil and

		for the free base			sludge.
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	$DT_{50, water}$ =3-5 days $DT_{50, whole\ system}$ =310-551 days			very persistent (vP)
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Pseudokirchneriella subcapitata*	OECD 201	NOEC	0.0085	mg/L	unicellular green alga, *Pseudokirchneriella subcapitata*
Daphnia sp. Reproduction Test	OECD 211	NOEC	0.026	mg/L	*Daphnia magna*
21-Day Fish assay/Fish sexual development test Japanese medaka (*Oryzias latipes*)	OECD 230/234	NOEC		µg/L	ongoing
Activated Sludge, Respiration Inhibition Test	OECD 209	EC_{50}: total respiration heterothrophic respiration nitrification respiration	 195.7 209.5 172.2	mg/L	NOEC for total, heterotrophic and nitrification respiration was therefore determined to be 98 mg/L.
Phase IIb Studies – ongoing/planned					
Toxicity to the sediment-dwelling organism *Chironomus sp*	OECD 218	BCF		L/kg	%lipids:
Nitrogen transformation test	OECD 216	DT50 $\%CO_2$			for all 4 soils
Collembola reproduction test	OECD 232	%effect		mg/kg	
Fish bioconcentration study,	OECD 305	NOEC		mg/kg	
Aerobic transformation in soil	OECD 307	NOEC		mg/kg	

2.3.6. Discussion on non-clinical aspects

In vitro ligand displacement assays revealed that enclomifene has a higher affinity for ERα than for ERβ. The major metabolite 4-OH enclomifene had similar affinity to the parent compound for ERα but much higher affinity for ERβ. The coactivator assay demonstrated that enclomifene had no significant agonist activity but rather that enclomifene and its 4-OH metabolites were potent anti-oestrogens.

In vivo results suggested that the rat is not a suitable species for investigating the PD of enclomifene in relation to secondary hypogonadism. This is due to the fact that, in the rat, enclomifene demonstrated suppression in testosterone production and LH secretion although to a lesser extent than clomifene and the Z-isomer (zuclomifene). This is most likely species-specific based on the bibliographic data pertaining to the opposite results in humans.

In contrast, in the baboon, administration of enclomifene resulted in a significant increase in circulating testosterone. This supports the rationale for the development of enclomifene for the treatment of secondary hypogonadism.

The secondary pharmacodynamic effects of enclomifene and zuclomifene were evaluated through a study to measure the uterotrophic response in ovariectomised mice. Both isomers showed minor macroscopic effects and no evidence of hyperplasia/hypertrophy in any tissue.

Only one formal safety pharmacology study was conducted by the applicant, in which it was demonstrated that the test-item did not inhibit hERG channel mediated potassium currents at the clinically relevant concentrations, suggesting that the likelihood of adverse effects on the cardiac QT interval is low. Further safety evaluations were conducted in the toxicology studies.

No pharmacodynamic drug interactions studies were conducted, and this was considered acceptable in view of the available information on clomifene.

In the single PK study in mice absorption of enclomifene was rapid. Tmax after a single oral dose of 40mg/Kg, was 1 hour.

In the six month rat study, absorption of enclomifene citrate was rapid, and the plasma levels of enclomifene (Cmax and AUC_{0-24}) increased with increasing dose on all sampling days. The increases in maximum exposure, as measured by Cmax, were approximately proportional to dose for the mid and high-dose groups on Days 14, 90 and 180, but the increase on Day 1 was greater than proportional to dose. For the increase in dose between the low and mid-dose, the increase in Cmax was less than proportional to dose on Days 1 and 14 and greater than proportional to dose on Days 90 and 180. For AUC_{0-24}, there were close to dose proportional increases from 5 to 10 mg/kg/day. For the increase from 0.5 to 5 mg/kg/day, the increase in AUC_{0-24} was variable, but this was possibly due to the limited number of samples with concentrations above the limit of quantitation for the low-dose group.

In the nine month dog study, On Days 1, 90, and 272, the increases in peak exposure, as measured by Cmax, and in total exposure, as measured by AUC_{0-24}, were non-linear and not directly proportional to dose.

No dedicated excretion studies for enclomifene were submitted, and this was considered acceptable as the applicant provided data from a clinical study (ZA-112, see also Section 2.4.2) using radiolabelled enclomifene citrate indicate that that the primary route of elimination of clomifene and enclomifene, is faecal.

In PK interaction studies the potential for drug interactions caused by CYP inhibition was found to be low except for CYP2D6. However, based on the reported steady-state concentrations of enclomifene after 6 months dosing with 25 mg enclomifene citrate (Wiehle et at 2013), that inhibition of CYP2D6, or substrate competition, would only be possible for short periods post daily dosing when the concentration of enclomifene in hepatic portal blood may reach micromolar levels.

Given that 4-OH enclomifene is the major and active metabolite of enclomifene it was important to study its inhibitory activity against the same range of cytochromes in human hepatocytes. Study XT135112 evaluated the potential of this metabolite 4 as an inhibitor of a range of human CYP450 enzymes. The greatest degree of inhibition was seen after pre-incubation with NADPH. However, in all but two cases, the IC50 values were greater than 2 µmolar. For CYP2C8 the IC50 was 0.77 µM and for CYP2D6 it was 0.88 µM. Both of these values are in excess of any time-averaged concentration of 4-OH enclomifene that would be expected in hepatic venous blood over the absorption phase and many-fold higher than maximal steady-state concentrations found in the systemic circulation. It was concluded that it is unlikely that the generation of 4-OH enclomifene will cause significant pharmacokinetic interactions with other drugs by inhibition of CYP450 activity.

The applicant states that in a similar series of studies. 4-OH enclomifene failed to show any inhibitory activity against transporter proteins. As with enclomifene, study XT148048 showed that the 4-OH metabolite may be

a substrate for P-gp. The CHMP however considered that there is no pharmacokinetic evidence to suggest any relevant effects *in vivo* and the clinical history of the use of clomifene also does not indicate any serious drug interactions of this type for enclomifene.

In conclusion the potential for enclomifene and its active metabolites to cause drug/drug interactions is low.

Pivotal repeat dose toxicity studies have been conducted in rats (study 1155-003) and dogs (study 1155-004 and study 10818-07). The major metabolite, 4-OH enclomiphene was measured only in the second dog study. The Applicant used TK data generated in other studies to estimate the exposure of the animals in the two other studies.

As 4-OH enclomifene was not measured in the pivotal long term clinical studies and following a request from the CHMP, the Applicant performed a PK analysis using data of the ongoing clinical study ZA-205. The Applicant used interim data generated after 12 weeks of dosing to determine multiples of exposure. In study 10818-07, this was determined as 4.3 in dogs at the highest dose of 10 mg/kg/day based on AUC values. In rats, using extrapolated TK data this was estimated to be 5.4 at the highest dose tested. Therefore as exposure to 4-OH enclomifene in the toxicity studies was greater than the levels determined in humans the lack of *in vivo* metabolism studies was considered acceptable by the CHMP.

Exposure to enclomifene in the rat 26 week oral study (1155-003) at the top dose was 10-20 fold higher than observed at the clinical dose in humans. However, a NOAEL could not be assigned due to test article-related organ weight decreases (prostate gland, pituitary gland and epididymides) that were recorded at all dose levels. However, the reduction in weight gain was considered to be due to reduced food intake rather tham compound-related toxicity. Furthermore, same reduction in weight gain was seen in the rat carcinogenicity study (1155-017) and was associated with prolonged survival over the control group.

In the dog 9 month study a NOAEL of 2 mg/kg/day (low dose), was assigned. As expected testosterones levels increased. Toxicities included organ weight changes (adrenal/liver/prostate gland), and delayed onset in puberty was also evident in high-dose animals which correlated with changes noted in testes and epididyides. Deaths in high dose animals were related to hepatotoxicity.

Ophthalmic abnormalities (cataracts) were widely reported. Following these findings, the Applicant commissioned a peer review of the eyes collected from the 9- month chronic toxicity study in dogs which concluded that these changes could more accurately be described as minimal lens fibre swelling. The peer reviewers also agreed that there was no progression in the lens fibre swelling between the interim 13-week necropsy and the terminal 9- month necropsy. There was no evidence of fibre fragmentation, lenticular disorganisation, or lenticular epithelial proliferation. Furthermore, it was stated that even though the area and density of the lenticular swelling increased, the histopathological features remained the same i.e. lens fibre swelling, and did not include morphologic features more characteristic of cataract formation including lens fibre disruption, degeneration and vocalisation.

Throughout the clinical trial program careful eye examinations with centrally adjudicated by a single expert blinded to therapy were conducted and there was no evidence that enclomifene leads to new or worsening of cataracts in the Phase II and III clinical trial population pool.

A further study in dogs (10818-08) was carried out to obtain more information on these findings in the eye and, therefore, extensive ophthalmologic investigations were performed throughout the study. No lenticular lesions were seen in either the control group or at the highest dose of 10 mg/kg (x6 clinical safety margin).

The CHMP concluded that the currently available data do not provide conclusive evidence that use of enclomifene is associated with development of new or progression of existing cataracts. It was therefore

recommended that ocular safety monitoring should be included in the Risk Management Plan in order to obtain further data on the matter.

No genotoxic potential was reported following a standard battery of testing. Following a 2-year rat carcinogenicity study it was reported that no test article-related macroscopic findings were present in neither sex nor any clinical signs were reported and hence it was concluded that the test article was not oncogenic.

In the mouse fertility study at the NOAEL (100 mg/kg), changes in sperm parameters, as well as increased resorptions and post-implantation loss were seen. Mouse PK data, indicates that in this study peak exposure is likely to be >100 ng/ml at 100 mg/kg. In comparison concentrations of enclomifene in clinical studies reached a mean Cmax of 10.63 ng/ml (14-day pharmacokinetic study ZA-001) and 15.6 ng/ml (6-week pharmacology study ZA-204) giving adequate clinical safety margins.

An Environmental Risk Assessment (ERA) Report was submitted, however one of the studies included in this report is still ongoing (OECD 211, fish life cycle) which does not allow for definitive conclusions to be drawn on the classification for toxicity.

The CHMP, taking into account the results of the clinical pharmacology studies (Section 2.4 in this report) considered that it is not possible to adequately assess the relevance of the non- clinical program as part of the risk assessment for enclomifene. This is due to the fact that the submitted data do not contain an adequate characterization of the human *in vivo* metabolite pattern in plasma. Without such data from a mass balance study, it is not possible to evaluate whether there are human major or unique metabolites which according to ICH M3R(2) that need to be specifically addressed.

This deficiency must be addressed and further studies to strengthen the bridge to the key pre-clinical studies, including pivotal repeat dose toxicity studies, in vivo genotoxicity and carcinogenicity testing by any available means (in silico, *in vitro*, *in vivo*).

2.3.7. Conclusion on the non-clinical aspects

A number of non-clinical pharmacology, pharmacokinetic and toxicology studies were submitted to support the use of enclomifene in the proposed indication. Due to the discrepancy in the metabolite profiling between non-clinical and studies conducted in humans, the non-clinical data does not contain an adequate characterization of the human *in vivo* metabolite pattern in plasma and are not considered sufficient to adequately assess the relevance of the non-clinical program as part of the risk assessment for enclomifene. Further studies would be required to link the non-clinical and human data.

The ERA submitted cannot be considered final prior to the conclusion of the ongoing fish cycle study.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community

were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Clinical Study No.	Description
Pivotal Studies (year)	
ZA-301 (2013)	Placebo-controlled, double-blind, 12-18 weeks treatment with enclomifene or placebo; 151 patients.
ZA-302 (2013)	Placebo-and active-controlled, double-blind, 12-18 weeks treatment with enclomifene or placebo; 181 patients.
ZA-304 (2014)	Placebo- and active-controlled, double-blind, 16 weeks treatment with enclomifene, AndroGel® 1.62% or placebo; 129 patients.
ZA-305 (2014)	Placebo- and active-controlled, double-blind, 16 weeks treatment with enclomifene, AndroGel® 1.62% or placebo; 127 patients.
Supportive data	
Phase 3	
ZA-300 (2014)	Open-label, 12 months treatment with enclomifene; 499 patients.
ZA-303 (2014)	Assessment of effects on bone-mineral density. Placebo-controlled, single-blind, 12 months treatment with enclomifene or placebo.

Clinical Study No.	Description
Phase 2	
ZA-003 (2007)	Placebo- and active-controlled, double-blind, 6 months treatment with enclomifene, AndroGel® 1% or placebo; 194 patients.
ZA-003 extension (2008)	Open-label, 12 months treatment with enclomifene; 104 patients who had completed ZA-003.
ZA-002 (2006)	Open-label; 14 days treatment with enclomifene in 15 healthy males to assess effects on serum testosterone.
ZA-201 (2009)	Active-controlled, open-label, 6 months treatment with enclomifene or Testim® in 12 patients to compare effects on spermatogenesis, serum testosterone and plasma LH and FSH.
ZA-204 (2012)	Active-controlled, single-blind, 6 weeks treatment with enclomifene or AndroGel® in 60 patients to compare effects on serum testosterone and plasma LH and FSH.
ZA-203 (2012)	Placebo- and active-controlled, double-blind, 3 months treatment with enclomifene or Testim® in 124 patients to compare effects on spermatogenesis, serum testosterone and plasma LH and FSH.
ZA-203 extension (2014)	Open-label, 12 months treatment with enclomifene or Testim® in 47 patients who had completed ZA-203.
ZA-202 (2012)	Assessment of effects on glycaemic control. Placebo-controlled, double-blind, 3 months treatment with enclomifene or placebo in 119 patients with HH and type 2 diabetes mellitus.

Phase 1	
ZA-001 (2004)	Assessment of initial tolerability, PK and PD. Placebo and –active controlled, 14 days treatment with enclomifene, placebo or AndroGel® in 52 patients with HH.
ZA-107 (2014)	Crossover study in 12 healthy subjects to evaluate effects of food on PK of single dose of 25 mg enclomifene.
ZA-110 (2014)	Crossover bioavailability study in 16 healthy subjects to compare two formulations of enclomifene (single doses of 12.5 and 25 mg).
ZA-109 (2014)	Dose-finding study for thorough QT/QTc study. Investigation of PK of single 125 and 250 mg doses of enclomifene in 9 healthy subjects.
ZA-108 (2014)	Thorough QT/QTc study. Double-blind, crossover study in 96 healthy subjects of three daily doses of enclomifene 25 mg or 250 mg, placebo or positive control.
ZA-105 (2014)	Assessment of hepatic impairment on PK of single 25 mg dose of enclomifene in 19 subjects. Open-label comparison of healthy subjects and those with hepatic impairment and poor CYP6D metabolism.
ZA-104 (2014)	Assessment of renal impairment on PK of single 25 mg dose of enclomifene in 26 subjects.

Clinical Study No.	**Description**
	Open-label comparison of healthy subjects and those with mild, moderate and severe renal impairment.
ZA-111 (2014)	Assessment of age (≥65 years) on PK of three daily doses of 25 mg enclomifene.
ZA-113 (2015)	Thorough QT/QTc study. Double-blind, crossover study in 106 healthy subjects of three daily doses of enclomifene 100 mg, placebo or positive control.
ZA-112 (2015)	Mass-balance study to investigate single 25 mg dose of [^{14}C] enclomifene in 6 healthy volunteers.
ZA-114 (2015)	Meta-analysis of metabolites from clinical studies of enclomifene.
ZA-115 (2015)	Crossover bioavailability study in 36 healthy subjects to compare two formulations of enclomifene (single dose of 25 mg)
ZA-116 (2015)	Cross-over bioavailability study in 36 healthy subjects to compare two formulations of enclomifene (single dose of 12.5 mg)
ZA-106 (2015)	Drug Interaction Study. Thirty healthy subjects administered three daily doses of enclomifene citrate or placebo followed by one of six CYP probes.

2.4.2. Pharmacokinetics

Absorption

Study **ZA-001** was a randomized, parallel group study conducted to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamic effects of enclomifene citrate in men with secondary hypogonadism.

Men with secondary hypogonadism, documented by low (< 250 ng/dL) or borderline low (250 to 350 ng/dL) total serum testosterone levels with normal LH and FSH levels, were randomly assigned to one of five treatment groups:

- Enclomifene citrate 12.5 mg orally once daily;
- Enclomifene citrate 25 mg orally once daily;
- Enclomifene citrate 50 mg orally once daily;
- Matching placebo orally once daily;
- AndroGel (testosterone gel) 1% 5 g applied topically once daily.

Results

Enclonfiene citrate plasma concentration on days 1 and 14 are presented in **Figure 3** and summary PK parameters by enclomifene dose in **Table 16**.

Figure 3. Mean Plasma Enclomifene Citrate Concentration Time Profiles on Days 1 and 14 in Study ZA-001

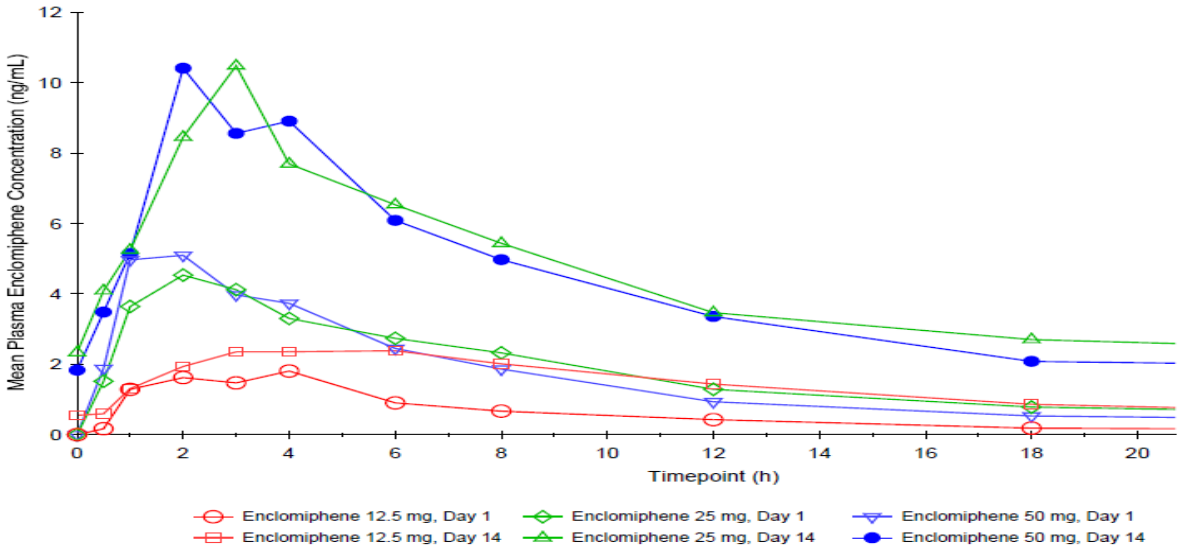


Table 16. Summary Pharmacokinetic Parameters for Plasma Enclomiphene by Enclomiphene Dose on Day 1 and Day 14 in Study ZA-001

Parameters	Day	Dose	N	Mean	SD	SE	Min	Median	Max	CV%
Tmax (hour)	Day 1	12.5 mg	5	3.6000	1.6733	0.7483	2.0000	4.0000	6.0000	46.4811
		25.0 mg	5	2.4000	1.1402	0.5099	1.0000	2.0000	4.0000	47.5073
		50.0 mg	4	2.0000	0.8165	0.4082	1.0000	2.0000	3.0000	40.8248
	Day 14	12.5 mg	5	4.2000	1.7889	0.8000	2.0000	4.0000	6.0000	42.5918
		25.0 mg	5	3.0000	0.7071	0.3162	2.0000	3.0000	4.0000	23.5702
		50.0 mg	4	2.5000	1.0000	0.5000	2.0000	2.0000	4.0000	40.0000
Cmax (ng/ml)	Day 1	12.5 mg	5	1.9770	1.7750	0.7938	0.6720	1.5400	5.0700	89.7822
		25.0 mg	5	4.7860	3.8758	1.7333	2.1000	3.8300	11.5000	80.9826
		50.0 mg	4	5.5600	1.0856	0.5428	4.1300	5.7300	6.6500	19.5258
	Day 14	12.5 mg	5	2.6842	1.6790	0.7509	0.5110	2.7600	5.1000	62.5524
		25.0 mg	5	10.6340	9.5829	4.2856	3.2900	6.2900	27.0000	90.1157
		50.0 mg	4	12.0925	5.7359	2.8680	5.3700	12.0000	19.0000	47.4338
T½ (hour)	Day 1	12.5 mg	5	7.9056	4.9135	2.1974	3.2747	7.7936	15.1408	62.1528
		25.0 mg	5	8.0848	2.0117	0.8997	5.7833	7.9971	11.1720	24.8825
		50.0 mg	4	6.5263	0.9160	0.4580	5.3878	6.6651	7.3871	14.0358
	Day 14	12.5 mg	5	9.3140	2.3982	1.0725	6.5512	8.3582	12.4543	25.7480
		25.0 mg	5	10.7314	2.5145	1.1245	7.9176	11.2987	13.2346	23.4316
		50.0 mg	4	9.6870	0.9214	0.4607	8.6011	9.7607	10.6254	9.5116
$AUC_{(0-24)}$ (hr.ng/ml)	Day 1	12.5 mg	5	13.6852	12.3443	5.5206	1.4717	10.2108	34.4866	90.2024
		25.0 mg	5	41.9225	34.4872	15.4231	9.4452	33.4381	100.0801	82.2641
		50.0 mg	4	38.2525	11.0499	5.5249	23.2863	40.4364	48.8510	28.8867
	Day 14	12.5 mg	5	34.0611	24.5600	10.9835	1.8622	36.9013	69.7163	72.1055
		25.0 mg	5	106.5200	77.6769	34.7382	29.5313	78.4035	231.2393	72.9224
		50.0 mg	4	99.7680	43.3353	21.6677	36.3181	115.6915	131.3707	43.4361

Influence of food

Study **ZA-107** was an open-label, randomised, single-dose, two-way crossover study to evaluate the effect of food upon the pharmacokinetics of Androxal.

12 healthy subjects each received a single dose of enclomifene citrate 25 mg in the fed and fasting state, separated by at least seven days.

The pharmacokinetic parameters for enclomifene and 4- hydroxyenclomifene are shown in **Table 17** and the statistical comparison of the relative bioavailability between the fasted and fed states in **Table 18.**

Table 17. Pharmacokinetic Parameters for Enclomifene and 4-Hydroxyenclomifene in Study ZA-107

Parameter	Enclomiphene		4-Hydroxyenclomiphene	
	Fasted State n = 12	Fed State n = 12	Fasted State n = 12	Fed State n = 12
C_{max} (ng/mL)	2.63 ± 3.21	4.66 ± 3.92	1.47 ± 0.82	2.28 ± 1.08
T_{max} (hr)[a]	2.00 (1 – 6)	2.50 (1 – 6)	6.00 (3 – 8)	5.00 (2 – 8)
AUC_{0-t} (ng•hr/mL)	27.3 ± 45.8	44.8 ± 52.5	18.9 ± 9.2	28.0 ± 12.2
$AUC_{0-\infty}$ (ng•hr/mL)	37.3 ± 68.1[b]	35.9 ± 12.1[b]	34.3[f]	44.5 ± 24.0[e]
k_e (hr^{-1})	0.0825 ± 0.0143[c]	0.0815 ± 0.0264[b]	0.0495 ± 0.0147[d]	0.0560 ± 0.0113[d]
$t_{1/2}$ (hr)	8.68 ± 1.76[c]	9.59 ± 3.81[b]	18.0 ± 11.1[d]	12.9 ± 2.9

[a] Values for T_{max} presented as median with range.

[b] n = 11 [c] n = 10 [d] n = 9 [e] n = 4 [f] n = 2

Table 18. Comparisons of Least Squares Means for Cmax and AUC0-t for enclomifene and 4-Hydroxyenclomifene in Study ZA-107

Analyte	Parameter	Ratio	90% CI	p-value
Comparison of Fed State to Fasted State				
Enclomiphene	$Ln(C_{max})$	202%	161% - 254%	0.0002
	$Ln(AUC_{0-t})$	203%	166% - 249%	0.0001
4-Hydroxy-	$Ln(C_{max})$	155%	133% - 180%	0.0004
..enclomiphene	$Ln(AUC_{0-t})$	149%	135% - 163%	< 0.0001

For enclomifene, the mean, median, and geometric mean values for Cmax and AUC0-t were higher for the fed state than for the fasted state. The median value for Tmax was slightly higher for the fed state (2.5 hours) than for the fasted state (2.0 hours) For 4-hydroxyenclomiphene, the mean, median, and geometric mean values for Cmax and AUC0-t were higher for the fed state than for the fasted state. The median Tmax value for the fed state was 5.0 hours with a range of 2 to 8 hours, and the median value for the fasted state was 6.0 hours with a range of 3 to 8 hours. The ratios of the least squares means for both log-transformed Cmax and log-transformed AUC0-t were above the 80% to 125% range considered as bioequivalent.

Distribution

Two *in vitro* studies were conducted to assess the degree of human plasma protein binding of enclomifene and its primary metabolite 4-OH enclomifene.

In study **XS-0493,** an in vitro assessment of the protein binding of Androxal and 4-OH enclomifene in human serum.

Enclomifene citrate and 4-OH enclomifene were added to human serum, and the in vitro serum protein binding was determined by the ultracentrifugation method; warfarin was included as a positive control.

The results showed that the mean in vitro serum protein binding ratio for enclomifene at a final concentration of 10µmol/L was 95.6% and 98.9% for 4-OH enclomifene.

As there was an error in the identification of 4-OH enclomifene, an additional study, **XS-0543** (an *in vitro* assessment of serum binding of 4-OH enclomifene in human serum) was conducted. This study showed that the mean *in vitro* serum protein binding ratio for 4-OH enclomifene at a final concentration of 10µmol/L was 99.4%.

Volume of distribution was compared in heathy subjects and subjects with varying degrees of renal impairment (study **ZA-104, Table 19**) following a single 25 mg dose, and in heathy subjects and subjects with moderate hepatic impairment (study **ZA-105, Table 20**).

Table 19. Descriptive Statistics for volume of distribution for enclomifene in patients with renal impairment-Study ZA-104)

Parameter	Group	Mean ± SD	%CV	Geometric Mean	Median	Range	n
V_z/F	Normal	24,125 ± 7,320	30.3	23,154	24,048	16,050 - 3,4973	8
(L)	Mild	19,216 ± 4,843	25.2	18,749	19,100	13,653 - 25,010	4
	Moderate	6,650 ± 2,108	31.7	6,279	6,805	2,703 - 9,573	8
	Severe	10,334 ± 4,977	48.2	9,529	8,435	6,572 - 18,473	5

Table 20. Descriptive Statistics for volume of distribution for enclomifene in patients with hepatic impairment (B)-Study ZA-105

Parameter	Group	Mean ± SD	%CV	Geometric Mean	Median	Range	n
V_z/F	A	18,111 ± 10,405	57.4	15,904	17,280	6,074 - 39,636	7
(L)	B	6,759 ± 1,717	25.4	6,584	6,313	4,652 - 9,015	5

Elimination

Study ZA-112 was an open-label, single dose study conducted to assess the absorption, metabolism, excretion and mass balance of radiolabelled enclomifene in healthy male subjects.

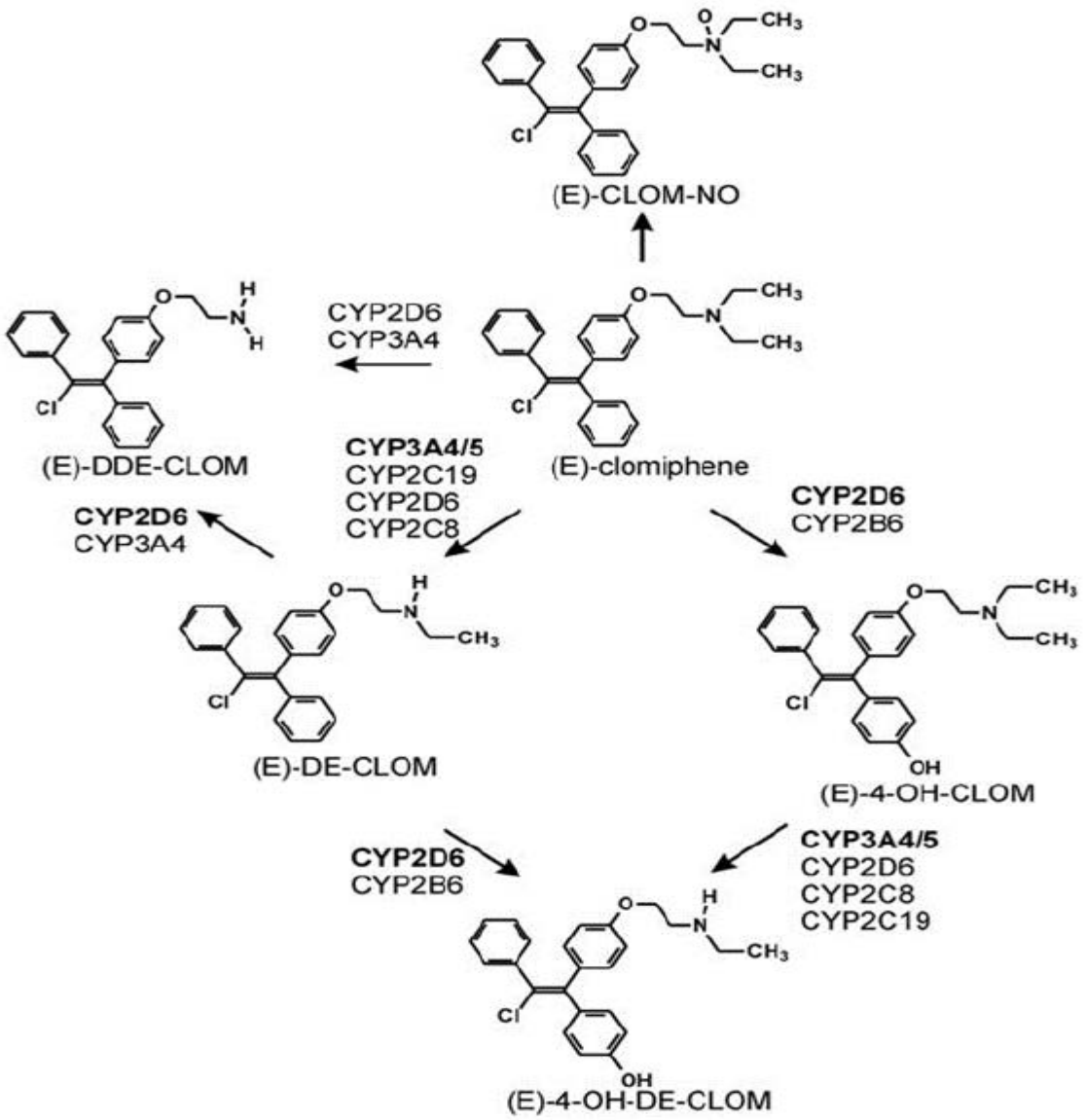
Six healthy male subjects between the ages of 19 and 60 years were enrolled in the study. On Day 1, a single 25 mg (~500 nCi) dose of [14C] enclomifene citrate was administered. Subjects fasted overnight for at least 8 hours before dosing and for at least 4 hours post-dose. Blood, urine, and faecal samples were collected for 168 hours post-dose (Day 8) to measure total radioactivity (plasma, whole blood, urine, and faecal samples).

Results showed a mean AUC0-t of 6696.0 ng Eq*hr/mL, mean Cmax of 157.7 ng Eq/mL, and median Tmax of 4 hours. Total radioactivity concentration equivalents in plasma remained quantifiable for all subjects through 168 hours post-dose. The majority of radioactivity was excreted into faeces (approximately 61.5% of the administered dose), with a smaller percentage excreted into urine (approximately 8.2% of the administered dose). The % partitioning of total radioactivity into whole blood relative to plasma at 4, 8, and 24 hours post-dose, was approximately 73%, indicating limited penetration of enclomifene and its metabolites into blood cells.

Metabolism

The metabolites of enclomifene have been reported in the literature using mass spectrometry to identify its metabolic pathways as summarised in **Figure 4.**

Figure 4. Pathway for enclomifene metabolites (Murdter et al, 2012)



Dose proportionality and time dependencies

Dose proportionality was not formally assessed. However in study **ZA-001** there appeared to be a higher that dose proportional increase in Cmax and systemic exposure of enclomifene between the 12.5mg and 25mg

strength. However between the 25mg and 50 mg strength there appeared to be a less than dose proportional increase suggesting saturation with increased doses.

Special populations

Impaired renal function

Study **ZA-104**, an open-label study to evaluate the pharmacokinetics and safety profile of Androxal in male subjects with impaired renal function. The study compared the PK profile of a single 25 mg dose of enclomifene citrate in groups of healthy subjects, and groups of subjects with mild, moderate and severe renal impairment (as determined by Cockcroft-Gault formula). Blood samples were taken pre-dose and at 1, 2, 3, 4, 6, 8, 12, 16 and 24 hours post-dose.

Statistical comparison of differences in bioavailability between healthy subjects and the groups with different degrees of renal impairment indicated higher exposure in subjects with moderate and severe renal impairment. Tmax and t1/2 were not affected significantly in subjects with any degree of renal failure.

- For the statistical comparison of relative bioavailability between normal subjects and subjects with mild renal impairment, the ratio of least square means ranged from 81.1% to 131%, indicating similar, but not identical exposure for the 2 groups.
- For the statistical comparison between normal subjects and subjects with moderate renal impairment, the ratio of least square means ranged from 214% to 251%, indicating higher exposure for moderately-impaired subjects than for normal subjects.
- For the statistical comparison between normal subjects and subjects with severe renal impairment, the ratio of least square means ranged from 137% to 191%, indicating higher exposure for the severely-impaired subjects compared to normal subjects.
- For the statistical comparison between normal subjects and all subjects with renal impairment, regardless of severity, the ratio of least square means ranged from 151% to 191%, indicating higher exposure for renally impaired subjects than for normal subjects.

Impaired hepatic function

Study **ZA-105**, an open-label study evaluating the pharmacokinetics and safety profile of Androxal in male subjects with impaired hepatic function. The study compared the PK profile of a single 25 mg dose of enclomifene citrate in groups of healthy subjects, CYP2D6 poor metabolisers (PM) subjects (with normal hepatic function) and subjects with moderate hepatic impairment (Child-Pugh criteria Class B). Blood samples were taken pre-dose and at 1, 2, 3, 4, 6, 8, 12, 16 and 24 hours post-dose.

Comparison of the healthy subjects and subjects with hepatic impairment showed that the least mean squares for Cmax and AUC (0-t) for enclomifene were significantly higher for subjects with hepatic impairment. Inter-subject variability was also higher for subjects with hepatic impairment (%CV >100), than for healthy subjects (%CV <60).

Elderly

Study **ZA-111** compared the PK profile of enclomifene between elderly (≥65 years) and adult subjects (18 to 45 years old (**Table 22)**.

Table 21. Pharmacokinetic parameters by age in study ZA-111

Parameter	Elderly Group n = 12	Adult Group n = 12
C_{max} (nM)	18.0 ± 12.4	14.9 ± 6.7
T_{max} (hr)[a]	3.00 (1 – 6)	4.00 (1 – 8)
AUC_{0-24} (nM•hr)	258 ± 220	209 ± 120
AUC_{0-t} (nM•hr)	389 ± 364	304 ± 194
AUC_{0-48} (nM•hr)	389 ± 364	304 ± 194

* Values for Tmax presented as median with range

In the elderly group, the subjects were stratified by 65-74 and 75-84 years. Results are shown in **Table 22.**

Table 22. Pharmacokinetic parameters by age in the elderly population in study ZA-111

Parameter	Age 65-74 years N=7			Age 75-84 N=5		
Mean ± SD	Enc	4-OH Enc	Combined	Enc	4-OH Enc	Combined
C_{max} (ng/mL)	2.7 ± 0.8	3.6 ± 1.9	14.1 ± 5.9	4.4 ± 3.4	5.7 ± 4.0	23.3 ± 17.6
T_{max} (hr)*	1 (1-4)	3 (2-6)	3 (1-6)	2 (1-4)	4 (2-4)	4 (2-4)
AUC_{0-24} (ng.hr/mL)	26.5 ± 13.8	54 ± 34.8	193.4 ± 114.3	48.3 ± 48	97.3 ± 82.9	349.6 ± 309.9
AUC_{0-t} (ng.hr/mL)	34.8 ± 20.7	80.9 ± 57.8	278.9 ± 183.3	67.6 ± 72.8	159.2 ± 143.8	544.4 ± 512.4
AUC_{0-48} (ng.hr/mL)	35.5 ± 19.9	80.9 ± 57.8	278.9 ± 183.3	68 ± 72.4	159.2 ± 143.8	544.4 ± 512.4

*T_{max} presented as median with range

Pharmacokinetic interaction studies

In vitro

Study **XT123158,** was an *in-vitro* evaluation of Androxal as an inducer of Cytochrome P450 expression in cultured human hepatocytes.

Results

CYP1A2: Negligible changes in activity or mRNA expression were seen.

CYP3A4: Concentration-dependent increases in activity (also seen for CYP3A5) and mRNA expression were observed and at 10 µM

CYP2B6: Negligible changes in activity were seen (<2.0 fold) bur concentration-dependent increases in mRNA expression were observed.

Study **XT125125** evaluated enclomifene as an inhibitor of Cytochrome P450 (CYP) enzymes in human liver microsomes.

Results

- Enclomifene exhibited metabolism-dependent (time and NADPH-dependent) inhibition of CYP2B6, CYP2C9, CYP2C19 and CYP2D6.

- CYP 2D6 was most sensitive to enclomifene with IC50 values of 1.2 µM (direct and time-dependent inhibition) and 0.13 µM after pre-incubation with NADPH.
- There was negligible evidence that enclomifene exhibited time- or metabolism-dependent inhibition of CYP1A2, CYP2C8 or CYP3A4/5.

Study **XT135112** evaluated 4-OH enclomifene as an inhibitor of cytochrome P450 (CYP) enzymes in human liver microsomes.

Results

4-OH enclomifene was found to exhibit direct inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5, metabolism-dependent (time and NADPH-dependent) inhibition of CYP2C9, CYP2C19 and CYP2D6 was exhibited and 4-OH enclomifene inhibited CYP2C8 with an IC50 of 0.82 µM with inhibition of all other cytochromes being less potent than this.

Study **XT138042** evaluated enclomifene as an inhibitor of human P-gp, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2K transporters and a substrate of human P-gp and BCRP transporters.

Results

The only significant inhibition seen was of the bidirectional transport of digoxin across Caco-2 cells, with an IC50 of 4.2 µM, indicating that enclomifene may be a inhibitor for P-gp. Enclomifene caused <50% inhibition of the remaining transporters examined.

Study **XT148036** evaluated 4-OH enclomifene as an inhibitor and a substrate of human P-gp, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2K transporters.

Results

4-OH enclomifene caused <50% inhibition of the all transporters examined.

In vivo

Study **ZA-106,** was an open-label, multiple dose study to assess the drug-drug interactions of Androxal with cytochrome P450 isoenzymes (CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) in healthy male subjects.

Thirty healthy male subjects were treated with either 25 mg Androxal citrate or placebo for three consecutive days. On Day 3 all subjects also received one of the CYP probe drugs. There was a minimum 7 day washout period after the third administration of study drug in each cycle before the first administration of study drug in the next cycle.

Blood samples were taken pre-dose of enclomifene or placebo on Days 1, 2 and 3. At Day 3, additional blood samples were taken at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after administration of the probe. The procedure was repeated, with interim washout periods, so that each probe was tested on all subjects.

Results

Androxal dosing had little effect on the Cmax and AUC0-t of bupropion, buprenorphine, warfarin, omeprazole and dextromethorphan indicating small potential for dug-drug interactions with CYP2B6, CYP2C8, CYP2C9, CYP2C19 and DYP2D6 respectively

When dosed with Androxal, the Cmax and AUC0-t of midazolam were approximately 27% and 31% lower with Androxal (**Table 23**), suggesting some potential induction of CYP3A4.

Table 23. Pharmacokinetic parameters and statistical comparison following 6 mg midazolam (CYP3A4) alone or with Androxal 25 mg once daily for 3 days

Parameter	Mean ± SD (% CV)		LSM Ratio	
	Androxal	Placebo	Androxal to Placebo (%)	90% CI
C_{max} (ng/mL)	23.7 ± 8.0 (33.6%)	32.7 ± 11.6 (35.3%)	73.0	59.4 to 89.6
AUC $_{0-t}$ (hr.ng/mL)	74.9 ± 28.7 (38.3%)	106 ± 29 (27.0%)	68.9	56.7 to 83.6

Consequences of possible genetic polymorphism

Study **ZA-105** also included a small number of poor CYP2D6 metabolisers (PM) subjects (but with normal hepatic function). Pharmacokinetic parameters in these patients is summarised in **Table 24.**

Table 24. Pharmacokinetic parameters of enclomifene in poor CYP2D6 metabolisers (Study ZA-105)

Parameter	Group A; Normal Subjects, n = 8	Group B; Moderate Hepatic Impairment, n = 8	Group C; CYP2D6 Poor Metabolizers, n = 3
C_{max} (ng/mL)	1.84 ± 0.91	7.33 ± 7.61	10.1 ± 1.0
T_{max} (hr)[a]	3.5 (3 - 6)[a]	4 (2 - 12)[a]	8 (4 - 8)[a]
AUC_{0-t} (ng•hr/mL)	19.0 ± 11.1	106 ± 142	130 ± 6
$AUC_{0-\infty}$ (ng•hr/mL)	25.1 ± 14.0[b]	59.2 ± 22.5[c]	-[c]

2.4.3. Pharmacodynamics

Mechanism of action

Enclomifene citrate is a selective oestrogen receptor modulator (SERM) which acts by blocking the oestrogenic suppression of the HPG axis. Cellular assays have demonstrated that the activity simulated by enclomifene is solely antagonistic. As a result, the pituitary secretes more LH and FSH, and this stimulates the testes to produce more testosterone. Enclomifene citrate appears to block the negative feedback inhibitory effects of oestradiol resulting in increased LH and FSH levels which stimulates endogenous serum testosterone production and spermatogenesis.

Primary and Secondary pharmacology

Primary pharmacology

In study **ZA-001** (described in Section 2.4.2 of this report) the pharmacodynamic effects of enclomifene citrate in men with secondary hypogonadism were investigated. Total and free serum testosterone levels were measured at baseline and after 14 days of treatment, approximately 24 hours after last dose of study medication, **Table 25**.

FSH and LH levels were also measured a at baseline and Day 15 (**Table 26).**

Table 25. Total and Free Serum Testosterone Concentration (ng/dL) Results in Study ZA-001

	Total Serum Testosterone (ng/dL) Results					
Parameter	**Enclomiphene 12.5 mg (N=10)**	**Enclomiphene 25 mg (N=11)**	**Enclomiphene 50 mg (N=11)**	**AndroGel® 1% 5 gm (N=10)**	**AndroGel® 1% 10 gm (N=10)**	**Placebo (N=10)**
Screening Mean (SD)	220.80(54.79)	244.18(55.41)	255.55(59.94)	234.20(65.71)	264.40(103.07)	267.90(57.66)
Baseline Day 1 Mean (SD)	242.90(102.41)	273.18(63.70)	295.18(109.63)	260.78(90.93)	245.60(103.39)	301.30(72.66)
Day 15 Mean (SD)	411.50(219.44)	520.09(160.30)	589.45(172.47)	473.01(289.31)	608.10(322.77)	300.10(108.56)
Day 15 Mean (SD) Change	*168.60 (145.75)*	*246.91 (141.16)*	*294.27 (192.35)*	*212.23 (264.63)*	*362.50 (398.64)*	*-1.20 (73.72)*
P Value vs. Baseline	*P=0.0053*	*P=0.0002*	*P=0.0005*	*P=0.0428*	*P=0.0183*	*P=0.9601*
P Value vs. Placebo	*P=0.0041*	*P<0.0001*	*P=0.0004*	*P=0.0437*	*P=0.0183*	--
Follow-Up Mean (SD)	351.80 (140.86)	458.00 (103.95)	579.27 (136.94)	256.00 (124.05)	354.10 (273.51)	276.20 (86.46)

	Free Serum Testosterone (pg/mL) Results					
Parameter	**Enclomiphene 12.5 mg (N=10)**	**Enclomiphene 25 mg (N=11)**	**Enclomiphene 50 mg (N=11)**	**AndroGel® 1% 5 gm (N=10)**	**AndroGel® 1% 10 gm (N=10)**	**Placebo (N=10)**
Baseline Day 1 Mean (SD)	9.58 (3.49)	9.30 (2.64)	9.18 (3.35)	10.66 (3.50)	16.42 (7.08)	12.38 (9.79)
Day 15 Mean (SD)	13.29 (7.42)	15.93 (7.07)	18.02 (5.12)	18.62 (10.98)	28.62 (14.94)	10.73 (4.22)
Day 15 Mean (SD) Change	*3.71 (5.70)*	*6.28 (6.17)*	*8.84 (6.26)*	*7.97 (10.05)*	*12.20 (16.33)*	*-1.65 (10.38)*
P Value vs. Baseline	*P=0.0698*	*P=0.0105*	*P=0.0009*	*P=0.0446*	*P=0.0425*	*P=0.6274*
P Value vs. Placebo	*P=0.1697*	*P=0.0525*	*P=0.0106*	*P=0.0565*	*P=0.0363*	--

Table 26. Luteinizing Hormone and Follicle Stimulating Hormone results in study ZA-001

	Enclomifene 12.5 mg n=5	Enclomifene 25 mg n=5	Enclomifene 50 mg n=5	AndroGel® 5 g n=5	AndroGel® 10 g n=5	Placebo n=5
	LH (MIU/mL) Mean (SD)					
Screening	4.18 (2.10)	3.92 (2.25)	4.81 (1.16)	4.76 (1.26)	2.68 (1.61)	5.56 (0.88)
Day 1	4.54 (2.00)	3.84 (1.21)	8.06 (3.88)	4.54 (0.82)	2.37 (1.70)	4.46 (1.98)
Day 11	7.48 (3.04)	8.46 (2.44)	11.20 (4.85)	2.56 (1.19)	0.51 (0.68)	4.98 (2.27)
Day 15	8.84 (4.53)	11.22 (7.03)	13.44 (5.85)	2.33 (0.93)	2.01 (1.84)	4.12 (1.84)
Follow up	5.46 (1.83)	8.72 (6.14)	11.36 (8.33)	5.04 (0.99)	3.93 (2.41)	5.04 (1.31)
	FSH (MIU/mL) Mean (SD)					
Screening	4.80 (1.34)	5.58 (2.50)	3.70 (1.82)	6.32 (33.19)	3.25 (1.84)	6.54 (2.98)
Day 1	5.04 (0.88)	5.44 (1.85)	4.48 (2.22)	5.98 (2.84)	3.18 (2.25)	6.56 (3.43)
Day 11	8.59 (2.89)	8.80 (4.18)	9.66 (3.46)	3.46 (2.80)	1.10 (1.12)	6.56 (3.47)
Day 15	9.18 (2.73)	9.94 (4.46)	11.67 (4.94)	3.64 (3.04)	2.56 (2.52)	6.22 (3.35)
Follow up	5.88 (0.71)	7.34 (2.48)	8.64 (5.57)	6.56 (2.56)	4.06 (3.03)	6.86 (3.54)

Study ZA-204 was randomised, single blind multi-centre phase 2 study conducted to determine the effects of three doses of Androxal (enclomifene) and AndroGel on average and maximum concentrations of testosterone and Luteinizing Hormone (LH), in men exhibiting morning testosterone ≤ 350ng/dL. This was assessed after six weeks of continuous administration. The primary efficacy endpoint was the 24-hour average and maximum testosterone concentration compared to baseline after 6 weeks of treatment (**Table 27**). The secondary endpoints were changes from baseline in LH and FSH, also at 6 weeks (**Tables 28-29**).

Table 27. Testosterone Values After 6 Weeks of Treatment with different doses of enclomifene in Study ZA-204

Dose mg/day (n)	Mean Baseline Morning T ng/dL (stdev)	Week 6 Mean Morning T	Week 6 24-hr Avg. T
6.25 (15)	243 (76)	405 (158)	392 (153)
12.5 (13)	298 (106)	485 (168)	461 (129)
25 (16)	248 (115)	541 (159)	587 (142)
Androgel (14)	293 (118)	452 (243)	544 (230)

Table 28. Effect of different doses of enclomifene on Mean Luteinizing Hormone in Study ZA-204

Treatment	Mean morning LH mIU/mL (SD)			
	Baseline	Week 2	Week 4	Week 6
Enclomifene 6.25 mg n=15	3.63 (1.55)	5.49 (2.69)	6.92 (3.59)	6.09 (3.30)
Enclomifene 12.5 mg n=13	4.82 (1.63)	8.6 (4.11)	7.20 (2.97)	8.22 (3.22)
Enclomifene 25 mg n=16	4.98 (3.45)	9.64 (5.04)	11.78 (7.39)	14.49 (10.45)
AndroGel® 5g n=14	3.57 (2.23)	2.00 (2.12)	1.88 (2.68)	2.16 (2.81)

Table 29. Effect of different doses of enclomifene on Follicle Stimulating Hormone in Study ZA-204

Treatment	Mean morning FSH mIU/mL (SD)			
	Baseline	Week 2	Week 4	Week 6
Enclomifene 6.25 mg n=15	4.61 (1.97)	6.18 (2.56)	6.45 (2.70)	5.69 (3.34)
Enclomifene 12.5 mg n=13	5.63 (2.26)	7.77 (3.03)	7.12 (266)	8.19 (3.25)
Enclomifene 25 mg n=16	6.31 (4.28)	11.41 (8.19)	12.34 (9.59)	13.45 (10.84)
AndroGel® 5g n=14	6.38 (2.79)	3.80 (3.06)	3.72 (3.66)	3.35 (3.34)

Study **ZA-002** was an open label, fixed dose, single centre, Phase 1 study to evaluate the changes in total testosterone from oral administration of enclomifene (trans-clomifene) citrate in healthy men with low and normal testosterone level. Enclomifene 25 mg was administered daily for 14 days. Total serum testosterone at Days, 14, 28 and 42 (post-treatment) were compared with baseline levels. Testosterone levels increased after treatment regardless of baseline testosterone status.

Secondary pharmacology

Study ZA-108:

A Thorough QT/QTc Study conducted primarily to determine the effects of once daily oral doses of Enclomiphene 25mg and 250mg for 3 days on the QTc interval at multiple time points after dosing. Assay sensitivity was established using moxifloxacin. 54 healthy male subjects were enrolled in to the study.

Results

The maximum mean ΔΔQTcF was 12.18 msec, and the maximum one-sided 95% UCB was 14.09 msec at 8 hours post-dose for Androxal250 mg. The values of mean ΔΔQTcF ranged from -1.28 msec to 1.54 msec for Androxal 25 mg treatment and from 7.70 to 12.18 msec for Androxal 250 mg treatment. The therapeutic dose values of UCB were all less than or equal to 3.43 msec.

Study ZA-113

A Thorough QT/QTc Study conducted primarily to determine the effects of once daily oral dose administration of Androxal® 100 mg for 3 days on the QTc interval at multiple time points after dosing. In addition, a

potential relationship between plasma concentrations of enclomifene and a hydroxylated derivative of enclomifene (4OH-enclomiphene) following Androxal 100 mg doses and change of QTc from the subject-specific and period-specific Baseline (ΔQTc) was explored.

Results

The maximum mean ΔΔQTcF was 4.4 msec, and the maximum one-sided 95% UCB was 5.85 msec, at 22.5 hours post-dose. The minimum mean ΔΔQTcF was 1.7 msec and the minimum UCB 3.18 msec. Within the observations for the Androxal dose, the values of ΔΔQTcF increased slightly with time after dose.

2.4.4. Discussion on clinical pharmacology

Pharmacokinetics

On the first day of administration, the time to maximal concentration was 4 hours for the 12.5mg strength and 2 hours for the higher doses. There appeared to be a higher than dose proportional increase in Cmax and systemic exposure of enclomifene between the 12.5 mg and 25 mg strength. However between the 25mg and 50 mg strength there appeared to be a less than dose proportional increase.

For enclomifene, the mean, median, and geometric mean values for Cmax and AUC0-t were slightly higher for the fed state than for the fasted state. The median value for Tmax was also slightly higher for the fed state (2.5 hours) than for the fasted state (2.0 hours). Similarly for 4-*OH*-enclomifene, the mean, median, and geometric mean values for Cmax and AUC0-t were higher for the fed state than for the fasted state. The median Tmax value for the fed state was 5.0 hours with a range of 2 to 8 hours, and the median value for the fasted state was 6.0 hours with a range of 3 to 8 hours. However, considering the wide therapeutic window these increases in plasma concentration are not of clinical consequence.

The enzymes responsible for its major metabolic transformation are CYP2D6 and CYP3A4/5. Its main metabolite appears to be 4-OH enclomifene formed primarily by CYP2D6 and to a lesser extent by CYB2B6. Enclomifene is also metabolised to desethyl enclomifene by CYP3A4 and CYP3A5; this is then further metabolised to 4-OH desethyl enclomifene, primarily by CYP2D6.

In vitro results suggest that enclomifene is a moderate inhibitor of CYP2D6, defined as causing a > 2-fold increase in plasma AUC. The results showed that time to maximal concentration in poor metabolisers delayed when compared to normal subjects (8 hours). Cmax was significantly higher (10.1ng/mL). Systemic exposure was also significantly higher, Three overweight subjects with CYP2D6 poor metaboliser genotype were included in another study. Cmax was increased approximately 5-fold, and AUC was increased approximately 7-fold. No safety findings indicative of toxicity were identified in these subjects. Based on these results, no dose adjustment of enclomifene in CYP2D6 poor metaboliser patients or with concomitant use of strong CYP2D6 inhibitors, is necessary.

Following administration of a single radiolabelled dose of enclomifene to healthy adult male subjects, the mean total radioactivity recovered after 7 days was approximately 71.0% of the dose; 61.5% of the dose was excreted in the faeces and 8.2% in the urine. The elimination t½ of enclomifene from plasma is 8-10 hours, indicating that ≥90% of the administered dose would be recovered within 14 days.

Based on the data presented by the applicant, it is not possible to evaluate whether there are human major or unique metabolites which according to ICH M3R(2) would need to be specifically addressed. In the ADME study, when comparing AUC for total radioactivity in plasma with the sum of the AUCs for the parent compound and the metabolites (4-OH enclomifene, desenclomifene and 4-OH desenclomifene), the latter

seem to account for approximately 3% of total drug exposure. There is no apparent explanation about this discrepancy. Additional information would therefore be required, possibly from another ADME study.

In older men (> 75 years) exposure to enclomifene appears to be slightly increased compared to men aged 65 - 74 years. The PK values for the adult group lay in between the two elderly age groups. Hence the increase in PK parameters noted in men aged >75 years is unlikely to be of clinical significance. Also, enclomifene is intended for use by men less than 60 years old; this is stated in the product information.

Studies were conducted in subjects with renal and hepatic impairment. In subjects with moderate and severe renal impairment Cmax and systemic exposure of enclomifene and its metabolite were significantly increased (the ratio of least square means ranged from 214% to 251% for the former and 137% to 191% for the latter). Considerable variability was noted in these groups and given the lack of any safety concerns in patients with renal impairment in the clinical trials, the observed pharmacokinetic differences were not considered sufficient to warrant dose adjustments.

Nevertheless, given that data are limited in patients with hepatic dysfunction, renal impairment but also CYP2D6 poor metabolisers and in patients older than 75 years old, further information in these populations would be required, for example through a population PK analysis.

Pharmacodynamics

In Study ZA-001, enclomifene increased total and free serum testosterone concentrations incrementally after 14 days dosing at all three investigated doses of 12.5 mg, 25 mg and 50 mg, in a less than dose proportional manner. This could be explained by the fact that treatment effect of enclomifene on testosterone is mediated through at least five intermediate steps. These include inhibition of the oestrogen receptor at the level of the hypothalamus and pituitary, release of LH from the pituitary gland, binding of LH at the leydig cell receptors, production of intra-testicular testosterone, and finally, release of testosterone into the systemic circulation.

Small statistically significant increases in LH and FSH were also noted in the enclomifene 12.5 mg, 25 mg and 50 mg groups compared to baseline and placebo, whereas small statistically significant or marginally significant decreases in LH and FSH were noted in the AndroGel 1% 5 mg and 10 mg groups compared to baseline and placebo.

Similar results were observed from the other two PD studies submitted (ZA-204 and Z-002) suggesting that enclomifene is able to stimulate endogenous testosterone production.

In terms of safety pharmacology, two QT studies are provided. One conducted with 25mg and 250mg enclomifene (study ZA-1080 and the other conducted with 100mg enclomifene (ZA-113).

The upper bound of the 1-sided 95% confidence interval for 25 mg dose for the time-matched mean effect of enclomifene on QTcI was less than 10 msec at all evaluated time-points. Similarly for the 100mg dose, the upper bound of the 1-sided 95%confidence interval for the time-matched mean effect of enclomifene on QTcI was less than 10 msec at all evaluated time-points indicating that the QT interval is not prolonged at these doses.

For the 250mg dose however, at 8 hours post-dose the maximum mean ΔΔQTcF was 12.18 msec, and the maximum one-sided 95% UCB was 14.09 msec indicating that there is prolongation of the QT interval with this dose which is however considerably higher than the daily recommended dose of 8.5 mg.

2.4.5. Conclusions on clinical pharmacology

The pharmacokinetics of enclomifene was investigated in a number of clinical studies. The information provided is considered incomplete and additional information would be required, to exclude the possibility of a unique human metabolite of significance. Identification of such a metabolite would also need to be thoroughly evaluated in non-clinical studies to investigate its potential chronic toxicity.

Further characterisation of the pharmacokinetic profile is also needed to inform the need for any dose adjustments in elderly patients, those with renal and hepatic impairment and poor CYP2D6 metabolisers.

The pharmacodynamic effect of enclomifene in stimulating endogenous testosterone has been demonstrated.

2.5. Clinical efficacy

2.5.1. Dose response studies

No formal dose response studies were submitted. Some information on dose response = was provided form studies ZA-001 and ZA-204 (described in Sections 2.3 and 2.4 of this report).

2.5.2. Main studies

ZA-304: A Randomized, Double Blind, Placebo-Controlled, Multi-Centre Phase III Study in Men with Acquired Hypogonadotropic Hypogonadism to Compare Changes in Testosterone and Sperm Concentration Following Treatment with 12.5 mg or 25 mg Androxal or AndroGel 1.62%

Methods

This study was a randomised, double-blind, placebo-controlled multicentre Phase 3 study to compare changes in sperm concentration and testosterone following treatment with 12.5 mg or 25 mg of Androxal, AndroGel 1.62%, or placebo (a double-dummy design was utilized) in overweight men with acquired hypogonadotropic hypogonadism and normal baseline sperm concentrations. The study required 10 clinic visits with one overnight stay and was approximately 5 months in duration.

Study Participants

Inclusion criteria

- Overweight (BMI 25 to 42 kg/m2 inclusive) males age 18 to 60 inclusive
- Previously or concurrently diagnosed as having secondary hypogonadism characterized as having at least 2 consecutive morning testosterone assessments < 300ng/dL, one of which must be confirmed at Baseline.
- LH < 9.4 mIU/mL (at Visit 1 only)

- Sperm concentration ≥ 15 million per millilitre (assessed at V2 and Baseline). V2 and Baseline measurements must have been at least 48 hours apart.
- Ability to complete the study in compliance with the protocol
- Ability to understand and provide written informed consent
- Agreement to provide a total of at least 4 semen samples in a sponsor-approved clinic on 4 separate occasions.

Exclusion criteria (selection)

- Any prior use of testosterone treatments (injectable, pelleted, transdermal or sublingual) within the last 6 months
- Use of spironolactone, cimetidine, Clomid, 5α-reductase inhibitors, aromatase inhibitors, hCG, androgen, estrogen, anabolic steroid, DHEA, or herbal hormone products during the study
- Use of Clomid in the past year
- Any clinically significant laboratory abnormality that did not have prior written sponsor approval. If the sponsor approved subject enrolment, this was not to be considered to be a protocol deviation.
- Uncontrolled hypertension or diabetes mellitus based on the Investigator's assessment at baseline. Subjects treated for Type II diabetes were allowed into the study if considered clinically stable by the investigator. Newly diagnosed diabetics needed to be treated for at least 48 hours before being enrolled in the study.
- A haematocrit >54%
- Symptomatic cataracts (nuclear sclerosis cataract or cortical cataract grade > 2 based on 0-4 scale or any evidence of posterior subcapsular cataract)
- Abnormal fundoscopy exam such as central retinal vein occlusion

Treatments

Subjects were randomized in a 1:1:1 ratio to one of 3 treatment arms:

• 12.5 mg Androxal daily, by mouth, plus placebo topical gel applied daily

• Placebo capsule, daily, by mouth plus placebo topical gel, applied daily

• Placebo capsule, daily by mouth plus AndroGel 1.62%, daily, applied topically

All subjects received both oral capsules and topical gel, of which one or both may have been placebo product. Study drug was administered and applied each morning, for a total of 16 weeks.

The AndroGel dose was adjusted (either up or down) per the manufacturer's instructions (at Weeks 2, 4, and 8).

The Androxal (or placebo capsule) dose was up-titrated to 25 mg daily, only at Week 4, if the subject's morning testosterone level was <450ng/dL. Placebo subjects were sham up-titrated.

Objectives

To compare the effects of 16 weeks of treatment with Androxal 12.5 mg, 25 mg, placebo capsules, AndroGel 1.62% or placebo gel on sperm concentration and testosterone in overweight men with acquired hypogonadotropic hypogonadism (confirmed morning T<300 ng/dL) and normal sperm concentration (≥ 15 million/mL). Subjects must not have previously been treated with testosterone products within the last 6 months.

Outcomes/endpoints

The Co-Primary endpoints were:

- The percentage change from baseline in mean sperm concentration after 16 weeks of treatment. Analyses compared Androxal to AndroGel 1.62%.
- Comparison of the proportion of subjects who obtain a successful testosterone level and sperm concentration. Subjects were considered successful if their mean sperm concentration remained ≥ 10 million/mL and their 24-hour testosterone levels stayed within the normal range [300-1,040 ng/dL] following 16 weeks of treatment. Androxal was compared to Placebo and AndroGel 1.62% in a pairwise fashion.

Secondary endpoints

- Change from baseline in LH and FSH after 16 weeks of treatment. Androxal was compared to AndroGel 1.62%
- Comparison of the proportion of subjects whose mean sperm concentration was found to be below 10 million/mL following 16 weeks of treatment with Androxal or AndroGel 1.62%.
- Comparison of mean 24-hour testosterone after 16 weeks of treatment. Androxal was compared to Placebo.
- Morning testosterone values assessed approximately one week post treatment. Androxal was compared to AndroGel 1.62%.
- Change from baseline in Haematocrit after 16 weeks of treatment. Androxal was compared to AndroGel 1.62%.

Other endpoints

- Comparison of the proportion of subjects whose mean sperm concentration became oligospermic (< 15 million/mL) following 16 weeks of treatment with Androxal or AndroGel 1.62%
- Comparison of the proportion of subjects whose mean sperm concentration became severely oligospermic (< 5 million/mL) following 16 weeks of treatment with Androxal or AndroGel 1.62%
- Comparison of the proportion of subjects whose mean sperm concentration dropped below 20 million/mL following 16 weeks of treatment with Androxal or AndroGel 1.62%.

Sample size

It was assumed that subjects treated with Androxal would have a 5% decrease in sperm concentration after 16 weeks of treatment while those treated with AndroGel would have a significant decrease in sperm concentration of 65%. The common standard deviation was assumed to be 85. A sample of 33 subjects per treatment group allows for 80% power of this endpoint. As this endpoint is historically not normally distributed, a 15% increase in the sample size to 38 subjects per treatment group allowed for adequate power if non-parametric methods were required for analysis.

The composite endpoint defines each subject as a success or failure based on their 16-week assessments of mean sperm concentration and 24-hour testosterone. If it is assumed that 70% of Androxal-treated subjects will have successful testosterone and sperm concentration at Week 16 while only 30% of subjects treated with AndroGel and only 20% of Placebo-treated subjects will meet both success criteria, a sample of 32 subjects will provide 90% power to detect a statistical difference between the proportions.

As it was expected that approximately 75% of the subjects treated with Androxal would be deemed a success with the composite endpoint, the lower limit on a 95% confidence interval on that proportion when the sample size is 40 is 59.64%.

Randomisation

Subjects were randomized by a web-based randomization system in a ratio of 1:1:1 to one of the following treatment arms:

• 12.5 mg Androxal daily, by mouth, plus placebo topical gel applied daily

• Placebo capsule, daily, by mouth plus placebo topical gel, applied daily

• Placebo capsule, daily by mouth plus AndroGel 1.62%, daily, applied topically

Blinding (masking)

This was a double-blind study.

Statistical methods

For all primary, secondary and tertiary analyses, comparisons between treatment groups entailed comparing Androxal (pooled dose groups) to placebo, and/or Androxal to AndroGel (pooled dose groups). AndroGel and Placebo were not compared. This was referenced by using ‘the pairwise fashion’ when discussing treatment group comparisons.

Safety analyses investigated treatment affect by the highest Androxal dose the subject was exposed to as well as pooled Androxal doses. For safety analyses, “the pairwise fashion” may have been used for brevity when discussing comparisons between treatment groups. Doses of AndroGel were not distinguished.

Statistical significance was declared if the two-sided p-value was $\leq$ 0.05.

All computations were performed using SAS, version 9.2 or higher.

Multiple Comparisons and Multiplicity

In order to control the risk of Type I error, analyses employed a sequential gatekeeping methodology for all primary and secondary endpoints.

The first endpoint was to be tested at a two-sided α of 0.05. If the hypothesis was rejected, then testing of the next endpoint was to continue at the 0.05-level. If at any point in the sequential testing the null hypothesis failed to be rejected then no further statistical conclusions were to be drawn.

Handling of Dropouts

In general, missing data was not replaced or imputed. However, for the primary and secondary efficacy variables, a last observation carried forward approach was used to impute missing data. If there were no data for post-baseline data for efficacy variables, a value of no change was imputed. Statistical significance was declared if the two-sided p-value was < 0.05.

Composite Endpoint

Subjects were defined as a success for the composite endpoint if:

• Week 16 24-hour testosterone Cavg was within the normal range [300, 1,040 ng/dL] and

• The mean of the end of study sperm concentration was at or above 10 million/mL

If a subject did not complete the 24-hour testosterone assessment the last assessment of testosterone, likely a morning testosterone, was used in this analysis. If there were no post-baseline determinations of testosterone the subject was considered a failure in this analysis. If there were no post-baseline semen collections then a last-observation was carried forward, including the mean baseline sperm concentration.

The proportion of subjects who successfully met each criterion at the end of the study for both sperm concentration and testosterone were summarized for each treatment group and compared between treatment groups using a Chi-Square test or Fisher's exact test.

Results

Participant flow

Patient disposition is summarised in **Table 30**.

Table 30. Patient disposition in Study ZA-304

Disposition	Treatment Group				
	Androxal		AndroGel 1.62%	Placebo	Total
	12.5 mg	25 mg			
Number of Patients Randomized	41	0	43	45	129
Maximum Dose Number of Patients Exposed	24	17	43	45	292
Number of Patients Completed Study	20 (83.3%)	17 (100.0%)	38 (88.4%)	39 (86.7%)	114 (88.4%)
Number of Patients Prematurely Discontinued from the Study	4 (16.7%)	0 (0.0%)	5 (11.6%)	6 (2.2%)	15 (11.6%)
Reasons for Premature Discontinuation from the Study:					
Adverse Events	1 (4.2%)	0 (0.0%)	2 (4.7%)	0 (0.0%)	3 (2.3%)
Lost to Follow-Up	0 (0.0%)	0 (0.0%)	1 (2.4%)	3 (6.7%)	4 (3.1%)
Withdrew Consent	3 (12.5%)	0 (0.0%)	1 (2.3%)	3 (6.7%)	7 (5.4%)
Hct>54%	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	1 (0.8%)
Populations Safety	24	17	43	45	129
ITT	24	17	43	45	129
PP	20	16	35	36	107

Recruitment

Study initiation date: January 17 2014

Study completion date: September 24 2014 (database lock)

Conduct of the study

The protocol was amended three times during conduct of the study. The most significant changes to the protocol were as follows:

• The following criteria for withdrawal from the study were added:

- Suspected thromboembolic event. Subjects who have a thromboembolic event ruled out may reinitiate treatment
- A need for major surgery arises. Enclomiphene therapy should be discontinued for 4 weeks prior to any major surgery
- If a need for major surgery arises, subjects will be discontinued from the study

An amendment was made in response to recommendations provided by the FDA in an advice letter dated April 28, 2014.

- The primary endpoints were revised to compare, for Androxal and AndroGel treated subjects:
  - Proportion of subjects with a mean sperm concentration below 10 million/mL
  - Percentage change from baseline in mean sperm concentration
- A secondary endpoint was added to assess morning testosterone levels at one week after end of treatment
- A secondary endpoint was deleted to investigate changes in BMI

A total of 128 protocol deviations occurred, the most commonly occurring one was assessment out of window. In most cases this involved visits being performed 8 or fewer days outside of the protocol-permitted windows or late blood draws for laboratory assessments or PK draws.

Baseline data

Subject baseline demographics of the subjects included in the study are shown in **Table 31**.

Table 31. Summary of baseline demographic information in study ZA-304 (ITT population)

Characteristic	Treatment Group Mean ± SD or N (%)			
	12.5 mg [n=24]	25 mg [n=17]	AndroGel [n=43]	Placebo [n=45]
Age (years)	48.3 ± 7.7	50.2 ± 7.2	47.4 ± 7.2	47.2 ± 9.0
Ethnicity				
Native Hawaiian or other Pacific Islander (Non-Hispanic or Non Latino)	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)
Asian (Non-Hispanic or Non-Latino)	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)
Black or African American (Non-Hispanic or Non-Latino)	4 (16.7%)	6 (35.3%)	9 (20.9%)	9 (20.0%)
Caucasian (Non Hispanic or Non Latino)	19 (79.2%)	11 (65.7%)	30 (70.0%)	34 (75.6%)
Caucasian (Hispanic or Latino)	1 (4.2%)	0 (0.0%)	2 (4.7%)	2 (4.4%)
Height (cm)	179.1 ± 7.9	177.7 ± 9.9	177.5 ±8.6	176.5 ± 6.7
Weight (kg)	107.0 ± 16.3	103.4 ±16.7	107.3 ±17.0	102.0 ± 16.3
BMI (kg/m^2)	33.4 ± 4.9	32.7 ± 3.8	34.0 ±4.4	32.6 ±4.3
Baseline Testosterone ng/dL	209.9 ± 46.2	194.0 ± 60.2	208.6 54.0	200.3 43.1
Mean Baseline Sperm Concentration (x10^6/mL)	70.5 ± 43.5	137.5 ± 116.2	78.9 ± 68.5	95.3 ± 90.8
Years Hypogonadic	1.3 ± 2.9	3.4 ± 3.4	1.5 ± 2.3	1.4 ± 2.5
Previous Testosterone Use	3 (12.5%)	4 (23.5%)	11 (25.6%)	8 (17.8%)
Populations				
Safety	19	25	42	41
ITT	19	25	42	41
PP	16	22	35	37

Numbers analysed

The Intention to Treat (ITT) set comprised the group of patients who were enrolled and randomized to treatment with study medication (primary analysis).

The per protocol (PP) population was defined as the subset of the ITT population who completed their Week 16 visit.

The safety population comprised all subjects that were administered even a single dose of study drug.

These populations are summarised in **Table 32**.

Table 32. Patient populations analysed in study ZA-304

	Androxal® 12.5 mg	Androxal® 25 mg	AndroGel 1.62%	Placebo	Overall
	Number of Subjects				
Safety Population	24	17	43	45	129
Intent-to-Treat (ITT) Population	24	17	43	45	129
Per Protocol Population (PP)	20	16	35	36	107

Outcomes and estimation

Sperm concentrations were all determined by a central laboratory. The mean sperm concentrations and change from baseline for each treatment group are shown in **Table 33**.

Table 33. Summary of Mean Sperm Concentration and Mean Percentage Change from Baseline in Sperm Concentration (ITT)

	Androxal N=41	AndroGel 1.62% N=43	Placebo N=45
Screening/Baseline Mean ± SD	98.30 ± 87.2	78.9 ± 68.5	95.3 ± 90.8
End of Treatment Mean ± SD	102.9 ± 96.8	32.7 ± 51.5	76.6 ± 47.3
Mean % Change ± SD	11.7 ± 80.3	-56.6 ± 48.2	4.1 ± 57.2
Median % Change	-3.3	-77.5	-10.0
Min/Max % Change	-100.0, 353.2	-100.0, 83.1	-73.8, 216.0
95% CI	-14.4, 37.7	-72.0, -41.2	-14.2, 22.4
P-Value (1)	0.9128	<0.0001*	0.3328*
P-Value (2)		<0.0001*	0.5482*

* Chi-Square test

Failure or success of treatment according to the pre-defined composite endpoint are summarised in **Table 34**.

Table 34. Composite Endpoint: Comparison of the Proportion of Subjects Who Obtain a Successful Testosterone Level and Sperm Concentration in study ZA-304

	Androxal N=41 N (%)	AndroGel N=43 N (%)	Placebo N=45 N (%)
Failures: Mean sperm concentration < 10M/mL or T not in normal range	16 (39.0)	36 (83.7)	43 (95.6)
Successes: Mean sperm concentration > 10M/mL and T in normal range [300-1040]	25 (61.0)	7 (16.3)	2 (4.4)
P-value		<0.0001* <0.0001**	<0.0001* <0.0001**

* Chi-Square test
** Fisher's Exact test

Proportion of Subjects with Mean Sperm Concentration below 10 million/mL Following Treatment

There were 19 (44.2%) AndroGel-treated subjects with a sperm concentration below 10 million/mL at the end of treatment, one (2.4%) Androxal-treated subject with a sperm concentration below 10 million/mL at the end of treatment and no Placebo-treated subjects had with a sperm concentration below 10 million/mL at the end of treatment.

Mean 24-Hour Testosterone at the End of Treatment

Mean 24-hour testosterone levels were within normal range for 12.5 mg Androxal and AndroGel treated subjects at the end of Treatment. The mean testosterone level for 25 mg Androxal-treated subjects was (298.0 ng/mL), for placebo it was (192.9 ng/dL). The proportion of subjects in the normal range [300-1040 ng/dL] was higher in the Androxal group (63.4%) in comparison to the AndroGel group (44.2%).

Effects on LH and FSH

Changes in levels of leuteinizing hormone and follicle stimula ting hormone during the course of the study are shown in **Figures 5** and **6** respectively.

Figure 5. Mean LH (mIU/mL) by study visit in ZA-304

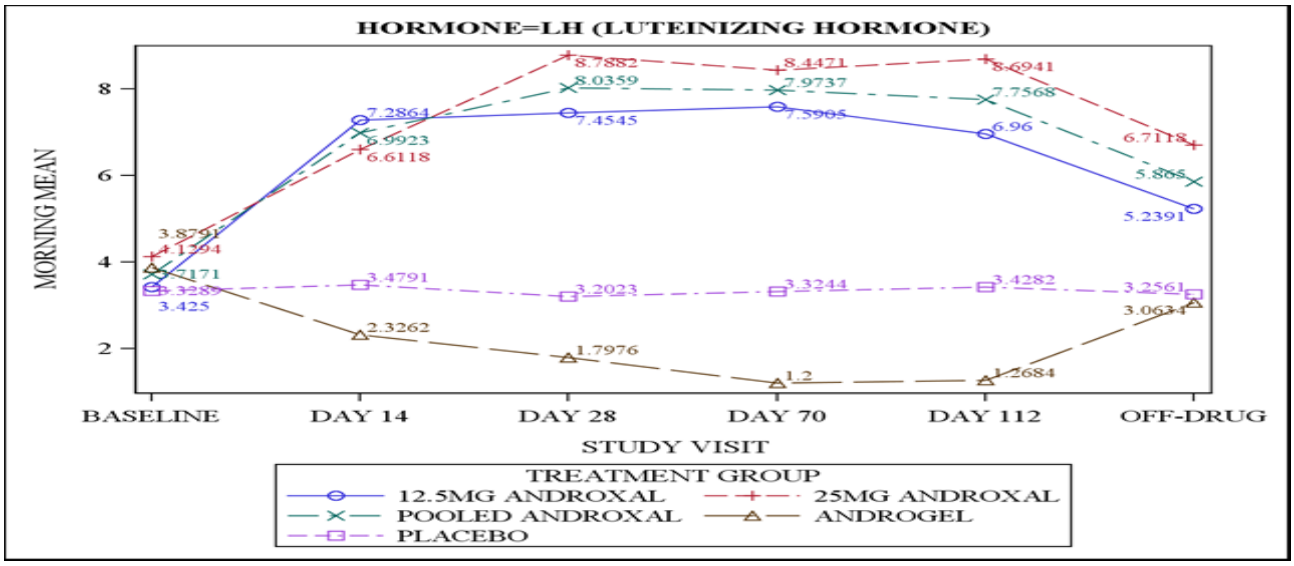
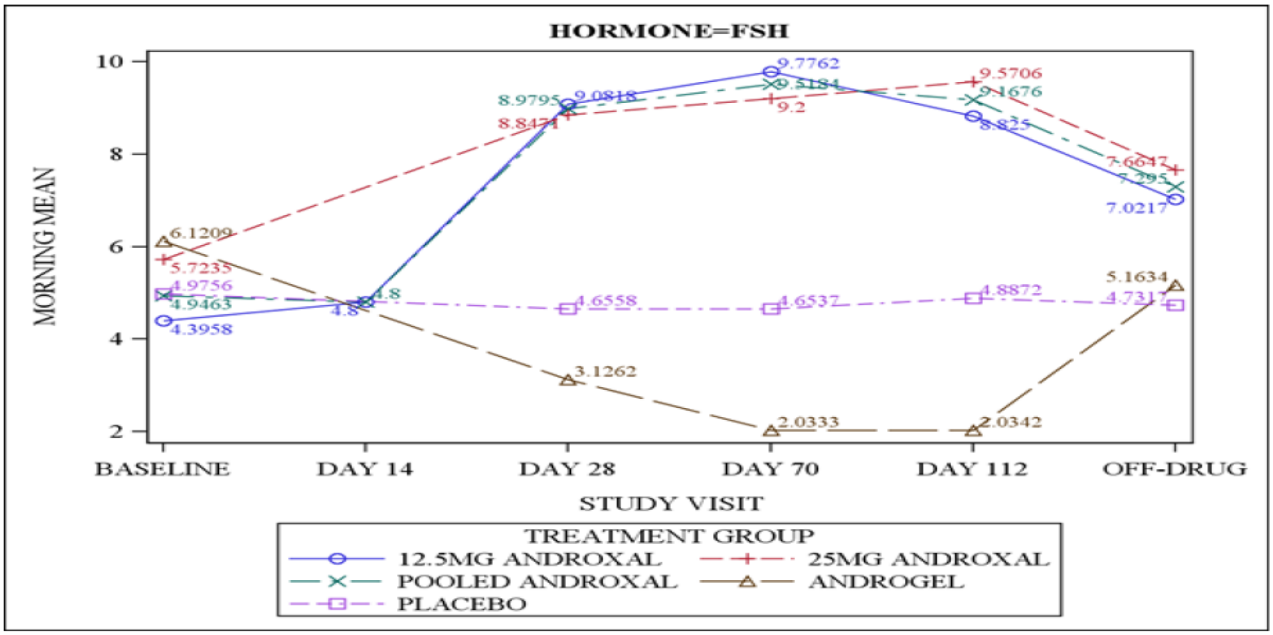


Figure 6. Mean FSH (U/L) by study visit in ZA-3054



ZA-305: A Randomized, Double-blind, Placebo Controlled, Multi-Center Phase III Study to Evaluate Normalization of Morning Testosterone Levels in Overweight Men with Acquired Hypogonadotropic Hypogonadism and Normal Sperm Concentration.

Study design was identical to ZA-304.

Participant flow

Patient disposition is summarised in **Table 35**.

Table 35. Patient disposition in Study ZA-305

Disposition	Treatment Group				
	Androxal		AndroGel 1.62%	Placebo	Total
	12.5 mg	25 mg			
Number of Patients Randomized	44	0	42	41	127
Maximum Dose Number of Patients Exposed	19	25	42	41	127
Number of Patients Completed Study	16 (84.2%)	24 (96.0%)	38 (90.5%)	39 (95.1%)	117 (92.1%)
Number of Patients Prematurely Discontinued from the Study	3 (15.8%)	1 (4.0%)	4 (9.5%)	2 (4.9%)	10 (7.9%)
Reasons for Premature Discontinuation from the Study:					
Adverse Events	1 (5.3%)	0 (0.0%)	1 (2.4%)	1 (2.4%)	3 (2.4%)
Lost to Follow-Up	1 (5.3%)	0 (0.0%)	1 (2.4%)	0 (0.0%)	2 (1.6%)
Withdrew Consent	1 (5.3%)	0 (0.0%)	2 (4.8%)	1 (2.4%)	4 (3.1%)
Hgb>18.5 g/dL	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	1 (0.8%)

Recruitment

Study initiation date: January 3, 2014

Study completion date: August 22, 2014 (database lock)

Conduct of the study

The same protocol amendments to study ZA-304 were applied.

A total of 375 deviations occurred the most commonly occurring one was assessment out of window.

Baseline data

Subject baseline demographics of the subjects included in the study are shown in **Table 36**.

Table 36. Summary of baseline demographic information in study ZA-305 (ITT population)

Characteristic	Treatment Group Mean ± SD or N (%)			
	12.5 mg [n=19]	25 mg [n=25]	AndroGel [n=42]	Placebo [n=41]
Age (years)	42.4 ± 10.5	49.5 ± 6.6	45.0 ± 8.2	47.4 ± 8.9
Ethnicity				
American Indian/Alaskan Native (Non-Hispanic or Non Latino)	0 (0.0%)	0 (0.0%)	1 (2.4%)	0 (0.0%)
Asian (Non-Hispanic or Non-Latino)	0 (0.0%)	0 (0.0%)	2 (4.5%)	1 (2.4%)
Black or African American (Non-Hispanic or Non-Latino)	7 (36.8%)	4 (16.0%)	11 (26.2%)	7 (17.1%)
Caucasian (Non Hispanic or Non Latino)	10 (52.6%)	19 (76.0%)	27 (64.3%)	30 (73.2%)
Black or African American (Hispanic or Latino)	0 (0.0%)	1 (4.0%)	1 (2.4%)	0 (0.0%)
Caucasian (Hispanic or Latino)	2 (10.3%)	1 (4.0%)	0 (0.0%)	3 (7.3%)
Height (cm)	176.0 ± 6.1	179.6 ± 7.0	178.9 ± 6.7	178.8 ± 7.8
Weight (kg)	104.2 ± 15.1	109.4 ±18.3	106.4 ±18.1	107.6 ± 18.8
BMI (kg/m^2)	33.6 ± 4.3	33.9 ± 4.9	33.1 ±4.6	33.5 ±4.4
Baseline Testosterone ng/dL	235.2 ±40.9	195.9 ±46.6	229.8 ±44.0	206.0 ±48.2
Mean Baseline Sperm Concentration (x10^6/mL)	80.3 ±51.1	78.1 ±59.1	75.1 ±45.8	80.5 ±62.3
Years Hypogonadic	1.1 ± 2.4	1.7 ± 2.7	1.5 ± 2.6	1.5 ± 3.0
Previous Testosterone Use	3 (15.8%)	8 (32.0%)	9 (21.4%)	10 (24.4%)
Populations				
Safety	19	25	42	41
ITT	19	25	42	41
PP	16	22	35	37

Numbers analysed

The ITT, Per Protocol and safety population were defined in the same manner as in study ZA-304.

These populations are summarised in **Table 37.**

Table 37. Patient populations analysed in study ZA-305

	Androxal® 12.5 mg	Androxal® 25 mg	AndroGel 1.62%	Placebo	Overall
	Number of Subjects				
Safety Population	19	25	42	41	127
Intent-to-Treat (ITT) Population	19	25	42	41	127
Per Protocol Population (PP)	16	22	35	37	110

Outcomes and estimation

Sperm concentrations were all determined by a central laboratory. The mean sperm concentrations and change from baseline for each treatment group are shown in **Table 38.**

Table 38. Summary of Mean Sperm Concentration and Mean Percentage Change from Baseline in Sperm Concentration (ITT) in Study ZA-305

	Androxal N=44	AndroGel 1.62% N=42	Placebo N=41
Screening/Baseline Mean ± SD	79.0 ± 55.2	75.1 ± 45.8	80.5 ± 62.3
End of Treatment Mean ± SD	88.6 ± 72.2	44.0 ± 43.3	72.9 ± 48.4
Mean % Change ± SD	15.2 ± 55.8	-32.8 ± 63.2	-7.6 ± 89.6
Median % Change	8.95	-39.49	-4.39
Min/Max % Change	-74.0, 197.0	-100.0, 174.4	-77.8, 478.8
95% CI	-2.22, 32.56	-53.61, -12.08	-21.46, 36.61
P-Value (1)	0.1820	0.0007*	0.5834*
P-Value (2)		0.003*	0.2007*

Failure or success of treatment according to the pre-defined composite endpoint are summarised in **Table 39.**

Table 39. Composite Endpoint: Comparison of the Proportion of Subjects Who Obtain a Successful Testosterone Level and Sperm Concentration in study ZA-305

	Androxal N=44 N (%)	AndroGel N=42 N (%)	Placebo N=41 N (%)
Failures: Mean sperm concentration < 10M/mL or T not in normal range	15 (34.1)	36 (83.7)	38 (92.7)
Successes: Mean sperm concentration > 10M/mL and T in normal range [300-1040]	29 (65.9)	7 (16.3)	3 (7.3)
P-value compared to Androxal		0.0025* 0.0047**	<0.0001* <0.0001**

* Chi-Square test
** Fisher's Exact test

Proportion of Subjects with Mean Sperm Concentration below 10 million/mL Following Treatment

There were 10 (23.8%) AndroGel-treated subjects with a sperm concentration below 10 million/mL at the end of treatment, one (2.3%) Androxal-treated subject had sperm concentration below 10 million/mL at the end of treatment and 1 (2.4%) Placebo-treated subject had sperm concentration below 10 million/mL at the end of treatment. The difference in proportions between Androxal and AndroGel was statistically significant (P=0.003).

Mean 24-Hour Testosterone at the End of Treatment

Mean 24-hour testosterone levels were within normal range for both Androxal and AndroGel treated subjects at the end of treatment. The mean for Placebo-treated subjects was below the normal range and statistically different to Androxal ($p<0.0001$). The proportion of subjects in the normal range [300-1040 ng/dL] was higher in the Androxal group (68.2%) that the AndroGel group (52.4%).

Effects on LH and FSH

Changes in levels of leuteinizing hormone and follicle stimula ting hormone during the course of the study are shown in **Figures 7**and **8**respectively.

Figure 7. Mean LH (mIU/mL) by study visit in ZA-305

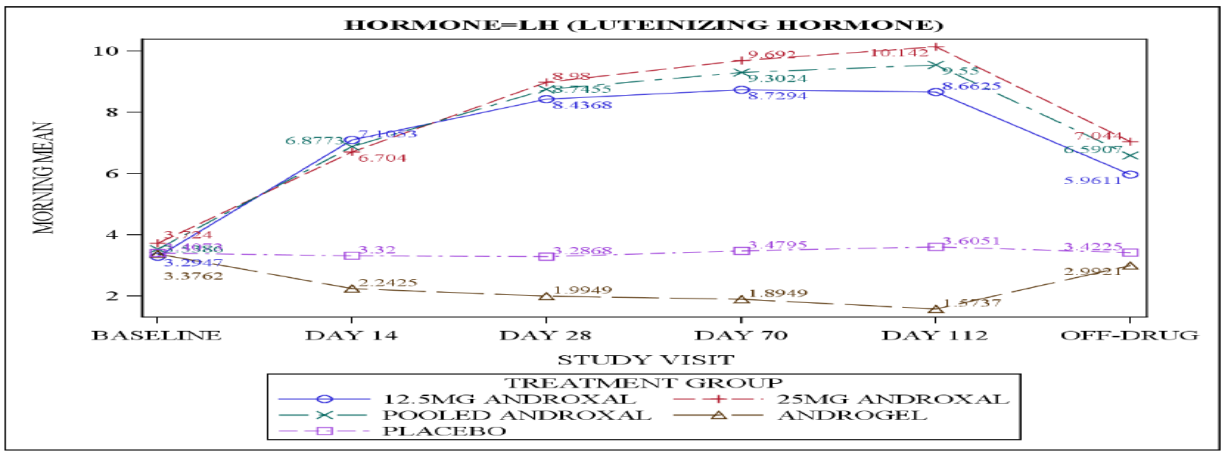
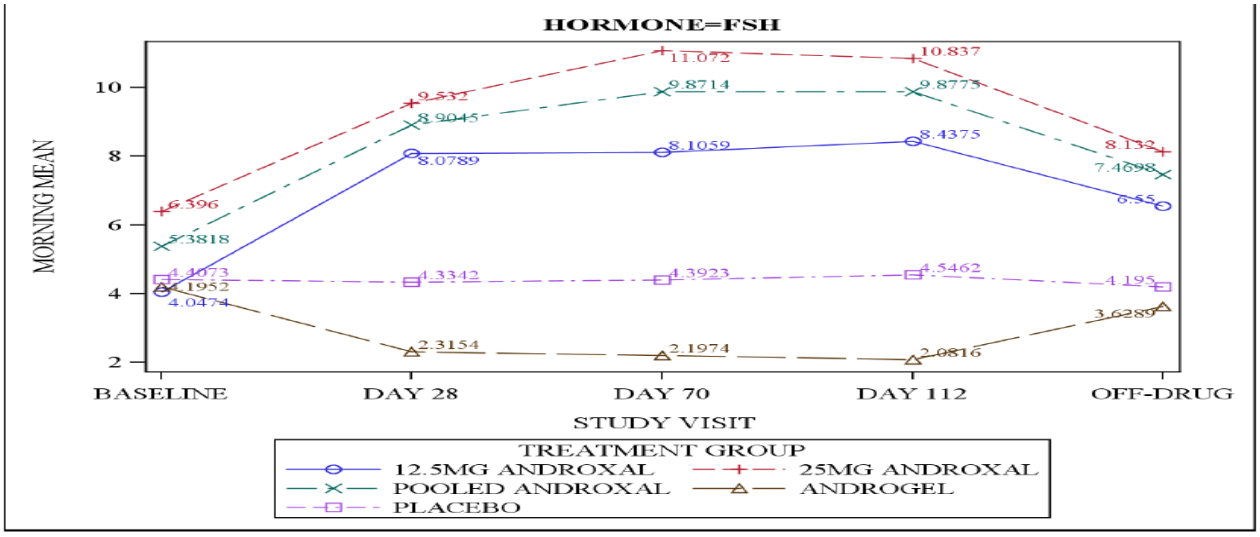


Figure 8. Mean FSH (U/L) by study visit in ZA-305



ZA-301: A Randomized, Double-blind, Placebo Controlled, Multi-Centre Phase III Study to Evaluate Normalization of Morning Testosterone Levels in Overweight Men with Acquired Hypogonadotropic Hypogonadism and normal Sperm Concentration.

Methods

This study was a phase III, randomized, double-blind, placebo-controlled multi-centre study to evaluate normalization of morning testosterone levels in overweight men with acquired hypogonadotropic hypogonadism and normal baseline sperm concentrations. The study required 10 to 12 clinic visits (2 for eye exams), and was approximately 4 to 5½ months in duration. Subjects were treated for 12-18 weeks.

Study Participants

Inclusion criteria

- Overweight (BMI 25 to 42 kg/m2 inclusive) males age 18 to 60 inclusive
- All clinical laboratory tests within normal ranges (any clinically significant deviation of laboratory results will require approval of sponsor)
- Previously or concurrently diagnosed as having secondary hypogonadism characterized as having 2 consecutive morning testosterone assessments < 300ng/dL, one of which must be confirmed at Baseline
- LH < 9.4 mIU/mL (at Visit 1 only)
- Sperm count ≥ 15 million per millilitre (assessed twice at least 48 hours apart)
- Ability to complete the study in compliance with the protocol
- Ability to understand and provide written informed consent
- Agreement to provide a total of up to 6 semen sample in a sponsor approved clinic on up to 6 separate occasions.

Exclusion criteria (selection)

- Any prior use of testosterone treatments within the last 6 months
- Use of spironolactone, cimetidine, Clomid, 5α-reductase inhibitors, hCG, androgen, estrogen, anabolic steroid, DHEA, or herbal hormone products during the study
- Use of Clomid in the past year
- Uncontrolled hypertension or diabetes mellitus based on the Investigator's assessment at baseline. Subjects treated for Type II diabetes will be allowed into the study. Newly diagnosed diabetics need to be treated for at least 48 hours before being enrolled in the study.
- Clinically significant abnormal findings at Screening (Visit 1) or Baseline, based on the Investigator's assessment

- A haematocrit >54% or a haemoglobin >17 g/dL (sponsor may approve enrolment of subjects with haemoglobin up to 17.5 g/dL if the subject is at a location with a high elevation)
- Use of an investigational drug or product, or participation in a drug or medical device research study within 30 days prior to receiving study medication.
- Known hypersensitivity to Clomid
- Symptomatic cataracts (nuclear sclerosis cataract or cortical cataract grade > 2 based on 0-4 scale or any trace of posterior subcapsular cataract)
- Abnormal fundoscopy exam such as central retinal vein occlusion

Treatments

Subjects were randomized in a 3:1 ratio to one of 2 treatment arms:

- 12.5 mg Androxal daily, by mouth, between 7:00 and 10:00 AM
- Placebo, daily, by mouth, between 7:00 and 10:00 AM

Study drug was administered each morning, for a total of 12 weeks. After 6 weeks of treatment (Visit 3), subjects who exhibited morning T<300ng/dL were up-titrated to 25 mg Androxal. Placebo subjects were sham up-titrated. Up-titrated subjects were treated for an additional 6 weeks (18 weeks in total) in order to evaluate any effects upon sperm concentration after 12 weeks of treatment at the higher dose.

Objectives

To evaluate normalization of morning testosterone levels in overweight men with acquired hypogonadotropic hypogonadism and normal baseline sperm concentrations

Outcomes/endpoints

Co-Primary Efficacy Variables

- Proportion of subjects with average serum concentration (Cavg) for T in the normal range after 12 weeks of treatment
- Proportion of subjects with a 50% or greater decrease in sperm concentration from baseline after 12 weeks of treatment

Secondary Efficacy Variable

- Comparison of Morning T between Androxal and placebo after 12 weeks of treatment
- The percentage of subjects with a maximum observed value (Cmax) of testosterone above 3 pre-determined limits:
  - Cmax ≥ 1500 ng/dL
  - Cmax ≥ 1800 ng/dL and < 2499 ng/dL
  - Cmax ≥ 2500 ng/dL

Other endpoints

• Changes in values from baseline in FSH and LH at weeks 6, 9, and 12, comparing Androxal to placebo
• Values for LH and FSH at Week 12
• Changes in HbA1c, FPG. Insulin and HOMA-IR
• Change in BMI
• Changes in sperm parameters (semen volume, sperm concentration, total count, morphology and motility)
• Values for dihydrotestosterone (DHT) and oestradiol, and the ratios Safety was assessed by physical and eye examinations, clinical laboratory tests and adverse event reporting)

Sample size

The co-primary endpoints of the study were the proportion of subjects with average serum concentration (Cavg) for testosterone in the normal range after 12 weeks of treatment and the proportion of subjects with a 50% or greater decrease in sperm concentrations from baseline after 12 weeks of treatment. A total sample size of 152 subjects (114 subjects in the Androxal treatment group and 38 subjects in the placebo treatment group) were required to adequately power both co-primary endpoints.

The proportion of subjects in the Androxal treatment group expected to have a Cavg for testosterone in the normal range after 12 weeks of treatment is 75%. Using the normal approximation to the binomial distribution, 113 subjects are required for the lower bound of the 95% confidence interval to be 67% or greater.

The proportion of subjects with a 50% or greater decrease in sperm concentrations from baseline after 12 weeks of treatment will be compared between treatment groups to determine whether Androxal is inferior to placebo in causing a 50% or greater decrease in sperm concentrations. Assuming that 1% of the placebo treatment group and 10% of the Androxal treatment group experience a 50% or greater decrease in sperm concentrations, a significance level of 0.05, a 20% non-inferiority limit (delta) and a 3:1 randomization ratio (Androxal: placebo), 114 subjects in the Androxal treatment group and 38 subjects in the placebo treatment group are required to have 80% power.

Randomisation

Subjects were randomized to either one capsule daily of 12.5 mg Androxal or placebo in a 3:1 ratio (Androxal: Placebo), for 12 weeks (18 weeks in up-titrated subjects).

Investigative sites were supplied with sequentially numbered study drug kits, the contents of which had been randomly assigned in block sizes of 4.

At Visit 2A, after the eligibility of each subject had been confirmed, the subject was assigned the lowest numbered kit available.

Investigational product was supplied to the site in packages containing one block of treatment kits (4 kits each, 3 Androxal kits and one placebo). Each kit contained 3 bottles each containing 45 capsules.

Blinding (masking)

This was a double-blind study.

Statistical methods

For all subjects included in this study, subject accountability, baseline demographic and medical history data were summarized for each treatment group. Summaries for quantitative variables include the sample size, mean, median, standard deviation, minimum, and maximum. Summaries for categorical variables include the number and percent of patients for each outcome. No formal hypothesis testing was performed to compare the treatment groups.

The proportion of subjects with a Cavg for testosterone within the normal range at each visit is summarized for each treatment group, along with the corresponding 95% confidence interval calculated from the normal approximation of the binomial distribution. If the lower limit of the 95% confidence interval for the Androxal treatment group at Week 12 is at least 67%, then the co-primary endpoint based on the Cavg for testosterone will have been achieved.

For the efficacy variables a last observation carried forward approach will be used to impute missing data. For determining testosterone Cavg in cases where 24-hour serial assessments are not available, the last assessment of morning testosterone will be utilized. If there are no post-baseline efficacy data then a value of no change will be imputed for the missing efficacy measure.

The proportion of subjects who experienced a 50% reduction in sperm concentrations from baseline to Week 12 is summarized for each treatment group and compared between treatment groups using Fisher's exact test. Additionally, the Androxal treatment group was assessed for non-inferiority compared to placebo in the incidence of a 50% reduction in sperm concentrations. The difference between the proportions (placebo minus Androxal) and corresponding 95% confidence interval was determined and compared to the equivalence limit of -20%. If the lower limit of the 95% confidence interval is greater than -20%, then Androxal will be concluded to be non-inferior to placebo in causing a 50% reduction in sperm concentrations.

The secondary efficacy variables included the change from baseline (Visit 2) to each visit for FSH and LH, and changes in HbA1c, FPG, insulin, HOMA-IR, BMI and sperm parameters (semen volume, sperm concentration, total count, morphology and motility). For each of these variables the treatment groups were compared using an Analysis of Variance.

No adjustment for multiple testing was employed.

Results

Participant flow

Patient disposition is summarised in **Table 40**

Table 40. Patient disposition in Study ZA-301

Disposition		Treatment Group			
		Androxal		Placebo	Total
		12.5 mg	25 mg		
Number of Patients Randomized		92	21	38	151
Number of Patients Treated		91	21	37	149
Number of Patients Completed Study		85 (92.4%)	19 (90.5%)	35 (92.1%)	139 (92.1%)
Number of Patients Prematurely Discontinued from the Study		7 (7.6%)	2 (9.5%)	3 (7.9%)	12 (7.9%)
Reasons for Premature Discontinuation from the Study:					
Adverse Events		0 (0.0%)	1 (50.0%)	1 (33.3%)	2 (16.7%)
Lost to Follow-Up		3 (42.9%)	0 (0.0%)	1 (33.3%)	4 (33.3%)
Withdrew Consent		3 (42.9%)	0 (0.0%)	0 (0.0%)	3 (25.0%)
Other		1 (14.3%)	1 (50.0%)	1 (33.3%)	3 (25.0%)
Populations	Safety	91	21	37	149
	ITT	92	21	38	151
	MITT	65	16	28	109
	PP	61	14	26	101

Recruitment

Study initiation date: August 23, 2012 (date of first patient visit)

Study completion date: February 6, 2014 (database lock)

Conduct of the study

The protocol was amended three times during conduct of the study, but changes were not considered major.

A total of 254 protocol deviations occurred, the most commonly occurring one was assessment out of window.

Baseline data

Subject baseline demographics of the subjects included in the study are shown in **Table 41.**

Table 41. Summary of baseline demographic information in study ZA-301 (ITT population)

Characteristic	Treatment Group Mean ± SD or N (%)			
	12.5 mg [n=92*]	25 mg [n=21]	Androxal [n=113]	Placebo [n=38]
Age (years)	47.2 ± 9.6	43.6 ± 10.1	46.5 ± 9.8	47.8 ± 9.5
Ethnicity				
Asian / Pacific Islander (Hispanic or Latino)	1 (1.1%)	0 (0.0%)	1 (0.9%)	0 (0.0%)
Asian / Pacific Islander (Non-Hispanic or Non-Latino)	1 (1.1%)	0 (0.0%)	1 (0.9%)	2 (5.3%)
Black or African American (Hispanic or Latino)	2 (2.2%)	0 (0.0%)	2 (1.8%)	0 (0.0%)
Black or African American (Non-Hispanic or Non-Latino)	9 (9.8%)	1 (4.8%)	10 (8.8%)	0 (0.0%)
Caucasian (Hispanic or Latino)	35 (38.0%)	16 (76.2%)	51 (45.1%)	20 (52.6%)
Caucasian (Non-Hispanic or Non-Latino)	44 (47.8%)	4 (19.0%)	48 (42.5%)	16 (42.1%)
Height (cm)	174.4 ± 6.2	177.30 ± 5.7	174.9 ± 6.1	175.8 ± 7.4
Weight (kg)	96.0 ± 14.8	103.6 ±19.5	97.4 ± 16.0	96.9 ± 14.4
BMI (kg/m^2)	31.5 ± 4.3	32.8 ± 5.0	31.8 ±4.4	31.3 ±3.8
Populations				
Safety	91	21	112	37
ITT	92	21	113	38
MITT	65	16	81	28
PP	61	14	75	26

Numbers analysed

The Intention to Treat (ITT) set comprised the group of patients who were enrolled and randomized to treatment with study medication (primary analysis).

The per protocol (PP) population was defined as the subset of the ITT population who completed their Week 16 visit.

The safety population comprised all subjects that were administered even a single dose of study drug.

These populations are summarised in **Table 42.**

Table 42. Patient populations analysed in study ZA-301

	Androxal® 12.5 mg	Androxal® 25 mg	Placebo	Overall
	Number (%) of Subjects			
Safety Population	91 (61.1%)	21 (14.1%)	37 (24.8%)	149
Intent-to-Treat (ITT) Population	92 (60.9%)	21 (13.9%)	38 (25.2%)	151
Modified Intent-to-Treat (mITT)	65 (60.0%)	16 (15.0%)	28 (26.0%)	109
Per Protocol Population (PP)	61 (60.0%)	14 (14.0%)	26 (26.0%)	101

Outcomes and estimation

Summary of Effect on Testosterone

At week 12 a 24-hour assessment of testosterone was conducted, measuring testosterone at the following time points after dosing: 0, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours. The mean of the measurements, Cavg, is a co-primary endpoint. Eighty nine (89) of the 113 (78.8%) subjects treated with Androxal in the ITT population achieved a Cavg in the normal range (300 – 1040 ng/dL) and the resulting 95% Wilson score confidence interval is: (70.3%, 85.3%). The lower limit on the 95% confidence interval on the proportion of subjects achieving a Cavg in the normal range exceeds 67% in the mITT and PP populations. Subjects were initially treated at 12.5 mg and could be up-titrated to 25 mg if their morning testosterone was lower than 300 ng/dL at Visit 3 (6 weeks). Only 21 subjects treated with Androxal were up-titrated. Of those subjects up-titrated, 14 (66.7%) experienced a Cavg in the normal range at 12 weeks.

Sperm Concentration

The primary efficacy analysis required a determination of subjects who experience a 50% or greater reduction in sperm concentration. This difference was calculated by comparing the mean of the pre-treatment sperm concentrations to the assessments made at the end of treatment. These results are summarised for the ITT and the PP populations in **Tables 43** and **44** respectively.

Table 43. Percentage of patients achieving > 50% Change from Baseline in Sperm Concentration (ITT population) in Study ZA-301

Analysis Method	Androxal	Placebo	95% Confidence Interval on Difference
All Assessments	n = 113 16 (14.2%)	n = 38 1 (2.6%)	(-19.4%, 0.5%)
All Assessments, Eligible Baseline	n = 112 16 (14.3%)	n = 38 1 (2.6%)	(-19.6%, 0.4%)
Last Two Observations, Eligible Baseline	n = 112 15 (13.4%)	n = 38 3 (7.9%)	(-14.6%, 8.4%)

Table 44. Percentage of patients achieving > 50% Change from Baseline in Sperm Concentration (PP population) in Study ZA-302

Analysis Method	Androxal	Placebo	95% Confidence Interval on Difference
All Assessments	n = 81 15 (18.5%)	n = 28 1 (3.6%)	(-25.2%, 0.8%)
All Assessments, Eligible Baseline	n = 81 15 (18.5%)	n = 28 1 (3.6%)	(-25.2%, 0.8%)
Last Two Observations, Eligible Baseline	n = 81 14 (17.3%)	n = 28 1 (3.6%)	(-23.8%, 1.9%)

Effects on LH and FSH

Changes in levels of leuteinizing hormone and follicle stimulating hormone during the course of the study are shown in **Table 45**.

Table 45. Summary of Change from Baseline in FSH and LH) by study visit in ZA-301

Change from Baseline	Androxal 12.5 mg	Androxal 25.0 mg	Androxal Pooled	Placebo
FSH				
Visit 3 (Week 6)	n = 89 5.2 ± 6.0 < 0.0001[1]	n = 20 1.7 ± 2.6 0.0078[1]	n = 109 4.6 ± 5.7 < 0.0001[1]	n = 35 0.4 ± 1.8 0.6300[1]
Visit 4 (Week 9)	n = 87 5.3 ± 7.1 < 0.0001[1]	n = 21 2.8 ± 3.1 < 0.0001[1]	n = 108 4.8 ± 6.6 < 0.0001[1]	n = 35 1.1 ± 2.1 0.0029[1]
Visit 5 (Week 12)	n = 86 4.8 ± 6.2 < 0.0001[1,2]	n = 21 3.2 ± 3.2 0.0002[1] 0.0003[2]	n = 107 4.4 ± 5.7 < 0.0001[1,2]	n = 36 0.3 ± 1.4 0.8161[1]
Visit 6 (Week 18)	NA	n = 16 2.7 ± 3.2 0.0033[1]	NA	n = 23 0.1 ± 1.8 0.8203[1]
Off Drug Visit (Week 13/19)	n = 86 2.5 ± 4.9 < 0.0001[1]	n = 21 2.2 ± 3.4 0.0043[1]	n = 107 2.5 ± 4.6 < 0.0001[1]	n = 35 0.1 ± 1.5 0.6594[1]

LH				
Visit 3 (Week 6)	n = 89 5.6 ± 4.8 < 0.0001[1]	n = 20 2.0 ± 2.6 0.0029[1]	n = 109 4.9 ± 4.7 < 0.0001[1]	n = 36 0.6 ± 1.8 0.2042[1]
Visit 4 (Week 9)	n = 87 5.5 ± 4.9 < 0.0001[1]	n = 21 3.4 ± 2.9 < 0.0001[1]	n = 108 5.1 ± 4.6 < 0.0001[1]	n = 35 1.1 ± 2.1 0.0017[1]
Visit 5 (Week 12)	n = 86 4.8 ± 4.9 < 0.0001[1,2]	n = 21 3.5 ± 3.8 < 0.0001[1,2]	n = 107 4.5 ± 4.7 < 0.0001[1,2]	n = 36 0.3 ± 1.4 0.3330[1]
Visit 6 (Week 18)	NA	n = 16 2.9 ± 3.3 0.0030[1]	NA	n = 23 0.1 ± 1.2 0.6040[1]
Off Drug Visit (Week 13/19)	n = 86 3.0 ± 4.4 < 0.0001[1]	n = 21 2.1 ± 3.3 0.0020[1]	n = 107 2.8 ± 4.2 < 0.0001[1]	n = 35 0.2 ± 1.5 0.4771[1]

Reference Appendix 16.2.8.6 and Table 14.2.5.1
[1] Within group test, change from baseline compared to 0
[2] Visit 5 only; Androxal versus Placebo Test

Study ZA-302: A Randomized, Double-blind, Placebo Controlled, Multi-Centre Phase III Study to Evaluate Normalization of Morning Testosterone Levels in Overweight Men with Acquired Hypogonadotropic Hypogonadism and normal Sperm Concentration.

Study design was identical to ZA-301.

Participant flow

Patient disposition is summarised in **Table 46.**

Table 46. Patient disposition in Study ZA-302

Disposition	Treatment Group			
	Androxal		Placebo	Total
	12.5 mg	25 mg		
Number of Patients Randomized	134	0	47	181
Number of Patients Exposed	112	22	47	181
Number of Patients Completed Study	99 (88.4%)	21 (95.5%)	45 (95.7%)	165 (91.2%)
Number of Patients Prematurely Discontinued from the Study	13 (11.6%)	1 (4.6%)	2 (4.3%)	16 (8.8%)
Reasons for Premature Discontinuation from the Study:				
Adverse Events	3 (2.7%)	0 (0.0%)	0 (0.0%)	3 (1.7%)
Lost to Follow-Up	2 (1.8%)	0 (0.0%)	0 (0.0%)	2 (1.1%)
Withdrew Consent	2 (1.8%)	1 (4.6%)	2 (4.3%)	5 (2.8%)
Other	6 (5.4%)	0 (0.0%)	0 (0.0%)	6 (3.3%)
Populations Safety	112	22	47	181
ITT	112	22	47	181
MITT	91	15	37	143
PP	78	13	37	128

Recruitment

Study initiation date: November 16, 2012

Study completion date: November 1, 2013 (database lock)

Conduct of the study

The same protocol amendments to study ZA-304 were applied.

A total of 375 deviations occurred the most commonly occurring one was assessment out of window.

Baseline data

Subject baseline demographics of the subjects included in the study are shown in **Table 47**.

Table 47. Summary of baseline demographic information in study ZA-302 (ITT population)

Characteristic	Treatment Group Mean ± SD or N (%)			
	12.5 mg [n=112]	25 mg [n=22]	Androxal [n=134]	Placebo [n=47]
Age (years)	44.6 ± 9.6	45.8 ± 8.6	44.8 ± 9.5	43.6 ± 10.5
Ethnicity				
American Indian/Alaskan Native (Non-Hispanic or Non Latino)	1 (0.9%)	0 (0.0%)	1 (0.7%)	0 (0.0%)
Black or African American (Non-Hispanic or Non-Latino)	8 (7.1%)	2 (9.1%)	10 (7.5%)	2 (4.3%)
Caucasian (Non Hispanic or Non Latino)	62 (55.4%)	9 (40.9%)	71 (53.0%)	27 (57.4%)
Black or African American (Hispanic or Latino)	1 (0.9%)	0 (0.0%)	1 (0.7%)	0 (0.0%)
Caucasian (Hispanic or Latino)	40 (35.7%)	10 (45.5%)	50 (37.3%)	18 (38.3%)
Other (Hispanic or Latino)	0 (0.0%)	1 (4.5%)	1 (0.7%)	0 (0.0%)
Height (cm)	178.5 ± 7.1	178.1 ± 7.2	178.4 ± 7.1	175.7 ± 8.6
Weight (kg)	105.2 ± 18.8	101.9 ±17.9	104.6 ±18.6	101.8 ± 21.1
BMI (kg/m^2)	32.9 ± 4.7	32.0 ± 4.7	32.7 ±4.7	32.8 ±5.1
Populations				
Safety	112	22	134	47
ITT	112	22	134	47
MITT	91	15	106	37
PP	78	13	91	37

Numbers analysed

Patient populations were defined as in Study ZA-301 and are summarised in **Table 48**.

Table 48. Patient populations analysed in study ZA-302

	Androxal® 12.5 mg	Androxal® 25 mg	AndroGel 1.62%	Placebo	Overall
	Number of Subjects				
Safety Population	19	25	42	41	127
Intent-to-Treat (ITT) Population	19	25	42	41	127
Per Protocol Population (PP)	16	22	35	37	110

Outcomes and estimation

Summary of Effect on Testosterone

One hundred and nine (109) of the 134 (81.3%) subjects treated with Androxal in the ITT population achieved a Cavg in the normal range (300 – 1040 ng/dL) and the resulting 95% Wilson score confidence interval was: (73.9%, 87.0%). The lower limit on the 95% confidence interval on the proportion of subjects achieving a Cavg in the normal range exceeded 67% in the mITT and PP populations.

Morning Testosterone

The effect of treatment on morning testosterone levels per visit are summarised in **Table 49**.

Table 49. Summary of Mean Change from Baseline in Morning Testosterone in study ZA-302

	Treatment Group Mean ± SD p-value			
Change from Baseline in Morning Testosterone	Androxal 12.5 mg	Androxal 25.0 mg	Androxal Pooled	Placebo
Visit 3 (Week 6)	n = 106 324.6 ± 159.6 < 0.0001[1]	n = 22 111.3 ± 130.4 0.0001[1]	n = 128 287.9 ± 174.3 < 0.0001[1]	n = 47 66.5 ± 144.7 0.0135[1]
Visit 4 (Week 9)	n = 102 323.8 ± 181.8 < 0.0001[1]	n = 21 212.8 ± 219.2 < 0.0001[1]	n = 123 304.9 ± 192.4 < 0.0001[1]	n = 47 64.0 ± 135.9 0.0491[1]
Visit 5 (Week 12)	n = 97 304.1 ± 192.9 < 0.0001[1,2]	n = 21 262.5 ± 230.8 < 0.0001[1] 0.0003[2]	n = 118 296.7 ± 199.8 < 0.0001[1,2]	n = 47 70.2 ± 135.3 0.0168[1]
Visit 6 (Week 18)	NA	n = 16 200.3 ± 199.8 0.0011[1]	NA	n = 31 41.1 ± 112.2 0.1805[1]
Off Drug Visit (Week 13/19)	n = 104 223.6 ± 181.4 < 0.0001[1]	n = 19 187.2 ± 195.6 0.0006[1]	n = 123 217.9 ± 183.3 < 0.0001[1]	n = 46 74.0 ± 143.6 0.0085[1]

Sperm Concentration

Results of the subjects who experiencd a 50% or greater reduction in sperm concentration in the ITT and the PP populations are presented in **Tables 50** and **51** respectively.

Table 50. Percentage of patients achieving > 50% Change from Baseline in Sperm Concentration (ITT population) in Study ZA-301

Testosterone Status	Androxal Pooled	Placebo
C_{avg} Normal Range [300-1040 ng/dL]	n = 134 109 (81.3%) (73.9%, 87.0%)	
C_{max} Normal Range [300-1040 ng/dL]	n = 134 124 (92.5%)[1]	n = 47 27 (57.5%)
$C_{max} \geq 1500$ ng/dL	n = 0	n = 0
$C_{max} \geq 1800$ ng/dL and < 2499 ng/dL	n = 0	n = 0
$C_{max} \geq 2500$ ng/dL	n = 0	n = 0

Table 51. Percentage of patients achieving > 50% Change from Baseline in Sperm Concentration (PP population) in Study ZA-301

	≥ 50% Decrease from Baseline n (%)		
Analysis Method	Androxal	Placebo	95% Confidence Interval on Difference
All Assessments	n = 91 15 (16.5%)	n = 37 2 (5.4%)	(-20.8%, 2.7%)
All Assessments, Eligible Baseline	n = 91 15 (16.5%)	n = 37 2 (5.4%)	(-20.8%, 2.7%)
Last Two Observations, Eligible Baseline	n = 91 16 (17.6%)	n = 37 0 (0.0%)	(-26.7%, -6.2%)

Effects on LH and FSH

Changes in levels of luteinizing hormone and follicle stimulating hormone during the course of the study are shown in **Table 52**.

Table 52. Summary of Change from Baseline in FSH and LH) by study visit in ZA-301

Change from Baseline	Androxal 12.5 mg	Androxal 25.0 mg	Androxal Pooled	Placebo
FSH				
Visit 3 (Week 6)	n = 89 5.2 ± 6.0 < 0.0001[1]	n = 20 1.7 ± 2.6 0.0078[1]	n = 109 4.6 ± 5.7 < 0.0001[1]	n = 35 0.4 ± 1.8 0.6300[1]
Visit 4 (Week 9)	n = 87 5.3 ± 7.1 < 0.0001[1]	n = 21 2.8 ± 3.1 < 0.0001[1]	n = 108 4.8 ± 6.6 < 0.0001[1]	n = 35 1.1 ± 2.1 0.0029[1]
Visit 5 (Week 12)	n = 86 4.8 ± 6.2 < 0.0001[1,2]	n = 21 3.2 ± 3.2 0.0002[1] 0.0003[2]	n = 107 4.4 ± 5.7 < 0.0001[1,2]	n = 36 0.3 ± 1.4 0.8161[1]
Visit 6 (Week 18)	NA	n = 16 2.7 ± 3.2 0.0033[1]	NA	n = 23 0.1 ± 1.8 0.8203[1]
Off Drug Visit (Week 13/19)	n = 86 2.5 ± 4.9 < 0.0001[1]	n = 21 2.2 ± 3.4 0.0043[1]	n = 107 2.5 ± 4.6 < 0.0001[1]	n = 35 0.1 ± 1.5 0.6594[1]
LH				
Visit 3 (Week 6)	n = 89 5.6 ± 4.8 < 0.0001[1]	n = 20 2.0 ± 2.6 0.0029[1]	n = 109 4.9 ± 4.7 < 0.0001[1]	n = 36 0.6 ± 1.8 0.2042[1]
Visit 4 (Week 9)	n = 87 5.5 ± 4.9 < 0.0001[1]	n = 21 3.4 ± 2.9 < 0.0001[1]	n = 108 5.1 ± 4.6 < 0.0001[1]	n = 35 1.1 ± 2.1 0.0017[1]
Visit 5 (Week 12)	n = 86 4.8 ± 4.9 < 0.0001[1,2]	n = 21 3.5 ± 3.8 < 0.0001[1,2]	n = 107 4.5 ± 4.7 < 0.0001[1,2]	n = 36 0.3 ± 1.4 0.3330[1]
Visit 6 (Week 18)	NA	n = 16 2.9 ± 3.3 0.0030[1]	NA	n = 23 0.1 ± 1.2 0.6040[1]
Off Drug Visit (Week 13/19)	n = 86 3.0 ± 4.4 < 0.0001[1]	n = 21 2.1 ± 3.3 0.0020[1]	n = 107 2.8 ± 4.2 < 0.0001[1]	n = 35 0.2 ± 1.5 0.4771[1]

Ancillary analyses

The CHMP requested information on the signs and symptoms of secondary hypogonadism for the subjects included in the pivotal studies (**Table 53**), as these were not part of the inclusion criteria in these studies.

Table 53. Baseline signs and symptoms of hypogonadotropic hypogonadism in enclomifene studies ZA-301, ZA-302, ZA-304 and ZA-305

Sign or Symptom	Enclomifene N=325 n (%)	AndroGel® N=85 n (%)	Placebo N=169 n (%)	Total N=579 n (%)
At least one sign or symptom	257 (79.1%)	69 (81.2%)	131 (77.5%)	457 (78.9%)
PHYSICAL				
Visceral (abdominal) obesity (BMI >30kg/m^2)	224 (68.9%)	63 (74.1)	111 (65.7%)	398 (68.7%)
Decrease in lean body mass and muscle strength	0	0	0	0
Hot flushes	0	0	0	0
Gynaecomastia	8 (2.5%)	2 (2.4%)	3 (1.8%)	13 (2.3%)
Osteoporosis and low trauma fractures	1 (0.3%)	1 (1.2%)	0	2 (0.4%)
SEXUAL				
Erectile dysfunction	32 (9.9%)	13 (15.3%)	23 (13.6%)	68 (11.7%)
Decreased libido and sexual activity	25 (7.7%)	3 (3.5%)	7 (4.1%)	35 (6.0%)
Fewer and diminished nocturnal emissions	0	0	0	0
MENTAL				
Changes in mood	27 (8.3%)	13 (15.3%)	17 (10.1%)	57 (9.8%)
Fatigue	20 (6.2%)	6 (7.1%)	14 (8.3%)	40 (6.9%)
Anger	0	0	0	0
Sleep disturbance	20 (6.2%)	3 (3.5%)	14 (8.3%)	37 (6.4%)
Diminished cognitive function	0	0	0	0
ENDOCRINE				
Insulin resistance	0	0	0	0
Type 2 diabetes	48 (14.8%)	26 (30.6%)	29 (17.2%)	103 (17.8%)
Metabolic syndrome[1]	157 (48.3%)	49 (57.7%)	84 (49.7%)	290 (50.1%)
Abdominal obesity, BMI >30	224 (68.9%)	63 (74.1)	111 (65.7%)	398 (68.7%)
Raised triglycerides	190 (58.5%)	34 (40.0%)	111 (65.7%)	335 (57.9%)
Reduced HDL cholesterol	68 (20.9%)	46 (54.1%)	55 (32.5%)	169 (29.2%)
Raised blood pressure	212 (65.2%)	54 (63.5%)	112 (66.3%)	378 (65.3%)
Raised fasting plasma glucose	151 (46.5%)	43 (50.6%)	75 (44.4%)	269 (46.5%)

[1] Metabolic syndrome requires, BMI > 30 along with at least two of the sub-symptoms (raised triglycerides, reduced HDL, raised blood pressure, raised fasting plasma glucose)

Sexual dysfunction and depression, both of which may be symptoms of hypogonadotropic hypogonadism, were further assessed by evaluating the medications that subjects were taking. The percentage of subjects taking medication for these conditions is shown in **Table 54**.

Table 54. Percentage of patients receiving treatment for sexual dysfunction and depression in enclomifene studies ZA-301, ZA-302, ZA-304 and ZA-305

Study	Erectile Dysfunction (%)	Depression (%)
ZA-301	0.7	2.8
ZA-302	2.2	7.0
ZA-304	6.2	14.8
ZA-305	3.9	10.6

Baseline biochemical markers of secondary hypogonadism of the subjects included in these studies were also submitted (**Table 55**).

Table 55. Baseline biological features of hypogonadotropic hypogonadism in enclomifene studies ZA-301, ZA-302, ZA-304 and ZA-305

Parameter (Mean ± SD)	Enclomifene N=324	AndroGel® N=85	Placebo N=168
Total Testosterone (ng/dL)	200.5 ± 64.2	219.1 ± 50.2	197.9 ± 55.9
Dihydrotestosterone (ng/dL)	52.7 ± 64.8	53.0 ± 38.6	60.4 ± 120.5
LH (mIU/mL)	3.4 ± 1.8	3.6 ± 1.5	3.2 ± 1.5
FSH (mIU/mL)	4.8 ± 2.9	5.2 ± 2.7	4.7 ± 2.9
Estradiol (pg/mL)	27.6 ± 12.2	26.6 ± 10.3	25.1 ± 9.4
SHBG (nmol/L)	27.6 ± 12.0	27.2 ± 13.6	26.5 ± 10.2
Sperm concentration (M/mL)	74.5 ± 71.7	76.7 ±62.0	80.4 ± 68.9
Weight (Kg)	102.9 ± 17.5	106.9 ± 17.4	102.3 ± 18.3
BMI (Kg/M^2)	32.6 ± 4.5	33.6 ± 4.5	32.6 ± 4.5

In addition, the proportions of subjects with baseline LH levels below 5 mIU/ml were also provided (**Table 56**).

Table 56. Subjects with baseline LH <5 mIU/ml in the enclomifene pivotal studies

Study	Treatment	Baseline LH < 5 mIU/mL n (%)
ZA-301	Enclomifene	94 (88.7%)
	Placebo	33 (91.7%)
ZA-302	Enclomifene	111 (82.8%)
	Placebo	43 (91.5%)
ZA-304	AndroGel®	31 (72.1%)
	Enclomifene	30 (73.2%)
	Placebo	40 (88.9%)
ZA-305	AndroGel®	35 (83.3%)
	Enclomifene	35 (79.5%)
	Placebo	36 (87.8%)

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 57. Summary of Efficacy for trials ZA-304, ZA-305, ZA-301 and ZA-202

Title: A Randomized, Double Blind, Placebo-Controlled, Multi-Center Phase III Study in Men with Acquired Hypogonadotropic Hypogonadism to Compare Changes in Testosterone and Sperm Concentration Following Treatment with 12.5 mg or 25 mg Androxal or AndroGel 1.62%			
Study identifier	**ZA-304**		
Design	Randomised, Double Blind, Placebo-Controlled, Multi-Centre Phase III Study in Men		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		16 weeks not applicable not applicable
Hypothesis			
Treatments groups	Enclomifene		12.5mg up-titrated to 25mg if required for 16 weeks, 41 subjects randomised
	AndroGel 1.62%		Dose not specified but used for 16 weeks, 43 subjects randomised
	Placebo		Placebo for 16 weeks, 45 subjects randomised
Endpoints and definitions	Co-Primary endpoint	The percentage change from baseline in mean sperm concentration treatment.	Analyses compared Androxal to AndroGel 1.62%.
	Co-Primary endpoint	Comparison of the proportion of subjects who obtain a successful testosterone level and sperm concentration (composite endpoint).	Subjects were considered successful if their mean sperm concentration remained ≥ 10 million/mL and their 24-hour testosterone levels stayed within the normal range [300-1,040 ng/dL] following 16 weeks of treatment. Androxal was compared to Placebo and AndroGel 1.62% in a pairwise fashion.
	Secondary endpoint	Change from baseline in LH and FSH after 16 weeks of treatment.	Androxal was compared to AndroGel 1.62%
	Secondary endpoint	Comparison of the proportion of subjects whose mean sperm concentration was found to be below 10 million/mL	Androxal was compared to AndroGel 1.62%

Database lock	24 September 2014			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat and Per protocol After 16 weeks of treatment			
Descriptive statistics and estimate variability	Treatment group	Enclomifene 12.5mg up-titrated to 25mg	AndroGel 1.62%	Placebo
	Number of subject	41	43	45
	Composite Endpoint: successes : Mean sperm concentration ≥ 10M/mL or T in normal range	25 (61.0%)	7 (16.3%)	2 (4.4%)
	<variability statistic>	<variability>	<variability>	<variability>
	The percentage change from baseline in mean sperm concentration treatment.	11.7	-56.6	4.1
	± SD	80.3	48.2	57.2

Title: A Randomized, Double Blind, Placebo-Controlled, Multi-Center Phase III Study in Men with Acquired Hypogonadotropic Hypogonadism to Compare Changes in Testosterone and Sperm Concentration Following Treatment with 12.5 mg or 25 mg Androxal or AndroGel 1.62%

Study identifier	**ZA-305**	
Design	Randomised, Double Blind, Placebo-Controlled, Multi-Centre Phase III Study in Men	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	16 weeks not applicable not applicable
Hypothesis		
Treatments groups	Enclomifene	12.5mg up-titrated to 25mg if required for 16 weeks, 44 subjects randomised
	AndroGel 1.62%	Dose not specified but used for 16 weeks, 42 subjects randomised
	Placebo	Placebo for 16 weeks, 41 subjects randomised

Endpoints and definitions	Co-Primary endpoint	The percentage change from baseline in mean sperm concentration treatment.	Analyses compared Androxal to AndroGel 1.62%.
	Co-Primary endpoint	Comparison of the proportion of subjects who obtain a successful testosterone level and sperm concentration (composite endpoint).	Subjects were considered successful if their mean sperm concentration remained ≥ 10 million/mL and their 24-hour testosterone levels stayed within the normal range [300-1,040 ng/dL] following 16 weeks of treatment. Androxal was compared to Placebo and AndroGel 1.62% in a pairwise fashion.
	Secondary endpoint	Change from baseline in LH and FSH after 16 weeks of treatment.	Androxal was compared to AndroGel 1.62%
	Secondary endpoint	Comparison of the proportion of subjects whose mean sperm concentration was found to be below 10 million/mL	Androxal was compared to AndroGel 1.62%
Database lock	22 August 2014		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat and Per protocol After 16 weeks of treatment			
Descriptive statistics and estimate variability	Treatment group	Enclomifene 12.5mg up-titrated to 25mg	AndroGel 1.62%	Placebo
	Number of subject	44	42	41
	Composite Endpoint: successes : Mean sperm concentration ≥ 10M/mL or T in normal range	29 (65.9%)	7 (16.3%)	3 (7.3%)

	The percentage change from baseline in mean sperm concentration treatment.	15.2	-32.8	-7.6
	± SD	55.8	63.2	89.6

Title: A Randomized, Double-blind, Placebo Controlled, Multi-Centre Phase III Study to Evaluate Normalization of Morning Testosterone Levels in Overweight Men with Acquired Hypogonadotropic Hypogonadism and normal Sperm Concentration.			
Study identifier	**ZA-301**		
Design	Randomised, Double Blind, Placebo-Controlled, Multi-Centre Phase III Study in Men		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 weeks not applicable 6 months
Hypothesis			
Treatments groups	Enclomifene		12.5mg up-titrated to 25mg if required for 12 weeks, 113 subjects randomised
	Placebo		Placebo for 12 weeks, 38 subjects randomised
Endpoints and definitions	Co-Primary endpoint	Proportion of subjects with average serum concentration (Cavg) for T in the normal range.	If the lower limit of the 95% confidence interval for the Androxal treatment group at Week 12 was at least 67%, then the co-primary endpoint based on the Cavg for testosterone would have been achieved.
	Co-Primary endpoint	Proportion of subjects with a 50% or greater decrease in sperm concentration from baseline	The difference between the proportions (placebo minus Androxal) and corresponding 95% confidence interval was determined and compared to the non-inferiority limit of -20%.
	Secondary endpoint	Comparison of Morning T between Androxal and placebo	
Database lock	6 February 2014		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat and Per protocol After 12 weeks of treatment		
Descriptive statistics and estimate variability	Treatment group	Enclomifene 12.5mg up-titrated to 25mg	Placebo

	Number of subject	113	38
	Proportion of subjects with average serum concentration (Cavg) for T in the normal range.	89 (78.8%)	
	Wilson score 95% confidence interval for a single arm	70.3%-85.3%	
	Proportion of subjects with a 50% or greater decrease in sperm concentration from baseline	ITT: 16 (14.2%) mITT: 15 (18.5%)	ITT: 1 (2.6%) mITT: 1 (3.6%)
	95% CI for difference between treatments		ITT: 95% CI: (-19.4-0.5%) mITT: 95% CI: (-25.2-0.8%)
	Comparison of Morning T between Androxal and placebo		

Title: A Randomized, Double-blind, Placebo Controlled, Multi-Centre Phase III Study to Evaluate Normalization of Morning Testosterone Levels in Overweight Men with Acquired Hypogonadotropic Hypogonadism and normal Sperm Concentration.			
Study identifier	**ZA-302**		
Design	Randomised, Double Blind, Placebo-Controlled, Multi-Centre Phase III Study in Men		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 weeks not applicable 6 months
Hypothesis			
Treatments groups	Enclomifene		12.5mg up-titrated to 25mg if required for 12 weeks, 134 subjects randomised
	Placebo		Placebo for 12 weeks, 47 subjects randomised
Endpoints and definitions	Co-Primary endpoint	Proportion of subjects with average serum concentration (Cavg) for T in the normal range.	If the lower limit of the 95% confidence interval for the Androxal treatment group at Week 12 was at least 67%, then the co-primary endpoint based on the Cavg for testosterone would have been achieved.

	Co-Primary endpoint	Proportion of subjects with a 50% or greater decrease in sperm concentration from baseline	The difference between the proportions (placebo minus Androxal) and corresponding 95% confidence interval was determined and compared to the equivalence limit of -20%.
Database lock	November 1 2013		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat and Per protocol After 12 weeks of treatment		
Descriptive statistics and estimate variability	Treatment group	Enclomifene 12.5mg up-titrated to 25mg	Placebo
	Number of subject	134	47
	Proportion of subjects with average serum concentration (Cavg) for T in the normal range.	109 (81.3%)	
	Wilson score 95% confidence interval for a single arm	73.9%-87.0%	
	Proportion of subjects with a 50% or greater decrease in sperm concentration from baseline	mITT: 17 (16.0%) PP: 15 (16.5%)	mITT: 2 (5.4%) PP: 2 (5.4%)
	95% CI for difference between treatments		mITT: -19.7-3.0% PP: -20.8-2.7%

Analysis performed across trials (pooled analyses and meta-analysis)

None.

Clinical studies in special populations

None.

Supportive studies

ZA-303: A single-blind, placebo-controlled multi-centre Phase 3 study to evaluate the effects on bone mineral density of administration of Androxal at 12.5 mg or 25 mg (after up-titration) for 52 weeks to overweight men with acquired hypogonadotropic hypogonadism.

Overweight men (BMI (BMI 25 to 42 kg/m2 inclusive) aged 18 to 60 years previously or concurrently diagnosed as having secondary hypogonadism and confirmed with morning testosterone <300ng/dL measured twice on separate days. One of the two TT levels must be confirmed at baseline. Subjects who fail this criterion may be enrolled in the placebo group were included in the study.

All subjects started treatment daily with:

- 12.5 mg Androxal daily, by mouth, between 7:00 and 10:00 AM

OR

- Placebo daily, by mouth, between 7:00 and 10:00 AM

Results

Change from baseline in mean testosterone levels and BMD are summarises in **Tables 58** and **59**.

Table 58. Change from baseline in mean testosterone levels in study ZA-303

	12.5 mg Androxal N=122	25 mg Androxal N=91	Placebo N=104
Baseline	226.7 (44.6)	198.9 (53.6)	362.7 (96.8)
Week 52	459.8 (145.1)	405.3 (174.3)	347.8 (107.5)
Mean Change	229.9 (140.0)	203.4 (155.7)	-6.6 (101.3)
P value (1)	<0.0001*	<0.0001*	0.5639**
P value (2)			<0.0001*

Table 59. Change from baseline in BMD in study ZA-303

	Treatment Group		
Visit	**12.5 mg Androxal N=122**	**25 mg Androxal N=91**	**Placebo N=104**
Total Hip			
Baseline (SD)	1.13 (0.12)	1.14 (0.12)	1.16 (0.14)
12 Months (SD)	1.13 (0.11)	1.15 (0.13)	1.16 (0.14)
Mean Percentage change (SD)	-0.01 (2.59)	-0.29 (2.74)	-0.75 (1.91)
P value (1)	0.3044*	0.4591*	0.0014*
P value (2)			0.0162*
Femoral Neck			
Baseline (SD)	0.99 (0.14)	1.04 (0.15)	1.06 (0.17)
12 Months (SD)	0.99 (0.13)	1.04 (0.16)	1.06 (0.17)
Mean Percentage change (SD)	-0.21 (3.31)	0.03 (3.53)	-0.68 (2.55)
P value (1)	0.5761**	0.9525**	0.0212**
P value (2)			0.1828**
L2-L4 Spine			
Baseline (SD)	1.25 (0.16)	1.29 (0.15)	1.32 (0.19)
12 Months (SD)	1.25 (0.16)	1.28 (0.15)	1.34 (0.19)
Mean Percentage change (SD)	-0.82 (3.22)	-0.38 (3.36)	0.03 (3.07)
P value (1)	0.0244**	0.4531**	0.6744*
P value (2)			0.1594*

P value (1) within group testing
P value (2) Compares change from baseline between pooled Androxal vs Placebo
* Wilcoxon Rank-Sum test
** t-test

ZA-202: A randomized, parallel, double-blind, placebo-controlled exploratory study conducted to evaluate the efficacy of Androxal® in improving glycaemic control in men with secondary hypogonadism or adult-onset idiopathic hypogonadotropic hypogonadism (AIHH) and type 2 diabetes mellitus with sub-optimum treatment.

There were significant increases from baseline (p=<0.0001) in morning testosterone for both Androxal treatment groups as summarised in **Table 60**.

Table 60. Change from Baseline in Mean Morning Testosterone After 3 Months of Treatment (Intent to Treat Population) in Study ZA-202

Treatment Group (n= # of subjects)	Baseline (Visit 2) HOMA-IR Ratio (stdev)	Month 3 (Visit 5) HOMA-IR Ratio (stdev)	Mean Change from Baseline to Visit 5 (stdev)	P-Value (change different from zero)
12.5 mg (n=38)	9.46 (10.92)	8.31 (7.36)	-1.15 (10.82)	0.4034
25 mg (n=35)	11.48 (9.23)	8.50 (6.46)	-2.98 (9.41)	**0.0477**
Placebo (n=43)	8.29 (5.76)	10.12 (12.22)	1.84 (10.93)	0.9811

Study ZA-203: This was a randomised, double-blind, placebo and active controlled, parallel, multi-centre, phase IIb study conducted to evaluate normalisation of morning testosterone levels in men with secondary hypogonadism with confirmed morning testosterone levels <250 ng/dL that wish to preserve their reproductive status and are not currently being treated with topical testosterone.

The primary endpoint was the change in morning total serum testosterone concentrations at 3 months for enclomifene compared to placebo and Testim (**Table 61**).

Table 61. Change in mean morning testosterone after 3 months of treatment (ITT) in Study ZA-203

Treatment Group	Baseline (Visit 2) morning T ng/dL (SD)	Month 3 (Visit 4) morning T ng/dL (SD)	Change from visit 2 to visit 4 ng/dL (SD)
12.5 mg (n=25)	217.2 (58.8)	471.9 (184.6)	258.5 (201.5)
25 mg (n=32)	209.8 (55.4)	405.8 (162.8)	197.3 (162.6)
placebo (n=26)	213.7 (74.9)	198.5 (72.6)	-16.9 (47.5)
Testim® (n=30)	210.0 (54.0)	462.6 (289.0)	253.7 (293.3)

ZA-203 (Extension)

This 12-month extension study recruited patients who had previously completed ZA-203. Out of the 124 patients who enrolled in ZA-203, 70 patients completed the study and 48 patients were recruited in the open label study.

Table 62. Mean total testosterone and change from baseline (ITT) in extension study ZA-203

Morning testosterone (ng/dL)	Enclomifene 12.5 mg	Enclomifene 25 mg	Enclomifene 12.5 mg and 25 mg	Testim®
Baseline	**n=17**	**n=20**	**n=37**	**n=10**
Mean (SD)	317.24 (133.42)	185.65 (54.58)	246.11 (117.91)	220.50 (57.07)
Median (Min,Max)	266 (158,628)	189 (42,295)	214 (42,628)	223.5 (104,314)
12 months	**n=8**	**n=15**	**n=37**	**n=5**
Mean (SD)	460.00 (152.09)	348.60 (171.66)	387.35 (170.46)	442.60 (181.28)
Median (Min,Max)	417.50 (306,657)	319 (156,689)	344 (156,689)	458 (207,645)
Change from baseline	**n=8**	**n=15**	**n=23**	**n=5**
Mean (SD)	158.63 (163.74) p=0.0289	164.27 (161.91) p=0.0015	162.30 (158.81) p<0.0001	215.40 (203.83) p=0.0774
Median (Min,Max)	114.50 (-31,360)	145 (-75,481)	125 (-75,481)	196 (-60,479)

Study ZA-003

This was a randomised, double-blind, placebo-controlled, and open-label, active-controlled study to evaluate the safety and efficacy of Androxal treatment in men with secondary hypogonadism. It was initially planned as a phase 3 trial but later considered by the applicant to be supportive.

194 adult male patients, aged 18-68, with a diagnosis of HH confirmed by morning total serum testosterone <300 ng/dL were enrolled in to the study.

Results

At Month 3, higher percentages of subjects in the Androxal groups (63.8% and 64.0% of subjects in the 12.5 mg and 25 mg groups, respectively) had morning total testosterone levels within the defined physiological range than did subjects in the placebo group (22.4% of subjects). The differences between Androxal and placebo were statistically significant (p <0.0001). Subjects using Androxal had statistically significant increases over the 6-month time period in mean testosterone levels compared with subjects using placebo. more subjects in the Androxal treatment groups (63.8% and 64.0% of subjects in the 12.5 mg and 25 mg groups, respectively) had total serum testosterone concentrations within the defined physiological range than did subjects in the Androgel group (54.2% of subjects). The differences between Androxal and Androgel were not statistically significant.

In terms of the International Index of Erectile Function (IIEF) questionnaire, only AndroGel® showed a significant improvement at every time-point for the libido component. In terms of erectile function, only the placebo group showed significant improvements at every time-point.

For Section 1 Sexual Desire of Derogatis Interview for Sexual Function (DISF-SRII M); all groups, including placebo, showed significant improvements throughout the study; AndroGel® showed a significant improvement from 2 months in terms of sexual desire.

For Male Sexual Distress Scale IV-A (MSDS); all groups, including placebo, showed a statistically significant improvement at every time-point throughout the study

ZA-003 (Extension)

This 12-month extension study recruited patients who had previously completed ZA-003. In ZA-003, adult males, aged 18-69 years, with a diagnosis of HH confirmed by morning total serum testosterone <300 ng/dL were recruited.

Out of the 194 patients who enrolled in ZA-003, 145 patients completed the study and 104 patients were recruited in the open label study.

Efficacy analyses were performed on the PP population, which comprised all patients who completed the 12-month treatment period, were at least 80% compliant with treatment and had no major protocol violations.

Table 63. The proportion of subjects at 1 year with morning total serum testosterone concentration within the normal physiological range (300 to 1040 ng/dL) (PP)m in Extension Study ZA-003

	Enclomifene 12.5 mg n=11	**Enclomifene 25 mg n=17**	**AndroGel® n=15**	**placebo n=13**	**Overall n=56**
n (%)	**8 (72.7)**	**11 (64.7)**	**6 (40.0)**	**10 (76.9)**	**35 (62.2)**
95% CI	-3.4, 68.9	-8.9, 58.3			
Enclomifene vs AndroGel®					
Chi-square test; p-value					
Enclomifene 12.5 mg		0.6571	0.0982	0.8130	
Enclomifene 25 mg			0.1622	0.4693	
AndroGel®				0.0490	

Study ZA-300

This was a phase III, multi-centre, open-label trial. 499 overweight males with acquired hypogonadotropic hypogonadism who had not been treated with injectable or pelleted testosterone or testosterone were enrolled into the study. Subjects were treated with 12.5 mg Androxal. Study drug was taken daily for 26 weeks.

Subjects returned to the clinic after 6, 16 and 26 weeks and for 2 follow up visits at 4 and 8 weeks after end of treatment. After 6 and 16 weeks of treatment (Visits 3 and 4), subjects who exhibited morning T<450ng/dL were up-titrated to 25 mg Androxal. Up-titrated subjects remained on the higher dose for the balance of the study

Mean morning total serum testosterone levels for both dose levels increased to within the normal physiological range from Week 6 and remained so until the end of treatment. Changes from baseline were statistically significant throughout the treatment period for both dose groups.

Table 64. Mean morning total serum testosterone and change from baseline by dose group and overall (ITT) in Study ZA-300

Morning testosterone (ng/dL)	12.5 mg n=216	25 mg n=283	All patients n=499
Baseline			
Mean (SD)	224.98 (45.33)	201.17 (46.57)	211.47 (47.48)
Median (Min, Max)	231 (50,301)	206 (35,318)	216 (35,318)
Last observation on treatment			
Mean (SD)	483.61 (163.67)	405.60 (155.02)	439.36 (163.31)
Median (Min, Max)	481.5 (84,937)	406 (14,1720)	437 (14,1720)
Change from baseline			
Mean (SD)	258.63 (161.12)	204.43 (153.99)	227.89 (159.24)
Median (Min, Max)	252 (-68,830)	194 (-93,1639)	221 (-93,1639)
p-value (1)	<0.0001	<0.0001	<0.0001
p-value (2)	<0.0001		

ZA-301 (Extension)

This 6-month extension study recruited patients who had previously completed the placebo-controlled pivotal studies ZA-301 and ZA-302. The study recruited adult males, aged 18-60 years, with a diagnosis of HH confirmed by morning total serum testosterone <300 ng/dL and LH <9.4 mIU/mL. In these pivotal studies, patients had been randomised to enclomifene citrate 12.5 mg (up-titrated to 25 mg at Week 6 if required), or placebo.

Results showed statistically significant increases in mean total serum testosterone levels at all visits for both dose levels of enclomifene citrate; this is notable as the mean baseline level for all patients was 357.61 ng/dL and this included patients who had previously been treated with placebo, for whom the mean baseline level was 251.89 ng/dL.

Analysis of mean levels one month post-treatment showed significant decreases from baseline in both treatment groups. The mean value had fallen below the normal physiological range in the group up-titrated to 25 mg enclomifene at six weeks. Analysis showed that this group had a lower mean baseline morning testosterone level and, at the end of treatment, a smaller proportion had achieved morning serum testosterone within the normal physiological range.

Table 65. Patients with testosterone in the normal range (ITT) in Extension Study ZA-301

	Enclomifene 12.5 mg	Enclomifene 25 mg	Enclomifene 12.5 mg and 25 mg
	Number of patients n (%)		
Baseline	70 (71.4)	38 (39.2)	108 (55.4)
Week 6	84 (94.4)	62 (63.3)	146 (78.1)
Week 16	73 (94.8)	64 (69.6)	137 (81.1)
Week 26	61 (91.0)	52 (63.4)	113 (75.8)
Week 30 (4 weeks post-treatment)	28 (34.2)	14 (16.1)	42 (24.9)

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Enclomifene is intended for the treatment of secondary hypogonadism in adult men. Four pivotal studies were submitted to demonstrate the efficacy of enclomifene in this indication. Two of the studies (ZA-304 and 305) were conducted with AndroGel (testosterone gel) as an active comparator. The other two studies (ZA-301 and 302) were placebo controlled studies. A number of supportive studies were also submitted. The number and choice of comparators in the submitted clinical studies to demonstrate efficacy were considered acceptable.

Subjects included in the four pivotal studies were overweight (BMI 25-42kg/m^2) with two consecutive morning testosterone values below 300ng/dl, LH below 9.4mIU/l and sperm concentrations greater 15 million/ml but not on signs and symptoms of secondary hypogonadism. The applicant considered that the most consistent sign of hypogonadotropic hypogonadism is obesity (Tajar 2010). The applicant stated that even though patients in the pivotal studies were not selected on the presence of specific signs and symptoms, over 79% of the patient population were found to have had such signs and symptoms. In addition, and as most of the patients included in the trials were obese, the applicant proposed to restrict the indication only to patients which had attempted diet and exercise.

The CHMP however noted that that for the majority of the subjects (approximately 70%) included in the studies, the dominant problem was obesity. Approximately 12% of the subjects had symptoms suggestive of erectile dysfunction in their medical history, 6.0% had symptoms suggestive of libido and approximately 10% symptoms of mood changes. Overall therefore it is questioned whether subjects were really hypogonadal but rather obese men who had not tried life-style modification and perhaps had low testosterone due to their obesity. Relevant guidelines such as the British Society for Sexual Medicine guidelines on the management of sexual problems in men consider that the diagnosis of treatable hypogonadism requires the presence of symptoms and signs suggestive of testosterone deficiency as well as biochemical evidence. It is therefore questionable, whether patients included in the enclomifene studies would in real life qualify for pharmacological treatment to restore their testosterone levels.

Restriction of the indication as proposed by the applicant for the treatment of hypogonadotropic hypogonadism (secondary hypogonadism) in adult men aged ≤60 years with a body mass index (BMI) ≥ 25 kg/m2, and which had not responded to diet and exercise could have been acceptable had the applicant been able to demonstrate an effect on signs and symptoms of secondary hypogonadism. Clinically meaningful

endpoints assessed through a validated, patient-reported questionnaire designed to assess the psychosexual function in terms of libido, erectile dysfunction were however not included. These are considered important for demonstrating a clinically significant benefit of a product intended for the treatment of secondary hypogonadism.

Considering that most patients in the trials were obese, the CHMP also noted that inclusion of physiologically-based endpoints such as reduction in body fat/ increase in muscle mass and strength/improvement in bone mineral density would have been important to provide information on the clinical benefit of enclomifene.

In terms of the initially applied indication regarding preservation of testicular function and spermatogenesis, the enclomifene studies were designed to demonstrate that that enclomifene does not cause a reduction in sperm concentration while restoring testosterone concentrations. In this regard, studies ZA-304 and 305 had two co-primary endpoints; percentage change from baseline in sperm concentrations and a composite endpoint comparing the proportion of subjects who obtain a successful testosterone level and sperm concentration. Sperm concentrations were to remain above 10 million/mL.

In studies ZA-301 and 302, non-inferiority to placebo in terms of effect on sperm concentration was chosen as an objective. This was because no change was expected in the placebo group and the applicant considered clinically meaningful to demonstrate non-reduction in sperm concentrations for patients wishing to preserve their reproductive status. However, the specified 20% non-inferiority margin was not appropriately justified as it is not clear whether a reduction of 20% difference between enclomifene and placebo would be clinically relevant. For the endpoint “proportion of subjects experiencing a 50% or greater reduction in sperm concentration appeared to have been chosen to account for inter and intra-individual variability.

Efficacy data and additional analyses

Study ZA-304

In study ZA-304, for the composite endpoint, results showed that treatment was successful in 61.0% of enclomifene treated subjects, 4.4% of placebo-treated subjects, and 16.3% of AndroGel-treated subjects. The mean testosterone level for 25 mg enclomifene -treated subjects was 298.0 ng/mL, and for placebo 192.9 ng/dL. The proportion of subjects in the normal range [300-1040 ng/dL] was higher in the enclomifene group (63.4%) in comparison to the AndroGel group (44.2%). LH and FSH increased in enclomifene treated subjects, there was no change LH and FSH levels in placebo treated subjects and in AndroGel-treated subjects levels of LH and FSH decreased.

At the end of treatment, after 16 weeks, AndroGel-treated subjects showed a decrease of more than 50% (-77.5%) in median sperm concentration from baseline that was highly significant in comparison with enclomifene-treated subjects (-3.3 %) and in comparison with placebo (-10%, p<=0.0001).

The change of mean sperm concentration in enclomifene-treated subjects increased from baseline but the difference was not statistically significant in comparison with placebo-treated subjects.

Study ZA-305

Similar results were reported in study ZA-305, with treatment termed successful in 65.9% of enclomifene treated subjects, 7.3%of Placebo-treated subjects, and 33.3%of AndroGel-treated subjects. The proportion of subjects with mean 24-hour testosterone levels in the normal range was higher in the enclomifene group (68.2%) compared to the AndroGel group (52.4%). LH and FSH increased in enclomifene treated subjects,

there was no change in LH and FSH level is in placebo treated subjects and in AndroGel-treated subjects levels of LH and FSH dropped.

AndrogGel-treated subjects showed a decrease of more than 30% in mean sperm concentration from baseline in comparison with enclomifene-treated subjects (p=0.0007). The median percentage of median change of sperm concentration in AndroGel-treated subjects (-39.49%) was statistically significant in comparison with those of enclomifene-treated subjects (8.95%) and placebo group (-4.39%, p=0.003).

Study 301

In study 301, 89 of the 113 (78.8%, 95% CI: 70.3%, 85.3%) subjects treated with enclomifene in the ITT population achieved an average testosterone concentration in the normal range. All visits showed that the change from baseline was statistically significant ($p < 0.05$) and that change was statistically significantly larger for enclomifene-treated subjects than those treated with Placebo ($p < 0.05$) at 12 Weeks (Visit 5). Subjects treated with enclomifene experienced a statistically significant increase over baseline in FSH and LH at each post-treatment assessment, which was sustained through the assessment 1 week off treatment. In addition, the changes were statistically significantly different from the change induced by Placebo at 12 weeks.

For the ITT population in this study, the difference between the proportion of subjects experiencing a 50% or greater reduction in sperm concentration in enclomifene treated patients was not significantly different than the proportion of subjects treated with placebo with the lower limit of the confidence interval (-19.4%) within the pre-specified margin of -20% equivalence limit difference that was pre-specified. Therefore, treatment with enclomifene was considered non-inferior to placebo with respect to changes in sperm concentration (above).

However, in the PP population in which 50 patients were excluded the lower limit of the confidence interval, -25.6%, did not demonstrate non-inferiority of enclomifene compared to placebo.

Study 302

In study 302, 109 of the 134 (81.3%, 95% CI: 73.9%, 87.0%) subjects treated with enclomifene in the ITT population achieved an average testosterone concentration in the normal range. As in study ZA-301, changes from baseline were statistically significant throughout all visits and in favour of enclomifene compared to placebo. Similar effects to ZA-301 were also reported for changes in LH and FSH.

In the ITT population, the difference between the proportion of subjects experiencing a 50% or greater reduction in sperm concentration in enclomifene treated patients was not significantly different than the proportion of subjects treated with placebo, with the lower limit of the 95% CI (-17.5%) within the pre-specified non-inferiority margin. As with study ZA-302, this was however not confirmed in the PP population in which the lower limit of the 95% CI was -20.8%.

Regarding the effect of enclomifene on sperm concentration, no real benefit on the preservation of sperm concentration was demonstrated as the results of non-inferiority with placebo are inconclusive. The applicant accepted this and further modified the claimed indication to hypogonadotropic hypogonadism (secondary hypogonadism) in adult men aged≤ 60 years with a body mass index (BMI) ≥25 kg/m^2 and which had not responded to diet and exercise.

The CHMP however noted that the proposed indication was not in line with the studied population as clinical features of secondary hypogonadism were not used to enrol these patients in the enclomifene trials.

Based on the submitted results, the CHMP accepted that enclomifene was shown to be able to restore testosterone levels in the patients included in the submitted studies. Enclomifene acts by blocking the oestrogenic suppression of the Hypothalamic-Pituitary-Gonadal (HPG) axis causing the pituitary gland to secrete more LH and FSH which then stimulates the testes to produce more testosterone. Therefore, in addition to increased testosterone levels, enclomifene also increases FSH and LH levels. The CHMP taking into account the novel mechanism of action of enclomifene considered that restoration of testosterone levels alone would not be sufficient to demonstrate a clinical benefit in the applied indication. The additional pharmacological effects of enclomifene compared to testosterone replacement therapy on the signs and symptoms of hypogonadism are not established. Available literature presented by the applicant to link normalisation of testosterone levels and a positive effect on symptoms of secondary hypogonadism relate primarily to testosterone replacement therapies and therefore cannot be assumed to apply equally to enclomifene.

Validated Patient Reported Outcomes (PRO) are currently lacking for the evaluation of treatments for secondary hypogonadism and which could have been potentially used in the submitted enclomifene trials. The CHMP however noted that in one of the submitted supportive studies (ZA-003), PROs were used including the International Index of Erectile Function (IIEF) questionnaire and in which only AndroGel (but not enclomifene) showed a significant improvement at every time-point for the libido component. Other PROs used in this study were not able to show any benefit for enclomifene compared to placebo.

2.5.4. Conclusions on the clinical efficacy

A number of important issues were identified during the evaluation of the clinical studies conducted with enclomifene.

There are concerns about the representativeness of the intended target population in the enclomifene clinical trial programme and whether pharmacological intervention would be the most appropriate measure to treat the population included in the clinical trials.

In terms of results, enclomifene has been shown to be able to normalise testosterone levels in the patients included in the clinical trials. However, this is not considered sufficient to conclude that this would translate into clinical meaningful benefits for patients with secondary hypogonadism. Unlike testosterone replacement therapy, enclomifene has been shown to have an effect on LH and FSH. Results showing an effect on the recognised signs and symptoms of the disease are therefore considered necessary in order to robustly demonstrate an effect in this indication.

The CHMP therefore concluded that the efficacy of enclomifene in the proposed indication has not been established.

2.6. Clinical safety

Patient exposure

A total of 1403 persons participated in Phase 2 and Phase 3 studies and were exposed to enclomifene. Of these, 482 persons were exposed to daily treatment with enclomifene citrate >6 months and 116 persons ≥12 months (**Tables 66 and 67**).

Table 66. Number of patients exposed to enclomifene per study pool

Study Pool	Enclomifene citrate	AndroGel®	Testim®	Placebo
Pool 1 (ZA-304, -305)	85	85	-	86
Pool 2 - Pivotal (ZA-301, -302, -304, -305)	324	85	-	168
Placebo-Controlled	557	133	33	288
Active-Controlled	288	147	33	163
Long-term	1055	48	10	152
Phase 2 and 3*	1403	147	39	391

Table 67. Duration of exposure to enclomifene.

Total exposed population		
Duration of exposure – direct exposure only (at least) using days in a month as 365.25/12=1 month of days	**Persons**	**Person time (days)**
<1 m	66	749
<1 m at least 1 day (< 26 days windowed)	51	322
1 m	1337	262370
1 m (≥26 days windowed)	1352	262797
3 m	1054	242929
3 m (≥80 days windowed)	1156	251655
6 m	482	153029
6 m (>154 days windowed)	877	222634
12 m	116	52647
12 m (>345 days windowed)	211	86342
Total person time	*1403*	*263119*

Adverse events

The most commonly reported non-serious TEAEs (summarised in **Table 68**) were in the SOC: Infections and infestations 257 (18.3%), Musculoskeletal and connective tissue disorders 158 (11.3%), Nervous system disorders 130 (9.3%) and Gastrointestinal disorders 127 (9.1%), Eye disorders 56 (4.0%), Reproductive system and breast disorders 52 (3.7%) and Vascular disorders 61 (4.3%).

Table 68. Non-serious TEAEs reported in the phase 2 and 3 pool

	enclomifene citrate N = 324	enclomifene citrate 12.5 mg N = 324	enclomifene citrate 25 mg N = 85	AndroGel N = 85	Placebo N = 168
Subjects with at Least One Treatment Emergent AE	116 (35.8%)	102 (31.5%)	21 (24.7%)	38 (44.7%)	61 (36.3%)
Headache	15 (4.6%)	12 (3.7%)	3 (3.5%)	4 (4.7%)	8 (4.8%)
Nasopharyngitis	7 (2.2%)	7 (2.2%)	0 (0)	1 (1.2%)	2 (1.2%)
Nausea	6 (1.9%)	5 (1.5%)	1 (1.2%)	1 (1.2%)	8 (4.8%)
Influenza	5 (1.5%)	5 (1.5%)	0 (0)	1 (1.2%)	1 (0.6%)
Upper respiratory tract infection	5 (1.5%)	5 (1.5%)	0 (0)	2 (2.4%)	4 (2.4%)
Diarrhoea	4 (1.2%)	4 (1.2%)	0 (0)	2 (2.4%)	2 (1.2%)
Muscle spasms	4 (1.2%)	4 (1.2%)	0 (0)	1 (1.2%)	1 (0.6%)
Dyspepsia	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	1 (0.6%)
Vomiting	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	4 (2.4%)
Dizziness	3 (0.9%)	3 (0.9%)	0 (0)	1 (1.2%)	3 (1.8%)
Fatigue	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	3 (1.8%)
Sinusitis	3 (0.9%)	2 (0.6%)	1 (1.2%)	0 (0)	4 (2.4%)
Laceration	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	0 (0)
Haematocrit increased	3 (0.9%)	3 (0.9%)	0 (0)	1 (1.2%)	0 (0)
Haemoglobin increased	3 (0.9%)	2 (0.6%)	1 (1.2%)	0 (0)	0 (0)
Weight increased	3 (0.9%)	2 (0.6%)	1 (1.2%)	2 (2.4%)	2 (1.2%)
Decreased appetite	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	0 (0)
Arthralgia	3 (0.9%)	3 (0.9%)	0 (0)	1 (1.2%)	1 (0.6%)
Libido decreased	3 (0.9%)	3 (0.9%)	0 (0)	1 (1.2%)	1 (0.6%)
Erectile dysfunction	3 (0.9%)	2 (0.6%)	1 (1.2%)	0 (0)	0 (0)
Nasal congestion	3 (0.9%)	3 (0.9%)	0 (0)	1 (1.2%)	0 (0)
Night sweats	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	2 (1.2%)
Rash	3 (0.9%)	3 (0.9%)	0 (0)	1 (1.2%)	1 (0.6%)
Hot flush	3 (0.9%)	3 (0.9%)	0 (0)	0 (0)	1 (0.6%)

The most frequently reported TEAEs that was considered at least possibly related to the study drug by the investigator (summarised in **Table 69**) were in the following SOCs: Psychiatric disorders 45 (3.2%), Nervous system disorders 40 (2.9%) and Gastrointestinal disorders 37 (2.6%).

Table 69. The most common TEAEs at least possibly related to enclomifene (phase 2 and 3 pool)

TEAE, at least possibly related (N=1403)*	Patients (N)	Patients (%)
Headache	23	1.6
Hot flush	16	1.1
Nausea	14	1.0
Muscle spasms	12	0.9
Dizziness	10	0.7
Fatigue	9	0.6
Haematocrit increased	9	0.6
Erectile dysfunction	8	0.6
Prostatic specific antigen increased	8	0.6
Increased appetite	8	0.6
Vision blurred	7	0.5
Aggression	7	0.5
Irritability	7	0.5
Acne	7	0.5

Serious adverse event / deaths / other significant events

The following serious adverse events met the regulatory definition of “Serious”. The following events occurred once unless otherwise stated: angina pectoris, bradycardia, food poisoning, biliary colic, cholelithiasis, appendicitis perforated, cellulitis, diverticulitis, road traffic accident, skin injury, cerebrovascular accident, transient ischaemic attack (x2), anxiety, nephrolithiasis (x2), urinary tract disorder, pulmonary embolism, coronary artery bypass, knee arthroplasty, deep vein thrombosis and hypotension.
SAEs related to thromboembolic events, are discussed in detail under Topics of Special Interest.

Deaths

Two deaths were reported during the trials. One of these was a case of ischemic stroke. The other subject died in a motor vehicle accident.

Topics of special interest

Cardiac events

In the placebo-controlled pool, cardiac disorders were reported in 5 (1.0%) of enclomifene-treated patients versus 1 (0.4%) of placebo-treated patients and there were a total of 12 cardiac events in the enclomifene cohort in all phase 2 and 3 trials as shown in **Table 70**.

Table 70. TEAEs in the Cardiac Disorders SOC - all phase 2 and 3 studies

	Enclomifene				AndroGel® N=147	Testim® N=39	Placebo N=391
	Total N=1403	6.25 mg N=16	12.5 mg N=1285	25 mg N=637			
Cardiac disorders	12 (0.9%)	0 (0)	8 (0.6%)	4 (0.6%)	1 (0.7%)	0 (0)	3 (0.8%)
Acute myocardial infarction	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3%)
Angina pectoris	1 (0.1%)	0 (0)	1 (0.1%)	0 (0)	0 (0)	0 (0)	2 (0.5%)
Arrhythmia	1 (0.1%)	0 (0)	0 (0)	1 (0.2%)	0 (0)	0 (0)	0 (0)
Atrial flutter	1 (0.1%)	0 (0)	0 (0)	1 (0.2%)	0 (0)	0 (0)	0 (0)
Atrioventricular block first degree	1 (0.1%)	0 (0)	0 (0)	1 (0.2%)	0 (0)	0 (0)	0 (0)
Bradycardia	2 (0.1%)	0 (0)	2 (0.2%)	0 (0)	0 (0)	0 (0)	0 (0)
Cardiac failure congestive	1 (0.1%)	0 (0)	1 (0.1%)	0 (0)	0 (0)	0 (0)	0 (0)
Coronary artery disease	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7%)	0 (0)	0 (0)
Palpitations	3 (0.2%)	0 (0)	3 (0.2%)	0 (0)	0 (0)	0 (0)	1 (0.3%)
Sinus tachycardia	1 (0.1%)	0 (0)	1 (0.1%)	0 (0)	0 (0)	0 (0)	0 (0)
Ventricular extrasystoles	1 (0.1%)	0 (0)	0 (0)	1 (0.2%)	0 (0)	0 (0)	0 (0)

QT prolongation

In the thorough QT Studies (see also section 2.3.2. of this report) , enclomifene was shown to have a small effect on the QT interval at the dose of 250 mg q.d. Concentrations were in excess of 11 times those of the maximum intended therapeutic dose, 25 mg.

Ophthalmic changes

Zuclomifene, the geometric isomer of enclomifene, appears to accumulate in the eyes of animals. In addition, Clomid is known to be associated with transient visual disturbance.

There were few visual disturbance TEAEs, and rates were similar to, or lower than in, the placebo group, with 1.6% of enclomifene patients versus 2.4% of placebo patients reporting eye disorders in the placebo-controlled trials. Blurred vision was the most common visual disturbance adverse event, and was reported by 14 patients (1.0%) in the enclomifene and 3 patients (0.8%) in the placebo group. 12 of the 14 reports of blurred vision in the enclomifene group were during dosing at the 12.5 mg dose.

Cataracts

In the phase 2 and 3 clinical trials, changes in cataracts of ≥ 1 step were reported in ophthalmic assessments but only changes of ≥ 2 steps were considered clinically relevant and are summarised in **Table 71**.

Table 71. Noteworthy Changes of Appearance of New of Worsening Cataracts from All Phase 2 and Phase 3 studies

	Enclomifene		**Comparator (AndoGel / Testim)**	**Placebo**
	12.5mg (N = 660)	**25mg (N = 621)**	**N = 172**	**N = 348**
Total events (%)	7 (1.1)	6 (1.0)	7 (4.1)	4 (1.1)
Events/100 subject years	2.1	1.7	12.4	2.5

Thromboembolism

Five cases of thromboembolic events were identified in enclomifene clinical trials. Four of these were in patients taking enclomifene (1- DVT and PE, 2-DVT only, 1-fatal ischaemic stroke), and one (myocardial infarction), was in the placebo group. The four patients who were taking enclomifene at the time of the event were noted to have other risk factors. Details of the reported cases are summarised in **Table 72**.

Table 72. Cases of thromboembolic events reported in the enclomifene trials

Patient; SAE; Age (yrs)	BMI (Kg/m2)	Dose	Days on Drug	Testo-sterone (ng/dL)	Oestr-adiol (pg/mL)	HCT (%)	Risk Factors
300- 02-029 DVT/PE 49	39.4	25	136	332	61.4	55.4	Hypertension, obesity, height (6'5"), standing at job, recent long trip, family history of clotting disorder
300- 17-007 DVT 54	27.4	12.5	53	593	35.5	50.1	5 hour flight 4 days previously, overweight, prior Testim® therapy with HCT=54% at study entry
303- 41-004 DVT 51	40.2	25	289	369	40.6	41.8	Knee surgery 43 days previously, obesity
304- 22-028 Stroke 59	41.8	12.5	34	478	53.8	47.8	Atrial fibrillation (untreated), obesity, diabetes, sleep apnoea, hyperlipidaemia
303- 41-044 MI 51	28.3	Placebo	231	550	26.2	46.1	Hyperlipidaemia, hypertension

Testicular disorders and erectile dysfunction

Erectile dysfunction is a common feature in men with hypogonadism. Incidence of erectile dysfunction was higher in enclomifene group compared to AndroGel and placebo groups (0.6 % in enclomifene group versus 0% in AndroGel and placebo groups) in the in phase 2 and 3 pool.

Testicular hypertrophy was reported more frequently in enclomifene group (0.1 % in enclomifene group versus 0% in AndroGel and placebo groups). Testicular pain was reported more frequently in enclomifene group compared to placebo (0.4% versus 0%) but less frequently compared to AndroGel (0.7%).

Breast disorders (including Gynecomastia and Breast cancer)

Breast enlargement was reported with a frequency of 0.2% in enclomifene group versus 0% in AndroGel and placebo groups (TEAEs at least possibly related). Gynaecomastia is reported with a frequency of 0.07 % in enclomifene group versus 0.7% in AndroGel group and 0% in the placebo group.

Psychiatric disorders

The AE Aggression in the phase 2 and 3 pool (TEAEs at least possibly related) was reported in 0.5% of the subjects in the enclomifene group versus 0% in the placebo group. Anxiety was reported with a similar frequency in the enclomifene group and in the placebo group (0.4%).

Vasodilatation – hot flushes

Hot flushes (TEAEs at least possibly related) were reported more frequently in the enclomifene group (1.1%) versus AndroGel (0%) and placebo (0.5%).

Laboratory findings

Increased Haematocrit

Increased testosterone is known to be associated with increases in haematocrit (Rhoden and Morgentaler, 2004), which is mediated by increased erythropoietin production by the kidney (Jockenhovel et al, 1997).

Tables 73 and **74**, show mean changes in haematocrit and haemoglobin in all phase 2 and phase 3 studies and the number and percentage of patients with a normal (39 or 40%, depending on study) haematocrit at baseline who transitioned to high (defined as >54% by the applicant)at the last visit.

Table 73. Mean changes in haematocrit in phase 2 and 3 studies

Hematocrit			
Treatment Group (n= # of subjects)	**Mean Change from Baseline to last value on drug % (SD)**	**P-Value (vs. Placebo)**	**NORMAL to HIGH n (%)**
Enclomifene 12.5mg (n=627)	1.2 (3.36)	<0.0001	21 (2.9%)
Enclomifene 25mg (n=637)	1.2 (3.05)	<0.0001	17 (2.5%)
Androgel 1.62% (n=139)	1.9 (3.43)		9 (6.1%)
Placebo (n=361)	0.0 (2.70)		1 (0.3%)

One case of polycythemia (patient had haematocrit of 55.4%) was reported as possibly related to enclomifene in the clinical development program. The subject developed deep vein thrombosis and bilateral pulmonary emboli during the trial ZA-300 and haematocrit was found elevated prior to the event.

Table 74. Mean changes in haemoglobin in phase 2 and 3 studies

Haemoglobin			
Treatment Group (n= # of subjects)	**Mean Change from Baseline to last value on drug gm/dL (SD)**	**P-Value (vs. Placebo)**	**NORMAL to HIGH N (%)**
Enclomifene 12.5mg (n=627)	0.3 (1.00)	<0.0001	39 (5.5%)
Enclomifene 25mg (n=637)	0.3 (0.91)	<0.0001	31 (4.6%)
Androgel 1.62% (n=139)	0.5 (0.95)		8 (5.4%)
Placebo (n=361)	0.0 (0.76)		6 (1.5%)

Serum Testosterone

Serum testosterone concentrations are an important efficacy parameter, but elevations above the desired normal range of 1040 ng/mL could have safety implications.

Testosterone excursions above the normal range were slightly higher in the patients treated with enclomifene than in in the placebo group but lower than in the AndroGel and Testim groups (**Table 75**).

Table 75. Proportion of patients who experienced a maximum testosterone >1040 ng/dL (phase 2 and 3 studies)

	Enclomifene				AndroGel®	Testim®	Placebo
	All doses (N=1403)	6.25mg (N=16)	12. 5 mg (N=712)	25 mg (N=675)	(N=147)	(N=39)	(N=391)
EXCURSIONS							
>1040 ng/dL	19 (1.4%)	0 (0.0%)	7 (1.0%)	12 (1.8%)	14 (9.5%)	4 (10.3%)	2 (0.5%)

Oestradiol

Enclomifene citrate and AndroGel showed statistically significant within-treatment increases in oestradiol throughout treatment and the mean change was significant compared to placebo at the end of treatment.

Table 76. Oestradiol transition from baseline to last observation on drug (Pool 2)

Estradiol		enclomifene citrate (N = 324) Baseline		
Visit	Ref. Range Indicator	HIGH	LOW	NORMAL
Last Observation On-Drug	HIGH	27 (8.3%)	0 (0.0%)	180 (55.6%)
	LOW	0 (0.0%)	0 (0.0%)	0 (0.0%)
	NORMAL	2 (0.6%)	0 (0.0%)	79 (24.4%)
		Placebo (N = 168) Baseline		
Visit	Ref. Range Indicator	HIGH	LOW	NORMAL
Last Observation On-Drug	HIGH	6 (3.6%)	0 (0.0%)	17 (10.1%)
	LOW	0 (0.0%)	0 (0.0%)	0 (0.0%)
	NORMAL	3 (1.8%)	0 (0.0%)	122 (72.6%)
		AndroGel (N = 85) Baseline		
Visit	Ref. Range Indicator	HIGH	LOW	NORMAL
Last Observation On-Drug	HIGH	8 (9.4%)	0 (0.0%)	30 (35.3%)
	LOW	0 (0.0%)	0 (0.0%)	0 (0.0%)
	NORMAL	1 (1.2%)	0 (0.0%)	41 (48.2%)

Increased PSA

Elevations of PSA are expected when the prostate gland is stimulated by testosterone. However, such elevations can also be a sign of prostate cancer. Patients in the enclomifene clinical trial programme were carefully monitored by repeated PSA measurements.

The mean changes in PSA in all Phase 2 and 3 studies is shown in **Table 77**.

Table 77. Mean change from baseline to last observation on drug for PSA and transitions in PSA (phase 2 and 3 studies)

	Enclomifene			AndroGel® n=147	Testim® n=39	Placebo n=391
	Total n=1403	12. 5 mg n=712	25 mg n=675			
Mean change from baseline (µg/L)	0.3 (1.0)	0.3 (0.7)	0.3 (1.1)	0.1 (0.3)	0.1 (0.3)	0.0 (0.3)
Change in PSA >75 µg/L	131 (9.3)	66 (9.3)	65 (9.6)	9 (6.1)	1 (2.6)	20 (5.1)

Safety in special populations and related to drug-drug interactions and other interactions

This was evaluated in a number of pharmacokinetic studies, described in Section 2.4.2 of this report.

Discontinuation due to adverse events

A total of 89 (6.3%) patients withdrew from the clinical trials due to adverse events, 74 (5.3%) of these patients were receiving enclomifene, 8 (5.4%) received AndroGel, 1 (2.6%) received Testim and 6 (1.5%) received placebo. The most common TEAEs leading to discontinuation in patients receiving enclomifene are summarised in **Table 78**.

Table 78. Most frequent TEAEs leading to study discontinuation (phase 2 and 3 studies)

Preferred Term	Enclomifene			AndroGel® n=147	Testim® n=39	Placebo n=391
	Total n=1403	12. 5 mg n=1285	25 mg n=637			
Vision blurred	4 (0.3%)	4 (0.3%)	0 (0)	0 (0)	0 (0)	1 (0.3%)
Muscle spasms	4 (0.3%)	3 (0.2%)	1 (0.2%)	1 (0.7%)	0 (0)	0 (0)
Headache	4 (0.3%)	4 (0.3%)	0 (0)	1 (0.7%)	0 (0)	1 (0.3%)
Aggression	4 (0.3%)	3 (0.2%)	1 (0.2%)	0 (0)	0 (0)	0 (0)
PSA increased	3 (0.2%)	1 (0.1%)	2 (0.3%)	0 (0)	0 (0)	0 (0)
Back pain	3 (0.2%)	3 (0.2%)	0 (0)	0 (0)	0 (0)	0 (0)
Erectile dysfunction	3 (0.2%)	3 (0.2%)	0 (0)	0 (0)	0 (0)	0 (0)
Hot flush	3 (0.2%)	1 (0.1%)	2 (0.3%)	0 (0)	0 (0)	1 (0.3%)

Post marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

The pooled clinical safety population includes 1403 subjects from 13 clinical trials (integrated database from phase 2 and phase 3 clinical trials). In the pivotal pool, 324 subjects had safety assessment. Subjects were treated for 12 to 18 weeks in the pivotal clinical trials. Exposure to more than 6 and 12 months was in 500 and 100 subjects respectively.

Overall, the size of the safety database was considered acceptable, even though rare adverse drug reactions may not have been detected because of the limited numbers exposed, in particular those exposed above 6 months.

Overall, most of TEAEs were mild or moderate in intensity. The incidence of TEAEs was similar between enclomifene and AndroGel groups (17.7% and 19.7% respectively) in the Phase 2 and 3 pool, compared to 8.7% in the placebo group. TEAEs were reported as “mild” in 12.1% of the subjects, moderate in 4.6% and severe in 0.9% in enclomifene group. There does not appear to be a notable difference between the frequency and type of event experienced by the patients in the long-term study pool compared to patients on short-term treatment.

It is difficult to conclude on the comparative safety profile between the 12.5 mg and 25 mg doses, as subjects were not randomized, but commenced treatment with 12.5 mg dose. In the clinical development program, adverse events appear to be more frequently reported for the lower dose.

The safety profile of enclomifene overall appeared similar to that of topical testosterone product AndroGel but differed in the incidence of hot flushes (known risk with the SERM clomifene) and erectile dysfunction which were more commonly reported with enclomifene. On the other hand a larger percentage of subjects treated with AndroGel experienced a shift to lower classification for sperm concentration after treatment.

Rates of PSA elevation were similar for the enclomifene and AndroGel groups, but slightly higher than observed for Testim and placebo. There was no evidence of a dose effect for enclomifene. No cases of prostate cancer were reported during the enclomifene clinical trials.

Few serious TEAEs were reported. The fatal case of ischaemic stroke with enclomifene occurred in a patient with multiple risk factors for stroke, including untreated atrial fibrillation. The other case of death was a patient, also randomized in enclomifene group, who died in a motor vehicle accident.

One of the thromboembolic events was reported in a patient with increased haematocrit. The two other cases of DVT occurred in subjects with multiple risk factors for thromboembolic events as they were overweight or obese (a BMI around 40 kg/m2), with comorbidities such as diabetes, cardiac disorders, dyslipidemia, haematocrit increased, sleep apnoea, hypertension, smoking, previous surgery. The applicant considers that one event also occurred in a placebo treated patient. This is not accepted as the reported event was a myocardial infarction and is not related to a thromboembolic event.

Despite the small numbers reported, it cannot be excluded that enclomifene increases the risk of thromboembolic events in the target population, especially as there were no cases reported for the patients on testosterone or placebo.

SERMs, such as clomifene, have been associated with thrombotic venous and arterial events. Subjects were treated for 12 to 18 weeks in the pivotal clinical trials and only 116 subjects were exposed more than 12 months in the clinical development program. The true incidence of these type of events could have been under-detected due to the limited number of subjects exposed, in particular above 6 months.

Increased testosterone is known to be associated with increases in haematocrit. Increase in haematocrit can be seen as a beneficial effect but an increase in red blood cell mass may increase the risk of thromboembolic events. Haematocrit increased is reported as TEAE at least possibly related in 0.6% of the subjects (compared to 1.4% in the AndroGel group and 0% in placebo group).

The applicant acknowledged the increased risk of VTE with the use of enclomifene and noted that the intended patient population are already at a higher risk of VTE. The applicant argued that in the long-term normalisation of testosterone levels would lead to beneficial effects such as reduction in weight and improvement in lipid levels. However, and as discussed in section on Clinical Efficacy in this report, there is no evidence that enclomifene provides clinically relevant benefits in these parameters (in the short or long-term as these were not specifically studied in the pivotal studies.

Furthermore, regarding the VTE risk, there is a potential that is further increased due to the oestradiol increases which were observed after long-term treatment with enclomifene.

In the placebo-controlled pool, the frequency of events in the Cardiac Disorders SOC events was low, reported for ≤1% of patients. Cardiac disorders were reported in 5 (1.0%) of enclomifene-treated patients versus 1 (0.4%) of placebo-treated patients. The five enclomifene-treated patients reported a variety of cardiac disorders: 1 AV block, 1 cardiac congestive failure, 2 palpitations, and 1 ventricular asystole.

In the overall enclomifene clinical trial programme, there also were very few cardiac adverse events. There were no notable differences between the frequencies of events reported in the treatment arms across the phase 2 and 3 study pools.

The possibility of cataract formation was a topic of special interest because of a safety signal in the non-clinical study in dogs. Visual symptoms have also been reported for clomifene including rare occurrences of cataracts and optic neuritis. In the phase 2 and 3 pool, the TEAEs “cataract” and “vision blurred” at least possibly related with treatment had a higher incidence in enclomifene group versus placebo (0.3% vs 0% and 0.5% vs 0%, respectively for each TEAE).

In subjects with moderate hepatic impairment, the potential consequences of higher plasma enclomifene concentrations on cardiac/vascular system, ophthalmic system, breast and reproductive system and bone in the context of chronic treatment is not known. The PK profile of enclomifene in subjects with severe hepatic impairment has not been investigated.

In subjects with moderate to severe renal impairment, the potential consequences of doubling of plasma enclomifene concentrations on cardiac/vascular system, ophthalmic system, breast and reproductive system and bone in the context of chronic treatment is not known.

Conclusions on the clinical safety

An increased risk of venous thromboembolism in patients treated with enclomifene has been reported in the clinical trials submitted in support of this application. This is a known concern in patients treated with testosterone replacement therapy and has also been associated with the mechanism of action of enclomifene as a modulator or the oestrogen receptor. Despite the small numbers reported, this risk is considered

important for patients known to be at increased risk due to common co-morbidities associated with the claimed indication.

Additional safety concerns for enclomifene are common to those associated with testosterone replacement therapy and include cardiac disorders, visual disturbances and increased laboratory parameters such as PSA and haematocrit.

Further studies would be required to characterise these risks, especially as available information on long term exposure is limited.

2.7. Risk Management Plan

The CHMP and PRAC, having considered the data submitted in the application were of the opinion that due to the concerns identified with this application, the risk management plan version 3.1 which was submitted in response to the second D180 joint overview PRAC/CHMP updated assessment report, cannot be agreed at this stage.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

The applicant declared that enclomifene is an isomer of clomifene and that they do not differ significantly in properties with regard to safety and efficacy.

The CHMP, based on the available data, considers that enclomifene is not a new active substance, as it is a constituent of a medicinal product previously authorised within the European Union. Clomifene is contained in the marketing authorisation of Clomid which has been authorised in the European Union.

2.10. Product information

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Enclomifene is intended for the treatment of secondary hypogonadism in adult men due to its mode of action as selective oestrogen receptor modulator (SERM)

Male hypogonadism results from insufficient secretion of testosterone by the testes and is characterised by low serum testosterone concentrations. Luteinising Hormone (LH), secreted by the pituitary, stimulates the Leydig cells of the testes to produce testosterone. Follicle Stimulating Hormone (FSH), also secreted by the pituitary, stimulates the process of spermatogenesis by the Sertoli cells of the testes. Testosterone is responsible for the normal growth and development of the male sex organs and for maintenance of secondary sex characteristics.

Symptoms associated with male hypogonadism include impotence and decreased sexual desire, fatigue and loss of energy, mood depression, increase in visceral fat, regression of secondary sex characteristics and osteoporosis.

The prevalence of hypogonadism in the EU has been reported in two population based studies to be between 2.1 and 12.8% (Haring, 2010; Tajar, 2012).

3.1.2. Available therapies and unmet medical need

Testosterone replacement therapy is the main alternative for the treatment of hypogonadism in men.

Secondary hypogonadism can be caused by a number of factors including disorders of both the hypothalamus and the pituitary. However, in males, obesity, stress and certain medications are the greatest contributors to this disorder, with obesity being the single greatest cause due to the negative feedback effects of oestrogen, which is produced by aromatase in fat tissue.

It is considered that there is no specific unmet medical need for enclomifene in overweight men with low testosterone, unless lifestyles measures such as diet and exercise have not been able to restore testosterone levels.

3.1.3. Main clinical studies

Four pivotal studies were provided to demonstrate the efficacy of enclomifene. Two of the studies (ZA-304 and 305) were conducted with AndroGel as active control. The other two studies were conducted as placebo controlled studies (ZA-301 and 302).

Studies **ZA-304** and **305** were randomised, double-blind, placebo-controlled multicentre Phase 3 studies conducted to compare changes in sperm concentration and testosterone following treatment with 12.5 mg or 25 mg of enclomifene, AndroGel or placebo in overweight men (BMI 25-42kg/m^2) with acquired hypogonadotropic hypogonadism and normal baseline sperm concentrations of $\geqslant$ 15 million/mL. Patients with

at least 2 consecutive assessments of morning testosterone below 300ng/dL, at baseline and LH less than 9.4mIU/mL at screening were recruited into the studies.

Studies **ZA-301** and **302** were randomised, double-blind, placebo-controlled studies conducted to evaluate normalization of morning testosterone levels in overweight men with acquired hypogonadotropic hypogonadism and normal baseline sperm concentrations treated with enclomifene. Overweight men (BMI 25-42kg/m2) with acquired hypogonadotropic hypogonadism, normal baseline sperm concentrations of $\geqslant$ 15 million/mL, at least 2 consecutive assessments of morning testosterone below 300ng/dL at baseline and LH less than 9.4mIU/mL at screening were recruited into the studies.

In addition to testosterone levels, the effect of treatment on sperm concentration was also evaluated in these studies as enclomifene was initially proposed as a treatment option for men with low testosterone wishing to preserve their reproductive status for which the applicant considered it will be clinically meaningful to demonstrate non-reduction in sperm concentrations.

3.2. Favourable effects

In study **ZA-304**, the proportion of subjects who had testosterone levels in the normal range [300-1040 ng/dL] was higher in the Androxal group (63.4%) in comparison to the AndroGel group (44.2%) and placebo group (4.4%). The same patterns were observed in study **ZA-305,** where subjects with normalised testosterone levels were 68.2%, 45.2% and 7.3% for the enclomifene, AndroGel and placebo groups, respectively.

In terms of the composite endpoint 'Comparison of the Proportion of Subjects Who Obtain a Successful Testosterone Level and Sperm Concentration (Mean Sperm Concentration ≥10 million/mL and 24-Hour Testosterone within Normal Range: 300-1040 ng/dL)'; in study **ZA-304**, 61.0% of subjects treated with enclomifene had mean sperm concentration > 10M/mL and T in normal range in comparison to 4.4% of placebo-treated subjects, and 16.3% of AndroGel-treated subjects. For study **ZA-305**, 65.9%of enclomifene treated subjects, 7.3% of placebo-treated subjects, and 33.3%of AndroGel-treated subjects had mean sperm concentration > 10M/mL and T in normal range.

The results of studies **ZA-301** and **ZA-302** suggest that after 12 weeks of treatment testosterone values are within normal range in more subjects on enclomifene when compared to placebo.

For study **ZA-301** in terms of sperm concentrations eighty-nine (78.8%) subjects treated with enclomifene in the ITT population achieved testosterone Cavg in the normal range. In study **ZA-302**, one hundred and nine (81.3%) subjects treated with enclomifene in the ITT population achieved testosterone Cavg in the normal range.

Subjects treated with enclomifene experienced an increase over baseline in FSH and LH at each post-treatment assessment.

In conclusion, the results of the four studies conducted suggest that testosterone levels may increase to normal range after treatment with a concurrent increase in LH and FSH levels but there is a lack of clarity regarding the efficacy of enclomifene on the signs and symptoms of secondary hypogonadism as this was not specifically studied.

3.3. Uncertainties and limitations about favourable effects

The subjects included in these studies were overweight (BMI 25-42kg/m2) with testosterone levels below 300ng/dl, LH below 9.4mIU/l and sperm concentrations greater 15 million/ml. However, subjects were selected and included into the studies based on their BMI rather than specific symptoms and signs of secondary hypogonadism. To overcome this issue, the applicant conducted a review of the medical history of the subjects to check for the signs and symptoms of secondary hypogonadism. This review suggests that for majority of the subjects included in the studies, the dominant problem was obesity (68.9%). A smaller percentage of subjects had symptoms suggestive of erectile dysfunction (approximately 12%), libido (6%) mood changes (approximately 10%). Overall, it cannot be concluded that the subjects included in the studies were truly hypogonadal or obese men who had not tried life-style modification such as diet and exercise and perhaps had low testosterone due to their obesity.

In addition, enclomifene when being developed was proposed for overweight men with secondary hypogonadism with sperm concentration >15 M/mL who wished to preserve their reproductive status. Therefore, it was considered important to demonstrate that enclomifene does not cause a reduction in sperm concentration while restoring testosterone concentrations. In study **ZA-301**, there was a statistically significant difference in the change in sperm concentration when compared with placebo which the applicant tries to disregard as being due to an artefact. The results indicate that testosterone concentrations are restored and sperm concentration appear to remain above 10 million/mL. However, the clinical relevance of these results remains unclear and it appears that enclomifene might not consistently preserve sperm function. However, it is noted that the applicant agreed to remove the reference to spermatogenesis from the indication.

Clinically meaningful end-points assessed through a validated, patient-reported questionnaire designed to assess the psychosexual function in terms of libido, erectile dysfunction were not included. These are considered important for demonstrating a clinically significant benefit of a product intended for the treatment of secondary hypogonadism

Overall, the results of all four studies are considered inconclusive as very few subjects included in the studies had predominant signs and symptoms of secondary hypogonadism at baseline. Therefore, although the results of the clinical studies suggest that testosterone levels are increased after administration of enclomifene, there is no evidence that enclomifene provides clinically meaningful benefits on the typical symptoms and signs of secondary hypogonadism especially as validated PRO measures were not studied and patients were not specifically recruited into the studies based on signs and symptoms of secondary hypogonadism.

Furthermore, physiologically-based endpoints such as reduction in body fat/ increase in muscle mass and strength/improvement in bone mineral density were not studied. This is unfortunate because overweight men were randomised into the study and an endpoint demonstrating reduction in body fat or BMI would have provided valuable information regarding the clinical benefit of enclomifene.

Therefore, it is not known if enclomifene provides any beneficial improvement in terms of reduction in body fat or increase in muscle mass strength and or improvement in bone mineral density.

3.4. Unfavourable effects

In terms of adverse events, the most frequently reported treatment emergent adverse events were headache, hot flushes, nausea and muscle spasms. The most common adverse events leading to discontinuation were blurred vision, muscle spasm, headache and aggression.

A number of adverse events that are known to be associated with testosterone replacement therapy were reported in the enclomifene clinical studies. These include venous thromboembolic events, cardiac disorders, increased haematocrit and PSA, eye disorders and psychiatric disorders. These events were reported with a higher frequency for enclomifene treated patients compared to the placebo group.

Four cases of thromboembolic events were reported, with one fatality occurring due to this. In contrast, no events were reported in patients treated with testosterone or placebo, even though the treated population included in these trials at an increased risk for such events.

The incidence of cardiac events in the enclomifene group was also slightly increased when compared to patients treated with testosterone (0.9% vs 0.7%).

3.5. Uncertainties and limitations about unfavourable effects

The safety population for Enclomifene comprised of 1403 persons who had received at least one dose of enclomifene in Phase 2 and Phase 3 studies. 482 patients were exposed to daily treatment with enclomifene citrate for duration greater than 6 months and 116 patients for duration greater than 12 months. The ability therefore to detect rare adverse reactions is limited, especially those that might occur following prolonged exposure to enclomifene.

Enclomifene is selective oestrogen receptor modulator (SERM). It should be noted that SERMs are associated with an increased risk of thrombo-embolic events (VTE). Thromboembolic events are also associated with testosterone replacement therapies due to increased erythropoiesis secondary to increasing testosterone and VTE are also associated with obesity. Therefore, it is unclear if the thromboembolic events occurred due to the increased testosterone and the resulting increased erythropoiesis or associated with class of drug or due to co-morbid conditions as the men included in the study were overweight or the result of an increase in oestradiol levels. Nevertheless, it is of concern that in a population with a high background incidence rate, such events were reported exclusively in the enclomifene treated patients. The exact magnitude of this risk remains unknown, especially as exposure to enclomifene over 12-months is currently limited.

3.6. Effects Table

Table 79. Effects Table for enclomifene for secondary hypogonadism in adult men aged ≤ 60 years with a body mass index (BMI) ≥25 kg/m

Effect	Short Description	Unit	Enclomifene	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	Enclomifene	Control	Uncertainties/ Strength of evidence	References
Testosterone concentration	Subjects within the normal range	%	ZA-304: 63.4% ZA-305: 68.2% ZA-301: 78.8% ZA-302: 81.3%	AndroGel ZA-304: (44.2%) Placebo: (4.4%) AndroGel ZA-305: (45.2) Placebo: (7.3%) Placebo Not provided	Due to mechanism of action normalisation of testosterone not sufficient to demonstrate a clinical meaningful effect.	ZA-304 ZA-305 ZA-301 ZA-302
Unfavourable Effects						
VTE	Patients experiencing adverse event	N	4	0		Pooled Phase 2 and 3 studies
Increased haematocrit		%	3%	AndroGel (6.1%) Placebo (0.3%)		
Increased PSA	Change in PSA >75 µg/L	%	9.3%	AndroGel (6.1%) Placebo (5.1%)	Clinical relevance of these increases is unknown	
Psychiatric disorders	TEAE at least possible related	N (%)	45 (3.2)			
Eye disorders	Patients with at least one TEAE	N (%)	56 (4.0)	AndroGel 4 (2.7%) Placebo 9 (2.3%)	Visual disturbances have been known to occur with clomifene	
Cardiac disorders	TEAEs in the cardiac disorders SOC	N %	12 (0.9)	Placebo 1 (0.7%)		

Abbreviations: VTE=Venous thromboembolism; PSA=Prostate Specific Antigen; TEAE: Treatment Emergent Adverse Event; SOC=System Organ Class

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The results of the studies suggest a trend towards normalisation of testosterone levels after treatment with a concurrent increase in LH and FSH with enclomifene. However these is no evidence that enclomifene will be clinically beneficial in the treatment of the symptoms and signs associated with secondary hypogonadism because of the failure to include physiologically-based endpoints such as reduction in body fat and increase in muscle mass or clinically meaningful end-points assessed through validated patient-reported questionnaires and designed to assess the psychosexual function in terms of libido, erectile dysfunction and the failure to include subjected with signs and symptoms of secondary hypogonadism. Therefore, a true clinical benefit of enclomifene in patients with secondary hypogonadism has not been established.

In terms of safety, safety concerns which are known to be associated with testosterone replacement therapy appear to also apply to enclomifene treatment. In addition, it is clear that there is an increased risk of thromboembolism. Even though the reported number of cases is low, these were only reported in patients receiving enclomifene. Furthermore, the true effect after long term treatment cannot be determined as there is limited data from patients exposed to enclomifene for more than 12 months.

There also appears to be a possible incomplete characterisation of the circulating metabolites in humans, therefore it is not possible to evaluate whether there are human major or unique metabolites which according to ICH M3R(2) need to be specifically addressed. Considering that the applied indication is for long term treatment rather that the short courses given for clomifene in the stimulation of ovulation, the preclinical studies are regarded as very important, especially regarding long term safety.

3.7.2. Balance of benefits and risks

The normalisation of testosterone levels need to be balanced against an increased risk of thromboembolic events and other risks associated with testosterone replacement therapies. Considering that the clinical relevance of enclomifene treatment has not been established due to the absence of evidence of a beneficial effect on the signs/symptoms of hypogonadism, the benefit/risk balance of enclomifene in overweight men with secondary hypogonadism is considered negative.

3.8. Conclusions

The overall B/R of EnCyzix is negative.

Divergent position is appended to this report.

4. Recommendations

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that enclomifene is an isomer of clomifene and is considered to not be a new active substance as it does not differ significantly in properties with regard to safety and/or efficacy from clomiphene contained in medicinal product previously authorised

within the European Union.

Outcome

Based on the CHMP review of data on quality, safety and efficacy for EnCyzix in the treatment of 'hypogonadotropic hypogonadism (secondary hypogonadism) in adult men aged ≤60 years with a body mass index (BMI) ≥25-40 kg/m2 which has been confirmed by clinical features and biochemical tests, and who have not responded to diet and exercise', the CHMP considers by majority decision, that the safety and efficacy of the above mentioned medicinal product is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the marketing authorisation for the above mentioned medicinal product. The CHMP considers that:

- The pivotal studies submitted showed a normalisation of testosterone levels in the patients included in these studies. However, due to the mechanism of action of enclomifene which has been shown to increase the levels of FSH and LH levels in addition to a direct antagonistic effect on the oestrogen receptors, 'normalisation of testosterone alone' is not sufficient to demonstrate efficacy. Further evidence would be required on the effects of enclomifene on the relevant physiological and clinical endpoints of secondary hypogonadism before a robust conclusion on the clinical benefits in this target population could be reached.
- Available data suggest that enclomifene is associated with a potentially high risk of venous-thromboembolism as a small number of these events, including one fatality, was reported in enclomifene treated patients but not in patients on the active comparators or on placebo. In addition, the available long-term safety data in non-clinical studies has not been shown to be sufficient to support the chronic treatment with enclomifene in males. This is due to the uncertainties with regard to the adequate characterisation of all the relevant human metabolites in the mass-balance study and all the relevant human metabolites should be demonstrated to be tested in the chronic toxicity studies. Therefore, as an effect of enclomifene on relevant clinical and physiological endpoints has not been adequately demonstrated, the identified risks and uncertainties around the safety profile of enclomifene outweigh its positive effect on serum testosterone levels.

Due to the aforementioned concerns, a satisfactory summary of product characteristics, labelling, package leaflet and risk management plan cannot be agreed at this stage.

The divergent position to the majority recommendation is appended to this report.

APPENDIX 1

Divergent position dated 25 January 2018

Divergent position – Encyzix (EMEA/H/C/004198)

Since serum testosterone is a well-established biomarker for clinically relevant effects on physiological parameters in patients with hypogonadism, it is not considered crucial to document such effects in the clinical studies. The effect of of emiclofene on s-testosterone concentrations was as least as high as the effect of Androgel which is an approved product for treatment of hypogonadism. This effect is fully supported by the mechanism of action of emiclofene.

Even though it is uncertain if the study population included in the pivotal studies is fully representative of the target population, the subjects had objectively defined hypogonadism and the study population is considered as a relevant population to study the effect of emiclofene on testosterone concentrations. A favourable effect of emiclofene is therefore considered as shown.

With respect to safety, both testosterone treatment and selective oestrogen receptor modulators have been associated with an increased risk of venous thromboembolism. 3 cases of DVT/PE and one fatal stroke were identified in enclomifene-treated patients. However all had, multiple risk factors for thromboembolism. Increased erythropoiesis secondary to increasing testosterone may also be a safety issue. These risks are considered to be mitigated by the proposed product information.

Therefore, with the provision of appropriate risk minimisation and post authorisation studies to further characterise the risks associated with the use of the product, the benefit –risk balance of enclomifene is considered positive for the treatment of patients with secondary hypogonadism who have not responded to diet and exercise interventions.

London, 25 January 2018

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