Early Mental Response - The EMRE Study

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Study Protocol

Clinical Study Protocol - English

Early mental response to hormonal treatment in transmen – The EMRE Study

EudraCT number:	2019-004349-34
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Date:	20220715
Sponsor:	Region Stockholm / Karolinska University Hospital, Stockholm, Sweden
Principal Investigator:	Mats Holmberg

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Signatures

Sponsor

I am responsible for ensuring that this protocol contains all the essential elements for conducting the study. I will provide the protocol and all other important study-related information to the responsible examiners so that they can complete the study in the right way. I am aware of my responsibility to keep the staff working on the study informed and educated.

Sponsor signature Date 220715

David Nathansson

Principal investigator

I have read this protocol and it contains all the essential parts to carry out the study. By signing, I agree to conduct the study in all its parts in accordance with this protocol, the informed consent, and to comply with the ICH GCP, the Declaration of Helsinki and the national and international regulations concerning the current clinical study.

I will share the minutes and all other important study-related information with the co-workers involved so that they can complete the study in the right way. I am aware of my responsibility to keep the staff working on the study informed and educated.

I am aware that quality control will take place of the study in the form of monitoring, audit and possible inspection.

Principal investigator signature Date 220715

Mats Holmberg

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List of used acronyms and abbreviations

Förkortning	**Förklaring**
AE	Adverse Event
AR	Adverse Reaction
CRF	Case Report Form
GCP	Good Clinical Practice
ICH	International Council for Harmonisation
ITT	Intention-to-treat
LVFS	Läkemedelverkets författningssamling (the Swedish medicial agency)
PP	Per Protocol analysis
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCS	Transgender Congruence Scale
SDI	Sexual Desire Inventory
BPAQ	Buss-Perry Aggression Questionnaire
BBQ	Brunnsviken Brief Quality of life scale
PERS-S	Perth Emotional reactivity Scale-Short Form
BIS-11	Baratt Impulsiveness Scale
MADRS	Montgomery–Åsberg Depression Rating Scale
Rosenberg	Rosenberg Self Esteem Scale

1. Synopsis

EudraCT number:	2019004349-34
Title:	Early mental response to hormonal treatment in transgender men – The EMRE Study
Brief background/Rationale/Purpose:	Gender dysphoria is a condition characterized by a perceived incongruence between the body and identity. For several decades now, this condition has been treated with cross-sex hormone therapy and surgery in order to change the body to be more congruent with the perceived gender identity. Patient satisfaction with this treatment is very high where an overwhelming majority of patients live the rest of their lives according to their perceived gender. A clinical observation, however, is that most patients experience that the congruence between the perceived gender and the assigned one improves very quickly on hormonal treatment. Long before any changes in the body have taken place. This may be partly due to a relief from having finally started treatment (i.e. a psychological / social explanation) but an alternative (and much more likely) explanation is that the hormonal treatment directly affects the brain. Since the cause of gender dysphoria is unknown today, this study is therefore a step in trying to clarify the mechanism. In addition, it is of value to be able to demonstrate the benefits of hormonal treatment in these patients. Finally, there is a basic research motive for this study. The effect of sex hormones on the brain is very well known from a clinical perspective but all the more unknown from a research perspective. This study will contribute knowledge in this area.
Aim:	Primary question: Does transsexual mens' experience of gender incongruence improve within 6 weeks of hormonal treatment? Long before any bodily changes occur. Secondary issue: Does transmens' experience of self-confidence, aggression, depression, anxiety, emotional reactivity, quality of life, impulsiveness and sexual desire change during 6 weeks of sex-hormone replacement therapy compared to placebo.

Study design:	Prospective, randomized, placebo controlled, double blind study
Study population:	Individuals assigned female sex at birth who have received the diagnosis transsexualism but who have not yet started hormonal therapy (transmen)
Number of studypersons	70
Inclusion criteria:	• Given written consent to participate in the study. • Transman diagnosed with transsexualism, as defined in ICD-10. • A desire for complete sex-confirming hormonal treatment • Approved for Nebido treatment by a clinically responsible Endocrinologist
Exclusion criteria: • Concomitant hormonal condition affecting the gonadal axis (e.g. CAH, PCOS, CAIS, PAIS, untreated thyroid disease, untreated hypercortisolism, etc.) • Disability that prevents the patient from fully participating in the study. • Treatment with steroid hormones (androgens, estrogens, progestogens or continuous treatment with per oral corticosteroids within the last three months. Previous use of hormone preparations without a doctor's prescription. • Laboratory samples which, according to responsible endocrinologist, fall significantly outside the normal reference range. • Anamnestic or investigational suspicion of breast cancer or existing or previous liver tumours. • Levels of P-ASAT, P-ALAT or P-GT at the screening time which is > 2 times the reference range • Hypersensitivity to the active substance or to any of the excipients • Ongoing pregnancy or wishes for an early pregnancy	
Test drug, dosage, administration:	Nebido 4 ml/1000mg administered intramuscularly. One single dose included in the study.

Effect evaluation:	Primary variable: Difference in score in Transgender Congruence Scale between inclusion and 6 weeks of treatment with testosterone or placebo Secondary variable: Difference in score in Rosenbergs Self-confidence Scale, Sexual Desire Inventory, Montgomery Åsberg Depression Rating Scale, Perth Emotional reactivity Scale-Short Form, Buss-Perry Aggression Questionnaire, Baratt Impulsivness Scale and Brunnsviken Brief Quality of life scale between inclusion, 2 weeks of treatment and after 6 weeks of treatment with testosterone or placebo
Study period:	Q 3 2022 – Q4 2025

2. Background information and rationale

2.1 Gender dysphoria is currently a psychiatric diagnosis in Sweden (8). To meet the criteria of the DSM-5 Diagnostic Manual, the patient must demonstrate an incongruity between their gender identity and their assigned gender that causes significant distress or impairment (1). The patient must also express a strong desire to be seen and treated as a member of a gender other than that assigned at birth and / or a strong desire to change their primary or secondary gender characteristics. The diagnostic criteria in ICD-10 are less specific but state that the patient must wish to live and be accepted as "the opposite sex" (i.e. opposite to that assigned at birth); and that this desire is usually accompanied by a desire to make the body more congruent with its gender identity, through hormone therapy and surgery (2). ICD-10 uses the term "transsexualism" rather than "gender dysphoria".

There is currently much debate in both the medical and LGBTQ communities about whether gender dysphoria should be depatologized and the term "transgender" used by the LGBTQ community contains a wider range of gender identities and behaviours than the diagnostic criteria for DSM-5 and ICD-10 (9). In fact, a large proportion of self-identified transgender people defines their gender as non-binary, and often do not seek gender-confirming healthcare either (10,11). For the purpose of this study, however, we limit ourselves to binary transgender people, by defining gender dysphoria (transsexualism) in accordance with the diagnostic criteria for ICD-10. Diagnostic criteria for gender dysphoria in children and adolescents differ from the criteria for adults (1,2). This study only concerns gender dysphoria in adults. And for practical reasons only trans gender men.

A meta-analysis conducted by Collin *et al* 2016 found that studies aimed at determining the prevalence of gender dysphoria have yielded very different results (11). Trans-related diagnoses as well as surgical and hormonal gender correction are rare, with an estimated incidence of 6.8 and 9.2 / 100,000 individuals, respectively. However, self-reported trans identity is much more common, with an estimated prevalence of 871/100 000 individuals. Gender dysphoria is associated with significant suffering, reduced quality of life and high self-reported disabilities (3,10). Comorbidity with other psychiatric diagnoses is high (12),

although part of the comorbidity is probably due to what stems from the dysphoria itself, in combination with minority stress and discrimination (3.12). E.g. Unemployment as a result of discrimination can lead to poverty and reduced access to care, which increases the risk of mental or physical health problems (12).

2.2 Gender-confirming hormone therapy

Hormone therapy (HB) is in most cases an important part of gender-confirming care of binary trans people (13,14). In transwomen (assigned men at birth), hormone therapy usually consists of estrogen - usually estradiol - in combination with an anti-androgen (5). Treatment regimens in the world differ depending on local tradition and legislation (5). In Europe, cyproterone acetate - a progestogen that also has antiandrogenic properties - is widely used (5,15). Other treatment options are spironolactone - a diuretic that has antiandrogenic properties - and GnRH agonists. In transgender men (assigned women at birth), treatment usually consists of testosterone alone (5). Method of administration is either topical gel or intramuscular injection. This study includes only transgender men, assigned female sex at birth and are consequently treated with testosterone.

2.3 Effects of estradiol on the brain

There are three main forms of estrogens in humans: estrone, estradiol and estriol (17). Estradiol is the predominant endogenous form, and the one commonly used as part of HB in trans women (5,17,18). In trans men, the total levels of estradiol are lowered to levels equivalent to those of testosterone administration, although estradiol is also a natural metabolite of testosterone. This lowering of estradiol levels also affects the brain. Estradiol is a steroid hormone that diffuses easily across the cell membrane (17). In the cell cytoplasm or nucleus, it binds to and activates estrogen receptors, which in turn regulate the expression of a large number of genes (19). There are also non-genomic pathways, which are less well understood (20). Estradiol also diffuses easily across the blood-brain barrier (BBB), in a process that is not saturable, so the estradiol levels in the central nervous system (CNS) mainly reflect the concentration in the blood (21). The effects of estrogens in the brain are significant, complex and incompletely understood. Cyr et al. (2000) states in their review article on drugs with estrogen-like effect and activity: "Changes in dopaminergic, cholinergic, GABAergic, glutamatergic and serotonergic neurotransmission through estrogen-mediated mechanisms have been clearly demonstrated." (22)

Estradiol and testosterone are both mood boosters (18). Fluctuating levels of estradiol, sudden falls in estradiol concentrations and persistent estradiol deficiency are all linked to mood swings. A proposed mechanism for the effect of estradiol on mood is that it increases the rate of degradation of monoamine oxidase. It also increases intraneuronal serotonin transport. Both measures increase the availability of serotonin and other monoamines in the synapses, thus affecting mood. Estradiol has also been shown to increase the availability of 5-hydroxytryptamine (serotonin) 3A binding sites in areas of the brain relevant to mood and cognition (18), and to increase binding to the serotonin 2A receptor - the most common serotonin receptor in the brain (23). This may explain the synergistic effect seen in some studies in which depression was treated with estradiol in combination with selective serotonin

reuptake inhibitors (SSRIs) (18). Estradiol also acts as an anti-inflammatory and antioxidant and has positive effects on cognitive function. It stimulates regeneration of damaged neurons and production of neurotransmitters.

In cis women, estradiol levels fluctuate depending on the menstrual cycle and the period of their life they are in (18). Periods during which estradiol fluctuates more drastically - puberty, pregnancy (especially the postpartum period) and perimenopause - are associated with an increased incidence of mood swings. A remarkably high percentage (50-80%) of women experience a certain degree of "postpartum blues". It has been suggested that this is caused by the sudden decrease in estrogen that follows postpartum.

The perimenopausal period has been clearly linked to depressive symptoms (18). This period is characterized by high estrogen variation with estrogen levels that are higher on average, but even more irregular than during other periods of life. Estradiol in the form of HRT is often prescribed during this period, mainly to relieve physical symptoms such as hot flashes and fatigue, but there is also evidence to support a positive effect of estradiol supplementation in the treatment of depression during perimenopause.

There is conflicting evidence for postmenopausal depression, when estradiol levels are low but stable, and estrone becomes the main form of estrogen. There are some studies that suggest that estradiol replacement therapy may relieve symptoms of depression in postmenopausal patients and that it may also increase the effect of fluoxetine - an SSRI antidepressant.

In a review of the last 30 years of estrogen and affective disorder research, Douma et al conclude: "Although other factors may be involved, there is a link between estrogen activity (whether it is determined by the actual level, rate of change, or cascades of subsequent events) and affective symptoms in women well established in the literature reviewed. "(18)

2.4 Effects of testosterone on the brain

Like estradiol, testosterone is a steroid hormone that diffuses easily across the BBB, as well as across the cell membrane (24). Its effects are mediated mainly via the androgen receptor (AR). There are also non-genomic pathways of androgenic activity, although they are less well known (20). AR is a nuclear receptor and acts as a transcription factor.

Testosterone can affect target cells directly or through conversion to the more potent form of dihydrotestosterone (DHT), or via conversion to estradiol (25). Both testosterone and DHT act directly on the androgen receptor, but DHT has a higher affinity for it. The relative concentrations of the enzymes that mediate the conversion of testosterone to DHT or estradiol (5-alpha-reductase and aromatase, respectively) vary between different tissues. In the central nervous system, aromatase is abundant in some nuclei, suggesting that the effects of testosterone can be mediated through conversion to estradiol there.

Serotonin plays a central role in mood, stress regulation and cognition (23). Testosterone affects serotonin signalling, although serotonergic neurons do not express androgen receptors. A PET study has shown that serum testosterone levels correlate with a proxy

measure of serotonin tone in healthy men, suggesting that men with high serum testosterone have a higher serotonergic tone. However, the clinical relevance of this is unclear.

Much of the research has focused on testosterone in relation to sexual behaviour and function (25). Several studies have found that testosterone plays an important role in sexual desire, sexual thoughts and sexual activity in men. The positive effects on male sexual function appear to be more pronounced in men with deficiency / hypogonadal men. Positive effects of exogenously administered testosterone on sexual function have also been shown in women who are androgen deficient due to natural or surgical menopause.

The correlation between testosterone and aggression has been thoroughly studied (25). Physically and verbally aggressive behavior has been suggested to correlate with high levels of plasma testosterone in men. However, the correlation seems to be more pronounced in young men and in men with very high levels of testosterone, while moderate supraphysiological levels of testosterone have not been linked to increased aggression.

As previously mentioned, both estradiol and testosterone are mood boosters (18). The symptoms of depression are very similar to the symptoms of hypogonadism in men, which can make it difficult to distinguish between these conditions (25). They share symptoms such as depression, fatigue, decreased libido, energy and general joy in life. While each condition has properties that the other lacks, many studies suggest a link between depression and hypogonadism in men. Lower testosterone levels have been found in depressed men compared to healthy controls, especially in cases of severe or treatment-resistant depression. The correlation between testosterone and depression in women has not been studied extensively, but low serum testosterone and other androgens have been associated with depression in women, especially in older women, and there are studies showing improvement in depressive symptoms with testosterone treatment. However, a study has shown that both high and low plasma concentrations of testosterone correlate with depression in women (26). In addition to depression, the correlations between several other mental illnesses and abnormal levels of testosterone have been studied (25). Low levels have been found in schizophrenia and chronic sleep disorders. High levels have been found in post-traumatic stress and have been associated with low stress tolerance in young men. Improvement of obsessive-compulsive disorder with antiandrogen therapy has been reported in some studies, as well as increased severity of Tourette-related symptoms with testosterone therapy.

2.5 Mental effects of hormonal therapy in trans men

Specifically in the trans-male group, increased sexual desire after HB has been reported in a number of publications. Whether these changes are due to hormone therapy or the fact that starting treatment is seen as a period of joy after an often long investigation period is not known. Decreased sensitivity / sensitivity after hormone insertion has also been reported.

3. Risk-benefit evaluation

The cause of gender dysphoria is today basically unknown. The condition is mainly considered congenital where abnormal development of the brain during the time gender

identity and phenotype develops is a theory. Clinically, this condition has been treated for several decades with adaptation of the body to the perceived identity with the help of e.g. gender-opposite hormone therapy and surgery. Patient satisfaction with this treatment is high and an overwhelming majority of these patients live the rest of their lives as the perceived gender.

After patients have been investigated and found to meet the diagnostic criteria for transsexualism (F64.0, ICD-10), they are referred to an endocrinologist for initiation of sex-opposite hormone therapy. In trans men, this treatment of testosterone consists either in the form of a daily supply of gel or in the form of long-acting injections (Nebido). An overwhelming majority are treated with Nebido.

A clinical observation is that patients experience a marked improvement in their gender incongruence already after a short period of gender-opposite treatment. I.e. long before any changes in the body occurred. The most likely explanation for this is a direct hormonal effect on the brain.

This issue is very important from a variety of perspectives. Partly regarding the question of what causes the actual condition of gender incongruence: Partly regarding which treatment works and finally partly from a basic research perspective where many of the sex hormones' effect on the brain are well known in clinics with poor research.

The benefit is thus very great with this study. Performing randomized, placebo-controlled studies on the trans group has long been considered impossible. However, thanks to the fact that this study has been planned in consultation with transgender people, a design has been constructed that enables comparison with placebo.

In terms of risks, patients receive an injection of Nebido (4 ml / 1000mg) or placebo. This is standard clinical treatment and when it is time for syringe no. 2 (after 6 weeks), the study is broken for the individual participant. For those receiving placebo, the study is also discontinued after 6 weeks of treatment and then they also receive Nebido according to clinical routine.

Risks with the injection are local tenderness at the injection site and transient cough associated with the injection. These risks are well known and transient.

No increased risks arise through participation in the study compared with standard clinical treatment.

4. Objectives

The aim of this study is to capture and describe the early effects on the brain of sex-opposite hormone therapy of trans men.

Primary variable: The difference in points on the Transgender Congruence Scale between inclusion, 2 weeks after treatment and 6 weeks after treatment with testosterone or placebo

Secondary variable: The difference in points on Rosenberg's self-confidence scale, Sexual Desire Inventory, Montgomery Åsberg Depression Rating Scale, Perth Emotional reactivity Scale-Short Form, Buss-Perry Aggression Questionnaire, Baratt Impulsivness Scale and Brunnsviken Brief Quality of life Scale between inclusion, 2 weeks of treatment and after 6 weeks of treatment with testosterone or placebo.

4.1. Primary aim

The primary purpose of this study is to study whether treatment with testosterone provides greater improvement in the experience of gender incongruence compared to placebo in trans men.

4.2. Secondary aim

The secondary purpose of this study is to describe changes in self-esteem, aggression, sexual desire, emotion, quality of life, impulsiveness and anxiety and depression during the first 6 weeks of hormonal treatment.

4.3. Primary variabel

Difference in points on the Transgender Congruence Scale between inclusion, after 2 weeks of treatment and after 6 weeks of treatment with testosterone or placebo.

4.4. Secondary variabel

Difference in points on Rosenberg's self-confidence scale, Sexual Desire Inventory, Montgomery Åsberg Depression Rating Scale, Perth Emotional reactivity Scale-Short Form, Buss-Perry Aggression Questionnaire and Brunnsviken Brief Quality of life Scale between inclusion, after 2 weeks of treatment and after 6 weeks of treatment with testosterone or placebo.

5. Study design and Procedures

5.1. Overall study design

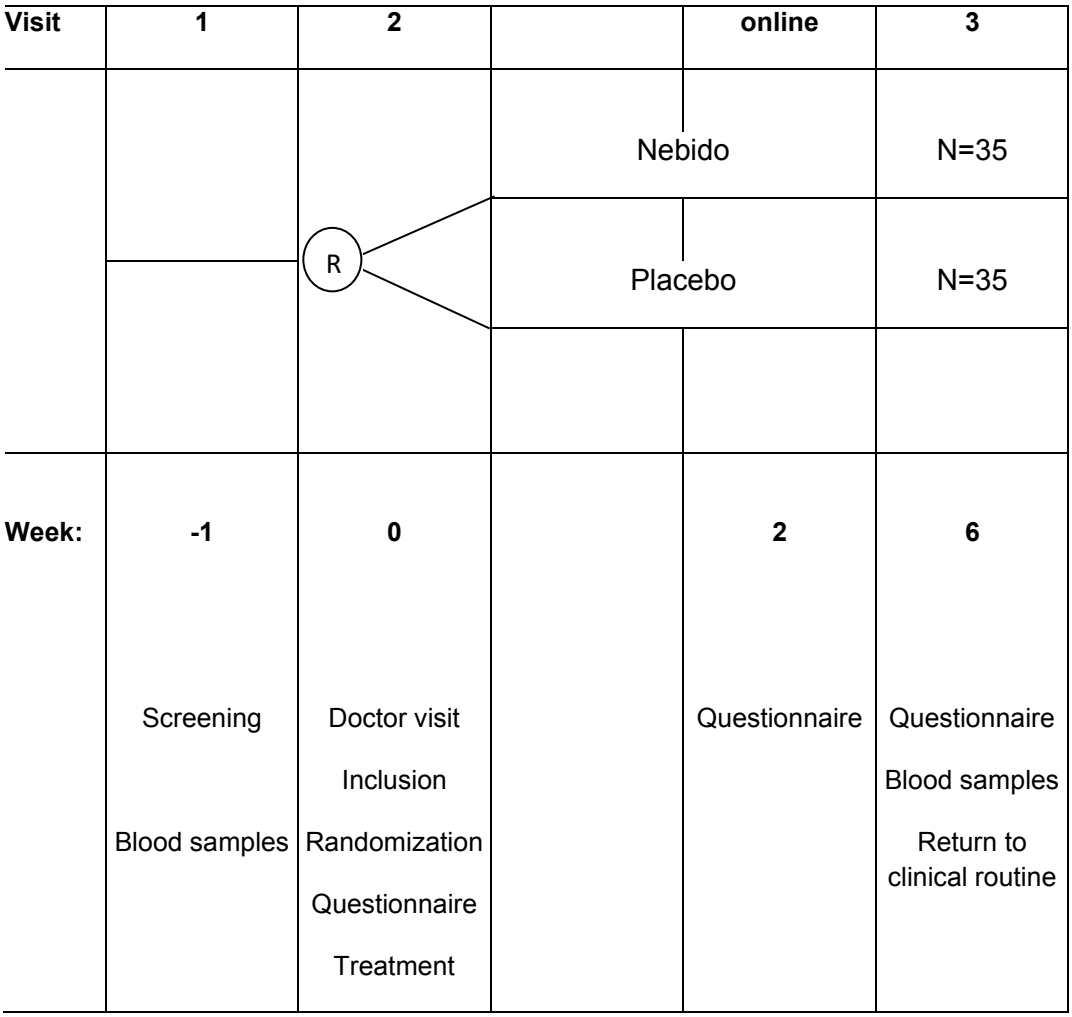
This is a prospective, randomized, double-blind placebo-controlled study of 50 individuals with assigned female sex at birth and recently diagnosed with transsexualism (F64.0)

The diagnosis of transsexualism is made by a psychiatrist, after which the patient is referred to an endocrinologist who makes his own assessment of the suitability of hormonal treatment.

There is a long, well-established clinical treatment of these patients with testosterone.

In the study, participants are treated with testosterone (Nebido) in standard dosage according to SmPC or placebo, both in injection form. Nebido treatment is started with two injections 6 weeks apart and every 12 weeks thereafter. To minimize deviations from clinical routine, the study length has therefore been set at 6 weeks, after which all patients are offered continued treatment according to clinical routine.

Figure 1



5.2. Procedures and flow chart

Table 1 Flow chart

Procedures	Visit 1	Visit 2 Inclusion	Online Week 2 (+- 3 days)	Visit 3 Week 6 (+- 3 days)
Incl/exclusion criteria	√			
Informed consent	√			
Medical history/current medication	√			
Blood samples	√			√
Randomization		√*		
Injection Nebido or placebo		√		
Filling form CRF		√		√
Filling of all scales (8 in total)		√	√	√
Unwanted events (AE & SAE)		√	√	√
Study ends				√

5.3. Biological sampling procedures

5.3.1. Handling, storage and destruction of biological samples

Sampling for safety and efficacy monitoring is performed according to standard at ANOVA before inclusion (before drug administration). The following assays include S-Testosterone, S-Estradiol, S-LH, S-FSH, S-SHBG, P-ASAT, P-ALAT, S-25-OH-Vitamin D, B-Hb, B-EVF, B-MCH, B-MCHC, B-LPK, B-TPK, P-Ca, P-CRP, P-Phosphate, fS-Insulin, P-Glucose, P-GT, B-HbA1c, P-HDL, fP-LDL, fP-Cholesterol, fP-Triglyceride, P-Na, S-Prolactin, S-fT3, S-fT4 and S-TSH. After 6 weeks of treatment, S-Testosterone, S-Estradiol, S-LH, S-FSH, S-SHBG are checked. All samples are analyzed at Karolinska University Hospital Laboratory. No samples are saved.

5.3.2. Total volume blood per study subject

The total volume of blood taken from each study subject during the study is a maximum of 40 ml.

5.4. Study end

The study ends when the last researcher has completed the last follow-up (LSLV).

The study may be terminated prematurely if it turns out that the treatment causes a large number of unwanted serious events or if the recruitment of research personnel cannot be fulfilled within a reasonable time limit. If the study is terminated prematurely, or temporarily stopped, the investigator shall immediately inform the researchers of this and ensure appropriate treatment and follow-up. The regulatory authority should be informed as soon as possible, but no later than within 15 days.

Decision on early termination of the study is made by the sponsor.

6. Selection of study subjects

6.1. Inclusion criteria

To be included in the study, the participant must meet the following criteria:

- The subject has given their written consent to participate in the study.
- Transgender people diagnosed with transsexualism, as defined in ICD-10.
- A desire for complete sex-confirming hormonal treatment
- Approved for Nebido treatment by a clinically responsible Endocrinologist

6.2. Exclusion criteria

Participants may not be included in the study if any of the following criteria are met:

- Concomitant hormonal condition affecting the gonadal axis (e.g. CAH, PCOS, CAIS, PAIS, untreated thyroid disease, untreated hypercortisolism, etc.)
- Disability that prevents the patient from fully participating in the study.
- Treated with steroid hormones (androgens, estrogens, progestogens or continuous treatment with per oral corticosteroids within the last three months. Previous use of hormone preparations without a doctor's prescription.
- Laboratory samples which, according to testers, fall significantly outside the normal reference range.

- Anamnestic or investigational suspicion of breast cancer or existing or previous liver tumors.
- Levels of P-ASAT, P-ALAT or P-GT at the screening time which is> 2 times the reference range Hypersensitivity to the active substance or to any of the excipients.
- Hypersensitivity to the active substance or to any of the excipients.
- Ongoing pregnancy or wishes for an early pregnancy.

6.3. Management of pregnancy

Nebido is contraindicated during pregnancy. For those participants who do not have penetrative sex with men and who do not plan any assisted reproduction within 9 months from the start of the study, this anamnestic task is sufficient. For those study participants who have penetrative sex with men, pregnancy tests are checked before inclusion if pregnancy is possible. An absolute requirement during the study period for these is also the use of a barrier method. Barrier method is not a requirement for a male partner who is vasectomized or if the included trans male has ligated the fallopian tubes.

6.4. Screening

That subjects meet all inclusion criteria and have no exclusion criteria is determined before inclusion, treatment or randomization takes place.

6.5. Discontinuation criteria

Subjects can terminate their participation in the study at any time without this having any consequences for the participant's continued treatment. The examiner / sponsor can at any time terminate the study for a researcher due to for example, unacceptable side effects or that the researcher does not follow the procedures in the study protocol. If the researcher interrupts the study, follow-up of the person will be performed according to the clinic's routine.

6.6. Handling of other medication during the study period

Permitted or prohibited medication during the study. Stable treatment with antidepressants, anxiety relievers, sleep medications, central stimulants, statins and blood pressure therapy is allowed during the study. Pain medication or antipyretics if needed are allowed within the study. New or discontinuation of psychoactive substances such as antidepressants, central stimulants or antipsychotics is noted but does not lead to exclusion. Drugs that affect the gonad axis as aromatase inhibitors or androgen receptor inhibitors are not allowed during the study. Cortisol synthesis inhibitors are also not allowed. Ongoing use of drugs (cannabis, central stimulants, opioids) is not permitted.

6.7. Management of possible interactions

Ongoing treatment with drugs that may interact with testosterone such as warfarin or, if necessary, medication or inhalation medication of corticosteroids does not lead to exclusion but is followed by special consideration of the possible consequences of interaction. New introduction of the above-mentioned substances also leads to increased vigilance with regard to possible interactions.

7. Trial drugs

7.1. Description of trial drug

Nebido 4 ml / 1000mg. Manufactured by Bayer AB. Each vial / vial of 4 ml solution for injection contains 1000 mg testosterone undecanoate equivalent to 631.5 mg testosterone.

Excipient with known effect: 2000 mg benzyl benzoate per ampoule / vial. Castor oil, refined.

Control consists of placebo.

Medicines and placebos are provided by Bayer AB

7.2. Dosage and route of administration

The drug is delivered by Bayer to Tamro AB, which labels and delivers to ANOVA. The drug can be stored at room temperature.

7.3. Packing, labeling, and handling

Läkemedlet levereras av Bayer till Tamro AB som märker och levererar till ANOVA. Läkemedlet kan förvaras i rumstemperatur.

7.4. Traceability and adherence to the treatment of the trial drug

Not applicable. Injection is given by a nurse.

7.5. Randomization

The subjects are included / randomized consecutively as they are found suitable to be included in the study. If a researcher interrupts their study participation, the researcher code will not be reused, and the researcher will not be allowed to be included in the study again. Randomization will be performed by Karolinska Trial Alliance (KTA).

7.6. Blinding

All participating research staff will be blinded to the treatment the patient is receiving. The drug and placebo will be labeled by Tamro AB, which will ensure that Nebido and placebo cannot be separated.

7.7. Breaking code

In connection with inclusion in the study, all participants will receive a card stating that they are part of a clinical drug trial with Nebido or placebo. This card contains contact information for ANOVA for contact during office hours Monday to Friday and for the principal examiner responsible for contact in the evenings / nights and weekends when the reception at ANOVA is closed. Code-breaking list for code-breaking is stored on ANOVA. If a situation requiring code breach arises, the sponsor will be contacted by the responsible principal examiner.

7.8. Treatment after the end of the study

Continued treatment according to clinical routine.

8. Assessment of efficacy and safety

8.1. Primary variable

The primary variable is the delta value in points on the Transgender Congruence Scale. Clarified expressed the difference in points on TCS at inclusion, after 2 weeks of treatment and after 6 weeks of treatment.

8.2. Secondary variable

Difference in points on Rosenberg's self-confidence scale, Sexual Desire Inventory, Montgomery Åsberg Depression Rating Scale, Perth Emotional reactivity Scale-Short Form, Buss-Perry Aggression Questionnaire, Baratt Impulsivness Scale and Brunnsviken Brief Quality of life Scale between inclusion, after two weeks of treatment and after 6 weeks of treatment with testosterone or placebo.

9. Management of adverse medical events

9.1. Definitions

9.1.1. Adverse Event (AE)

AE: Any adverse medical event or deterioration of an existing medical condition in a researcher who has received a trial drug, whether or not it is causally related to treatment, i.e. may be an unfavorable and undesirable sign (including an abnormal laboratory finding), symptom or disease that is temporally associated with the use of a trial drug, whether or not it is related to the trial drug.

9.1.2. Adverse Drug Reaction (ADR)

In clinical use before a new drug is approved or before its new uses are approved, and especially when the therapeutic dose cannot be determined, any adverse and unintended

response to a drug, regardless of dose, should be considered adverse drug reactions ADR (Adverse Drug Reactions). The phrase "response" to a drug means that a causal link between a drug and a side effect is at least a reasonable possibility, i.e. that the relationship cannot be ruled out.

9.1.3. Serious Adverse Event (SAE)

Any adverse event occurring at any dose:

- results in death
- is life threatening
- causes hospitalization or extended hospitalization
- causes permanent or significant disability or impairment
- results in a congenital injury / malformation

Medical and scientific assessment should be performed to determine if an event is "serious" and if it would lead to reporting in other situations, such as important medical events that may not be directly life-threatening or result in death or hospitalization, but may endanger the researcher or may require intervention to prevent one of the other results given in the definition above. These should also normally be considered as SAEs.

9.1.4. Suspected, unexpected, serious adverse reaction (SUSAR)

SUSAR: A reaction / event that is unexpected, serious and suspected to be caused by the treatment, i.e. side effects not included in the Summary of Product Characteristics (SmPC).

9.2. Assessment of adverse medical events

9.2.1. Assessment of causation

The investigator is responsible for determining whether there is a causal link between AE / SAE and the use of a trial drug.

The AEs that are suspected of having a connection with the investigational medicinal product will be followed up until the researcher has recovered or been well cared for and is well on his way to recovery (see also section 9.4).

All AEs should be categorized as probably related, possibly related or not related as defined below:

Probably related: Clinical event, including abnormal responses from laboratory tests, occurring within a reasonable time after administration of the intervention / trial drug. It is unlikely that the event can be attributed to an underlying disease or other drug, but it is most likely caused by the trial drug and the occurrence is reasonable in connection with the use of the trial drug.

Possibly related: Clinical event, including abnormal responses from laboratory analyzes, occurring within a reasonable time after administration of the intervention / trial drug. The event can be explained by the trial drug and the occurrence is reasonable in connection with the use of the trial drug, but there is not sufficient information to establish the relationship. The event can be explained by the underlying disease or other drugs.

Not related: Clinical event, including abnormal responses from laboratory tests, but which is not reasonable in relation to the use of the intervention / trial drug. The event is unlikely to be related to the intervention / trial drug and may be explained by other drugs or underlying disease.

9.2.2. Assessment of intensity

Any adverse medical event should be classified by the examiner as mild, moderate or pronounced.

Mild: The event is relatively mild and transient in nature but does not affect the researcher's normal life.

Moderate: The event causes deterioration of function but does not affect health. The event may be unpleasant enough and interfere with normal activities but does not completely hinder them.

Pronounced: The event causes impairment of function or ability to work or poses a health risk to the researcher.

9.3. Reporting and recording of adverse medical events

During the study visit after six weeks, undesirable events (AE) are registered. All AEs that occur during the study and that are observed by the investigator / research nurse or reported by the researcher will be registered in the CRF regardless of whether they are related to the investigational medicinal product or not. Assessment of causation, intensity and whether AE is judged to be an SAE or not is done by the examiner directly in CRF. As a minimum, a description of the event is registered for each AE / SAE (diagnosis / symptoms if there is no diagnosis), start and stop dates, causal relationship, intensity, whether AE is judged to be an SAE or not, measures and outcome.

9.3.1. Reporting of adverse medical events (AE)

All AEs must be registered in the CRF within 3 days as above (paragraph 9.3).

9.3.2. Reporting of serious adverse medical events (SAE)

Serious adverse medical events (SAEs) are reported to the sponsor on a special SAE form within 24 hours of the examiner becoming aware of SAEs.

9.3.3. Reporting of suspicious, unexpected, serious adverse reactions (SUSAR)

The SAEs that are assessed by the sponsor as SUSAR are reported on a CIOMS form to the Norwegian Medicines Agency (Eudra Vigilance database) according to the specified time frames.

As the sponsor does not have the opportunity to report directly in the EudraVigilance database, the Medical Products Agency is asked to help with this when a SUSAR occurs in

Sweden. Reporting then takes place on a CIOMS form which is sent to the Medical Products Agency by post. As these reports contain personal data, they should not be sent to the Medical Products Agency by regular e-mail. SUSARs that are reported should, if possible, be reported blinded, i.e. with an indication of which trial drug the subject experienced side effects. Placebo should only be reported if it is suspected that some component of the placebo preparation has caused the side effect.

SUSARs that are fatal or life-threatening are reported as soon as possible and no later than within 7 days after the occurrence has become known to the sponsor. Relevant follow-up information is then submitted within a further 8 days. Other SUSARs are reported as soon as possible and no later than within 15 days after they have come to the sponsor's notice.

9.4. Follow-up of incidents (AE / SAE)

Patients are treated with only one injection. That is, discontinuation of treatment is not possible. Should the patient suffer a serious event after the first injection, it will primarily be handled by a doctor at ANOVA, and secondarily by a doctor at the nearest emergency department. All AEs will be followed up by the responsible examiner until the patient has stabilized or recovered. AE will be reported after patient safety is ensured.

9.5. Annual Safety Report (DSUR)

The annual safety report to the Medical Products Agency will be submitted by the sponsor.

9.6. Reference safety information

As reference safety information, the product summary comes from Fass dated: First approval: 2004-10-15; Renewed approval: 2008-11-25 to be used.

9.7. Analysis population

The study will include 70 individuals with assigned female sex (trans men) who have recently been diagnosed with transsexualism (F640) and who have a desire for sex-opposite hormone therapy in the form of Nebido injections.

9.8. Statistical analysis

9.8.1. Statistical method

Patients with active substance and placebo will be compared with the Mann-Whitney U test. Associations between variables will be analyzed with Spearman's rank test Spearmans rank test.

Significance test is two-sided and the significance level is 0.05. Study results will be reported as differences in median values with associated interquartile range and p-values.

All patients who underwent randomization and received a dose (1000mg / 4ml) of Nebido are included in the ITT population. All patients who received a dose are included in the safe population.

9.8.2. Loss

With good knowledge of the population, we expect a very low dropout rate.

9.9. Dimension calculation

Based on the publication Owen-Smith AA et al, 2018 “Association between Gender Confirmation Treatments and Perceived Gender Congruence, Body Image satisfaction, and Mental Health in a Cohort of Transgender Individuals. J Sex Med 15:591-600” and other consumptions, the hormonal treatment arm’s average increase during six weeks of treatment is estimated to six steps in the Transgender Congruence Scale from 30 to 36 with a standard deviation of 4,5. The placebo arm is estimated to have an average increase of 2 steps with a standard deviation of 6,5. If you test the following with a Mann-Whitney U-test and a power of 0,8 it will result in a total of 66 patients (33 Hormonal treatment and 33 Placebo). With a drop out of 5% 70 patients will be recruited to the study.

A clinical assessment is that a rapid change in the experience of gender dysphoria is very common. Long before any changes have taken place in the body.

As the study design follows clinical routine, we expect very little dropout.

9.10. Monitoring

To ensure that the study is conducted according to the protocol, that data is collected, documented and reported according to ICH-GCP and current ethical and regulatory requirements, the study will be monitored by an independent monitor before the study begins, during the study and after the study ends. . The monitoring is performed according to the study's monitoring plan and aims to ensure that the researchers' rights, safety and well-being are met and that the data in CRF are filled in, correct and in accordance with the source data. Monitoring will take place by Karolinska Trial Alliance (KTA).

9.11. Source data

The examiner must keep source documents for each researcher in the study. A document on what is classified as source data in the study must be in the investigator's file (Investigator Site File, ISF). The tester must ensure that all source documents are available for monitoring and other quality control.

9.12. Deviations or serious violations

Violations and deviations from trial protocols, GCP and other regulations that in a significant way directly affect, or in all probability would affect, researchers in Sweden or the scientific

value of the trial must be reported immediately within 7 days (from knowledge) to LV. It is the sponsor's responsibility to assess the consequences of deviations that have occurred, and thus also decide whether LV should be informed.

Minor deviations that do not affect the integrity or safety of the researchers, or significantly affect the scientific value of the trial are documented in the trial documentation of the main investigator and the sponsor.

9.13. Audit and inspections

Authorized representatives of the sponsor and competent authorities may perform audits or inspections at the test site, including source data verification. The examiner must ensure that all source documents are available for audit and inspection. The purpose of an audit or inspection is to systematically and independently review all study-related activities and documents, to determine if these activities were performed, registered, analyzed and reported correctly according to the protocol, Good Clinical Practice (GCP) and applicable regulations.

10. Ethics

10.1. Compliance with the protocol, GCP and regulations

The study will be conducted in accordance with the study protocol, ICH-GCP E6 (R2), the latest version of the Declaration of Helsinki and applicable regulatory requirements. This is to ensure the safety and integrity of the researchers as well as the quality of the data collected.

10.2. Ethical review of the study

The final study protocol, including the final version of the informed consent form and other information provided to the researchers, has been approved by the ethics review authority (Dnr 2022-03016-1). The Ethics Review Authority shall be informed of any amendments to the Protocol in accordance with applicable requirements.

10.3. Procedure for obtaining informed consent

The principal examiner (or the person who has been delegated the task) must at each examination site ensure that the researcher is given full and adequate oral and written information about the study, its purpose, possible risks and benefits as well as inclusion / exclusion criteria. Researchers must also be informed that they are free to cancel their participation in the study at any time without having to state the reason. The researcher must be given the opportunity to ask questions and be allowed time to consider the information provided. If the person chooses to participate, the researcher and the investigator sign the consent. A copy of the research person's information and the signed consent is provided to the research person. The researcher's signed and dated informed consent must be obtained before performing any study-specific activity in the study. Each researcher who participates in the study will be identified by a researcher number on a research person identification list. The researcher agrees that monitors and inspectors have access to their medical records. If

new information is added to the study, the researcher has the right to decide again whether he / she want to continue his / her participation.

10.4. Data protection

If any part of the data processing is performed by any other organization, within or outside the EU, appropriate agreements and / or other documentation will be established, to ensure that the processing is carried out in accordance with the provisions of the Data Protection Regulation and other relevant legislation, before any data transfer.

The content of the informed consent form complies with relevant privacy and data protection legislation. In the research person's information and the informed consent form, the research people will be given complete information on how the collection, use and publication of their study data will take place. The research person's information and the informed consent form will explain how study data is stored in order to maintain confidentiality in accordance with national data legislation. All information processed by the sponsor will be “pseudonymized” and identified with the Study Code.

The informed consent form will also explain that for verification of data, authorized representatives of the sponsor, as well as the relevant authority, may require access to parts of hospital or study records relevant to the study, including the researcher's medical history.

10.5. Insurance

All participants are insured by Stockholm County Council having taken out a patient injury insurance with the County Council's Mutual Insurance Company, Löf.

11. Significant change to the study

Significant changes to the signed protocol are only possible through approved protocol amendments and by agreement of all responsible persons. Details of non-material changes shall be clearly noted in the amended minutes.

In the event that significant changes to the protocol (e.g. change of main purpose, primary or secondary variables, method of measuring primary variable, change of investigational medicinal product or dosage) will be made during the study, approval from the Ethical Review Authority and the Medical Products Agency must be obtained before implementing the amendments. A change that concerns a new review site, new reviewer and / or a new research person information shall only be approved by the Ethical Review Authority.

Non-significant changes must be registered and entered in the documentation when it is then submitted, for example in any subsequent notification of a significant change or in connection with the reporting of End of Trial.

12. Collection, handling and archiving of data

Researchers participating in the study are coded with specific researcher numbers. All research persons are registered on a research person identification list (subject enrollment

and identification list) which links the research person's name and social security number with a research “personal number”.

All data must be registered, managed and stored in a way that enables correct reporting, interpretation and verification. Complete sample folder, as well as source documents will be archived for at least 10 years after the study is completed. Source data in patient record systems are stored and archived in accordance with the regulations of each hospital region.

12.1. Case Report Form (Researcher Form)

A paper Case Report Form (CRF) is used for data collection. The examiner must ensure that the information is registered and that any corrections in CRF are made in accordance with what is stated in the study protocol and in accordance with the instructions. The examiner must ensure that the registered information is correct, complete and that reporting takes place according to the timelines predetermined. The tester signs the completed CRF. A copy of the completed CRF will be filed at the trial site.

If an examination / test has not been performed and the task does not exist, select ND (Not done) or NK (Not known). If the question is irrelevant, write NA (Not applicable). Correction in paper CRF is done by drawing a line over the incorrect information. Then write the correct information next to the incorrectly filled in information, sign and date the correction.

13. Notice that the study has been completed, reporting and publication

No later than 90 days after the end of the study, the Medical Products Agency must be informed by submitting a "Declaration of End of Trial Notification".

Within one year after completion of the study, the results are analyzed, a clinical study report with individual data is prepared and the study results are also reported to the EudraCT database.

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15. Appendix

a) Transgender Congruence Scale (TCS) – svensk översättning

b) Perth Emotional Reactivity Scale-Short Form (PERS-S) – svensk översättning

c) Sexual Desire Inventory (SDI) – svensk översättning

d) Buss-Perry Agression Questionnaire (BPAQ) – svensk översättning

e) Rosenberg Self Esteem Scale (RSES)– svensk översättning

f) Montgomery Åsberg Depression Rating Scale (MADRS-S)

g) Brunnsviken Brief Quality of life Scale (BBQ)

h) Baratt Impulsiveness Scale (BIS-11)