Protocol for study titled: "Transdermal versus oral induction of puberty in Turner syndrome using 17β-estradiol – a comparative pragmatic study"

FULL/LONG TITLE OF THE STUDY

Transdermal versus oral 17β-estradiol for pubertal induction in girls with Turner syndrome (TS) – a comparative pragmatic study by the Turner syndrome working group (TSWG) of the European Society for Paediatric Endocrinology (ESPE).

SHORT STUDY TITLE / ACRONYM

TSWG study of transdermal versus oral estrogen for pubertal induction in TS

PROTOCOL VERSION NUMBER AND DATE

Version 1.0, 12/07/2020

RESEARCH REFERENCE NUMBERS

IRAS Project Number:	XXXXXX
SPONSOR Number:	None as yet. University of Glasgow and/or NHS Greater Glasgow and Clyde to be asked to sponsor the UK arm of this international study.
FUNDER Number:	Funding for building proposed database has been obtained by colleagues in Netherlands on behalf of the European Society for Paediatric Endocrinology Turner Syndrome Working Group.

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Date:
...../...../.....

.....

Name (please print):

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Position:

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Chief Investigator:

Signature: Date:
...../...../.....

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Name: (please print):

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Sponsor	For the UK, University of Glasgow and/or NHS Greater Glasgow and Clyde will be approached
Joint-sponsor(s)/co-sponsor(s)	For participating countries outside UK, sponsorship arrangements will be made separately according to national guidelines
Funder(s)	A £15,000 grant has been awarded in April 2020 from the European Registries for Rare Endocrine Conditions, in response to a proposal titled, "Creating an interdisciplinary registry for girls and women with Turner syndrome: a platform for international collaboration in research and care". Note that funding will also be required to pay the salary of the person who will manage and oversee this database during the study period
Key Protocol Contributors	Please see above, under "Steering Committee"
Committees	Please see above

STUDY SUMMARY

Study Title	Transdermal versus oral 17β-estradiol for pubertal induction in girls with Turner syndrome (TS) – a comparative pragmatic study by the Turner syndrome working group (TSWG) of the European Society for Paediatric Endocrinology (ESPE)
Internal ref. no. (or short title)	TSWG study of transdermal versus oral estrogen for pubertal induction in TS
Study Design	Pragmatic comparative open study comparing oral and transdermal pubertal induction
Study Participants	Girls with Turner syndrome aged ≥11.0 years requiring pubertal induction
Planned Size of Sample	84 patients in each treatment arm – transdermal and oral = 168 total
Follow up duration	Follow up will be for at least the duration of induction: 3 years in most girls, 3 ½ years in especially short girls, and 2 years in the case of older girls receiving a shortened induction protocol. However, where possible, follow up will be continued until final adult height is achieved (usually around 16 years)
Planned Study Period	Eight years (including set-up and recruitment)
Research Question/Aim(s)	Is transdermal induction of puberty in girls with Turner syndrome superior to oral induction in terms of: Height gain during the 3-year induction period? This is the primary outcome measure

	Adult height, cardiovascular health (systolic blood pressure, aortic root dilatation), uterine health (uterine shape and size), bone health (bone mineral density), patient satisfaction and acceptability (adherence to each regime). These are secondary outcome measures
Brief synopsis of study	Most girls with Turner syndrome (TS) require pubertal induction with estrogen, followed by long term replacement. However, no adequately powered prospective studies comparing transdermal with oral 17β-estradiol administration exist. This reflects the difficulty of securing funding to study a rare condition with relatively low morbidity/mortality when competing against conditions such as cancer and vascular disease. The TS Working Group of the European Society for Paediatric Endocrinology (ESPE) has reached a consensus for two 3-year pubertal inductions regimens – one transdermal and one oral. A shorter regimen for the minority of girls presenting aged >14 years has also been devised. Prerequisites include suitable 17β-estradiol tablets and matrix patches to allow the delivery of incremental doses based on body weight. We wish to carry out an international prospective cohort study with single centre analysis in which clinicians and families are invited to choose either of the agreed regimens, usually starting at 11 years. We hypothesise that pubertal induction with transdermal estradiol will result in better outcomes for some key parameters. Data analysis will include assessment of the demographics and drop-out rates of patients choosing either oral or transdermal preparations; and appropriate analysis of outcomes including pubertal height gain, final height, adherence/acceptability, uterine health (assessed with pelvic ultrasound) cardiovascular health (blood pressure measurement and cardiac ultrasound, and bone health (assessed with DXA). This proposed model of prospective data collection according to internationally agreed protocols aims to break the current impasse in obtaining evidence-based management for TS. (See Donaldson et al [21])

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this study)	**FINANCIAL AND NON FINANCIALSUPPORT GIVEN** See above. Funding has been obtained to curate the proposed database in the context of the international disorders of sex development (i-DSD), managed by Professor

	Faisal Ahmed, Child Health Section, University of Glasgow School of Medicine.
	Funding will be required to support a data manager, responsible for coordinating the running of the study both in the UK and mainland Europe

ROLE OF STUDY SPONSOR AND FUNDER

The sponsors (in the UK, University of Glasgow or NHS Greater Glasgow and Clyde) will be asked to support this pragmatic study. There is no perceived risk inherent in this proposed study since girls with TS requiring estrogen for pubertal induction, together with their families, are being asked to choose one of two approved regimens which are already in use in current medical practice.

ROLES AND RESPONSIBILITIES OF STUDY MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

The Turner Syndrome Working Group of the European Society for Paediatric Endocrinology has 38 members of which 7 are members of the TSWG Steering Group – these members are responsible for study management. TSWG had requested pharmacy support from Alder Hey Children's Hospital.

The Turner Syndrome Support Society in the UK is aware of and supportive of the study (see Donaldson et al [21])

PROTOCOL CONTRIBUTORS

Please see above. The seven Members of the TSWG Steering Group are contributors. Three other colleagues have been invited to join the protocol contributor group. Two are from the UK and are Emma Jane Gault and Catrin Barker. Emma Jane Gault has expertise in Research Governance and ran the UK Turner study examining the effect of early vs late pubertal induction and the anabolic steroid on final height in TS (Gault EJ et al, British Medical Journal 2011 [27]). Catrin Barker is a pharmacist with expertise in paediatric medicines management and optimisation. The third colleague is Professor Marleen van Gelder, from Radboud University in The Netherlands who is experienced in health care statistics.

KEY WORDS:

Turner syndrome; puberty; 17β-estradiol; oral pubertal induction regimen; transdermal pubertal induction regimen, estradiol delivery, estradiol administration

Figure 1: Recruitment process for TSWG study of transdermal versus oral 17β-estradiol for pubertal induction in TS

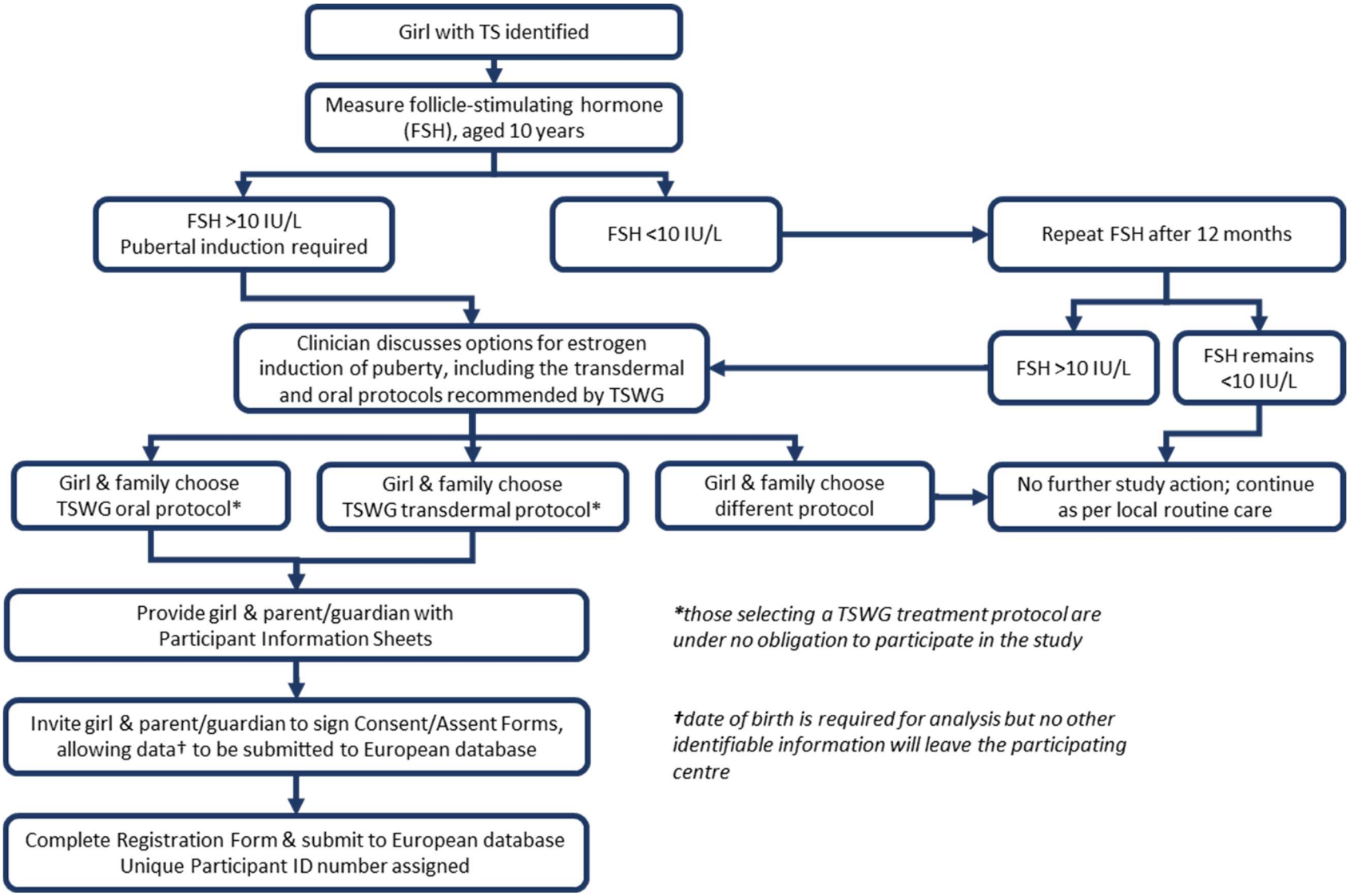
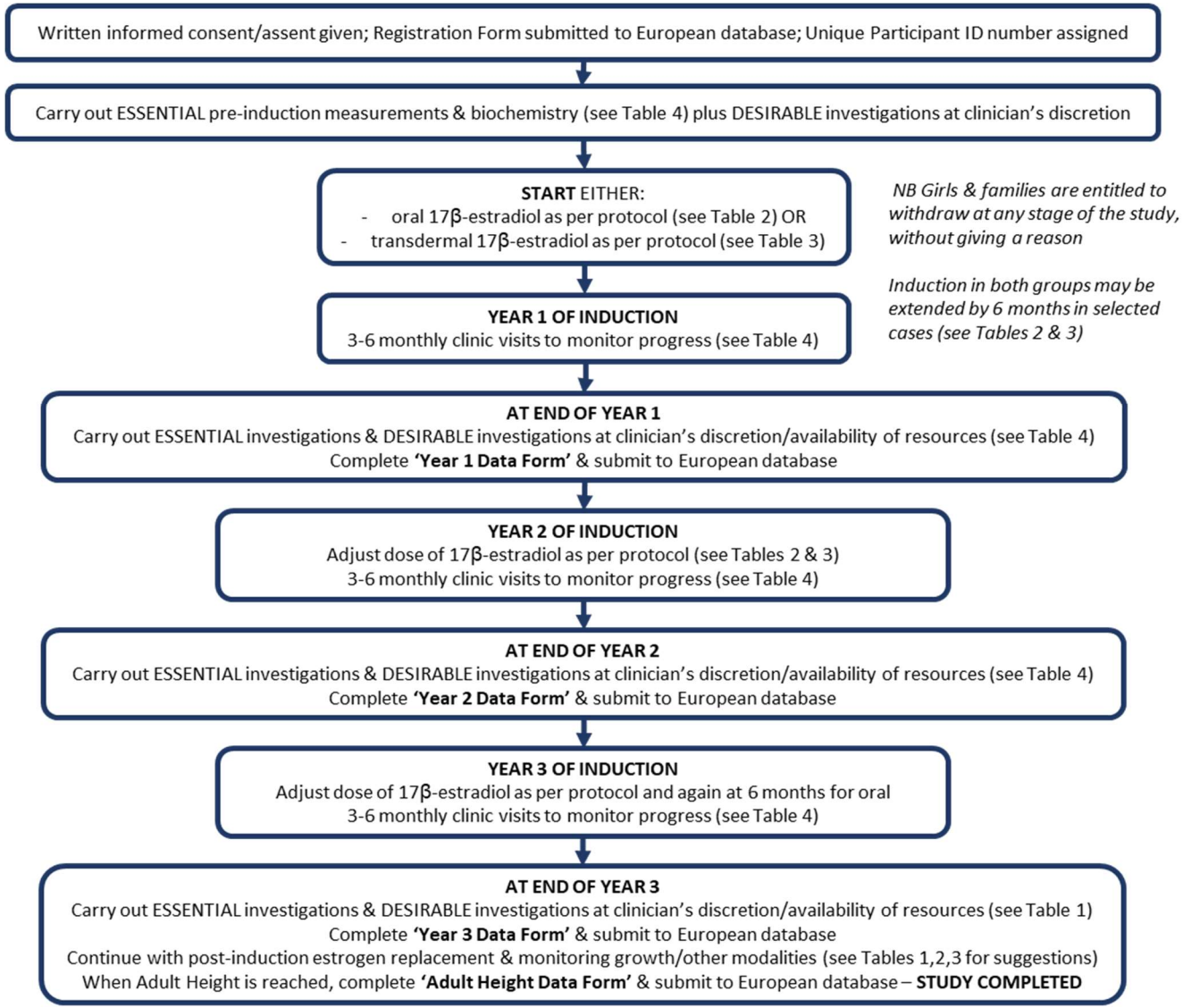


Figure 2: Study process for TSWG study of transdermal versus oral 17β-estradiol for pubertal induction in TS



Study Protocol

1 BACKGROUND

Turner syndrome (TS) is a condition in which there is loss of the second sex chromosome (either X or Y) and/or abnormality of the second X chromosome in at least one major cell line in a phenotypic female. Features include short stature and primary ovarian insufficiency, accompanied by typical phenotypic traits and a variety of associated cardiac and renal malformations in a minority [1,2].

TS carries a significant morbidity and mortality [3,4]. Long-term health consequences for cardiovascular disease and osteoporosis are particularly important. Problems such as aortic dissection (arising particularly in girls with previously repaired aortic coarctation or bicuspid aortic valve), hypertension, atheroma and increased risk of metabolic syndrome [5,6,7,8,9,] give rise to a marked increase in mortality rate (standardised mortality ratio 3) and a life expectancy 13 years less than the general population, a finding similar to patients with diabetes. However, since TS has a relatively low prevalence, affecting about 1 in 2000 live female births [1], there is little awareness of the condition and its implications.

Appropriate, physiological sex steroid replacement is essential in the optimal management of patients with TS from puberty through to the age of normal menopause. Estrogen induction of puberty is usually required in TS, a retrospective Italian study showing that spontaneous onset of periods (menarche) occurred in only 84 of 522 (16.1%) patients [10]. Puberty is a highly important process both physically and psychologically. Over 50% bone mineral accrual occurs during puberty & the early post-pubertal years [11]. Moreover timely pubertal induction is important for self-esteem, psychosexual development and quality of life.

The European Society of Human Reproduction and Embryology (ESHRE) has developed clinical practice guidelines for the management of women with primary ovarian insufficiency (POI) [12]. Clinical practice guidelines for the care of girls and women with TS were published after the International Turner Syndrome Meeting in Cincinnati [13]. Matthews and colleagues from the United Kingdom (UK) have described a consensus from the British Society for Paediatric Endocrinology and Diabetes on hormone supplementation for pubertal induction in girls with POI [14]. Christin-Maître published a review on the use of hormone replacement in females with endocrine disorders [15]. However, the Cincinnati guidelines do not cover pubertal induction in depth, and the other papers mentioned do not deal specifically with TS.

While transdermal estrogen treatment has theoretical advantages over oral treatment and has been used in Sweden for decades (16), the evidence for any superiority is lacking. Matthews et al write, “There is a real paucity of carefully constructed, randomised controlled clinical trials in girls undergoing induction of puberty. The evidence base is derived mainly from expert experience, a small number of observational studies and very few controlled trials on small study populations. In addition, studies of treatment acceptability and patient adherence are lacking” [14]. Thus, while transdermal delivery of physiological 17β-estradiol estrogen may be superior to oral ethinylestradiol in terms of cardiac, bone and uterine health in POI [17,18,19], adequately powered studies are needed to address these as well as other elements such as growth during pubertal induction, adherence and acceptability [20].

The Turner Syndrome Working Group (TSWG) of the European Society for Paediatric Endocrinology (ESPE) proposes a large prospective international pragmatic study in which girls and families are invited to choose either an oral or transdermal protocol, both of which have been agreed by TSWG and comparing outcomes with each regimen. This proposal has been peer-reviewed and published in the journal Hormone Research in Paediatrics [21]. The paper emphasizes that the proposal is not a randomised clinical trial which would be logistically impractical, considering the patient numbers required for adequate power would demand international recruitment, making such a study unrealistically costly to fund. However, rigorous planning to collect and compare prospective data on girls treated according to defined, disciplined protocols and with agreement on primary & secondary outcomes together with expert statistical analysis of the outcomes, will allow valid conclusions to be drawn in an area where current evidence is very poor.

Of note, this pragmatic proposal lies within the scope of what has been described by Torgerson as a “Patient Preference Trial” [22].

2 RATIONALE

Aim: To compare the outcome and acceptability of transdermal and oral pubertal induction in TS, using the natural estrogen 17β-estradiol in both regimens.

3 RESEARCH QUESTION/AIM(S)

Research question: Is transdermal 17β-estradiol induction superior to oral induction in terms of key parameters including pubertal height gain and adult height, cardiac (e.g. blood pressure), uterine and bone health, and in acceptability?

Study hypothesis: Induction of puberty using transdermal 17β-E2 will result in better outcomes for some key parameters.

3.1 Objectives

The study objectives are to compare outcomes between the oral and transdermal TSWG regimens.

3.2 Outcomes

The primary outcome measure is height gain in cm during the pubertal induction period, since this can be accurately determined in the context of an international framework. Secondary outcome measures include final adult height, systolic blood pressure, aortic root diameter, uterine size and shape, bone mineral density, adherence and dropout rate.

Table 1 Outcome measures to be analysed at the end of pubertal induction in girls with Turner syndrome using either an oral (see Tables 2a and 2b) or transdermal (see Tables 3a and 3b) 17-β estradiol regimen approved by the Turner Syndrome Working Group of ESPE.
Induction period is normally 3 years (plus 6 months for very short girls), exceptionally 2 years for girls presenting late (e.g. >14 years), minimum follow up is during induction period but where possible, follow up is to final adult height.

Primary outcome measure
Pubertal height gain, i.e. increase in height in centimetres during the (usually) 3-year pubertal induction period
Secondary outcome measures
▪ Final adult height in centimetres & population-specific SDS
▪ Change in population-specific height SDS between start and end of pubertal induction
▪ Breast development (clinical assessment - Tanner B4-5 reached - Satisfactory breast shape (ie not tubular, no overdevelopment of areolae) according to attending clinician (graded as satisfactory or unsatisfactory)
▪ Cardiovascular health - Systolic & diastolic blood pressure at the end of induction (lowest of three readings or mean values if recorded by ambulatory 24-hour blood pressure monitoring - Change in aortic root diameter and aortic size index between start and completion of induction
▪ Patient satisfaction; adherence/compliance with treatment - Percentage drop-outs from either regimen - Average of good, reasonable and poor rating with adherence during pubertal induction (3 years ± X months) - Patient satisfaction with shape and size of breast (scoring 1-5 where 5=best) - Patient satisfaction with mode of administration (scoring 1-5 where 5=best)
▪ Uterine health and sex hormone profile at the end of induction - Uterine length and volume, endometrial thickness - Uterine shape and fundo-cervical ratio - LH and FSH - Serum 17-β estradiol

▪ Skeletal age and bone health - Change in bone age/chronological age per year - Change in size-adjusted whole body lumbar spine and hip bone mineral areal density (BMAD) (z-score) before and after induction
▪ Metabolic profile Number of patients in each group in whom all parameters are normal at the end of induction for: - Liver function - Cholesterol, triglycerides, low and high density lipoproteins, apoprotein A1, B and A1:B ratio - Fasting glucose and insulin (HOMA)

4 STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYIS

4.1 Identification of patients and enrolment process (see Figure 1)

At 10-11 years clinicians should, as part of normal clinical practice, assess the need for pubertal induction by measuring the hormone follicle-stimulating hormone (FSH), levels of >10 IU/L indicating a degree of primary ovarian failure necessitating estrogen replacement.

Clinicians from European countries and beyond who wish to participate in the study will counsel the families of girls requiring pubertal induction as to the options. These include either of the oral and transdermal regimens agreed and recommended by the TSWG of ESPE; and alternative regimens.

This is an open, non-randomised study comparing two regimens which are regarded as best practice. Clinicians will be asked to help families make an informed choice regarding either or neither of the oral or transdermal regimens recommended by TSWG. Choice will depend partly on the availability of the recommended transdermal or oral 17β-estradiol preparations, but clinicians are entitled to request appropriate, treatment from their hospitals and pharmacies which comply with Cincinnati and UK guidelines [13,14]. In the event of a family choosing either of the TSWG-recommended regimens, written informed consent and assent will be requested by the clinician for participation in the study and the European central registry will be contacted.

This consent is for anonymised data (**with the exception of date of birth, which is required for analysis**) before, during and after pubertal induction to be sent to the designated European centre and entered into an independent ESPE-approved database under the supervision of an ESPE TSWG member trained in Good Clinical Practice.

In the UK, NHS Research Ethics Committee (REC) & Research and Development (R&D)/HRA approvals will be sought, enabling participating centres to recruit and enroll patients into the study.

Outside the UK, participating centers will need to follow their national regulatory procedure and gain ethical approval for patients and families to give written consent.

Since the proposal is not a randomised clinical trial, but an observational cohort study it should not need to be registered as the former. Any medications given for pubertal induction would be prescribed and funded in the usual way for the country concerned.

4.2 Age, duration and protocols for oral and transdermal 17β-estradiol induction of puberty in TS

Induction of puberty will be from 11 years. Specific age of beginning induction, and duration of induction are at the discretion of the clinician in consultation with the family. Initiation of treatment in eligible patients should normally begin at between 11-12 years, consistent with the Cincinnati and UK guidelines [13,14]. Pubertal induction should normally be carried out over a 3-year period. However, a later induction age will be necessary in girls presenting >12 years. Also, a faster induction period (e.g. 2 years) should be considered in girls who are older (e.g. >14 years) at the time of starting induction due to late diagnosis.

4.2.1 Oral induction

The agreed oral three-year induction protocol, derived from the Dutch regimen of Bannink et al [23], together with a final (fourth) year, of maintenance treatment is shown in Table 2a. A 2-year protocol for older girls (e.g. aged >14 years) in whom a shorter induction period is desired by the clinician and family, is given in Table 2b

Dose is based on body weight and is recalculated at the beginning of each year.

17-β estradiol will be given in its micronized (e.g. Estrace) or hemihyrate (e.g. Cetura) form using 0.5 mg tablets which can be halved by the family.

Alternatives such as esters - 17-β estradiol valerate (e.g. Climaval and Zumenon) and hemihyrate preparations (e.g. Elleste solo and Estrofem) are acceptable but only available as 1 mg tablets so that participating centres would therefore need their pharmacy to prepare a suitable dose from a 1 mg tablet.

- Girls receiving <0.25 mg daily with any preparation would also need their medication prepared by the pharmacist. Girls receiving <0.25 mg daily with any preparation will require their pharmacy to prepare the correct dose in capsule form, as currently practiced in The Netherlands using Cetura. (*Documentation from Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, available on request*). In patients requiring ≥0.25 mg daily the dose will be rounded to the nearest 0.25 mg. Evening administration is recommended, to mirror the transdermal regimen.

Table 2a Three-year oral 17β-estradiol regimen for pubertal induction and, in italics, a suggested first post-induction year [21]. The dose is calculated according to body weight at the beginning of each year and given before sleep each night.

Year (months)	Dose of estradiol [microgram/kg/day]
1 (0-12)	5
2 (13-24)*	7.5
3 (25-30)	10
3 (31-36)	15
*4 (37-48)***	*20†*

**Girls in whom low final height is anticipated or who are markedly short at the start of induction (defined as ≤3 standard deviations below the population mean) may be offered a slower induction by receiving an extra 6 months of 7.5 microgram/kg/day between Year 2 and Year 3.*
***Morning serum estradiol sample is recommended at the beginning of Year 4.*
†This post-induction dose equates to about 1 mg daily. However, the dose required will vary between 1 and 4 mg daily, depending on patient sensitivity, most patients requiring at least 2 mg daily.

Table 2b Two-year oral 17β-estradiol regimen for pubertal induction in girls presenting late (e.g. >14 years) and judged by clinician and family to require a shorter induction period. Year 3 corresponds to post-induction dose. The dose is calculated according to body weight at the beginning of each year and given before sleep each night.

Year (months)	Dose of estradiol [microgram/kg/day]
1 (0-6)	5
1 (7-12)	7.5
2 (13-18)	10
2 (19-24)	15
3 (25-36)	*20†*

During the fourth year of maintenance treatment, estradiol dose will vary between 1-4 mg daily, depending on patient sensitivity, most patients requiring at least 2 mg daily.

4.2.2 Transdermal induction

Table 3 shows the agreed regimen, adapted from the Swedish protocol of Ankarberg-Lindgren [16] in which an overnight patch is applied for 10-12 hours during the first two years of induction. Dosage is weight-based and increased in accordance with weight at the clinic visit. Patches are applied on a healthy part of the skin (buttocks, belly or lower part of back), cutting the appropriate dose as described [21]. A new patch is applied on a different part of the skin. During the third year of induction the dose is split during the 24-hour period, cutting the desired patch piece in half to give two pieces, applying both pieces overnight then removing one in the morning. The stability at 21 and 35°C and uniformity of estradiol distribution has recently been demonstrated for Evorel, Estraderm and Oesclim (50µg, 50µg and 25µg per 24 hours) patches when cut into 8 pieces [24]. Therefore, while a new 25µg patch is normally required each day for older girls, those with a low body weight may receive a second piece during the next 24 hours.

Table 3a Three-year protocol for transdermal 17β- estradiol pubertal induction regimen and a suggested first post-induction year. The dose at the beginning of each year is calculated according to body weight.

	Weight (kg)	50 µg patch	25 µg patch	Duration of treatment, Instructions
Year 1*				
	< 40 kg	1/16 of patch (3.1 µg)	1/8 of patch (3.1 µg)	10-12 hours (overnight, to mimic the normal diurnal variation)
	40-55	1/12 of patch (4.2 µg)	1/6 of patch (4.2 µg)	
	>55	1/8 of patch (6.2 µg)	¼ patch (6.2 µg)	
Year 2				
	< 40 kg	1/8 of patch (6.2 µg)	1/4 of patch (6.2 µg)	10-12 hours (overnight)
	40-55	1/6 of patch (8.3 µg)	1/3 patch (8.3 µg)	
	>55	¼ of patch (12.5 µg)	½ patch (12.5 µg)	
Year 3				
	< 50 kg	1/3 of patch (16.7 µg)	2/3 of patch (16.7 µg)	Cut the desired dose into two halves; attach both parts to the skin in the evening. After 10-12 hours (overnight) remove one part, keep the second part during the day. Remove second part in the evening, before evening application of a two further half patches. This results in a diurnal dose variation, with higher dose nighttime.
	50-65	3/8 patch (18.8 µg)	3/4 patch (18.8 µg)	
	>65	1/2 of patch (25 µg)	1 patch (25 µg)	
Year 4*	***Post-induction dose***			
	Not applicable	*50 – 75 (-100) µg/24h* *(Often ~1 ug/kg/24h)*		*Patch continuously attached to the skin. Apply whole patch three times weekly, changing after 2 days* *Serum sample for estradiol target to be drawn in the morning after the previous evening's dose, target range 150–450 pmol/L*

**Girls in whom low final height is anticipated may receive an extra 6 months of the Year 1 dose*
***Year 4 suggests a post-induction dose*
***Serum estradiol sample to be drawn early of Year 4 in the morning using the post-induction dose and a new patch applied the evening before, target range 150–450 pmol/L [25]*

Table 3b Two-year transdermal 17β-estradiol regimen for pubertal induction in girls presenting late (e.g. >14 years) and judged by clinician and family to require a shorter induction period. Year 3 corresponds to suggested post-induction dose. The dose at the beginning of each year is calculated according to body weight.

	Weight (kg)	50 µg patch	25 µg patch	Duration of treatment, Instructions
Year 1 (0-6 months)				
	< 40 kg	1/16 of patch (3.1 µg)	1/8 of patch (3.1 µg)	10-12 hours (overnight, to mimic the normal diurnal variation)
	40-55	1/12 of patch (4.2 µg)	1/6 of patch (4.2 µg)	
	>55	1/8 of patch (6.2 µg)	¼ patch (6.2 µg)	
Year 1 (7-12 months)				
	< 40 kg	1/8 of patch (6.2 µg)	1/4 of patch (6.2 µg)	10-12 hours (overnight)
	40-55	1/6 of patch (8.3 µg)	1/3 patch (8.3 µg)	
	>55	¼ of patch (12.5 µg)	½ patch (12.5 µg)	
Year 2				
	< 50 kg	1/3 of patch (16.7 µg)	2/3 of patch (16.7 µg)	Cut the desired dose into two halves; attach both parts to the skin in the evening. After 10-12 hours (overnight) remove one part, keep the second part during the day. Remove second part in the evening, before evening application of a two further half patches. This results in a diurnal dose variation, with higher dose nighttime.
	50-65	3/8 patch (18.8 µg)	3/4 patch (18.8 µg)	
	>65	1/2 of patch (25 µg)	1 patch (25 µg)	
Year 3	***Post-induction dose***			
	Not applicable	*50 – 75 (-100) µg/24h (Often ~1 ug/kg/24h)*		*Patch continuously attached to the skin. Apply whole patch three times weekly, changing after 2 days Serum sample for estradiol target to be drawn in the morning after the previous evening's dose, target range 150–450 pmol/L*

**Girls in whom low final height is anticipated may receive an extra 6 months of the Year 1 dose*
***Year 4 suggests a post-induction dose*
*** Serum estradiol sample to be drawn early of Year 3 in the morning using the post-induction dose and a new patch applied the evening before, target range 150–450 pmol/L [25]*

4.3 Oral progesterone administration

An oral progestogen such as Dydrogesterone (9β,10α-pregna-4,6-diene-3,20-dione) 10 mg; micronised progesterone 100 mg; and medroxyprogesterone acetate 5 mg initially [13] is recommended but depends on local availability. A duration of 10 days each calendar month is recommended, in keeping with the Cincinnati guidelines [13].

To allow maximum time for uterine and breast development with unopposed estrogen, pubertal staging and where possible pelvic ultrasound examination should be carried out at the time of first breakthrough bleeding so that endometrial thickness and uterine size can be determined [21].

Depending on the assessment, a decision should be taken to either introduce progesterone, or delay doing so and review after 6 months (or earlier if breakthrough bleeding becomes problematic).

Features suggesting postponing progesterone treatment include: Tanner stage B3 breast development or less: uterine length < 5 cm, i.e. the 50th centile for general Scottish population aged 13 years [26], fundo-cervical ratio ≤ 1 (i.e. infantile or cylindrical configuration) and endometrial thickness ≤ 2 mm. Features suggesting that progesterone should be introduced include Tanner B4-5 development, uterine length ≥ 5 cm, fundo-cervical ratio > 1 (pubertal or pear-shaped configuration) and endometrial thickness > 2 mm.

4.4 Concurrent treatment with growth hormone (GH) and oxandrolone

It is anticipated that most girls will be receiving both GH therapy at the time of pubertal induction and to continue this until around 16 years of age, or when height velocity is < 2 cm/year . Participating centres are likely to give standard GH doses for TS - 1.4 mg/m^2/day, equivalent to 50–65 microgram/kg/day [26] and modified according to individual GH responsiveness. Insulin-like growth factor-1 (IGF-I) should be measured at the beginning of induction and then annually as per the Cincinnati guidelines [13], aiming for values between +1.5 and +2.5 SDS and modifying GH dosage to keep IGF-1 below +3 SDS.

The weak anabolic steroid Oxandrolone is not currently manufactured in Europe but is available although expensive to import from the United States of America and Australia. Consequently, although shown to improve adult height in TS in the dose of 0.05 mg/kg/day (maximum dose 2.5 mg daily) [27], Oxandrolone tends to be reserved for girls who respond poorly to GH despite good compliance [28].

No standard approach to either GH or Oxandrolone treatment can be imposed on participating centres, and variations in these treatment options will be factored into the data analysis.

4.5 Data collection before and during pubertal induction

Table 4 shows the recommended information to be collected before, during and at the end of pubertal induction, and until adult height, i.e. height velocity < 2 cm/year.

TABLE 4 Recommended measurements before, during and at the end of pubertal induction with either transdermal or oral 17-β estradiol grouped according to priority – essential and desirable.

Variable	Before induction (± 6 months)	3-6 monthly visits*	End of Years 1 & 2	End of Year 3	Comment
ESSENTIAL					
Karyotype	X				From time of diagnosis
Auxology Height (cm)	X	X	X	X	Previous growth data will be requested, to enable calculation of a) height velocity, using 12 (±1) month interval, during previous year; and GH responsiveness – derived from height/weight status one year prior to, at the start of, and one year after starting GH.
Weight (kg)	X	X	X	X	
Height velocity (cm/yr)	X		X	X	
Measured parental heights (where available)	X				
Breast and pubic hair stage (1-5)	X	X	X	X	
Systolic & diastolic blood pressure (mm Hg)	X	X	X	X	Lowest of three consecutive readings using Dinamap V100 or similar device
Adherence to treatment		X	X	X	Scored according to estrogen doses missed per month as **good** (≤ 2) **moderate** (≤ 4) and **poor** (> 4)
Adverse events/illness		X	X	X	All adverse events (e.g. injury, intercurrent infection) irrespective of relevance to growth and puberty
FSH, LH	X			X	
Free T4 and TSH	X			X	
Liver function	X		X	X	
HbA1c	X			X	
Concurrent treatment	X	X	X	X	GH, L-T4, Oxandrolone
DESIRABLE					
Final adult height					Measurement once height velocity < 2cm/year
Bone age	X		X	X	Assessed using 'BoneXpert', where available
Sitting height	X	X	X	X	
Echocardiography	X			X	Including measurement of aortic size index
Pelvic ultrasound	X		X	X	Uterine length, shape and volume, endometrial thickness
DXA BMC	X			X	Hip and lumbar spine z-score Total body BMD
Fasting lipid profile	X			X	Cholesterol, triglycerides, low and high-density lipoproteins
Coagulation studies	X			X	
Serum estradiol	X			X	Beginning of Year 4 (see Table 2)
Serum IGF-I	X		X	X	Keep +1.5 -2.5 SD of reference range
24h continuous ambulatory blood pressure	X			X	
Fasting blood glucose and insulin	X			X	

Abbreviations

*FSH = follicle stimulating hormone; LH = luteinising hormone; fT4 = free thyroxine; TSH = thyroid stimulating hormone; DXA = dual X-ray absorptiometry; BMC = bone mineral content; BMD = bone mineral density; GH = growth hormone; L-T4 = levo-thyroxine. *4-monthly visits are recommended; however 3-6 monthly may be more suitable for some participating centres.*

It should be noted that measurements such as height, weight, pubertal stage and blood pressure are marked as 'essential' and are within the scope of any paediatric service caring for girls with TS. By contrast, 'desirable' measures should be undertaken at the clinician's discretion, and take account of the resources available in the participating centre.

Since height gain during induction is the agreed primary outcome measure, previous height and weight data from one year before, at the time of and one year after the start of GH treatment and at least yearly thereafter, together with GH dose and possible oxandrolone treatment, are required to assess GH responsiveness [29,30].

Karyotype (normally available from time of diagnosis) is essential information.

At each visit, measurement of height, weight, blood pressure, pubertal stage, adherence (scored according to the number of estrogen doses estimated by the family to have been missed per month), recording of adverse events, and concurrent treatment are required.

Note that the family will be asked at each visit about **any** adverse events including minor injury, intercurrent infection, middle ear problems and gastrointestinal symptoms, and summarised at the end of each year by the clinician.

Annual biochemistry for thyroid function and liver function, FSH, LH and estradiol levels will be carried out as part of normal clinical practice.

Echocardiogram to define the anatomy of the heart and great vessels, with measurement of aortic root diameter at the annulus, and aortic size index (aortic root diameter divided by body surface area), is recommended before and at the end of induction.

Pelvic ultrasound, measuring uterine length, fundo-cervical ratio, uterine shape (infantile, cylindrical and pubertal) and endometrial thickness are also recommended before and after induction.

Dual X-ray absorptiometry (DXA), with measurement of bone mineral density at the lumbar spine and hip is recommended before and at the end of induction, the measurements being converted to standard deviation scores. Due to different machines in different centres, with no possibility to harmonise the data from the range of equipment, the patients' individual change in bone mineral density standard deviation score will be the used variable in the study.

As stated above, these investigations depend on local availability and are not prerequisites for participation in the study.

4.6 Data analysis

When the target number of patients has been enrolled (see below), and a sufficient number (84 in each arm) have completed the full 3-year induction, the following analyses between the transdermal and oral groups will be conducted:

- Demographics of patients choosing oral or transdermal estradiol preparations, differences at baseline
- Numbers of patients in each treatment arm, their centres and countries
- Adherence to each of the treatment arms including % of dropouts
- Propensity score matching to reduce bias due to confounding factors e.g. 45,X karyotype, mid-parental height, height at the beginning of pubertal induction, duration of prior GH treatment, GH responsiveness and treatment or not with oxandrolone.
- Use of Directed Acyclic Graphs to establish the minimally sufficient set of confounders for each outcome parameter, entering these into multivariable regression models

The minority of girls undergoing a 2-year induction will be analysed separately and their outcomes compared with the majority of girls undergoing the full three-year induction

5 STUDY SETTING

This is a multicentre, international study which includes the UK. The study setting will be in endocrine clinics and centres within the UK, other European countries, and beyond. Treatment administration and data collection will be carried out as indicated above. A single European centre will receive

anonymised data from study participants at enrolment and at the end of each year of the pubertal induction regimen chosen.

Participating centres and countries are likely to have at least some ESPE members. Centres must have facilities for recording the essential data required for good management in TS (see section 5.5).

6 SAMPLE AND RECRUITMENT

6.1 Eligibility Criteria

6.1.1 Inclusion criteria

- Karyotype-proven Turner syndrome
- Pubertal induction for primary ovarian failure required (FSH > 10 IU/L)
- Age ≥ 11 years at time of induction
- Written informed consent/assent, as applicable

6.1.2 Exclusion criteria

There are no formal exclusion criteria. However, note will be taken in the analysis of patients with accompanying health problems such as hypothyroidism, coeliac disease and inflammatory bowel disease, all of which have an increased prevalence in TS. Moreover, problems with adherence/compliance will also be taken into account when analysing the data.

6.2 Sample size

Mean (SD) height gain during three years of pubertal induction in the UK clinical trial of Gault et al [26] in which TS girls were treated with a consistent dose of GH has been calculated as 12.2 (3.5) cm. A mean difference of 2.5 cm between transdermal and oral regimens is considered clinically significant, giving a fractional effect size (f) of 2.5/3.5 = 0.71. Using the Lehr formula where $n=21/f^2$ gives the number of patients in each arm of the study required for 90% power at the 5% level [31], the number would be 42. However, given the covariates including age at diagnosis, karyotype, age at starting growth hormone therapy, differing dosage regimens, and use or not of Oxandrolone amongst the enrolled patients, a recruitment target of twice the power calculation, i.e. up to 84 in each treatment arm, is considered desirable so as to adjust for potential confounding factors. Note that this target of 84 in each treatment arm applies to girls undergoing the full 3-year induction period and does not include girls undergoing the shortened induction period.

6.3 Recruitment

Members of the European Society for Paediatric Endocrinology (ESPE) will be invited to participate in the study, which will mean a) securing approval from national bodies to engage in a pragmatic study; b) obtaining ethical approval to seek informed consent from participating families; and c) to ensure that appropriate 17β-estradiol preparations are available. NB the latter is a prerequisite for compliance with current guidelines [13,14]

ESPE members wishing to, and being able to, participate will approach families as described above.

6.3.1 Sample identification

See above. Patients will be identified from the clinics of participating centres and countries.

6.3.2 Consent

Informed consent will be obtained from girls and their families for anonymised data to be sent to a central European registry. Please see Flow Sheet and section 5.1 for details. Information sheets will be given to families in the language of the country concerned, and at least two weeks will be allowed to elapse between the initial approach and signing of the consent form.

7 ETHICAL AND REGULATORY CONSIDERATIONS

In the UK, we are asking the MHRA to accept this study as a pragmatic work rather than a randomised clinical trial. Once we have MHRA acceptance that this is so, we will develop the necessary study documents, including participant information sheets and seek the relevant NHS REC & R&D/HRA approvals to consent patients.

Date of birth is a prerequisite for data analysis. This is the only potential identifier which will be sent to the European centre. It will be linked with a unique study number so as to distinguish between patients with the same date of birth.

7.1 Assessment and management of risk

There is no risk inherent in this study since girls with TS who require pubertal induction and the paediatrician will be advised to use one of two approved regimens.

7.2 Research Ethics Committee (REC) and other Regulatory review & reports

A favourable opinion from the UK Health Departments Research Ethics Service (NHS REC) & NHS R&D/HRA approvals will be sought for UK centres participating in the study and will involve the study protocol, participant information sheets and informed consent/assent forms. In the UK, members of BSPED and of the parent and family support group, TSSS, will be made aware of and updated on the progress of this proposal.

Regulatory Review & Compliance

Assent from MHRA that this a pragmatic study, and approval from NHS REC & R&D/HRA for data to be sent to a European centre, will be obtained before any UK site can enrol patients into the study.

Once the Sponsor organisation has been agreed, its Pharmacovigilance procedures for safety reporting and Independent Data Monitoring Committee requirements will be followed. If an IDMC is required, it will be established before recruitment begins and a Charter will be agreed, to outline the committee's remit, roles and responsibilities for the ongoing review of data, in order to protect participant safety and ensure validity of the results.

Amendments

Once approved, any subsequent amendments will be discussed with the Sponsor representative, categorised as Substantial or Non-substantial and processed/approved accordingly, before being implemented.

7.3 Peer review

The study proposal has been published in a quality paediatric endocrine journal after extensive independent peer review, necessitating two revisions (see Malcolm Donaldson, Berit Kriström, Siska Verlinde, Janielle van Alfen-vanderVelden, Aneta Gawlik and Theo Sas on behalf of the Turner Syndrome Working Group for the European Society for Paediatic Endocrinology. Pubertal induction in girls with Turner syndrome; a proposed modern strategy. Hormone Research in Paediatrics 2019;91(3):153-163. doi: 10.1159/000500050) [21].

7.4 Patient & Public Involvement

Patients, families and parent support groups will be informed of the study outcome once the data have been analysed. The UK Turner Syndrome Support Society was consulted about the paper published in Hormone Research [21] and will continue to be kept informed of the proposal.

7.5 Protocol compliance

Protocol deviation, non-compliance (including dropout) will be recorded by the European centre. Since this is an open, pragmatic observational study, serious protocol breaches are not envisaged.

7.6 Data protection and patient confidentiality

All investigators and study site staff will comply with the requirements of the General Data Protection Regulation, as well as the requirements of the study's Sponsor, with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

Personal information will be confined to participating centres with the exception of date of birth which is needed by the European centre. The data will be kept secure with a unique study number and no one but the enrolling physician will have access to patient name and address.

Concerning publication of the study, results will be presented only for groups, therefore it will not be possible to identify data from an individual girl with TS.

Data will be stored for at least ten years after completion of the study.

The designated European centre will be the data custodian.

7.7 Indemnity

This important aspect of the proposal will be addressed once the study category has been confirmed and a Sponsor organisation agreed.

7.8 Access to the final study dataset

This important aspect of the proposal will be addressed once the study category is confirmed and the Sponsor organisation agreed.

8 DISSEMINATION POLICY

8.1 Dissemination policy

This important aspect of the proposal will be addressed once the study category is confirmed and the Sponsor organisation agreed.

8.2 Authorship eligibility guidelines and any intended use of professional writers

This important aspect of the proposal will be addressed once the study category is confirmed and the Sponsor organisation agreed.

9 REFERENCES

1. Donaldson MDC, Gault EJ, Tan KW, Dunger DB. Optimising management in Turner syndrome – from infancy to adult transfer. Archives of Disease in Childhood 2006; 91:513-520
2. Ranke MB, Saenger P. Turner's syndrome. Lancet 2001;358(9278):309-14.
3. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. Journal of Clinical Epidemiology 1998; 51(2):147-158.
4. Schoemaker MJ, Swerdlow AJ, Higgins CD, Wright AF, Jacobs PA, on behalf of the United Kingdom Clinical Cytogenetics Group. Mortality in Women with Turner Syndrome in Great Britain: A National Cohort Study. J Clin Endocrinol Metab 2008; 93(12):4735-4742.
5. Carlson M, Silberbach M. Dissection of the aorta in Turner syndrome: two cases and review of 85 cases in the literature. J Med Genet 2007; 44(12):745-749.
6. Ostberg JE, Donald AE, Halcox JPJ, Storry C, McCarthy C, Conway GS. Vasculopathy in Turner Syndrome: Arterial Dilatation and Intimal Thickening without Endothelial Dysfunction. J Clin Endocrinol Metab 2005; 90(9):5161-5166.
7. Ho VB, Bakalov VK, Cooley M, Van PL, Hood MN, Burklow TR et al. Major Vascular Anomalies in Turner Syndrome: Prevalence and Magnetic Resonance Angiographic Features. Circulation 2004; 110(12):1694-1700.
8. Wong SC, Cheung M, Zacharin M. Aortic dilatation and dissection in Turner syndrome: what we know, what we are unclear about and what we should do in clinical practice? Int J Adolesc Med Health. 2014;26(4):469-88.
9. Calcaterra V, Brambilla P, Maffè GC, Klersy C, Albertini R, Introzzi F, Bozzola E, Bozzola M, Larizza D. Metabolic syndrome in Turner syndrome and relation between body composition

and clinical, genetic, and ultrasonographic characteristics.Metab Syndr Relat Disord. 2014;12(3):159-64.
10. Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. J Clin Endocrinol Metab. 1997 Jun;82(6):1810-3.
11. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, Bonjour JP. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab. 1992 Oct;75(4):1060-5
12. The ESHRE Guideline Group on POI, Webber L, Davies M, Anderson R et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Human Reproduction 2016; 31: 926-937
13. Gravholt CH, Andersen NH, Conway GS et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting Eur J Endocrinol. 2017 Sep;177(3):G1-G70.
14. Matthews D, Bath L, Högler W, Mason A, Smyth A, Skae M. Hormone supplementation for pubertal induction in girls. Arch Dis Child 2017;102 (10): 975-980.
15. Christin-Maitre S. Use of Hormone Replacement in Females with Endocrine Disorders. Horm Res Paediatr 2017;87:215–223.
16. Ankarberg-Lindgren C, Kriström B and Norjavaara E. Physiological estrogen replacement therapy for pubertal induction in girls: a clinical observational study. Horm Res Paediatr. 2014;81(4):239-44.
17. Langrish JP, Mills NL, Bath LE, Warner P, Webb DJ, Kelnar CJ et al. Cardiovascular Effects of Physiological and Standard Sex Steroid Replacement Regimens in Premature Ovarian Failure. Hypertension 2009; 53(5):805-811.
18. O'Donnell RL, Warner P, Lee RJ, Walker J, Bath LE, Kelnar CJ, Wallace WHB, Critchley HOD. Physiological sex steroid replacement in premature ovarian failure: randomized crossover trial of effect on uterine volume, endometrial thickness and blood flow, compared with a standard regimen. *Human Reproduction* 2012;27(4):1130-1138.
19. Crofton PM, Evans N, Bath LE, Warner P, Whitehead TJ, Critchley HO, Kelnar CJ, Wallace WH. Physiological versus standard sex steroid replacement in young women with premature ovarian failure: effects on bone mass acquisition and turnover. Clin Endocrinol (Oxf). 2010 Dec;73(6):707-14.
20. Nabhan ZM, Dimeglio LA, Qi R, Perkins SM, Eugster EA. Conjugated oral versus transdermal estrogen replacement in girls with Turner syndrome: a pilot comparative study. J Clin Endocrinol Metab 2009; 94: 2009-2014.
21. Malcolm Donaldson, Berit Kiström, Siska Verlinde, Janielle van Alfen-vanderVelden, Aneta Gawlik and Theo Sas on behalf of the Turner Syndrome Working Group for the European Society for Paediatric Endocrinology.Pubertal induction in girls with Turner syndrome; a proposed modern strategy. Hormone Research in Paediatrics 2019;91(3):153-163.
22. Togerson D. Understanding controlled trials: What is a patient preference trial? BMJ 1998; 316:360.
23. Bannink EMN, van Sassen C, van Buuren S, de Jong FH, Lequin M, Mulder PGH, de Muinck Keizer-Schrama SMPF. Puberty induction in Turner syndrome: results of oestrogen treatment on development of secondary sexual characteristics, uterine dimensions and serum hormone levels. Clinical Endocrinology 2009; 70: 265–273.
24. Ankarberg-Lindgren C, Gawlik A, Kriström B, Mazzanti L, Ruijgrok EJ, Sas TCJ. Estradiol matrix patches for pubertal induction: stability in cut pieces at different temperatures. Endocrine connections 2019 (*in press)*
25. Fanelli F, Gambineri A, Belluomo I, Repaci A, Di Lallo VD, Di Dalmazi G, et al. Androgen profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in healthy normal-weight ovulatory and anovulatory late adolescent and young women. J Clin Endocrinol Metab. 2013 Jul; 98(7): 3058–67.
26. Griffin IJ, Cole TJ, Duncan KA, Hollman AS, Donaldson MDC. Pelvic ultrasound measurements in normal girls. Acta Paediatrica 1995;84(5): 536-543.
27. Gault EJ, Perry RJ, Cole TJ, Casey S, Paterson WF, Hindmarsh PC, Betts P, Dunger DB, Donaldson MDC on behalf of the British Society for Paediatric Endocrinology and Diabetes.

The effect of oxandrolone and timing of pubertal induction on final height in Turner syndrome: a randomised, double blind, placebo-controlled trial. Brit Med Journal 2011; 342:d1980.
28. Sas TCJ, Gault EJ, Bardsley MZ, Menke LA, Frericks K, Perry RJ, Otten BJ, de Muinck Keizer-Schrama SMPF, Timmers H, Wit JM, Ross JL, Donaldson MDC. Safety and efficacy of Oxandrolone in growth hormone treated girls with Turner syndrome – evidence from recent studies and recommendations for use. Hormone Research in Paediatrics 2014;81:289-297.
29. Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, Albertsson-Wikland K, Price DA. Prediction of Long-Term Response to Recombinant Human Growth Hormone in Turner Syndrome: Development and Validation of Mathematical Models. J Clin Endocrinol Metab 2000;85(11):4212-8.
30. Kriström B, Dahlgren J, Niklasson A, Nierop AFM, Albertsson-Wikland K. The first-year growth response to growth hormone treatment predicts the long-term prepubertal growth response in children. BMC Medical Informatics and Decision Making 2009;9(1):1.
31. Cole TJ. 1997. Sampling, study size and power. In: Margetts BM, and Nelson M, editors. Design concepts in nutritional epidemiology. 2nd ed. Oxford: Oxford University Press. p 64-86.

10 APPENDICES

To be completed once study category confirmed and Sponsor organisation agreed.