UNIVERSITI KEBANGSAAN MALAYSIA
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Title:

Bayesian Pragmatic Trial for Pubertal Induction in Turner Syndrome: TRansformation Initiative For Efficient Clinical TriAl Design Advancement in RarE Diseases (TRIFECTA-DARED framework)

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1.0 PROTOCOL SYNOPSIS

Abbreviated Title	Bayesian Pragmatic Trial for Pubertal Induction in Turner Syndrome: Transformation Initiative For Efficient Clinical TriAl Design Advancement in RarE Diseases (TRIFECTA-DARED framework)
Trial Phase	Phase II
Clinical Indication	Pubertal Induction in Adolescents and young women with Turner syndrome as evidenced by normal breast, uterine development and healthy bone mineral profile
Trial Type	Interventional
Type of Control	Active control (Oral Estradiol Valerate (Progynova)) without placebo
Route of administration	Oral (Estradiol Valerate (Progynova)), Transdermal gel application (17β Estradiol, Oestrogel)
Trial Masking	No Blinding /Masking
Treatment Groups	17β Estradiol group (transdermal gel): Transdermal 17β Estradiol gel (Oestrogel) administered 0.75 mg twice a week for month 1, then 0.75 mg daily for the next 6 months, 1.5 mg daily for another 6 months and 3.0 mg daily for another 6 months Estradiol Valerate group (oral tablets): Oral tablets of Estradiol Valerate administered at 1.0 mg twice a week for 1 month then 1.0 mg daily for 6 months then 2.0 mg daily for 6 months followed by 4.0 mg daily for another 6 months
Number of trial subjects	At least 12 concurrent trial participants per Progynova and Oestrogel groups (n_{total}=24 participants) based on Bayesian sample size calculation

Estimated Duration of Trial	2 years (24 months), starting from the date the first subject signs the informed consent until the date the last subject completes the last follow-up
Duration of Participation	The participants will be enrolled for 19 months (1 ½ years). The enrolment date will be Day 1 (baseline) of the trial, 1st follow-up at Month 7, 2nd follow-up at Month 13, and final follow-up at Month 19.
Randomization Ratio	The participants will be randomised to oral Estradiol Valerate (Progynova) or transdermal 17β estradiol gel (oestrogel) at a 1:1 ratio based on stratified blocked randomisation of varying block sizes.

SECTION 1

INTRODUCTION

Turner syndrome (TS) is a condition that occurs in females who have either a complete or partial absence of the second X chromosome, resulting in only one X chromosome. This condition can result in various physical characteristics such as a distinct facial appearance, neck webbing, short stature, and lymphedema, as well as medical issues like ovarian insufficiency, sensorineural hearing loss, congenital cardiovascular disease, renal anomalies, some neurodevelopmental disorders, and an increased risk of thyroid and celiac disease. TS affects around 25 to 50 out of every 100,000 females, with a wide range of possible symptoms. The karyotype (number and appearance of chromosomes) in TS can range from complete 45 X to various forms of mosaicism where the individual has a normal cell line (46,XX or 46,XY) and an abnormal second or third cell line.(1)

The objective of pubertal induction is to replicate the natural pubertal process and achieve normal timing and extent of breast and uterine development, as well as adolescent growth spurts. This is accomplished by using low initial doses of oestrogen which are gradually increased. The patient's clinical response, particularly linear growth, breast development and uterine development, is closely monitored throughout the process. The standard treatment for patients with TS involves using estrogen replacement therapy, often combined with growth hormone. The primary goal of estrogen therapy is to initiate puberty, promote healthy secondary sexual development, and provide other significant physiological benefits of estrogen, such as uterine maturation and bone mineralization. (1,2)

Low-dose estrogen can be used over approximately three years, similar to the normal pubertal process. Ideally, the dosage should mimic the physiological level of the hormones as produced by normal ovaries. The dosage can be increased at six months intervals to mimic the normal pubertal tempo until the adult dosing is reached. The choices of estrogen are 17ß-estradiol, ethinylestradiol, and conjugated equine estrogens. Estrogen can be administered via oral or transdermal routes. Both routes provide normal plasma concentrations of estradiol and have similar effects in suppressing gonadotropin concentrations. However, transdermal estrogen has fewer systemic complications because it avoids first-pass metabolism by the

liver. It can achieve higher plasma levels of circulating estradiol with a lower treatment dose, thus resulting in fewer circulating estrogen metabolites. Both regimes effectively prevent bone loss and produce favourable lipid and lipoprotein changes. (2,3,4)

According to a randomised controlled trial, oral and transdermal 17ß-estradiol in gradual doses can produce oestrogen concentrations similar to those seen in a normal oestrogen milieu. However, there were notable differences in bio-oestrogen and total estrone and estrone sulphate concentrations between the two administration routes, with transdermal 17ß-estradiol resulting in much higher levels of these compounds. This suggests that transdermal administration may lead to a more physiological oestrogen environment than the oral route. Moreover, because transdermal oestrogen avoids first-pass hepatic metabolism, it is more effective and better tolerated, with fewer side effects such as breast tenderness and erratic vaginal bleeding. In addition, transdermal oestradiol replacement can restore mean femoral neck bone mineral density (BMD) to normal levels in young women with spontaneous primary ovarian deficiency. (3,4,5)

Currently, there is limited research on the effectiveness of various hormone replacement therapy (HRT) regimes in treating estrogen deficiency in adolescents and young adults, specifically those with Turner Syndrome. A pilot study in Indianapolis found that transdermal HRT resulted in faster spine bone accrual and increased uterine growth compared to orally administered conjugated estrogen. Another study indicated that both oral and transdermal 17ß-estradiol resulted in comparable increases in uterine size for those with Turner Syndrome, which was similar to normal women. (6,7)

The research gap is the significant need for more knowledge and understanding of Turner syndrome patient care. Specifically, there is a notable absence of studies comparing the efficacy of oral estradiol valerate and transdermal 17ß-estradiol in managing this population, as well as a need for clear references for the ideal dosage of estrogen replacement therapy and pubertal induction. Importantly, this gap is further compounded within the context of Turner syndrome patients in Malaysia, as no specific research has been conducted in this population. Addressing these research gaps is crucial for improving the care for individuals with Turner syndrome, not just in Malaysia, but globally and ensuring that appropriate, evidence-based treatment protocols are developed and implemented.

SECTION 2

LITERATURE REVIEW

2.1 Hormone Replacement Therapy (HRT)

Hormonal replacement therapy (HRT) is vital in managing young females with ovarian insufficiency. The rationale for hormonal treatment, specifically estrogen, in this population is for the attainment of secondary sexual characteristics, menstrual induction, pubertal growth spurt generation, acquisition of adequate bone mineral mass, and promotion of sufficient uterine development. Low-dose estrogen can be used over approximately three years, similar to the normal pubertal process. Ideally, the dosage should mimic the physiological level of the hormones as produced by normal ovaries. The dosage can be increased at six months intervals to mimic the normal pubertal tempo until the adult dosing is reached (2).

Continuous estrogen replacement is required to avoid symptoms of estrogen deficiency. The choices of estrogen are 17ß-estradiol, ethinylestradiol, and conjugated equine estrogens. Estrogen can be administered via oral or transdermal routes (3). Both routes provide normal plasma concentrations of estradiol and have similar effects in suppressing gonadotrophin concentrations (4). However, transdermal estrogen has fewer systemic complications because it avoids first-pass metabolism by the liver (5). It can achieve higher plasma levels of circulating estradiol with a lower treatment dose, thus resulting in fewer circulating estrogen metabolites (6). Both regimes effectively prevent bone loss and produce favourable lipid and lipoprotein changes (7). Conjugated equine estrogen (CEE) is no longer recommended due to its higher incidence of thromboembolic and cardiovascular events, as documented in post-menopausal females (8). Besides, Wakatsuki et al demonstrated a higher plasma level of triglycerides and smaller LDL particles that are less impervious to oxidation in CEE recipients than transdermal 17β estradiol recipients (9). This is further supported by Zaiem et al who showed in their small-scale meta analysis (n=4) that CEE is associated with a higher LDL-C level than transdermal and oral 17β estradiol in Turner Syndrome individuals (10). Again, these findings are in agreement with Ho and coworkers who showed a reduction in atherosclerotic vascular disease (ASVD) risk in the transdermal 17β estradiol recipients compared to the CEE group (11). Furthermore, Shah and colleagues showed increased level of estradiol and a higher rate of successful feminization in adolescent girls with hypogonadism who received transdermal 17β estradiol than the CEE recipients in their small RCT (n=20, age

range: 12-18 years)(12). As a result, CEE is now considered inferior to either transdermal or oral 17β estradiol in terms of its safety profile.

A randomised controlled trial has shown that both oral and transdermal 17ß-estradiol in titrating doses produce close-to-normal estrogen concentrations. However, despite similar estrogen concentrations, bio-estrogen and total estrone and estrone sulfate concentrations were much higher after oral 17ß-estradiol, proposing that the transdermal route leads to a more physiological estrogen milieu than the oral formulation (4). In addition, by avoiding first-pass hepatic metabolism, transdermal estrogen maintains its effectiveness with a better tolerability profile due to fewer side effects like breast tenderness and erratic vaginal bleeding (6). In addition, transdermal estradiol replacement restores mean femoral neck bone mineral density (BMD) to normal in young women with spontaneous primary ovarian deficiency (13). Furthermore, transdermal 17β-estradiol is associated with a significant reduction in the thromboembolic event risk compared to oral estradiol preparation in post-menopausal females (8). This is further corroborated by a small-scale meta-analysis (n= 4 studies) by Zaiem et al. which showed the superiority of transdermal 17β estradiol over oral estradiol in reducing fasting blood glucose and total cholesterol and in elevating bone mineral density (9). Besides, Taboada et al. showed that transdermal estradiol administration resulted in estradiol, estrone, and bioestrogen concentrations that were closer to the physiological levels of those three compounds than when estradiol was administered orally in Turner Syndrome (14). All this evidence demonstrates the possible superiority of transdermal 17β estradiol over oral estradiol. However, a definitive conclusion is thwarted by the small sample size employed by each study. Besides, the only available systematic review and meta-analyses conducted by Zaiem et al. on this topic also suffered from many methodological weaknesses, such as:

1) combining trials that have different methodological qualities (no blinding of the participants and outcome assessors and no allocation concealment performed in all trials, one trial (Gravholt et al.) is a non-randomized trial, and one trial by Shah et al. has severe participant attrition) based on the GRADE assessment system for quality of the assessed trials included in the meta-analysis;

2) the included trial used different regimens for the oral estradiol route (3 out of 4 trials combined oral estradiol with conjugated estrogen or norethisterone acetate);

3) the included trials utilised different doses for oral and transdermal estradiol routes;

4) the included trials have different follow-up times (in 3 out of 4 trials, the follow-up durations were too short (12 months or less));

5) the included trials' participants have different age distributions at trial recruitment (one trial (Gravholt et al. 1998) recruited mid-age TS adults (30 years and above), whilst, for the other trials, the mean ages of participants at baseline in both oral and transdermal routes were less than 18 years old);

6) No metaregression technique was performed to control the confounding effects of these different trial qualities (as evidenced by high I^2 statistics indicating high trial heterogeneity, which may compromise the reliability of the pooled results) and other potential confounding variables (for instance, the concurrent use of growth hormone, which may impact the bone mineral density (BMD) of the TS patients treated with either the transdermal or oral form of estrogen);

7) the authors did not report the differential efficacy of these two routes of estradiol administration on pubertal induction parameters (uterine size, time to withdrawal bleeding occurrence, and breast development as assessed by the Turner staging system).

8) Overreliance on surrogate markers such as bone mineral density (BMD), fasting glucose and lipid profiles as outcome measures. No direct comparisons of hard clinical outcome measures between the oral and transdermal forms of oestrogen replacement therapy were performed, such as breast development (Tanner stage B4 and above), time to the first menstrual initiation (defined as time to first withdrawal bleeding occurrence) and normalization of uterine dimensions (the uterine length and fundal AP diameter).

9) The presence of publication bias was not assessed. As a result, the exclusion of trials with negative findings could not be ruled out;

10) No pooled results for the safety profiles of both transdermal and oral routes of oestrogen replacement were provided (e.g., rates of local skin reaction for transdermal oestrogen and liver function abnormalities for both forms of oestrogen replacement therapies).

In fact, Zaiem et al. concluded at the end of their report that a well-designed RCT that satisfactorily addresses all the aforementioned methodological shortcomings is warranted to

provide more concrete evidence of the differential efficacy between the two different routes of estrogen replacement therapy in Turner syndrome. Consequently, it can be surmised that there is still clinical equipoise on the best routes of administration for estrogen replacement therapy that may optimize the rates of successful pubertal induction (as defined by overt clinical endpoints such as normalization of uterine dimension, shorter time to withdrawal bleeding occurrence, and successful breast development (Tanner Stage B4 and above)) in TS patients.

Patients with a uterus need progestogen supplementation. After 1 to 2 years of estrogen replacement therapy, progestin should be added to prevent endometrial hyperplasia. (15) There are several types of progestin, and each has different effects on various receptors like progesterone, androgen, glucocorticoid, and mineralocorticoid receptors. Therefore, some progestin will have not only progestational effects but also anti-androgenic, anti-mineralocorticoid, and/or glucocorticoid-agonistic action (2). Once progestin is added, many women prefer the ease of use of a pill containing both estrogen and progestin. Thus, another accepted option is to use combined oral contraceptive pills (COCP) (2).

Few studies compare the efficacy of different HRT regimes in treating adolescents and young adults with estrogen deficiency and mainly concentrate on Turner Syndrome (TS) only. A pilot study in girls with TS in Indianapolis demonstrated that there is faster bone accrual at the spine and increases in uterine growth in those who received transdermal preparation compared to conjugated oral estrogen recipients (16). Another study looking at the increment of uterine size after oral vs transdermal 17ß-estradiol in TS showed that both groups achieved similar increases in uterine size comparable to normal women(17).

Most studies have enlightened more on the effect of HRT regimes in postmenopausal women. Hillard et al. found no significant difference between oral and transdermal estrogen on bone density and bone turnover (18). Both were equally effective in preventing osteoporosis in postmenopausal women (7). Moreover, a randomised control trial found no difference in reducing hot flushes for both oral and transdermal estrogen (19).

The aim of this study is to compare the efficacy of different estrogen replacement regimes in adolescents and young women with estrogen deficiency in induction of puberty (breast and uterine development), attainment of withdrawal bleeding, improvement in bone mineral density and also the patients' satisfaction regarding the treatment received.

2.2 Safety profile of estrogen-only replacement therapy

The safety profile of estrogen-only hormone replacement therapy was primarily investigated among post-menopausal women. The details from various study sources (meta-analysis, RCTs, observational studies and case series) are summarized as follows.

2.2.1 General safety information

In a Bayesian meta-analysis by Derzko and colleagues, the use of 1.5 mg estrogel was associated with statistically-significant relative risk of adverse events (occurrence of any of these AEs: post-menopausal bleeding, headache, breast pain, infection, nausea, rash and vaginitis) than placebo (RR: 1.58 (95% CI 1.19, 2.00)) (20). However, the use of the lower-dose 0.75 mg oestrogel was not significantly associated with AEs compared to placebo (RR:1.21 (95% CI 0.87, 1.61). Besides, both 0.75 and 1.5 mg oestrogel were not significantly associated with AEs-associated treatment discontinuation compared to placebo ($RR_{0.75\ mg\ oestrogel}$: 2.21 (95% CI:0.59, 5.89); $RR_{1.5\ mg\ oestrogel}$: 2.27 (95% CI:0.61, 6.09)) (20). Hence, oestrogel use does not result in a higher probability of treatment discontinuation secondary to AEs occurrence than placebo.

In another meta-analysis by Canonico et al., oral estrogen was significantly associated with venous thromboembolism (VTE) (OR: 2.5 (95% CI 1.9, 3.4)), but not transdermal estrogen (OR: 1.2 (95% CI 0.9, 1.7)) (21). The findings were further corroborated by a large UK population-based cohort study based on a cohort of 955 582 post-menopausal females aged 50 to 79 years old from the United Kingdom's General Practice Database (period of recruitment 1st January 1987 until 1st March 2008) (22). The authors found similar findings where oral estrogen was significantly associated with VTE (RR: 1.49 (95% CI: 1.37, 1.63)). However, the risk of VTE was not elevated in transdermal estrogen users (RR: 1.01 (95% CI 0.89–1.16)). Hence, transdermal estrogen is deemed safer than oral estrogen in terms of VTE occurrence.

2.2.2 Cardiovascular Disorders

An elevated probability of cardiovascular accident (CVA) and DVT has been documented with estrogen-only therapy. Besides, an increased risk of venous thromboembolism (VTE) (RR: 1.63 (95% CI 1.40–1.90)), DVT (RR: 2.09 (95% CI 1.35,3.23)), stroke (RR: 1.24 (95% CI 1.03, 1.48), but not MI (RR: 1.17 (95% CI 0.80, 1.71)), has been documented in oral estrogen users relative to transdermal oestrogen recipients (23). Should any of these occur or be suspected, estrogen with or without progestin therapy should be withheld promptly.

Other concomitant arterial vascular disease risk factors such as hypertension, diabetes mellitus, tobacco use, hypercholesterolemia, and obesity and/or (VTE) should be managed accordingly.

Cerebrovascular Accident (CVA) / Stroke

In the Women's Health Initiative (WHI) estrogen-only sub-trial, a statistically significant increased risk of CVA was documented in females aged 50 to 79 years old receiving 0.625 mg conjugated estrogen (CE) daily compared to similar-age-group females who were in the placebo arm (incidence rate: 45 versus 33 per 10,000 women-years, HR: 1.33 (95% CI 1.05, 1.68), Absolute excess risk per 10000 women-years: 10.9 (95% CI 1.6, 10.3)) (24). The heightened risk was shown in year 1 and remained increased throughout the study. Based on a subgroup analysis of women aged 50-59 years old, no elevated risk of CVA was found in 0.625 mg CE-alone recipients compared to similar age women administered with placebo (18 versus 21 per 10,000 women-years, HR: 0.89 (95% CI 0.47, 1.69), Absolute excess risk per 10,000 women-years: 2.0) (24). However, the risk of CVA was increased in 0.625 mg CE recipients aged 60-69 years old compared to placebo recipients of similar age (84 versus 54 per 10,000 women-years; HR: 1.62 (95% CI 1.15, 2.27); Absolute excess risk per 10,000 women-years: 20.0), but not in women aged 70-79 years old (66 versus 52 per 10,000 women-years; HR: 1.21 (95% CI 0.84, 1.75); Absolute excess risk per 10,000 women-years: 17.0).

In the WHI combined estrogen-progestin sub-trial, a statistically significant increased risk of CVA was documented in women aged 50 to 79 years old receiving 0.625 mg CE daily plus 2.5 mg MPA compared to 50-to-79-year-old women administered with placebo (33 versus 25 per 10,000 women-years; HR: 1.31 (95% CI 1.03, 1.68); Absolute excess risk per 10,000 women-years: 7.8 (95% CI 0.8, 14.2)) (24). The heightened risk was demonstrated after the first year of observation and remained elevated throughout the study (24). Based on these results, estrogen plus progestin therapy should be promptly halted if CVA is suspected or

occurs in the post-menopausal women. Nevertheless, CVA occurrence in young children or adolescents with TS receiving estrogen-based pubertal induction therapy is perhaps low due to the young age factor. Nonetheless, further well-designed studies are required to prove this assumption.

Coronary Heart Disease

In the WHI estrogen-sole sub-trial, no statistically-significant overall effect on coronary heart disease (CHD) events (defined as nonfatal MI, silent MI, or CHD death) was reported in women receiving estrogen-alone compared to placebo (HR 0.95 (95% CI 0.79, 1.16)) (25). Subgroup analyses of women 50 to 59 years of age suggest a statistically non-significant reduction in CHD events (daily 0.625 mg CE-alone recipients compared to the placebo group) in women with less than 10 years since menopause (8 versus 16 per 10,000 women-years; HR 0.48 (95% CI 0.20, 1.17)) (24).

In the WHI estrogen plus progestin sub-study, there was a statistically non-significant increased risk of CHD events reported in women receiving daily CE (0.625 mg) plus MPA (2.5 mg) compared to women receiving placebo (41 versus 34 per 10,000 women-years; HR 1.23 (95% CI 0.99, 1.23)) (24). An increase in relative risk was demonstrated in year 1, and a trend toward decreasing relative risk was reported in years 2 through.

In postmenopausal women with documented heart disease (n_{CE+MPA} = 1380, $n_{placebo}$ = 1383) average 66.7 years (SD 6.7 years) of age), in the Heart and Estrogen/Progestin Replacement Study [HERS] trial, treatment with daily combination of 0.625 mg CE and 2.5 mg MPA did not confer statistically-significant benefits against non-fatal MI or CHD-associated mortality compared to placebo (HR: 0.99 (95% CI 0.80, 1.22)) (26). During an average follow-up of 4.1 years, treatment with CE plus MPA did not also improve other cardiovascular outcomes (revascularization rate, coronary artery bypass graft surgery rate, hospital admission rates due to angina or congestive heart failure and others) in postmenopausal women with established coronary heart disease ($p>0.05$). There were more CHD events in the CE plus MPA-treated group than in the placebo group in year 1, but not during the subsequent years. Around 2,321 women from the original HERS trial then consented to take part in an open-label extension of HERS, HERS II that has an additional average follow-up period of 2.7 years, resulting in a mean total follow-up of 6.8 years overall. The CHD event rates were also found to be similar among women in the CE plus MPA group and the placebo group in this HERS II trial (27).

Venous Thromboembolism

In the WHI estrogen-alone sub-study involving 10739 women of 50-79 years of age, the VTE (DVT and PE) risk was increased for women receiving daily 0.625 mg CE-alone compared to placebo (30 versus 22 per 10,000 women-years; HR 1.32 (95% CI 0.99, 1.75)) (28). However, the effect was marginally insignificant. Based on a more in-depth analysis, the risk of DVT attained statistical significance (23 versus 15 per 10,000 women-years; HR 1.47 (95% CI 1.06, 2.06)) whilst the risk of PE remained statistically insignificant (14 versus 10 per 10,000 women-years; HR 1.37 (95% CI 0.90, 2.07)) (28). The increased VTE risk was the greatest during the first 2 years of follow-up. Statistically-significant interactions between estrogen use and other VTE risk factors (e.g. age, BMI and others) were absent.

In another WHI sub-trial investigating the effects of estrogen plus progestin on VTE development in 16608 women aged 50-79 years old, a statistically significant 2.06-fold greater hazard of VTE was reported in women assigned to 0.625 mg CE plus 2.5 mg MPA daily than the placebo recipients (35 against 17 per 10,000 women-years; HR 2.06 (95% CI 1.57, 2.70)) (29). They also found statistically significant higher risks of VTE with increasing age and being overweight (HR 3.80 (95% CI 2.08, 6.94)) or obese (HR 5.61 (95% CI 3.12, 10.11)). Besides, the presence of Factor V Leiden further raised venous thrombosis risk (HR 6.69 (95% CI 3.09, 14.49)). The increased VTE risk was shown during the first year of the study and remained elevated throughout the whole study period (29). These findings were further supported by the results from the HERS trials, which showed higher incidence of venous thromboembolic events in the combined CE-MA group compared to placebo recipients (HR: 2.89 (95% CI 1.50, 5.58)) (26). Estrogen plus progestin therapy should be promptly halted if VTE occurs or is suspected. If possible, estrogen should be halted for a minimum of 4 to 6 weeks prior to any surgical types associated with a raised thromboembolic risk, or during the extended period of immobility.

Apart from those, the presence of familial hereditary thrombophilia should also be screened for before initiating estrogen replacement therapy. In the Malaysian context, Abdullah and co-workers demonstrated 5.6% heterozygous FVL mutation in 71 healthy Malaysian Indians (30). Besides, they did not find any homozygous FVL or G2021A prothrombin mutations in their samples. However, Mohd Yusoff and colleagues did not find any factor V Leiden mutation in 46 Kelantanese Malays experiencing recurrent spontaneous abortions (31). Nevertheless, Ayadurai, Muniandy and Omar (2009) found FVL prevalence of

about 8 out of 87 (9.2%) women with active protein C resistant (APC-R) recurrent pregnancy losses (32). Following racial stratification, the investigators found FVL prevalence rates of 1% (3/287) in Malays, 6.1% (5/82) in Indians and none in Chinese. The majority of the rest of the APC-R cases were due to protein S deficiency (6.9%), protein C deficiency (51.7%), combined proteins C and S deficiencies (6.9%) and 1.1% with anti-thrombin deficiency (32). These findings underscore the importance of familial thrombophilia screening in our TS population prior to formal inclusion into the trial and subsequent estrogen replacement therapy initiation.

2.2.3 Malignant Neoplasms

2.2.3.1 Endometrial Cancer

In a meta-analysis by Grady et al. unopposed estrogen usage was associated with 2.3-fold (95% CI 2.1, 25) increased risk of endometrial carcinoma among females with functioning uterus and this is dependent upon treatment duration (a) <1 year: RR= 1.4 (95% CI 1.0, 1.8); b) 1-5 years: RR= 2.8 (95% CI 2.3, 3.5); c) 5-10 years: RR= 5.9 (95% CI 4.7, 7.5); d) >10 years: RR= 9.5 (95% CI 7.4, 12.3)) and the dose of estrogen (a) 0.625 mg: RR= 3.4 (95% CI 2.0, 5.6); b) 1.25 mg and above: RR= 5.8 (95% CI 4.5, 7.5)) (33). The findings were further supported by the large nationwide Million-Women Study which confirmed the association between unopposed estrogen use and endometrial cancer in post-menopausal women without prior history of cancer or hysterectomy (RR: 1.45 (95% CI 1.02, 2.45), p=0.04) (34). These observations were further scrutinised and verified by Sjogren, Morch and Lokkegaard in their systematic review where they identified unopposed estrogen use as one of the risk factors of endometrial cancer development (35). Hence, clinical surveillance, which includes directed or random endometrial sampling when indicated, in all women using estrogen-alone is imperative.

So far, there is no evidence that the use of natural estrogen leads to a different endometrial risk profile than synthetic estrogen of equivalent estrogen dose. Adding a progestin to estrogen therapy in postmenopausal women has been shown by Grady and colleagues and in the Million-Women study to decrease the risk of endometrial carcinoma.

Breast Cancer

The most pivotal randomized clinical trial providing information about breast cancer in estrogen-alone users is the WHI sub-trial of daily 0.625 mg CE only. In the WHI estrogen-alone sub-study, after an average follow-up of 7.1 years (SD 1.6 years), daily CE-only regime

was not significantly associated with a raise in invasive breast cancer risk (HR: 0.80 (95% CI 0.62, 1.04), p = 0.09) or overall breast cancer (HR: 0.82 (95% CI 0.65, 1.04), p = 0.10) (36). The authors concluded that the use of unopposed estrogen was not associated with raised breast cancer cases in post-menopausal women with a prior history of hysterectomy.

On the hand, information about breast cancer incidence in combined estrogen-progestin users was provided by the WHI sub-study of daily 0.625 mg CE combined with 2.5 mg MPA (37). After a mean follow-up of 5.6 years (SD 1.3 years), a significant increased risk of invasive breast cancer was found in women who was allotted to the combined daily CE-MPA group (Unweighted HR: 1.24 (95% CI 1.01, 1.54), p=0.003), as well as overall breast cancer cases (Unweighted HR: 1.24 (95% CI 1.02, 1.50), p<0.001). Among women who reported 5 or more years of prior use of hormone therapy, the hazard ratio of invasive breast cancer was 2.27 (95% CI 1.00, 5.15), and the absolute risk was 54 versus 24 cases per 10,000 women-years for combined CE-MA recipients and placebo users, respectively. Among women who reported no prior use of hormone therapy, the hazard ratio of invasive breast cancer was 1.09 (95% CI 0.86, 1.39), and the absolute risk was 40 per 10,000 women-years in the combined CE-MPA group versus 36 cases per 10,000 women-years in the placebo recipients. In the same sub-trial, invasive breast cancers were larger (mean tumor size in cm (CE+MPA vs placebo): 1.7 (SD 1.1) vs 1.5 (SD 0.9), p=0.04), were diagnosed at a more advanced stage (metastatic / regional tumours: 25.4% vs 16.0%, p=0.04) and with greater proportions of being node positive (25.9% versus 15.8%, p = 0.03) in the combined CE-MPA group than the placebo group. No significant differences were found with respect to other prognostic factors such as histological characteristics, tumour grades, ER and PR status between the CE+MPA and placebo groups. However, the risks are assumed to be much more reduced if estrogen replacement therapy is used in young women such as TS patients requiring pubertal induction therapy.

Ovarian Cancer

The WHI estrogen plus progestin sub-study reported a statistically non-significant increase in ovarian cancer risk. After an average monitoring duration of 5.6 years, the relative risk for ovarian cancer in the combined CE and MPA group with respect to placebo was 1.58 (95% CI 0.77, 3.24) (38). The absolute risk for CE plus MPA versus placebo was 4 versus 3 cases per 10,000 women-years. Besides, the investigators also demonstrated that current or recent

use of estrogen therapy was significantly associated with serous (OR=1.63, 95% CI 1.27, 2.09) or endometroid (OR=2.00, 95% CI 1.17, 3.41) ovarian cancer subtypes in their case-control study. In addition, the use of estrogen plus progestin and estrogen-only products, in particular for 5 or more years, has been linked with an increased in ovarian cancer risk in some epidemiologic studies. Nonetheless, the associative effect of the duration of estrogen with ovarian cancer risk is inconsistent across all epidemiologic studies since several studies report no significant association.

2.2.4 Probable Dementia

In the WHIMS estrogen-only ancillary study of WHI, a sample of 2,947 hysterectomized women aged 65 to 79 years old was randomly allotted to either daily 0.625 mg CE-only or placebo (39). Following a mean follow-up of 5.2 years, 28 women in the estrogen-alone group and 19 women in the placebo group were diagnosed with probable dementia. The relative risk of probable dementia for CE-alone versus placebo was 1.49 (95% CI 0.83, 2.66). The absolute risk of probable dementia for CE-alone versus placebo was 37 versus 25 cases per 10,000 women-years.

In the WHIMS estrogen plus progestin auxiliary study of WHI, a sample of 4,532 postmenopausal females aged 65 to 79 years old was randomly assigned to daily 0.625 mg CE in combination with 2.5 mg MPA or placebo (39). After a mean duration of follow-up of 4 years, 40 women in the CE and MPA group and 21 women in the placebo group had been diagnosed with probable dementia. The relative risk of probable dementia for CE plus MPA versus placebo was 2.05 (95% CI 1.21,3.48). The absolute risk of probable dementia for CE plus MPA versus placebo was 45 versus 22 cases per 10,000 women-years (39). The detrimental effects of both estrogen-only and combined estrogen-progestin replacement therapies were further verified by Espeland and colleagues who showed statistically significant worse global cognitive function, measured by the Modified Mini-Mental State Examination (3MSE) scores, in CE users (mean difference (SE): 0.26 points lower (0.13), p=0.04) and combined CE-MPA users (mean difference (SE): 0.21 points lower (0.08), p=0.006) compared to placebo in 2808 post-menopausal women aged 65-79 years old (40).

After combining the data from the two populations in the WHIMS estrogen-only and estrogen plus progestin ancillary studies as pre-specified in the original WHIMS protocol, the reported overall relative risk for probable dementia was 1.76 (95 %CI, 1.19-2.60). Since both

auxiliary analyses were carried out in females aged 65 to 79 years old, it is thus uncertain whether such findings are relevant to younger postmenopausal females. However, Pourhadi and co-workers recently showed an increased dementia rate among estrogen-progestin users (HR: 1.24 (95% CI 1.09, 1.35)) compared to age-matched controls that include younger post-menopausal females (41). The effects persisted irrespective of regimen types (cyclic or continuous) or the onset of dementia (late or early). Nevertheless, only those who received estrogen-progestin combination at the age 55 years or less exhibited persistent association between estrogen progestin use and dementia.

2.2.5 Gallbladder Disease

In an analysis of the WHI trial data by Cirillo and co-workers, increased hazard of gallbladder disease and surgery was observed in estrogen-only (CEE) (HR: 1.67 (95% CI, 1.35-2.06)) or combined estrogen-progestin (HR: 1.59 (95% CI, 1.28-1.97)) users. Besides, estrogen users had high risks of developing cholecystitis and gallstones (CEE: HR: 1.86 (95% CI 1.48,2.35); combined estrogen-progestin regime: HR: 1.68 (95% CI 1.34-2.11)) (42). These findings were further corroborated by HERS trial results which a higher hazard of gallbladder disease in women receiving the combined estrogen and progestin regimen (HR 1.38 (95% CI 1.00, 1.92)) (26). Therefore, caution should be exercised when prescribing estrogen-containing HRT, especially in those with a high risk of gallbladder diseases.

2.2.6 Hypercalcemia

Estrogen administration may potentially cause severe hypercalcemia in individuals with stage IV breast cancer with bone deposits. This was firstly observed by Beckett in 1969 who showed 7 out of 36 post-menopausal breast cancer patients with bone metastasis experienced hypercalcemia following estrogen administration (43). A more recent review by Coelingh Bennink and colleagues summarised the evidence for estrogen use in breast cancer and they demonstrated hypercalcemia as one of the common adverse events occurred in diethylstilbesterol (DES) recipients (44). Hence, extra considerations must be borne in mind when prescribing estrogen to those with heightened risks of hypercalcemia.

2.2.7 Visual Abnormalities

Retinal vascular occlusion has been reported among estrogen recipients. Several case reports have previously documented the possible link between oral estrogen use and retinal vein occlusion (RVO) or retinal artery occlusion (RAO) (45-46). However, a multiple cohort study (205 304 estrogen-containing female hormone therapy (e-FHT) users vs 755 462 matched controls) showed no significant elevation of hazards of either RVO (HR: 1.07; 95% CI, 0.82-1.39; P = 0.65) or RAO (HR: 1.17; 95% CI: 0.83,1.65; P = 0.36) among estrogen users compared to the age-and-race-matched controls (47). Hence, we can fairly assume that estrogen use does not cause elevated risk of retinal vascular thrombosis.

2.2.8 Addition of a Progestin in non-hysterectomised females

Previous research in 1970s demonstrated an elevated risk of endometrial hyperplasia and endometrial cancer in estrogen-only HRT recipients (48-49). Therefore, progestin addition for 10 or more days within a cycle of estrogen administration, or daily if a continuous regimen of combined progestin-estrogen is used, is recommended since it reduces endometrial hyperplasia incidence (50-51). Nevertheless, the risk of breast cancer may be elevated when estrogen is combined with progestin in an HRT regimen, especially in the presence of prolonged duration of use (52). Hence, caution should exercised so that the benefits will outweigh the risks when prescribing combined estrogen-progestin therapy in non-hysterectomised females.

2.2.9 Elevated Blood Pressure

Previous research documented that significant blood pressure elevation can be ascribed to the differences in the modes of estrogen delivery (53). This is further supported by a recently-published population-based cohort study (112 240 Canadian females aged 45 years and above who used estrogen-only HRT postmenopausal symptoms alleviation) which showed a higher risk of hypertension in oral estrogen users than the transdermal (HR: 1.14 (95% CI 1.08, 1.20)) and vaginal estrogen (HR: 1.19 (95% CI 1.13, 1.25)) users (54). Besides, the length of estrogen use and the cumulative estrogen dose were significantly predictive of hypertension. Based on these findings, the mode of estrogen delivery is thus associated with the development of hypertension.

2.2.10 Hypertriglyceridemia

In females with antecedent elevated triglyceride level, estrogen use may be associated with severe hypertriglyceridemia which may result in pancreatitis (55). Estrogen treatment should be interrupted if hypertriglyceridemia-induced pancreatitis occurs.

2.2.11 Liver Derangement and/or Prior Episode of Cholestatic Jaundice

Estrogen has long been known to be metabolised by the liver (56). Hence, females with hepatic impairment may inadequately metabolise estrogen, resulting in an increased serum estrogen concentration level. Besides, women with a previous history of cholestatic jaundice due to prior use of estrogen or pregnancy, extra care is warranted. In the presence of recurrence, estrogen must be promptly withheld.

2.2.12 Hypothyroidism

Estrogen use may result in inflated thyroid-binding globulin (TBG) levels (57). Those without abnormal thyroid function may offset the increased TBG by producing more thyroid hormone, thus keeping the free T4 and T3 serum levels within the normal range. Individuals who are reliant on thyroid hormone replacement therapy and are also recipients of estrogen may need additional doses of their thyroid-replacement medication. Their thyroid function should be regularly examined so that the thyroid function parameters can be maintained within an optimal range.

2.2.13 Fluid Retention

Estrogens may lead to significant edema via transcapillary retention of albumin, resulting in the extracellular fluid volume expansion (58). Women with illnesses that might be impacted by fluid retention, for instance cardiac or renal impairment, require cautious monitoring when estrogen-only regimen is administered.

2.2.14 Hypocalcemia

Estrogen replacement therapy should be judiciously and cautiously administered in patients with hypoparathyroidism since estrogen-induced low serum calcium concentration may transpire. It was first demonstrated in a case report in 1975 where a 35-year-old female experienced tetanic episodes after being treated with estrogen-containing oral contraceptives (59). Therefore, serum parathyroid hormone should be measured prior to starting estrogen replacement therapy.

2.3 Study Rationale

2.3.1 Trial Rationale from the Clinical and Biological Perspectives

Adequate ovarian function is vital to promote secondary sexual characteristics and uterine development. However, in patients with premature ovarian insufficiency (POI) of various causes, including genetic disorders like Turner syndrome, Complete gonadal dysgenesis, and Swyer syndrome, the ovaries are impaired thus, they have insufficient endogenous estrogen production to promote breast and pubic hair growth, uterine development and initiation and normal regulation of menses. Lack of estrogen can lead to vasomotor symptoms and psycho-affective, urogynecology, and sexual changes. Estrogen is important for bone health and cardiovascular risk reduction in women. There are no proven, effectively established regimes of hormone replacement therapy (HRT) for pubertal induction and maintenance in these affected young women.

2.3.2 Rationale for Chosen Subject Population

Turner Syndrome patients are selected as the trial participants since TS is the most prevalent condition (1 in 2000) among those with gonadal dysgenesis. The inclusion of oestrogen-deficient participants afflicted with other conditions, such as Swyer Syndrome or Premature Ovarian Insufficiency, will make the data analyses more complex since we need to conduct multiple subgroup analyses to identify the effects within each specific disease entity. This procedure will be made more complicated if the number of successfully-recruited participants is small due to the high attrition rate. Hence, we plan to recruit only TS patients for this trial to ensure sample homogeneity.

We will also include mosaic TS patients since excluding such a subpopulation of TS patients will make our sample size smaller. However, we will perform a subgroup analysis for this particular TS subpopulation. We believe a single subgroup analysis will not affect the study power much since we are confident that we will be able to recruit a sufficient number of mosaic TS patients as our trial participants.

2.3.3 Rationale for Hormone Replacement Therapy (HRT) Choices

Based on our literature review, there is still a knowledge gap with respect to whether estradiol valerate (Progynova) or 17ß-estradiol (Oestrogel) is the best HRT regime for pubertal

induction in Turner Syndrome. Conjugated equine estrogen (CEE) will not be investigated in this trial since a number of previous research have conclusively shown the superiority of estradiol over CEE (see Section 2.1). Hence, this trial is designed to provide a conclusive answer to this unanswered research gap.

2.3.4 Rationale for the Trial Design and Statistical Analysis

A hybrid real-world (RWD) and concurrent trial design was chosen due to the rarity of Turner Syndrome. The double-dummy design could not be opted due to inavailability of dummy (placebo) gel or tablets (60). As a result, the ascertainment bias could not be ruled out, particularly during trial outcome assessment.

We will also employ the Bayesian statistical paradigm and Bayesian Hierarchical Model (BHM) for statistical analysis to reduce the impacts of possible small sample sizes for this study. By using the Bayesian paradigm, the valuable findings from the previously published small-size trials can be represented by a prior distribution. This will be combined with our trial's data that are summarised in the form of likelihood. Using Bayes' theorem, posterior updates of the estimates of each trial endpoint can be obtained by multiplying the likelihood (i.e. information in our trial's data) with the prior distribution (valuable information/findings about the trial endpoint from prior research). In this case, we "borrow" the strength of information from previous research to make our estimates of the trial endpoint parameters (i.e. mean difference in uterine length (measured in cm) between the Progynova and Oestrogel groups at 2 years) more precise (61).

2.3.5 Rationale for the Choices of Efficacy Endpoints and Safety Parameters

The Tanner Staging for breast development was chosen since this is a primary endpoint of interest that is frequently monitored during clinical management for TS patients. Withdrawal bleeding status and time to the first withdrawal bleeding were selected since no previous research has investigated the distinctive effects of different HRT regimes on these two endpoints. More frequent measurement points (six-monthly follow-ups instead of yearly or end-of-trial measurements) are employed to obtain more granular information about the impacts of different HRT regimes on each trial endpoint.

Besides, the safety profiles of both estrogen-only preparations have not been extensively investigated in female TS adolescents and young women requiring long-term HRT

for pubertal induction. As previously elaborated, the safety features of both estrogen-only preparations were characterized in post-menopausal females aged 50 to 79 years old in the majority of studies. Hence, we believe this trial will provide further evidence on the safety characteristics of both estrogen preparations in female TS adolescents and young women. Besides, a composite safety outcome could also be constructed and this can be analysed using the win-ratio method (62), resulting in increased statistical power (63). However, the individual component of the composite safety outcome may not have similar clinical importance to the trial participants and the individual component of the composite safety outcome might not be similarly affected by the interventions, two chief pre-requisites for the validity of a composite outcome (64-65). Therefore, we choose to analyse each safety parameter separately.

2.4 Conceptual Framework

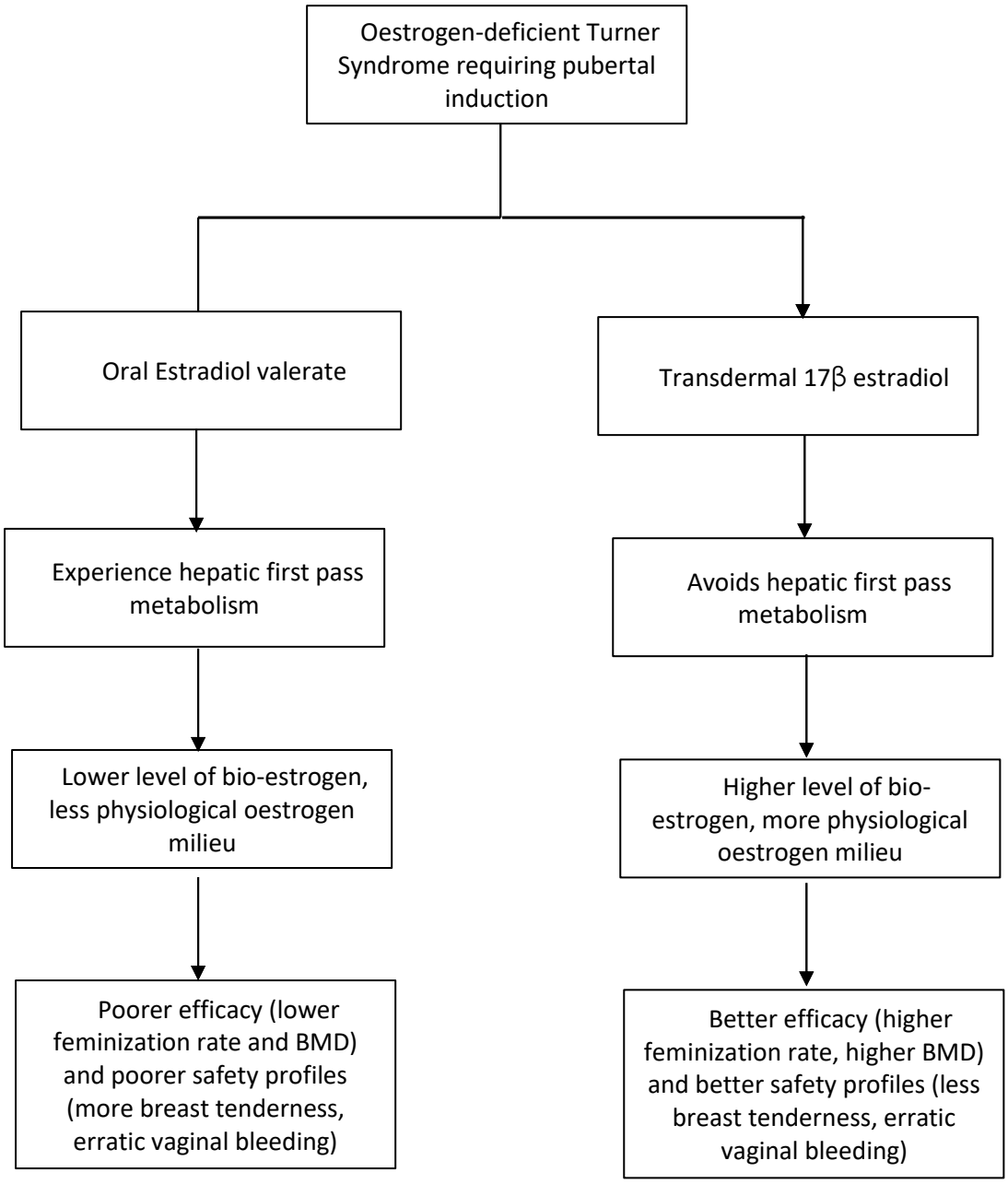


Figure 1: The biological framework elucidating the impacts of different HRT regimes (oral estradiol valerate versus transdermal 17β estradiol) on pubertal induction in Turner Syndrome patients

SECTION 3
TRIAL OBJECTIVES

3.1 General Objective

To compare the efficacy and safety of different estrogen replacement regimes in pubertal induction in adolescents and young women with Turner Syndrome

3.2 Specific Objectives

1. To compare the proportions of complete breast development in adolescents and young women with Turner Syndrome who receive two different HRT regimes (Oral estradiol valerate (Progynova) or Transdermal 17β estradiol (Oestrogel)).
2. To compare the mean differences in uterine dimensions in adolescents and young women with Turner Syndrome receiving two different HRT regimes (Oral estradiol valerate (Progynova) or Transdermal 17β estradiol (Oestrogel)).
3. To compare the proportions of adolescents and young women with Turner Syndrome who attain uterine withdrawal bleeding after receiving two different HRT regimes(Oral estradiol valerate (Progynova) or Transdermal 17β estradiol (Oestrogel)).
4. To compare the timing of uterine withdrawal bleeding in adolescents and young women with Turner Syndrome who received two different HRT regimes (Oral estradiol valerate (Progynova) or Transdermal 17β estradiol (Oestrogel)).
5. To compare the differences in the proportions of adverse events (nausea, vomiting, hypersensitivity, hypertension, new-onset headache, lower abdominal pain, vaginal bleeding and discharge) or mean adverse event parameters (serum triglyceride) in adolescents and young women with Turner Syndrome who receive two different HRT (Oral estradiol valerate (Progynova) or Transdermal 17β estradiol (Oestrogel)).

3.3 Research Questions

1) Is there a difference in the proportions of complete breast development in adolescents and young women with Turner Syndrome who receive two different HRT regimes (Oestrogel or Progynova)?

2) Is there a difference with regard to the mean uterine dimensions in Turner Syndrome adolescents and young women receiving two different HRT regimes (Oestrogel or Progynova)?

3) Is there a difference with respect to the proportions of Turner Syndrome adolescents and young women who attain withdrawal bleeding after receiving two different HRT regimes (Oestrogel or Progynova)?

4) Is there a difference in the timing of withdrawal bleeding since the first day of HRT in adolescents and young women with Turner Syndrome who receive two different HRT regimes (Oestrogel or Progynova)?

5) Are there differences in the proportions of adverse events (nausea, vomiting, hypersensitivity, hypertension, new-onset headache, lower abdominal pain, vaginal bleeding and discharge) or mean adverse event parameters (serum triglyceride) in adolescents and young women with Turner Syndrome who receive two different HRT regimes (Oestrogel or Progynova)?

3.4 Research Hypotheses

In the Bayesian statistical framework, H1 and H2 are known as hypotheses 1 and 2, respectively, not the null and alternative hypotheses. The Bayesian paradigm is a separate school of thought in statistics and, therefore, does not adhere to the null hypothesis significant testing (NHST) methodology subscribed by the p-value-based frequentist paradigm.

1) H1: There is no significant difference between the two estrogen replacement therapy regimes (Oestrogel or Progynova) with respect to the proportions of complete breast development in adolescents and young women with Turner Syndrome.

H2: There is a significant difference between the two estrogen replacement therapy regimes (Oestrogel or Progynova) with respect to the proportions of complete breast development in adolescents and young women with Turner Syndrome.

2. H1: There is no significant difference between the two estrogen replacement therapy regimes (Oestrogel or Progynova) in terms of the mean uterine dimensions in adolescents and young women with Turner Syndrome.

H2: There is a significant difference between the two estrogen replacement therapy (Oestrogel or Progynova) regimes in terms of the mean uterine dimensions in adolescents and young women with Turner Syndrome.

3. H1: There is no significant difference in terms of the proportions of adolescents and young women with Turner Syndrome who attain withdrawal bleeding in the two estrogen replacement therapy regime groups (Oestrogel or Progynova).

H2: There is a significant difference in terms of the proportions of adolescents and young women with Turner Syndrome who attain withdrawal bleeding in the two estrogen replacement therapy regime groups (Oestrogel or Progynova).

4. H1: There is no significant difference between the two estrogen replacement therapy regimes (Oestrogel or Progynova) in terms of the time to withdrawal bleeding in adolescents and young women with Turner Syndrome.

H2: There is a significant difference between the two estrogen replacement therapy regimes (Oestrogel or Progynova) in terms of the time to withdrawal bleeding in adolescents and young women with Turner Syndrome.

5. H1: There are no significant differences in the proportions of adverse events (nausea, vomiting, hypersensitivity, hypertension, new-onset headache, lower abdominal pain, vaginal bleeding and discharge) or mean adverse event parameters (serum triglyceride) in adolescents and young women with Turner Syndrome receiving two different estrogen replacement therapy regimes (Oestrogel or Progynova).

H2: There are significant differences in the proportions of adverse events (nausea, vomiting, hypersensitivity, hypertension, new-onset headache, lower abdominal pain, vaginal bleeding and discharge) or mean adverse event parameters (serum triglyceride) in adolescents and young women with Turner Syndrome receiving two different estrogen replacement therapy regimes (Oestrogel or Progynova).

SECTION 4
METHODOLOGY

4.1 Study Design

This is a Phase II Hybrid Concurrent + Historical/Synthetic Active Controlled Randomized, Parallel-arm, Open-Label Exploratory Clinical Trials with a 1:1 control-to-intervention ratio for label expansion since both Oestrogel and Progynova have received the US Food and Drug Authority (FDA) and Malaysian National Pharmaceutical Regulatory Agency (NPRA)'s approval for hormone replacement therapy in post-menopausal women (NPRA 2022; 2023).

4.2 Study Locations

1. Pediatric and Adolescent Gynecology (PAG) Clinic, Dept of Obstetrics & Gynaecology (O&G) at Hospital Canselor Tuanku Muhriz (HCTM).

2. Paediatric and Adolescent Gynaecology (PAG) Clinic, Hospital Tunku Ampuan Besar Tuanku Aishah Rohani (HTABTAR), Hospital Pakar Kanak-Kanak (HPKK), UKM.

4.3 Study Duration.

1st November 2024 – 31st October 2027 (36 months)

4.4 Reference population

The population of Turner Syndrome adolescents and young women aged 11 to 30 years in Malaysia who have not received pubertal induction therapy.

4.5 Source population

Turner Syndrome adolescents and young women aged 11 to 30 years who have not received pubertal induction therapy and are attending clinical follow-up at HCTM and HPKK.

4.6 Sampling frame

The source population and those who fulfil the trial's eligibility criteria.

4.7 Sampling method

A purposive sampling method will be employed due to the scarcity of the number of potential Turner Syndrome patients fulfilling the eligibility criteria.

4.8 Study subjects

Those who fulfil 4.6 and also consent to trial participation.

4.9 Eligibility criteria

Inclusion criteria

1. Females aged 11-30 years old with karyotype-verified (45, X or other similar karyotypes) and clinically confirmed Turner's syndrome prior to the time of pubertal induction
2. Confirmed estrogen deficiency with primary ovarian failure (high level of follicular-stimulating hormone (FSH > 25 IU/L))
3. Patients who have not undergone pubertal development (no breast development and an underdeveloped uterus).
4. HRT-naive TS patients
5. Breast Tanner Stage of 2 or less.
6. Patients on Growth Hormone (GH) will be allowed entry into the study.
7. Consented to trial participation (from individual TS patients (if aged 18 and above) or the parents or guardians (for under-18 TS patients) with the individual's assent)

Exclusion Criteria

1. Patients with signs of spontaneous puberty
2. Contraindications to trial products (e.g., hypersensitivity to any components of the HRT) based on the most recent version of the British National Formulary (BNF 85)
3. Previous history of exposure to estrogen treatment.
4. Concomitant use of other drugs that affect the bone mineral density (BMD) of the participants (e.g. Bisphosphonates or prolonged use of systemic corticosteroids). Vitamin D supplementation and short corticosteroid usage are allowed.
5. Acute or chronic hepatic disease
6. Patients with untreated hypothyroidism
7. Inflammatory bowel disease (Ulcerative Colitis, Crohn's disease) and coeliac disease
8. Cigarette smoking patients
9. Severely obese patients based on the following criteria:
   a) For TS patients aged 11-17 years old: Based on the WHO chart with BMI > 95th percentile
   b) For TS patients aged 18 years until 30 years: BMI of 37.5 or above based on the Malaysian Clinical Practice Guideline for the Management of Obesity (66))
10. Unknown abnormal genital bleeding
11. Porphyria
12. Recent involvement with clinical research studies (previous 6 months) investigating new HRT formulations

4.10 Outcome Measures

4.10.1 Primary Outcomes

There are four primary domains that will be measured at 4 separate time points:

4.10.1.1: Breast Development

There are two main outcomes that will be measured for this domain:

i) proportions of Turner Syndrome patients achieving Tanner Stage B3 and above at months 1, 7, 13 and 19 after Oestrogel or Progynova initiation;

ii) Median time to achieving Tanner Stage B4 and above (in days)

4.10.1.2 Uterine Development

Two main outcomes will be measured for this domain:

i) Uterine length (in cm) measured at month 1, 7, 13 and 19 by ultrasound (USS) after Oestrogel or Progynova initiation.

ii) Anteroposterior fundal diameter (in cm) measured at month 1, 7, 13, and 19 by ultrasound (USS) after Oestrogel or Progynova initiation.

4.10.1.3 Withdrawal Bleeding

Two main outcomes will be measured for this domain:

i)Proportions of TS subjects achieving self-reported withdrawal bleeding (defined as spotting or bleeding episodes that occur at least for one day during the intake of the estrogen regimen; average duration of withdrawal bleeding: 3-5 days) (67-68) at month 1, 7, 13 and 19 after Oestrogel or Progynova initiation.

ii) Median time to withdrawal bleeding (in days) after the initiation of Oestrogel or Progynova initiation

4.10.1.4 Endocrinological Profile (Safety Parameter)

1. Serum Triglyceride levels at month 1, 7, 13 and 19 after Oestrogel or Progynova initiation (safety parameter)

4.11 Sample size calculation (Frequentist)

1) General Information and Assumptions

The sample size was calculated using PS Software Version 3.1.6 (Plummer and Dupont, 2018). All sample size calculations were performed with $\alpha = 0.05$, $1-\beta = 0.80$ and the ratio of controls to cases (m) =1. The dropout rate is considered 5% for all endpoints. We also assume that 17β-estradiol and estradiol valerate dosages are equivalent.

Table 1: Sample size calculation for the HRT in Turner Syndrome Trial Efficacy Endpoint Parameters

Objectives	Endpoints	Parameters (reference)	Assumed Effect Size*	Sample size	Final sample size, including drop out rate (n_{total+})
1: Breast development	i) Proportions of subjects achieving at least Tanner Stage B3 at 1 year	Estradiol Valerate group:0.56 based on rough extrapolation of graph 4a (page 331 of Schonhoff et al) (69)	In the Estrogel group, we assume a higher proportion: 0.80 Hence: P_1-P_0 = 0.24	66 subjects per group[a]	70 subjects per group (140 subjects)
	ii) Time to Tanner Stage ≥B4 (in days)	Median time to Tanner Stage ≥B4 in 17β estradiol group: 733 days (Labarta et al) (70)	Median time to Tanner Stage ≥B4: 365 days Additional parameters: 1) Accrual time (A) for patient recruitment: 1095 days (3 years) 2) Additional follow-up time: 730 days (2 years)	41 subjects per group[b]	44 per group (88 subjects)
2. Uterine development	i) Uterine length (cm)	Uterine length (SD): 0.96 cm (Kim et al) (71).	A moderate standardised effect size was chosen based on Cohen's d = 0.50	64 subjects per group[c]	68 per group (136 subjects)

			Mean difference (δ) = 0.96 x 0.50 = 0.480		
	ii) AP Fundal Parameter (cm)	Uterine Fundal AP Diameter (SD): 0.54 cm (Kim et al) (71)	A moderate standardised effect size was chosen based on Cohen's d = 0.50 Mean difference (δ)= 0.54 x 0.50 = 0.270	64 subjects per group[c]	68 per group (136 subjects)
3. Proportion of TS achieving withdrawal bleeding[d]	-	-	-	-	-
4. Time to withdrawal bleeding[d]	-	-	-	-	-

[a]Based on two-proportion formula, Fisher's exact test; [b]Based on the log-rank test procedure (73) [c]Based on t-test; [d]Cannot be calculated due to the absence of estimates from prior studies;[*]Based on clinically important difference

Table 2: Sample size calculation for the HRT in Turner Syndrome Trial Safety Endpoint Parameters

Safety Parameters	**Endpoints**	**Parameters (reference)**	**Assumed Effect Size***	**Sample size**	**Final sample size, including drop out rate (n_{total+})**
1: Lipid profile	Serum Triglyceride Level (mmol/L)	SE: 5.5 (Shah et al, in mg/dL) (72) SD was obtained using this formula = sqrt (5.5^2 x 5) = 12.298 Conversion of SD to SI unit (mmol/L) = 12.298 mg/dL*0.0113 = 0.1390 mmol/L	Assumed mean difference (δ) = 0.36 mmol/L (assumed to be half the effect size that Shah et al reported) (74)	4 subjects per group	5 subjects per group (10 subjects)

*Based on clinically important difference

2) Methodology for determining standardised effect size (Cohen's d)

Cohen's d (74) is given by

$$d = \frac{\underline{x}_1 - \underline{x}_2}{\sqrt[2]{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}}}$$

where n_1 = sample size for group 1, n_2 = sample size for group 2, s_1^2 is the variance for group 1 and s_2^2 is the variance for group 2. $\underline{x}_1$ is the sample mean for group 1 , and $\underline{x}_2$ is the sample mean for group 2, and both can be considered as unbiased estimators for the population means of groups 1 and 2 (μ_1 and μ_2, respectively).

However, if we assume n_1 and n_2 are the same and 1 and 2 are relatively very small compared to n_1 and n_2, the denominator of the formula for Cohen's d (pooled SD) can be reduced to

$$\sigma_{pooled} \approx \sqrt[2]{\frac{s_1^2 + s_2^2}{2}}$$

Since we only have an estimate of standard deviation from one group only from previous literature, we have to assume $s_1^2 = s_2^2$. Hence,

$$\sigma_{pooled} \approx \sqrt[2]{\frac{2s_1^2}{2}} = \sqrt[2]{s_1^2} = s_1$$

Hence, the SD used for calculating sample size is from one group only. Based on Cohen's d, the magnitude of effect size can be classified as follows:

d = 0.20 (small effect size)

d = 0.50 (moderate effect size)

d = 0.80 (large effect size)

In our study, we shall assume a moderate effect size (d=0.50). However, according to Thompson (75), the effect size should not be rigidly interpreted and adhered to. In our case, we use Cohen's d just to facilitate the calculation of the mean difference (δ) between the groups after consulting the clinicians involved in this study to ensure that the effect size also represents a clinically significant difference between the two intervention groups.

3) Sample Size Calculation Based on the Minimum Detectable Alternative (δ)

The sample size can also be estimated based on the minimum detectable alternative (δ). In this case, we assumed, at the most, we could only obtain n=30 subjects per group (n_{total} = 60, m=1). The study power and type I error rate are fixed at 0.80 and 0.05, respectively. Hence, the minimum detectable alternative (δ) for each parameter for each trial objective is as follows:

Table 3: The minimum detectable alternative (δ) for each efficacy endpoint parameter of the HRT in the Turner Syndrome Trial when n=30 per group (n_{total} = 60, without 5% drop-out rate included)

Objectives	Endpoints	Parameters (reference)	Minimum detectable alternative (δ)
1: Breast development	i) Proportions of subjects achieving at least Tanner Stage B3 at 1 year	Estradiol Valerate group:0.56 based on rough extrapolation of graph 4a (page 331 of Schonhoff et al. 2011)	The proportion of subjects achieving Tanner stage ≥ B3 at 1 year should be ≤0.185 or ≥ 0.906. Minimum lower limit difference: 0.56-0.185 = 0.375 Minimum upper limit difference: 0.906-0.56 = 0.346
	ii) Time to Tanner Stage ≥B4 (in days)	Median time to Tanner Stage ≥B4 in 17β estradiol (oral) group: 733 days Additional parameters: 1) Accrual time (A) for patient recruitment: 1095 days (3 years) 2) Additional follow-up time: 730 days (2 years) All estimates are taken from (Labarta et al. 2012)	The median time to Tanner Stage ≥B4 in the estrogel group should be 325 days or less. It means that the estrogel should demonstrate a median time of around 10.8 months to show there is a significant difference between the intervention groups in terms of this parameter if the median time to Tanner Stage ≥B4 for the 17β estradiol (oral) is around 24.4 months.
2. Uterine development	i) Uterine length (cm)	Uterine length (SD): 0.96 cm (Kim et al. 2012).	The MDA (δ) should be ≥+0.706 or ≤-0.706 for the difference in the mean uterine length between the 2 intervention groups to be significant

	ii) AP Fundal Parameter (cm)	Uterine Fundal AP Diameter (SD): 0.54 cm (Kim et al. 2012)	The MDA (δ) should be ≥+0.397 or ≤-0.397 for the difference in the mean AP Fundal Parameter between the 2 intervention groups to be significant
3. Proportion of TS achieving withdrawal bleeding[d]	-	-	-
4. Time to withdrawal bleeding[d]	-	-	-

[a]Based on two-proportion formula, Fisher's exact test; [b]Based on the log-rank test procedure (Schoenfeld & Richter (1982)) (73);[c]Based on t-test; [d]Cannot be calculated due to the absence of estimates from prior studies;[*]Based on clinically important difference

Table 4: The minimum detectable alternative (δ) for each safety endpoint parameter of the HRT in the Turner SyndromeTrial when n=30 per group (ntotal = 60, without 5% drop-out rate included)

Objectives	Endpoints	Parameters (reference)	Minimum detectable alternative (δ)
1: Lipid profile	Serum Triglyceride Level (mmol/L)	SE: 5.5 (Shah et al, in mg/dL) (74) SD was obtained using this formula = sqrt (5.5^2 x 5) = 12.298 Conversion of SD to SI unit (mmol/L) = 12.298 mg/dL*0.0113 = 0.1390 mmol/L	The MDA (δ) should be ≥+0.102 or ≤-0.102 for the difference in the mean serum triglyceride level between the 2 intervention groups to be significant

Based on the MDA (δ), the greatest threat to achieving statistical significance is for both parameters for objective 1. This verdict can be explained as such: the difference in the proportions of subjects achieving Tanner stage B3 and above at 1 year should be very large to achieve statistical significance. For instance, if transdermal 17β estradiol (Oestrogel) is found to be inferior to oral estradiol valerate (Progynova), the proportion of trial participants achieving Tanner stage B3 and above at 1 year in the transdermal 17β estradiol (Oestrogel) group should be 0.185 or less (a proportional difference of 0.375). However, if transdermal 17β estradiol (Oestrogel) is superior to oral estradiol valerate, the proportion of trial participants achieving Tanner stage B3 and above at 1 year in the Oestrogel group should be at least 0.906 or more (a proportional difference of 0.346).

4) Final Verdict based on Frequentist sample size calculation

Without considering the attrition rate, the largest sample size required for this trial is 66 subjects per group ($n_{total,\ without\ attrtition}$= 132). After considering a 5% dropout rate, n_{total} is 140 subjects. Hence, the final sample size for this trial is 70 Turner Syndrome subjects per group.

However, based on the MDA (δ), if we could only achieve a sample size of n=30 per group (n_{total}=60), then differences between the groups need to be larger. The greatest possibility of not achieving statistical significance is thus for objective 1 (parameter) which is detecting the difference in the proportions of TS participants achieving Tanner Stage B3 and above.

5) Sample size calculation based on Bayesian paradigm (presentation slides)

The sample size calculation under the Bayesian paradigm was performed based on the methods outlined in the previous work by Kuzmann and colleagues (76) and Lindley (77). Besides, the concept of study power was substituted with assurance (78) and the sample size was based on maximizing the utility function (79). However, based on Stallard and colleagues, the optimal sample size for phase II clinical trials should be at least $n_{phase\ II\ trial}$ = $n_{phase\ III}$ trial * 0.03 (multiplier) (80). However, it was deemed that the multiplier was too small, leading to an underpowered trial. Hence, the multiplier value was increased to 0.20.

The sample size calculation was based on the uterine development parameter and the procedure is outlined in the slides below:

BAYESIAN SAMPLE SIZE CALCULATION (UTERINE DEV PARAMETER, DECISION THEORETIC)

- Based on work by Kunzmann et al (2021) and Lindley (1997; original reference). So far applicable only for continuous variable. Hence, for illustration purpose, we use uterine development parameter since it has the largest sample size for continuous efficacy end point for this trial.
- Based on a decision problem and using the parameter values for calculating phase III trial's sample size.
- Two fundamental concepts: utility function (decision-theoretic paradigm; Barnet 1999) and assurance (Wilson et al 2022; brief details included in the notes).
- To simplify the calculation, we shall only use utility function for calculating the sample size. A simple utility function that is a trade-off between the trial cost and the probability of rejecting H_0 is given by:

$$U = aU_1 + (1 - a)U_2,\ 0<a<1$$

- U_1 = the cost of the trial ($U_1 = -(1+bn)$) and $U_2 = 1$ if H_0 is rejected and $U_2 = 0$ otherwise. For U_2 we assume p = 0.80 if the sample size n=70 and p = 0.75 if n=60. Hence, we need to solve these two simultaneous linear equations to obtain a and b:

$$-a(1+70b) + 0.80\ (1-a) = 0 \quad \text{and} \quad -a\ (1+60b) + 0.75\ (1-a) = 0$$

- a = 0.3103, b = 0.0111 [**Solution: Based on Gaussian Elimination, general purpose method to solve n simultaneous linear equations**]
- Hence, $U = 0.6897U_2 - 0.3103\ (1+0.0111n)$

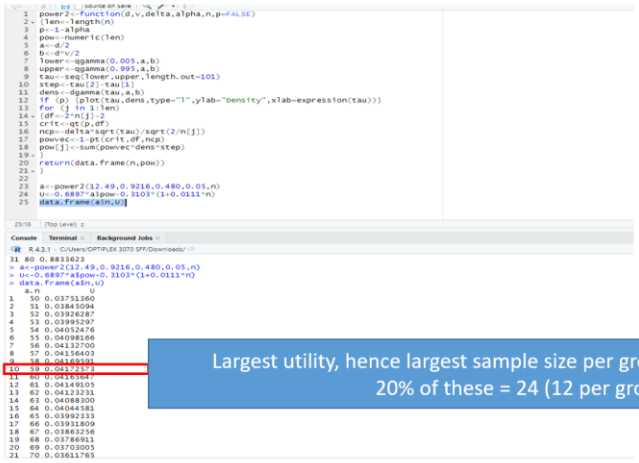
SAMPLE SIZE CALCULATION (BASED ON THE BAYESIAN SETTING)

- Instead of using $\sigma^2 = 0.9129$ (uterine development, Kim et al 2012), we give $\tau = 1/\sigma^2$ a prior distribution of Gamma (d/2, dv/2), where we assume v = 4. Then, we can use the R function below to find the d value that best suits the gamma distribution:

```
> d<-9:14
> rbind(d,pgamma(1/9,d/2,2*d))
          [,1]         [,2]         [,3]         [,4]         [,5]         [,6]
d 9.00000000 10.00000000 11.00000000 12.00000000 13.0000000 14.00000000
  0.08858747  0.07492149  0.06357214  0.05409099  0.0461322  0.03942441
> d<-seq(12,13,0.2)
> rbind(d,pgamma(1/9,d/2,2*d))
          [,1]         [,2]         [,3]         [,4]         [,5]         [,6]
d 12.00000000 12.20000000 12.40000000 12.60000000 12.80000000 13.0000000
  0.05409099  0.05238706  0.05074142  0.04915181  0.04761609  0.0461322
> d<-seq(12.4,12.6,0.02)
> d<-seq(12.4,12.6,0.02)
> rbind(d,pgamma(1/9,d/2,2*d))
          [,1]         [,2]         [,3]         [,4]         [,5]         [,6]
d 12.40000000 12.42000000 12.44000000 12.46000000 12.48000000 12.50000000
  0.05074142  0.05057997  0.05041908  0.05025875  0.05009897  0.04993975
          [,7]         [,8]         [,9]        [,10]        [,11]
d 12.52000000 12.54000000 12.56000000 12.58000000 12.60000000
  0.04978107  0.04962294  0.04946536  0.04930831  0.04915181
> d<-seq(12.48,12.50,0.01)
> rbind(d,pgamma(1/9,d/2,2*d))
          [,1]         [,2]         [,3]
d 12.48000000 12.49000000 12.50000000
  0.05009897  0.05001929  0.04993975
```

SAMPLE SIZE CALCULATION (BASED ON THE BAYESIAN SETTING)

- Based on Bayesian sample size calculation proposed by Kunzmann et al (2021) and Lindley (1997).
- Calculated using R. More details are given in the full protocol



```
power2<-function(d,v,delta,alpha,n,p=FALSE)
{len<-length(n)
p<-1-alpha
pow<-numeric(len)
a<-d/2
b<-d*v/2
lower<-qgamma(0.005,a,b)
upper<-qgamma(0.995,a,b)
tau<-seq(lower,upper,length.out=101)
step<-tau[2]-tau[1]
dens<-dgamma(tau,a,b)
if (p) {plot(tau,dens,type="l",ylab="Density",xlab=expression(tau))}
for (j in 1:len)
{df<-2*n[j]-2
crit<-qt(p,df)
ncp<-delta*sqrt(tau)/sqrt(2/n[j])
powvec<-1-pt(crit,df,ncp)
pow[j]<-sum(powvec*dens*step)
}
return(data.frame(n,pow))
}

a<-power2(12.49,0.9216,0.480,0.05,n)
U<-0.6897*a$pow-0.3103*(1+0.0111*n)
data.frame(a$n,U)
```

```
31 80 0.8833623
> a<-power2(12.49,0.9216,0.480,0.05,n)
> U<-0.6897*a$pow-0.3103*(1+0.0111*n)
> data.frame(a$n,U)
   a.n         U
1   50 0.03751360
2   51 0.03845094
3   52 0.03926287
4   53 0.03995297
5   54 0.04052476
6   55 0.04098166
7   56 0.04132700
8   57 0.04156403
9   58 0.04169591
10  59 0.04172573
11  60 0.04165647
12  61 0.04149105
13  62 0.04123231
14  63 0.04088300
15  64 0.04044581
16  65 0.03992333
17  66 0.03931809
18  67 0.03863256
19  68 0.03786911
20  69 0.03703005
21  70 0.03611761
```

Largest utility, hence largest sample size per group is 59 (n_{total} = 118); 20% of these = 24 (12 per group)

List of abbreviations:

a) SE: Standard error

b) SD: Standard deviation

Since our trial is based on the Bayesian design and analysis, the final sample size required for the concurrent Progynova and Oestrogel trial participants is 12 subjects per group (n=24). The sample size will be augmented by the historical Progynova and Oestrogel users identified from our TS patient registry through the HCTM Electronic Medical Records. Thus far, the number of identified TS patients already on Progynova-only or Oestrogel-only regimes for pubertal induction is 28 and 11, respectively

4.12 Recruitment process

The overall process of participant recruitment at each trial site is represented by Figure 1 below:

Figure 2: The overall process for participant recruitment at each trial site

4.13 Participant Screening and Randomization

After the patients consented to trial participation, they will be screened for trial eligibility. Each patient will be assigned a unique screening number before randomisation and intervention assignment. Each screening number must be used once for each patient, even in patients screened multiple times.

Eligible study participants will be randomised in a 1:1 fashion to one of the two intervention groups (oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel)) using a block randomisation scheme of varying block

A suitable vendor will be identified to carry out the randomisation procedure to protect against any accidental breach of randomisation codes and maintain allocation concealment, which will minimise selection bias. So far, we have identified two possible vendors [redacted] who will assist in the trial participant randomization [redacted]

4.14 Allocation Concealment and Masking

The vendor will conduct central randomisation and the randomisation sequence will be uploaded to the electronic data capture software, REDCAP. After confirming the study eligibility of the study participants, the recruiter will fill in the study participants' clinical details in the REDCAP and the vendor will perform the randomisation and the treatment allocation will only be notified REDCAP to the recruiter to maintain allocation concealment. The recruiter will then inform the pharmacist dispensing the Oral Estradiol Valerate and Transdermal 17β Estradiol. The pharmacist will subsequently notify the interventionists (clinicians in charge of the trial participants, exclusive of the trial investigators), who will first demonstrate to the trial participants the appropriate ways to use the interventions. There will be two pharmacists who will be involved in the storing and labelling of the interventional agents (including placebo) at the central trial locations (Hospital Canselor Tuanku Muhriz (HCTM) and Hospital Pakar Kanak-Kanak (HPKK UKM; also known as Hospital Tuanku Ampuan Rohani, UKM).

The study participants investigators, data handlers, healthcare givers (including physicians/specialists in charge, nurses and other support staff), outcome assessors and the trial statistician will not be masked to the identities of the assigned intervention.

4.15. Clinical Protocol

4.15.1 Trial Procedures

For all eligible Turner Syndrome patients that fulfil the trial eligibility criteria, they will be randomised into two groups:

Group A: Oral Estradiol Valerate (Progynova) group
Group B: Transdermal 17β Estradiol gel (Oestrogel) group

They will receive the treatment according to the protocol below (Table 5).

Table 5: The estrogen regimes based on the allotted HRT types

Duration	**Oral**	**Transdermal**

	Estradiol valerate Group	17β Estradiol Group
Twice a week for a month	1.0 mg	0.75mg (1/2 ruler)
Once daily for 6 months	1.0 mg	0.75mg (1/2 ruler)
Once daily for 6 months	2.0 mg	1.5mg (1 ruler)
Once daily for 6 months until total 2 years	4.0 mg	3.0mg (2 rulers)

The detailed clinical procedural steps for this trial are given in Figure 3 below:

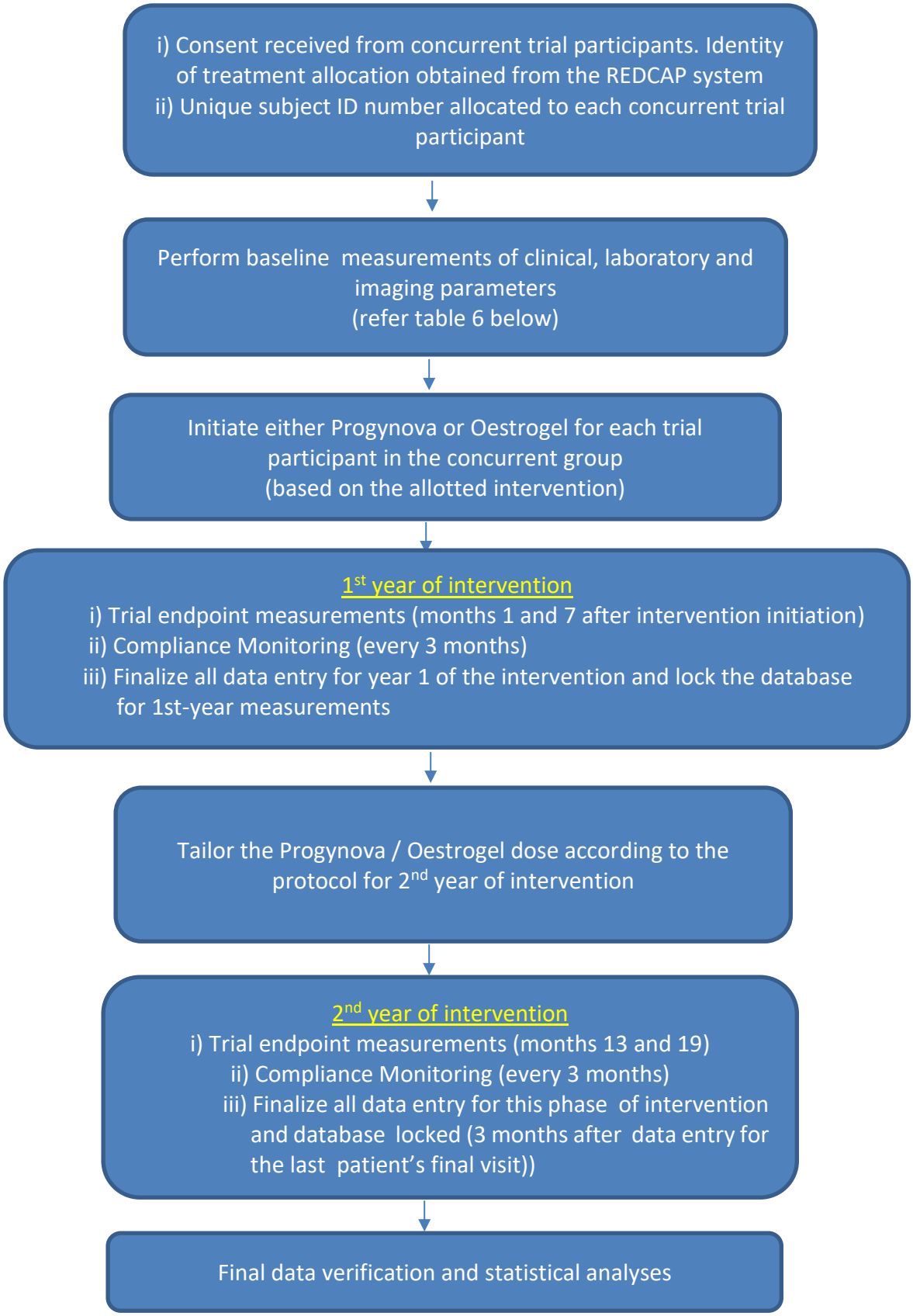


Figure 3: The overall process for the conduct of the trial

Table 6. Planned Measurements of Clinical, Laboratory, Imaging and Quality of Life Parameters for each participant for the entire trial duration (the allowable observation window is ± 7 days from the actual measurement time points).

Variables	**Timepoints**			
	Pre-intervention (Baseline)	Month 1	Month 7 and Month 13	Month 19
Karyotype	X			
Sociodemographic Profile	X			
a) Clinical Parameters				
Height (cm)	X	X	X	X
Weight (kg)	X	X	X	X
BMI (kg/m^2)	X	X	X	X
Height increment rate (cm/year)	X			X
Vital signs (SBP, DBP, HR, RR, Temperature)	X	X	X	X
Tanner Breast Stage	X		X	X
Pubic hair development	X			
Withdrawal Bleeding			X	X
Compliance Monitoring		X	X	X
Safety Monitoring		X	X	X
Concomitant Medication		X	X	X
b) Laboratory Parameters				
Serum FSH, LH, prolactin	X			
Serum Estrone	X		X	X

Serum Estrone / Estradiol ratio	X		X	X
Serum SHBG	X		X	X
Free T4 and TSH	X			X
Liver Function Test (LFT)	X			X
Fasting Blood Glucose	X		X	X
HbA1c	X			X
Fasting Lipid Profile (Total Cholestrol, LDL, HDL and Triglycerides)	X		X	X
Imaging				
Pelvic Ultrasound (Uterine Length, AP Diameter, Endometrial lining presence and endometrial thickness (ET)	X		X	X

For trial endpoints that may result in poor inter-rater agreement (e.g., Tanner Staging for breast development, USS Uterine images that are difficult to classify), training sessions will be provided to the raters (O&G registrars with at least 5 years of experience working in the O&G setting) to standardize rating procedures. This will be followed by a trial endpoint realignment meeting to discuss difficult cases and assessments of inter-rater agreement for all trial endpoints with poor inter-rater agreement. Any rater that exhibits poor inter-rater agreement will be excluded as a clinical assessor for the trial endpoints. Statistical procedures that will be employed to evaluate inter-rater agreement are elaborated in detail in Section 4.20.4. To assess the patient's withdrawal bleeding status and time to the first withdrawal bleeding, a monitoring log (diary) will be provided to each trial participant (Appendix IV). The severity of withdrawal bleeding will be graded by the number of pads/tampons used per HRT cycle, as advocated by previous research (81-83). The operational definition of spotting by Zhang et al will be utilised for this research (84).

All laboratory tests will be conducted in laboratories with Good Laboratory Practice (GLP) certification or at least are accredited with the MS ISO 15189:2014 and MS ISO/IEC 17025 (testing and calibration laboratories) credentials by Standards Malaysia.

4.15.2: Study Instruments (captured REDCAP)

The REDCAP system (eCRF) will capture the following information for each trial participant

1. Socio-demographic profile

2. Baseline blood investigations: FSH, LH, Estradiol, and DNA Karyotyping.

3. Ultrasound measurement of uterine size and endometrial thickening: before and after treatment commenced at 6-monthly intervals until attainment of first withdrawal bleed (according to regime protocol). Pelvic ultrasound is used to measure the uterus (length, width, depth, volume) and endometrial thickening (in the sagittal plane). Two readings will be obtained for each, and the average will be taken.

4. Tanner staging:

   a) breast development: before and after treatment commenced, at intervals of 6 months.

   b) pubic hair development: before treatment only (for baseline information only).

   Breast and pubic hair (for baseline information only) development will be assessed using Tanner Staging (Appendix 1), also known as Sexual Maturity Rating (SMR), developed by Marshall and Tanner. This is an objective classification system that is used to document and track the development and sequence of secondary sex characteristics of children during puberty. The descriptions for each Tanner stage for breast and pubic hair development are given below:

   <u>Breast development:</u>

   Stage 1: No glandular breast tissue palpable

   Stage 2: Breast bud palpable under the areola (1st pubertal sign in females)

   Stage 3: Breast tissue palpable outside areola; no areolar development

   Stage 4: Areola elevated above the contour of the breast, forming a “double scoop” appearance

   Stage 5: Areolar mound recedes into a single breast contour with areolar hyperpigmentation, papillae development, and nipple protrusion

   <u>Pubic Hair development:</u>

   Stage 1: No hair

Stage 2: Downy hair

Stage 3: Scant terminal hair

Stage 4: Terminal hair that fills the entire triangle overlying the pubic region

Stage 5: Terminal hair that extends beyond the inguinal crease onto the thigh

5. The date of first withdrawal menses and the time duration from treatment commencement, and the details of this withdrawal bleed: duration, menstrual flow, and number of pads used per day.

4.15.3 Labelling, Packaging, Storage, Accountability and Return of Clinical Supplies and Interventional Agents

4.15.3.1 Investigational Medicinal Products (IMPs)

It is the responsibility of the investigators at each trial site to ensure satisfactory and adequate handling, supply, storage, distribution and usage of IMPs according to the trial protocol and all relevant rules and regulations. The clinical supplies provided by the sponsor (UKM) are detailed below (Table 7).

Table 7. The descriptions of clinical supplies.

Product Name	**Dosage Form**	**Manufacturer**
Oral estradiol valerate (Progynova®)	Tablet, 1 and 2mg	Bayer (Leverkusen, Germany)
Transdermal 17β estradiol (Oestrogel®)	Non-greasy, non-staining clear and colourless transdermal gel. It is provided in an 80 g bottle with a metered dose pump. Each pump pack delivers 64 metered doses. Each 1.25 grams of estradiol gel contains 0.75 mg (as hemihydrate)	Besins Healthcare (Monaco, France)

4.15.3.2 Packaging and Labeling of the Clinical Supplies

The conditions for IMP storage (temperature, humidity) will be provided on the trial package. Packaging will be performed by Besins Healthcare for both the transdermal 17β-estradiol (Oestrogel) gel. Oral estradiol valerate (Progynova) will be purchased from Bayer (Leverkusen, Germany). All packaging and labelling processes will adhere to the Good Manufacturing Practices (GMP) and the Good Clinical Practice (GCP) guidelines, as well as all Malaysian legal requirements. All IMP packages will have a tear-off part that needs to be affixed to the study documents upon IMP dispensation by the investigators/pharmacists to the study participants at each trial site.

4.15.3.3 Storage and Handling Requirements

All clinical supplies (IMPs) shall be stored at a secured and locked facility at each trial centre and under the conditions stated on the labels (storage temperature: 1) Oral Estradiol valerate (Progynova): below 30^{o}C; 2) transdermal 17β estradiol (Oestrogel): between 20^{o} to 25^{o}C, temperature excursion (fluctuation) is permitted to 15^{o} to 30^{o}, with access restricted to the principal investigators/local pharmacists/authorized personnel in charge of storing and distributing the clinical supplies at the specific trial site. The principal investigators/pharmacists should maintain an updated temperature log to provide evidence of satisfactory storage of clinical supplies (IMPs) for the entire duration of the trial.

The receipt and dispensing of clinical supplies (IMPs) shall be compulsorily documented by the principal investigator/local pharmacists/authorized personnel at each trial site. The principal investigator/local pharmacists/authorized personnel should sign the receipt forms for clinical supplies (IMPs). The details and records of the delivery of clinical supplies (IMPs) to the trial site, the cataloguing, listing and counting of IMPs, inventory maintenance at the trial site and the return of unused IMPs to the Sponsor shall also be documented by the principal investigator/pharmacist/authorized personnel at each trial site. The details shall include batch numbers, dates, quantities and the unique numbers allotted to the IMPs and study participants. Clinical supplies must be used by the investigators for trial purposes and administered only to enrolled trial participants.

4.15.3.4 Discard, Destruction, Returns and Reconciliation

It is the investigator's responsibility to maintain and safeguard the accuracy of the records for clinical supplies provided by the Sponsor at each trial site. The investigators at each trial site should also document the number of IMPs allocated to each study participant and the number of IMPs returned by each subject at the end of the trial.

The Sponsor shall provide the investigators with documentation forms (including format) that should be filled in to assess the drug accountability or return (or discard and destruction). Any unused IMPs must be returned to the Sponsor for accountability and returned upon trial conclusion.

4.15.4 Patient Compliance

Study participants will be required to bring used and unused oral estradiol valerate (Progynova) blister packs and transdermal 17β-estradiol (Oestrogel) tubes during each follow-up visit. Participant compliance will be evaluated by counting the remaining tablets or measuring the length of the tubes containing transdermal 17β-estradiol (Oestrogel). The compliance rate at each visit should not be below 80% to be considered as adequate compliance. Apart from that, they will also be required to supply the photographs (in a suitable format) of their blister packs and gel tubes as objective evidence of their compliance. Reminders will also be sent periodically about the schedule for their next follow-up visits to maximise attendance at their monitoring visits.

4.15.5 Protocol Deviation

Protocol deviations are defined as

1. Not meeting/fulfilling any of the eligibility criteria (inclusion and exclusion criteria) during subsequent reviews after the patients have been enrolled into the trial.
2. Consumption or use of concomitant medications that are forbidden during the clinical trial
3. No show during the visit dates or visits are performed outside the acceptable visit windows (± 1 week)
4. Non-adherence to the study protocol
5. Medication Storage / Administrative Issues

For condition no 3, visit windows should be calculated by assuming 1 month is equivalent to 30 days and 1 week is equivalent to 7 days.

4.15.6 Participant Withdrawal and Loss to Follow-Up

Trial participants who withdraw from the study will not be replaced with new participants. A participation withdrawal is operationally defined as a scenario in which the participant does not return for a trial final visit as scheduled in the trial protocol. The statistical treatment of participants who withdraw from the study is outlined in Section 4.19.1: Statistical Analysis Plan for Efficacy Endpoints.

A participant may withdraw from the trial or rescind their consent to trial participation at any time, and for any reason, without prejudicing the clinical management and treatment they may receive in the future. Participants will be asked to complete all pending research procedures prior to trial withdrawal.

If the trial participant wishes to withdraw due to intervention-associated Adverse Events (AEs), the participant will be requested to undergo follow-up until the resolution of AEs before the investigator completes the end-of-study electronic Clinical Record Form (eCRF) on REDCap. All information associated with participant withdrawal will be documented on the eCRF, including whether the decision to withdraw from the trial was made by the

participant or the investigator at the trial site. One of the following will be documented as the reason for trial withdrawal:

i) AEs (state the actual AEs)

ii) Serious AEs (state the actual SAEs)

iii) Death

iv) Lost to follow-up

v) Major Protocol Deviation

vi) Trial termination by Sponsor

vii) Investigator / Clinician-In-Charge Decision

viii) Consent withdrawal by the trial participant (state reason)

ix) Others

Participants who withdraw from the trial may request that their collected but untested samples be destroyed. In this case, the investigator must document this. All the results obtained before such a request is made should be discarded. All samples collected must thus be destroyed. If such a request is not made, the investigator is allowed to retain and use the collected data before consent is withdrawn.

Participants are deemed lost to follow-up if they repeatedly fail to attend their trial visits without explicitly requesting to withdraw from the trial, or if they cannot be contacted by their respective trial sites. In this case, the following steps should be taken to resolve the problem:

i) Multiple attempts should be made to contact the participants and missed visits should be rescheduled. Counselling should be given to the participants on the importance of attending the follow-up visits and the investigator should also explore whether the participants are still interested in being part of the trial.

ii) Prior to concluding that the participants are lost to follow-up, at least three phone calls/ text messages should be made and a registered letter should be sent to the patient's last known home address to re-establish communication with the participants. These attempts to re-establish communication with the participants should be documented, signed and dated in the patient's eCRF and medical records.

iii) After the final attempt to contact the participant is unsuccessful, the participant is considered to have been lost to follow-up and withdrawn from the trial.

4.16 Interventional Safety Assessment

4.16.1 Adverse Events (AEs) / Serious Adverse Events (SAEs): Definitions

For this study, the study investigators define adverse events as "an abnormal sign, symptom, laboratory test, syndromic combination of such abnormalities, untoward or unplanned occurrence (e.g., an accident), or any unexpected deterioration of concurrent illness" (85). For serious adverse events (SAEs), they are defined according to the USA Code of Federal Regulations Title 21, Section 312.32, which states that "adverse events result in the following outcomes:

1) Death;

2) Life-threatening AEs;

3) Inpatient hospitalisation or prolongation of existing hospitalisation;

4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect" (86).

Other significant medical events are also considered SAEs if the events compromise study participants and require medical or surgical intervention to prevent one of the outcomes above. As an example, allergic bronchospasm requiring intensive treatment in an emergency setting to prevent the outcomes above.

4.16.2: The likelihood of AEs / SAEs: Classification of causality

The likelihood of AEs and SAEs is classified based on the modified Naranjo et al recommendations as follows (87):

a) Unrelated: The AEs / SAEs are unlikely to be related to oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application.

b) Possible: The connection between AEs / SAEs and oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) cannot be excluded with absolute certainty:

i) There is a temporal relationship between oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application and the occurrence of AEs / SAEs.

ii) However, there is an alternative factor (e.g., characteristic of the patient's disease / clinical state) that can possibly or likely explain such a relationship, or a significant uncertainty exists over the cause of AEs / SAEs

c) Probable: The connection between AEs / SAEs and oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) has a high degree of certainty

i) There is a temporal relationship between oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application and the occurrence of AEs / SAEs

ii) AEs / SAEs disappear or decrease upon the withdrawal of oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application, but do not reappear upon subsequent oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application

iii) Alternative causes cannot reasonably explain the relationship between oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application and the occurrence of AEs / SAEs.

e) Definite: The AEs / SAEs are obviously linked to oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application with the occurrence of AEs / SAEs

i) There is a temporal relationship between oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application and the occurrence of AEs / SAEs

ii) AEs / SAEs disappear or decrease in severity upon the withdrawal of oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application and reappear on subsequent oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) application.

iii) No other alternative causes.

4.16.3 Methods for assessing and recording AEs / SAEs

The study protocol will be halted at any moment of the study period if the study participants develop any sudden (expected or unexpected) severe complications / AEs. The withdrawal of a study participant from the trial will be documented on the adverse event page of the CRF and the participant will be further followed up for the study outcomes and included in the analysis as per the original randomisation group (intention-to-treat analysis). All AEs / SAEs will be recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5 and the US FDA's Toxicity Grading Scale for Healthy Adults and Adolescents Volunteers Enrolled in Preventive Vaccine Clinical Trials criteria (88-89). The statistical analysis plan for interventional safety assessment is given in section 4.20.

4.16.4 Reporting of AEs / SAEs to the Institution Review Board (IRB) and Malaysian National Pharmaceutical Regulatory Agency (Malaysian NPRA)

The safety reporting window will start from the first administration of the intervention (Progynova/Oestrogel) randomly allotted to the first trial participant at baseline (first patient first visit (FPFV)) until 30 days after the last administration of the randomly allotted intervention to the last trial participant. All AEs will be recorded in the eCRFs in the REDCap system. AEs of grade 3 and above will be reported to the UKM Human Ethics Committee (IRB) and the Data Monitoring Committee (DMC, a subcommittee of the IRB) within 5 business days or in accordance with the IRB guidelines for other trial centres. All SAEs (including Serious Unexpected Suspected Adverse Events (SUSARs)) will be reported within 24 hours of occurrence (expedited reporting) to the UKM Human Ethics Committee. The reporting procedures for SAEs should adhere to the following steps:

i) The SAEs initial report form should be filled in by the assessors (O&G registrars) and reviewed and signed by the clinical PI (Associate Professor Ani Amelia Dato Zainuddin) or the co-investigator (Professor Nur Azurah Abdul Ghani) at the trial centres.

ii) The SAEs initial report should then be submitted to the UKM Ethics Committee (expedited).

iii) SAEs should be reviewed and followed to resolution by the O&G registrars / PI/co-investigator in charge of the study participants.

iv) A subsequent follow-up report will be submitted that documents any additional important information, including the resolution of SAEs.

The UKM Ethics Committee will then be responsible for relaying all the information on the SAEs experienced by the individual participants (recorded in the Individual Case Study Report (ICSR)) to the Malaysian NPRA. Besides, the aggregate reporting of the SAEs will be analysed and prepared by an independent statistician (see Section 4.20.1) and submitted to the Data Monitoring Committee for further adjudication and submission to the Malaysian NPRA.

4.16.5 AEs / SAEs follow-up at the end of the study period

If AEs/SAEs occur or are still ongoing at the end of the study, trial participants will continue to be followed up and monitored until AEs/SAEs resolve, unless the PI deems no further follow-up is necessary. The follow-up may take the form of 1) additional subject visits to the trial centre/hospital, 2) telephone calls to the subjects, and 3) additional reporting in the form of letters from the treating physicians.

4.16.6 Study Halting Criteria

Participant enrolment and allocation and institution of interventions will be stopped if one of the following occurs:

a) Death related to the intervention (oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel)) application

b) Any participant experiences bronchospasm, laryngospasm, or anaphylaxis within 24 hours post-intervention (oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel)) usage or application

c) Any SAE related to interventions (oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel))

d) Any AE of grade 3 and above or any SAE that cannot be obviously implicated by other causes

e) Any study participant who develops abscess/ulceration/erosion at the site(s) of transdermal 17β estradiol Oestrogel application

4.16.7 Management of AEs linked to oral Estradiol Valerate (Progynova) and transdermal 17β estradiol (Oestrogel)

Oral estradiol valerate (Progynova) administration or transdermal 17β estradiol (Oestrogel) should be promptly halted if the following AEs occur during the oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) use:

i) Pregnancy or lactation

ii) Undiagnosed vaginal bleeding, defined as excessive vaginal blood loss that warrants tampon, pad, or pantyliner use

iii) Known or suspected breast cancer

iv) Known or suspected premalignant conditions or malignancies, if they are influenced by sex steroids

v) Presence or history of hepatic tumours (e.g. hepatic adenoma)

vi) Severe liver disease resulting in cholestatic jaundice or deterioration of liver function

vii) Acute arterial thromboembolic events (myocardial infarction or stroke) or a recent history of myocardial infarction or stroke

viii) Active deep vein thrombosis, thromboembolic disorders, thrombophlebitis, or a recent history of deep vein thrombosis, thromboembolic disorders, or thrombophlebitis

ix) New onset of migraine-type headache or unusually severe headache that occurs for the first time

x) Severe hypertriglyceridemia that leads to pancreatitis

xi) Any hypersensitivity (anaphylaxis, angioedema) to the oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) components

For other AEs, withhold Progynova or Oestrogel until the AEs have fully disappeared if the severity is Grade 3 and above based on the CTCAE Version 5 classification. Reintroduce oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel), and if AEs recur, permanently stop oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel), and the trial participant will be withdrawn.

4.16.8 Dermatological Toxicity

A) Grade 1 CTC-AE maculopapular rash/desquamation

Definition

Macules / Papules covering 10%< Body Surface Area (BSA) with or without symptoms (pruritus, burning, tightness).

Management

Oestrogel / Progynova will be continued and the study participant will be treated with hydroxyzine 100mg/day for 8 days.

B) Grade 2 CTC-AE maculopapular rash/desquamation

Definition

Macules/papules covering 10 - 30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental ADL; rash covering > 30% BSA with or without mild symptoms.

Management

1) The participant will be clinically managed with hydroxyzine 100 mg/day for 8 days and with prednisone for 8 days (1 mg/kg for the first 2 days, 0.5 mg/kg for the next 2 days, then 20 mg/day for the next 2 days, and 10 mg/day for the last 2 days).

2) If grade 2 maculopapular rash/desquamation remains despite symptomatic treatment, Progynova / Oestrogel will be stopped.

B) Grade 3-4 CTC-AE of any dermatological toxicity

Definitions

1) Macules/papules covering >30% BSA with moderate or severe symptoms; limiting self-care ADL

2) Macules/papules covering >30% BSA with moderate or severe symptoms; limiting self care activity of daily living (ADL)

3) Skin sloughing covering 10 - 30% BSA with associated signs (e.g., erythema, purpura, epidermal detachment and mucous membrane detachment)

Management

Consult a dermatologist for diagnosis and immediately stop the interventions.

4.16.9 Liver toxicity

A) Baseline Liver toxicity grade 1 (Baseline Serum Alanine Aminotransferase (ALT) & Aspartate Aminotransferase (AST) ≤ 3 Upper Limit of Normal (ULN) or Gamma GT ≤ 2.5 ULN or bilirubin ≤ 1.5 ULN)

Management

1) In case of grade 2 liver enzymes increased, maintain Progynova / Oestrogel

2) In case of grade 3 CTC-AE liver enzymes increase, interrupt Progynova/Oestrogel until return to grade 1 or to baseline level, then resume.

3) In case of grade 4 liver enzymes increased, halt Progynova / Oestrogel.

B) Baseline liver toxicity grade 2 (Baseline Serum Alanine Aminotransferase (ALT) & Aspartate Aminotransferase (AST) transaminases > 3 and ≤5 ULN, gamma GT > 2.5 and ≤ 5 ULN, bilirubin > 1.5 and ≤3 ULN)

Management

In case of liver enzymes increased ≥ 20% from baseline, halt Progynova / Oestrogel until return to baseline level, then resume.

C) Any grade 4 liver toxicity

Management:

Stop Progynova / Oestrogel indefinitely.

4.16.10 Cardiac-related toxicity

Management

At each follow-up visit, cardiac symptoms need to be carefully elicited during history taking and clinical examination.

If a cardiac event is suspected based on these symptoms; dyspnoea, thoracic chest pain, increased blood pressure, the following steps should be undertaken by the clinicians in charge of the study participants:

1) Perform an ECG: If there is any change compared to the previous ECG(s) readings, a cardiologist's advice should be sought.

2) Obtain serum troponin level: If the result exceeds the Upper Limit of Normal (ULN) (more than the 99^{th} percentile of upper reference limit (local-assay dependent)), a cardiologist's advice must be sought.

3) For blood pressure increase (hypertension): Start or adapt existing antihypertensive medications. If hypertension persists, a cardiologist must be consulted and the cessation of study intervention must be considered based on the risk-benefit ratio for the study participant.

4.16.11 Endocrinological toxicity

A) Hypertriglyceridemia Grade 1 and 2 (Grade 1: 1.71 – 3.42 mmol/L; Grade 2: >3.42 – 5.7 mmol/L)

Management:

1) If grade 1, maintain the same dose or stop the assigned estrogen replacement therapy until serum triglyceride level returns to normal (at physician's discretion).

2) If grade 2, stop the assigned estrogen replacement therapy until serum triglyceride level returns to grade 1 or less. Consider starting fenofibrate (267 mg daily) if serum triglyceride is above 5 mmol/L - 10 mmol/L if the 10-year CVD risk is above 8% based on the SCORE2-ASIA risk prediction model.

B) Hypertriglyceridemia Grade 3 and 4 (Grade 3: >5.7 – 11.4 mmol/L; Grade 4: >11.4 mmol/L)

Management:

1) Discontinue the assigned estrogen therapy and start fenofibrate 267 mg daily immediately if serum triglyceride level is above 10 mmol/L.

2)For those with serum TG between >5.7 mmol/L and 10 mmol/L, start fenofibrate 267 mg daily if the 10-year risk of CVD event is above 8% based on the SCORE2-ASIA risk prediction model (90).

3) Reintroduce the assigned estrogen therapy at one lower dose level after hypertriglyceridemia returns to grade 1 and below. Permanently halt the assigned intervention if hypertriglyceridemia grade 3 and above reoccurs.

4.16.12 Haematological toxicity

A) Thromboembolic events (TEE)

I) Grade 1 (Superficial venous Thrombosis) or Grade 2 (uncomplicated DVT)

Management

1) No medical intervention is required for Grade 1 TEE. Continue assigned intervention.

2) Perform serum D-Dimer and venous ultrasonography to confirm DVT. Medical intervention using appropriate anticoagulants is required for grade 2 TEE (No INR monitoring for rivaroxaban, apixaban; regular serum platelet count is required for heparin use due to heparin-induced thrombocytopenia). Temporary discontinuation of the IMP until TEE reduces to grade 1 is required. Rechallenge with 25% reduction in the originally assigned intervention dose. Permanently halt the assigned intervention if grade 2 TEE recurs.

3)Consult a haematologist to further refine the patient management.

II) Grade 3 (uncomplicated pulmonary embolism (PE), non-embolic cardiac thrombus) or Grade 4 (Life threatening TEE – complicated PE, cerebrovascular event, arterial insufficiency, with or without hemodynamic or neurological instability)

Management

1)Confirm PE with CT Pulmonary Angiography (CTPA) or V/Q Scan or CT Brain for cerebrovascular event. Medical intervention using appropriate anticoagulants is required for Grade 3 TEE. Rechallenge with 50% reduction in the originally assigned intervention dose if Grade 3 reduces to grade 1. Permanently stop the assigned intervention if Grade 3 TEE recurs.

2) URGENT medical intervention (fibrinolytic therapy with tissue plasminogen activator (tPA)) is warranted for Grade 4 TEE. Refer cardiothoracic surgeons for urgent catheter-guided pharmacomechanical therapy (physical thrombus destruction or dissolution plus catheter-guided low-dose thrombolysis) in the presence of complicated massive PE. ICU admission is warranted (consult intensivist). Stop the assigned interventions permanently.

4.17 Operational Definitions of Study Variables and Data Standards

The operational definitions for important trial variables are given below (Table 8).

Table 8: The Operational Definitions of important trial variables

Variables	**Definitions**
Demographic Profiles	

Age	Age of the Turner Syndrome patients at trial enrolment. This will be recorded in years and months. Continuous variable.
Gender	Gender of the participant. Since the participants are all females, this will be recorded as a single-group categorical variable.
Ethnicity	The racial identity of the participants. This will be recorded as one of the following multinomial categories: Malay, Chinese, Indian, Other Ethnicities.
Education level	The highest education level of the participant at the time of enrolment. This will be recorded as one of the following ordinal categories: Primary, Secondary, Pre-college (Matriculation/STPM/A-Level/IB), Undergraduate Degree, Post-Graduate Degree
Socioeconomic Status	The socioeconomic status of the family of the trial participants. This will be ordinally recorded as low (B40), intermediate (M40) and high (T20). The income range for each category will be based on the Household Income and Basic Amenities Survey 2019 by the Department of Statistics Malaysia.
Employment	The employment status of the participants at the time of trial enrolment. If the participants are employed during the course of the trial, the participant's employment status will not be changed since it represents the participant's baseline employment status. This will be recorded as a binary variable (yes, no) should include if the participant is a student as well

Clinical Parameters	
Weight	The weight of the participants in kilograms measured at baseline, months 3, 6, 12, 15, 18, 21 and 24 This will be recorded as a continuous variable.
Height	The height of the participants in centimetres measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be recorded as a continuous variable.
BMI	The Body Mass Index (BMI) of the participants in kg/m^2 measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be initially recorded as a continuous variable and subsequently categorized according to the WHO classification.
Systolic Blood Pressure (SBP)	The SBP of the participants in mmHg measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be recorded as a continuous variable.
Diastolic Blood Pressure (DBP)	The DBP of the participants in mmHg measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be recorded as a continuous variable.
Heart Rate (HR)	The HR of the participants in beats/minutes measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be recorded as a continuous variable.
Respiratory Rate (RR)	The RR of the participants in breaths/minutes measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be recorded as a continuous variable.
Temperature	The temperature of the participants in Celsius measured at baseline, months 3, 6, 12, 15, 18, 21 and 24. This will be recorded as a continuous variable.

Trial Endpoints	
Tanner Staging (Breast development)	See Section 4.15.2 for classification. It is measured as a 5-category ordinal variable at baseline, months 6, 12, 18 and 24.
Uterine Length	Self- explanatory. Measured from the fundus of the uterus to the lower margin of the cervix. It is measured in cm at baseline, month 6, 12, 18 and 24. It is treated as a continuous variable.
Uterine Antero-posterior (AP) Diameter	The width of the uterus from the anterior (front) to posterior (back) uterine wall at the largest diameter. It is measured in cm at baseline, months 6, 12, 18 and 24. It is treated as a continuous variable.
Uterine Withdrawal bleeding	Uterine bleeding occurring during the treatment with either estrogen. It is treated as a binary categorical variable (yes / no).
Time to uterine withdrawal bleeding	The time from the initiation of oral estradiol valerate (Progynova) or transdermal 17β estradiol (Oestrogel) until the first uterine withdrawal bleeding occurs. It is measured in days and is treated as a continuous variable.

4.18 Data Collection and Study Monitoring

4.18.1 Data Collection

The trial investigator/coordinator at each trial site must record the data collected in the electronic record form (eCRF) (REDCAP) developed, provided and maintained by the Sponsor (UKM). Details on each trial endpoint must also be accurately recorded in the participant's medical records since the information documented in the records will be regularly cross-checked with information in pCRF and eCRF for data consistency. Besides, double data entry procedure (i.e. the same patient information will be entered twice in another duplicated eCRF) will be carried out to minimize errors in data entry.

4.18.2 Study Monitoring (Audits)

The Sponsor will perform a six-monthly internal audit to ensure all research activities at each trial centre comply with the trial protocol and the GCP principles. The eCRFs that have been thoroughly verified and monitored at the final visit for each trial participant will be

locked and transferred to the Sponsor's Data Management Team. At the conclusion of the trial, a copy of each completely-endorsed eCRF will be shared with each respective trial site.

In addition, the Malaysian National Pharmaceutical Regulatory Agency (NPRA) might also want to inspect each or one particular trial site. In this case, the site investigator must promptly notify the Sponsor (UKM) of such requests. The investigator must therefore furnish the NPRA with relevant documents and access to the trial site. Besides, the IMP provider, Besins Healthcare, may also conduct an independent periodic external audit for the purposes mentioned above.

4.18.3 Data Management and Standards

Each trial dataset will be stored in triplicate in separate folders within the protected Amazon Cloud Drive. This will ensure there are two backup files for every original trial dataset. The information entered in the eCRF will be regularly and systematically evaluated in three ways:

1. Online with programmable system-based automatic check
2. Offline by the Data Management Personnel
3. Based on error messages obtained via additional validation programs or database listings.

Each error message in data entry (e.g. incorrectly entered BMI values of 398 kg/m2) will be captured by Data Clarification Forms and the investigators will be promptly notified to rectify the issues. The investigators are then required to sign the final eCRF electronically. As part of Quality Assurance, the Data Manager will then check the accuracy of the data for each efficacy and safety endpoint before the database is locked. Any subsequent changes to the database at this stage will require the explicit written approval of the Principal Investigator (UKM) and the principal statistician (UKM).

Concomitant medications should be coded according to the WHO Drug Reference List based on the Anatomical Chemical Classification System. The MedDRA classification system will be used to code all other comorbidities.

The trial data acquisition and handling will conform to the Clinical Data Interchange Standards Consortium (CDISC) guidelines. The types and fields for the data entered in the REDCAP database will be specified according to the Clinical Data Acquisition Standards Harmonization (CDASH) standards. The conversion of trial data to standard data tables will follow the Study Data Tabulation Model (SDTM). On the other hand, the primary efficacy variables will be derived based on the Analysis Data Model (ADAM) standards.

4.19 Ethical issues

4.19.1 Ethical Approval and Trial Registration

The trial will be conducted in accordance with the Declaration of Helsinki (64th World Medical Association General Assembly, 2013) and International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines. Upon the finalisation of the protocol version, ethical approval will be sought and obtained from the UKM Research Ethics Committee (UKM-REC) and the participating institutions' IRBs. The trial protocol will be subsequently registered with the ClinicalTrials.gov and the Malaysian National Medical Research Register NMRR) registries and the document containing the full trial protocol will be uploaded to both registries.

4.19.2 Participant Consent

Signed and dated informed consent will be obtained from parents or legal guardians for under-18 participants (and participant's assent), along with the dated signature of the person conducting the informed consent process. A copy of the signed informed consent will be given to each participant before trial commencement. Any new information regarding the IMPs (e.g. new information on the safety and efficacy of the IMPs) that may affect the participant's willingness to continue participating in the trial will be conveyed to the participants, parents or legal guardians in a timely fashion. The disclosure of such information will be conveyed and documented in a revised patient information sheet (PIS) and consent form that should be signed and dated by the parents or legal guardians for all participants. The revised PIS and consent form should receive approval from the UKM-REC and respective IRBs before use. Hence, the general format of the consent form will comply with the general requirements of each IRBs, the National Pharmaceutical Regulatory Agency (NPRA) laws and regulations and the Sponsor (UKM).

4.19.3 Data Protection and Participant Confidentiality

All participant data will be made accessible to the primary investigators and collaborators of the trial only. The participants are only identifiable by their unique trial identification number. All information that may identify individual participants, including the participant's hospital registration number (RN) and National Identity Card (NIC), will be stored in a separate password-protected database system that can only be accessed by the principal investigator and collaborators of this trial. However, for safety monitoring, the participant's identifying information can only be divulged by cross-referencing the participant's unique randomisation number with the subject identification number. This can only be performed upon obtaining written approval and at the discretion of the trial's principal investigator (UKM).

The participants' trial data will only be published in summary group measures, and therefore, it is unfeasible to identify the individual Turner Syndrome patients. If individual participants' trial data are needed as part of the publication requirements, the participant's identifying information will be removed.

To ensure the integrity of record keeping and prevent further accidental/non-accidental breaches of patient information, a data entry and access log will be created on the data management system (REDCAP). Each trial personnel (participant recruiters, assessors, interventionists, trial principal investigators and collaborators, trial coordinators) will be given

a unique ID and password for REDCAP access that can only be used by that trial personnel. This information should not be shared with other trial personnel and upon discovery of any access sharing with other trial personnel or outside individuals, the access to the REDCAP system for such trial personnel will be promptly rescinded. The person will then be disbarred from the trial due to trial misconduct.

4.19.4 Indemnity

Clinical trial insurance provided by an appropriate insurer/underwriter (e.g. no-fault compensation insurance coverage provided by the Great Eastern General Insurance (Malaysia) Incorporation or Chubb Insurance (Malaysia) Incorporation) will be purchased to protect against any foreseen/unforeseen liabilities related to research activities at the trial site. Additional research funding will be secured from the Faculty of Medicine, UKM to cover the cost of the trial insurance premium. The projected premium costs will be based on participant numbers and study duration, and the justification will be aligned with the institutional risk-management policies.

Upon approval, these funds will be ring-fenced in the trial budget and disbursed directly to the insurer before the first participant visit, thereby ensuring uninterrupted coverage from study initiation until the end of the follow-up period.

4.20 Statistical Analysis Plan (SAP)

4.20.1 Personnel

There will be three statisticians involved in the data analyses and safety data monitoring: the principal trial (study) statistician, the data monitoring committee (DMC) statistician and an independent statistician (contractually hired throughout the trial duration). The study statistician (Dr Muhammad Irfan) will be responsible for the overall design, conduct, analyses and reporting of final trial data. The DMC statistician (Prof Shamsul Azhar, an experienced triallist and a clinician) and the other members of DMC in the UKM ethics committee will be in charge of assessing the interim reports produced by the independent statistician so that recommendations on the trial conduct can be made (e.g. whether the trial can be halted prematurely or should be modified due to obvious efficacy or serious safety concern). The independent statistician, who will be hired later, will be tasked with producing interim reports on safety and efficacy parameters that the DMC members will appraise for further evaluation. The rationale for our hierarchical organisation of statistical personnel is based on the recommendations by Pocock in 2014 that guarantee the impartiality of trial conduct, design, data analysis and interpretations, and reporting of results (91).

4.20.2 Main Statistical Paradigm: Bayesian Paradigm

Since this trial includes both data from historical patients who have already received Progynova/Oestrogel and concurrent trial participants who will be randomly assigned to Progynova or Oestrogel, synthetic control methods (92) will be used to analyse the data using a Bayesian paradigm. The recency of data from historical participants and the data obtained

from trial participants make the synthetic method based on the Bayesian paradigm the best way to combine the information from both data sources to obtain a better posterior summary of each trial endpoint (92).

To address potential imbalance in confounder profiles due to the lack of randomization in the historical cohort, propensity score matching will be used to match Progynova/Oestrogel users in the historical cohort with concurrent trial participants. However, due to the potential high-dimensional nature of the inclusion of many confounders, machine learning methods such as Variational Autoencoders (VAE) based on the Variational Bayes (VB) and Generative Adversarial Network (GAN) will be utilized to create the propensity scores (93-94). To account for the effect of patient drift due to unmeasured confounders and possible changes of the measured confounders across time – a known limitation of synthetic control methods - simulated values will be generated from different statistical distributions with varying parameters for categorical and continuous unmeasured confounders. These unmeasured confounders will be included in the model and their impacts on the biasedness of the estimates will be analyzed using the method proposed by Jiang et al. (95) or based on Power / Commensurate Prior Distribution within the Bayesian paradigm (96-97) to account for heterogeneity in data sources (i.e historical data is given lower weight (discounted) compared to internal trial information).

To combine information from historical Progynova/Oestrogel users and concurrent trial participants, the Bayesian Hierarchical Model (BHM) will be used. As an illustration, the data generation process for this trial is represented in Figure 4 below (adapted from (92)):

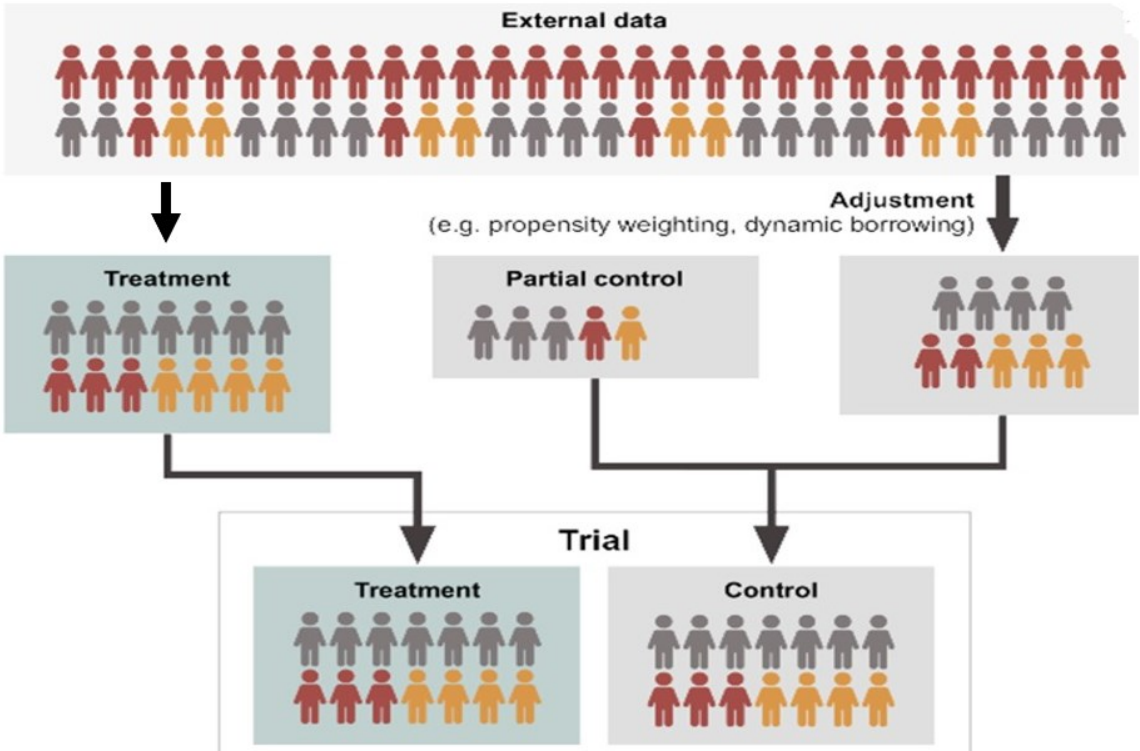


Figure 4. Sources of data and proposed synthetic control methods to combine the external data generated by historical Progynova / Oestrogel users with concurrent trial participants

The proposed BHM is represented by the schematics below (Figure 5) (adapted from (98)):

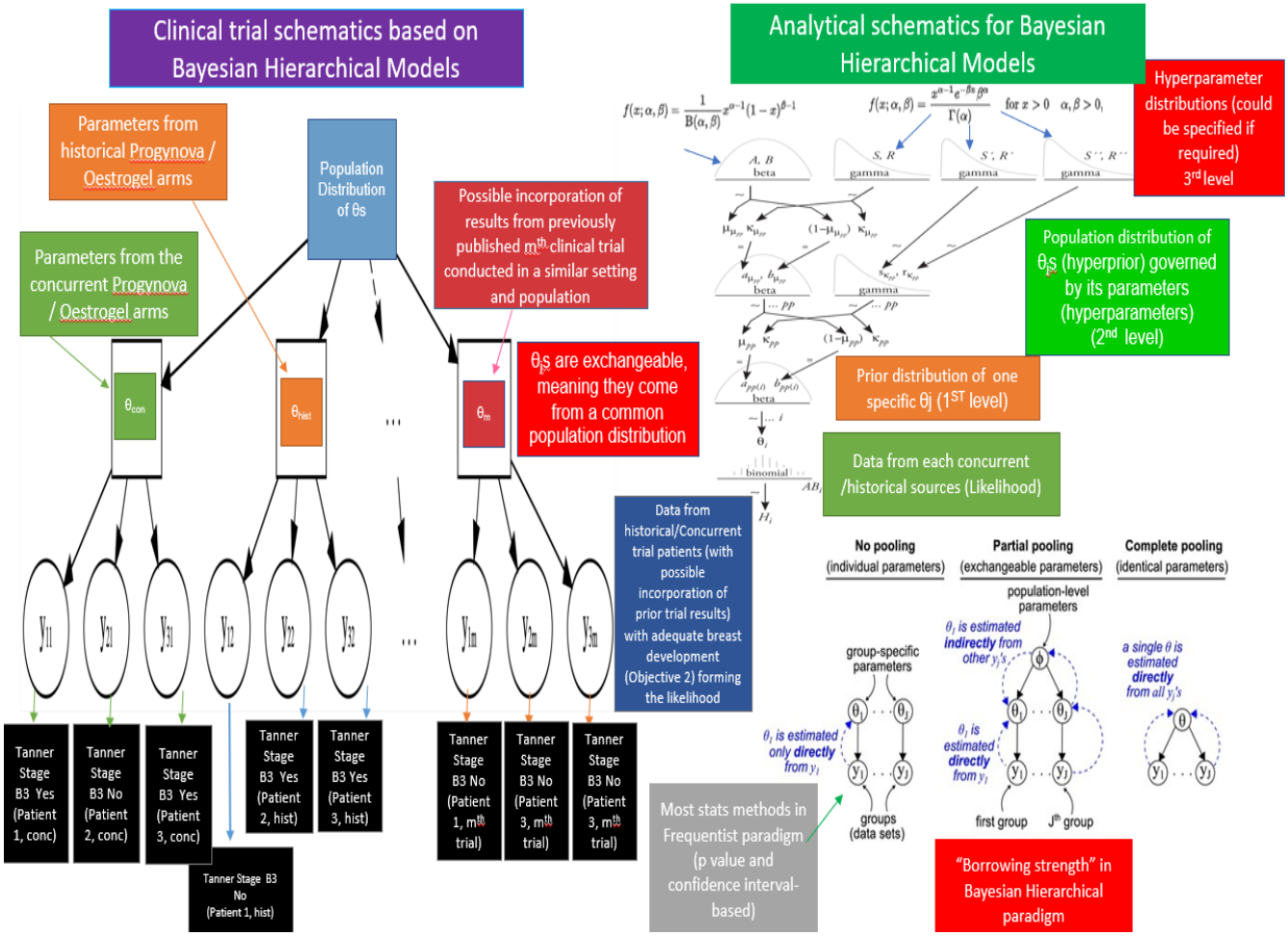


Figure 5. Schematic of the BHM to combine the information from historical Progynova/Oestrogel users with the concurrent trial participants who will be randomly assigned to Progynova/Oestrogel.

Appropriate prior distributions for each trial endpoint will be elicited and constructed to represent the findings from the previous trials (prior information). The prior information will be combined with the information in our trial data (likelihood) to obtain posterior updates for each trial endpoint parameter. To obtain the full conditional distribution (FCD) for each trial endpoint parameter, Markov Chain Monte Carlo (MCMC) techniques (Gibbs Sampling or Metropolis-Hastings algorithm) will be utilised. The procedures will be implemented in R Version 4.3.1 (R Core Team, 2023) using the Rjags version 4.14 (Plummer 2022) package. Hamiltonian Monte Carlo (HMC) algorithm will also be carried out using RStan or Integrated Laplace Approximation (INLA) method using R-INLA. Bayes factor will be computed to test statistical hypotheses and for statistical model selection. Posterior predictive checks will also be employed as part of model-checking procedures under the Bayesian paradigm. If the results obtained using the Bayesian paradigm conform with those obtained under the frequentist analyses, the veracity of such findings will be made more strengthened.

4.20.3 Main Statistical Analysis Steps for Efficacy Endpoints

Data will be cleaned for inconsistencies, wrong data entry and missing observations. Multiple imputation will be performed to impute the missing observations under the Missing At Random (MAR) assumption. The analysis will adhere to the intention-to-treat (ITT) principle as the major analytical principle, but the modified ITT (i.e. the analysis will adhere to the ITT principles, but the participants who prematurely withdraw from the trials due to non-treatment related reasons such as withdrawal of consent not related treatment efficacy or safety, non-intervention related deaths will be excluded from the analyses) and per-protocol analyses will also be performed as part of sensitivity analyses. No interim analyses will be performed.

Descriptive analyses will then be carried out to explore the data. Normally-distributed continuous variables (age, BMI, hormonal levels, uterine length and volume) will be summarised in mean (SD) or median (IQR) if the variables do not follow normal distributions. Categorical variables (Tanner Stage, HRT-associated adverse events) will be described in frequency and percentage. The normality of the continuous variables will be graphically assessed using histograms with overlying Gaussian (normal) distribution curves and subjectively using the Shapiro-Wilks test and Fisher's skewness and kurtosis coefficients (threshold ± 1.96).

For univariable analysis, the association between different types of HRTs and proportions of subjects achieving Tanner Stage B3 and above will be investigated using the exact version (Mehta & Patel algorithm) of chi-square. The differences in the mean (median) uterine length and volume between those receiving different HRT types will be examined using either the independent or Mann-Whitney test. All statistical test assumptions (homogeneity of variance, normality of the variables) will also be evaluated and reported. The Kaplan-Meier method will be employed to obtain the median times to Tanner stage B3 and above in both the oral estradiol valerate (Progynova) and Transdermal 17β estradiol (Oestrogel) groups. The equality of median times to Tanner stage B3 and above in both groups will be evaluated using the log-rank test.

For multivariable analysis, multiple logistic regression will be performed to evaluate the association between HRT types and Tanner Stage B3 or higher. On the other hand, the association between different HRT types and uterine length and volume will be investigated using multiple linear regression. Sequential model-building strategies will be employed to assess the relevance of the predictors in the model based on the sequential inclusion and exclusion of variables with $p<0.25$ at the simple logistic/linear regression level and clinically significant predictors. Multicollinearity will be assessed using inter-variable correlations (threshold: ≥ 0.75), Variance Inflation Factor (VIF) (threshold VIF ≥ 4), Tolerance (≤0.25) and Collinearity diagnostics (Condition Index VIF ≥ 30). The presence of statistical interaction will be investigated by assessing the significance of clinically plausible multiplicative interaction terms.

The residual versus predictor value scatter plot and Durbin-Watson statistics will be utilised to assess the multiple linear regression assumptions (normality, heteroscedasticity, independence and linearity of the residuals). For the multiple logistic regression model, the Hosmer-Lemeshow test will be carried out to assess the model's overall fit, and the

percentage of correct classification will be obtained to assess the model's accuracy. The area under the receiver operating curve (ROC) for the sensitivity versus 1-specificity plot will be obtained to assess the discriminatory properties of the model. The deviance (threshold ± 4) and dfBeta (threshold ± 1) will be used to evaluate the presence of influential observations. If present, they will be removed if the values are clinically implausible (i.e. incorrectly measured values) and retained if they are correctly measured observations.

No subgroup analyses will be performed. For these frequentist analyses, two-tailed statistical tests will be employed and the significance threshold will be fixed at 0.05. The results will be reported in accordance with the 2010 and 2022 CONSORT outcome reporting guidelines.

4.20.2 Statistical Analysis for Safety Parameters

A separate dataset will be created for safety parameters. The Medical Dictionary for Regulatory Activities (MedDRA) coding scheme will be used for coding the AEs. Results will be presented for each intervention group and the whole patient population. Separate results will be presented for those randomised but who do not receive the allotted interventions. All subjects who receive the allotted interventions at Day 1 and have follow-up data will be included in the analysis for safety parameters.

The following descriptive analytical measures will be carried out and presented for each time point (Day 1, Months 6, 12, 18, 24):

1. Frequency (percentage) of study participants with at least one AE

2. Frequency (percentage) of study participants with at least one SAE (death, non-fatal SAEs)

3. Frequency (percentage) of study participants with at least one grade 3 or 4 SAE

4. Frequency (percentage) of study participants with at least 1 AE that results in trial discontinuation

In addition, the incidence rate for each AE/SAE (number of study participants with the relevant AE or SAE at the end of each relevant timepoint/total number of study participants at each relevant timepoint * 100) and its associated 95% confidence interval will be calculated and presented.

4.20.3 Statistical Analysis for Compliance Monitoring

The medication adherence (compliance) of study participants will be descriptively summarised as the number (percentage) of participants who achieved at least 80% compliance at the end of each time period, including the baseline (Day 1).

4.20.4 Statistical Analysis for Assessing Inter-rater Agreement

Fleiss Kappa will be used to assess the inter-rater agreement for categorical trial endpoints (e.g., Tanner Staging for breast development). For continuous trial endpoints, the intraclass correlation coefficient will be computed. Any rater that causes the inter-rater agreement to fall below 0.60 for Fleiss Kappa and/or ICC will be excluded from being the assessor for the clinical trial endpoints (99-100).

4.23 Plans for Dissemination of Results

Upon trial completion, the trial results will be uploaded to the clinicaltrials.gov registry within 6 months of the last patient visit to facilitate early dissemination of trial results. The final and cleaned-up version of the trial dataset containing individual clinical-trial participant data (IPD) for all study variables will be deposited in a public repository such as the Harvard Dataverse data repository (available from: https://dataverse.harvard.edu/) to ensure transparency in trial reporting and compliance with the clinicaltrials.gov data sharing policies. The trial results will then be published as presentations at scientific conferences and a complete manuscript publication, even if the trial has to be prematurely halted. All authors will be provided with the results for all primary and secondary endpoints before publication. Upon the acceptance of the manuscript, the principal investigators and research collaborators from all trial sites allow the publication of key sections of this protocol (trial objectives, the eligibility criteria of the subjects, the design of the trial (including revisions), the statistical analysis plan (including amendments) and the originally planned measurements of efficacy and safety parameters.

Authorship eligibility will depend on fulfilling ALL the criteria below:

1. Significant contributions to the conception and design of the trial, trial implementation, data acquisition, statistical analysis and data interpretation;

2. Significant contributions to the drafting of the manuscript

3. Final endorsement for the publication of the final manuscript version

The final decision on the order of the authors' names will be subject to actual participation and contributions to the trial and manuscript or conference proceeding write-ups. The primary (first) author shall be the person who contributes most significantly to all three criteria above. The primary (first) author shall be responsible for safeguarding and preserving the trial data's integrity, the soundness of the trial methodology, data analysis and the manuscript's overall scientific content.

The corresponding author will be responsible for all communications during manuscript submission, the peer-review process and after publication. The responsibilities include responding to queries by the journal editors and peer reviewers and managing communications related to ethical oversight, data sharing requests and any post-publication clarifications. Besides, the corresponding author shall also be the principal point of contact for any future queries related to trial results and design presented in the accepted manuscript for future readers.

SECTION 5

EXPECTED RESULTS

4.1 Flow diagram for participant recruitment (modified CONSORT diagram for hybrid RWD-concurrent group trial)

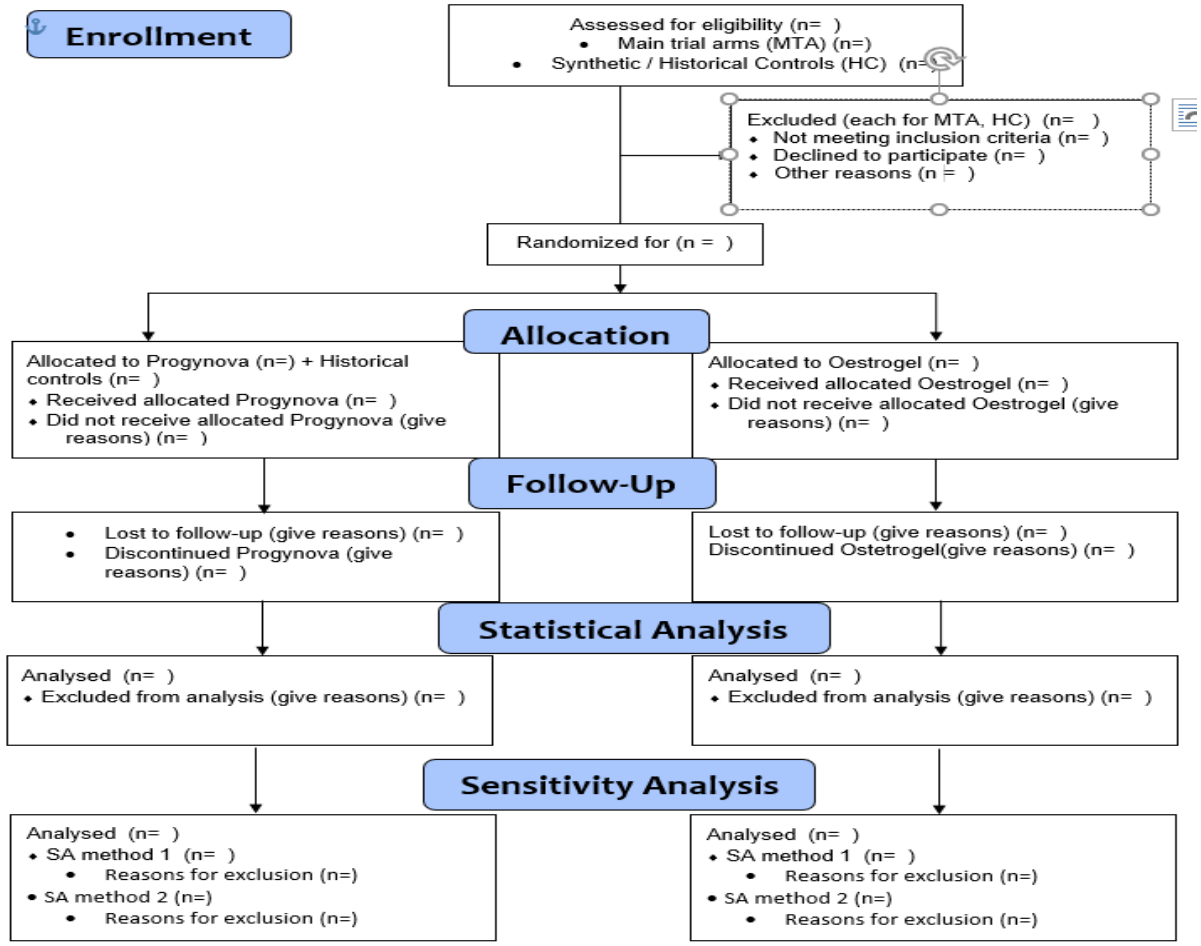


Figure 4: The modifiedCONSORT diagram depicting the procedural flow of trial participants

4.2 Baseline Characteristics of Study Participants

BASELINE CLINICODEMOGRAPHIC PROFILES

Table X: The baseline characteristics of trial participants (n=)

Factors	Progynova (n=) Mean (SD) / n (%)	Oestrogel (n=) Mean (SD) / n (%)	Bayes Factors	95% Credible Intervals
Age (years)				
Ethnicity				
Malay				
Chinese				
Indian				
Others				
Socioeconomic Status				
Low (B40)				
Intermediate (M40)				
High (T20)				
Height (cm)				
Weight (kg)				
BMI (kg/cm²)				
Concomitant Medications				
Cat 1				
Cat 2				

Tanner Stage (Breast)				
B1				
B2				
B3				
B4				
B5				
Tanner Stage (Pubic Hair)				
Stage 1				
Stage 2				
Stage 3				
Stage 4				
Stage 5				
Withdrawal Bleeding				
Yes				
No				
bleeding (days)				
Uterine length (cm)				
Uterine AP Diameter (cm)				
Lumbar BMC (gram)				
Hip BMC (gram)				
Lumbar BMD (g/cm²)				
Hip BMD (g/cm²)				
Total body BMD (g/cm²)				
AEs				
Yes				
No				
Serious AEs				
Yes				
No				

4.3 Comparisons of trial efficacy endpoints for binary outcome (dummy table for one efficacy endpoint)

EFFICACY ENDPOINTS: CATEGORICAL ENDPOINTS

Table XX: The determinants of normal breast development (Tanner stage ≥B4) at the completion of trial interventions (n=)

Determinants	Simple logistic regression			Multiple logistic regression		
	β (SE)	Crude Odds Ratio (95% Bayesian CI)	Bayes Factors	β (SE)	Adjusted Odds Ratio (95% Bayesian CI)	Bayes Factors
Age (years)						
Intervention						
Progynova						
Oestrogel						
Use of Growth Hormone						
No						
Yes						

4.4 Comparisons of trial efficacy endpoints for continuous outcome (dummy table for one efficacy endpoint)

EFFICACY ENDPOINTS: CONTINUOUS ENDPOINTS

Table XX: The determinants of BMD (g/cm^2) at the completion of trial interventions (n=)

Determinants	Simple linear regression				Multiple linear regression			
	Crude β (SE)	95% CI β	t-statistics (df)	p-value	Adjusted β (SE)	95% CI β	t-statstics (df)	p-value
Age (years)								
Intervention								
Progynova								
Oestrogel								
Use of Growth Hormone								
No								
Yes								
BMI (kg/m²)								
Underweight								
Normal								
Overweight								
Obese								
Body fat mass (gram)								
Skeletal muscle mass (gram)								

4.5 Comparisons of time to withdrawal bleeding between intervention groups

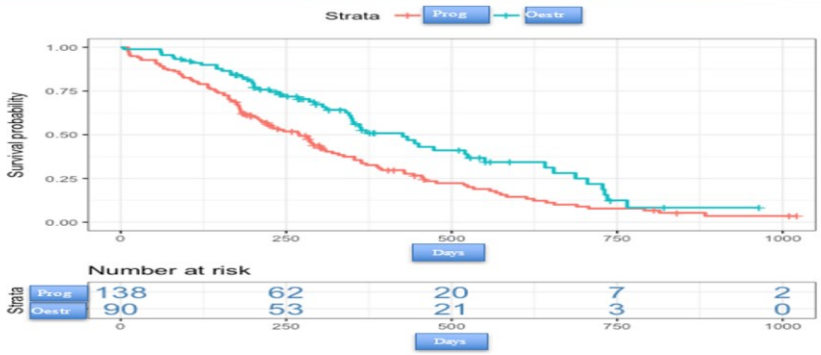


Table XXX: Time to withdrawal bleeding in different intervention groups

Variables	Median survival (days)	95% Bayesian CI	Bayes Factors
HRT regimes			
Progynova			
Oestrogel			
Ethnicity			
Malay			
Chinese			
Indian			
Others			

4.6 Comparisons of safety endpoints between Intervention groups

4.6 Comparisons of safety endpoints between Intervention groups

Table XXXX: The comparisons of adverse events between the intervention groups (n=)

Factors	Progynova (n=)	Oestrogel (n=)	χ^2 statistics / t statistics (df)	p-value	Incidence rate (95% CI)
	n (%)	n (%)			
Hypersensitivity					
Yes					
No					
Nausea					
Yes					
No					
Vomiting					
Yes					
No					
Hypertension					
Yes					
No					
Severe headache (new onset)					
Yes					
No					
Breast pain/tenderness					
Yes					
No					
Lower abdominal pain					
Yes					
No					
Vaginal Bleeding					
Yes					
No					
Vaginal Discharge					
Yes					
No					
Serum Triglyceride level (mmol/L) (mean (SD))					
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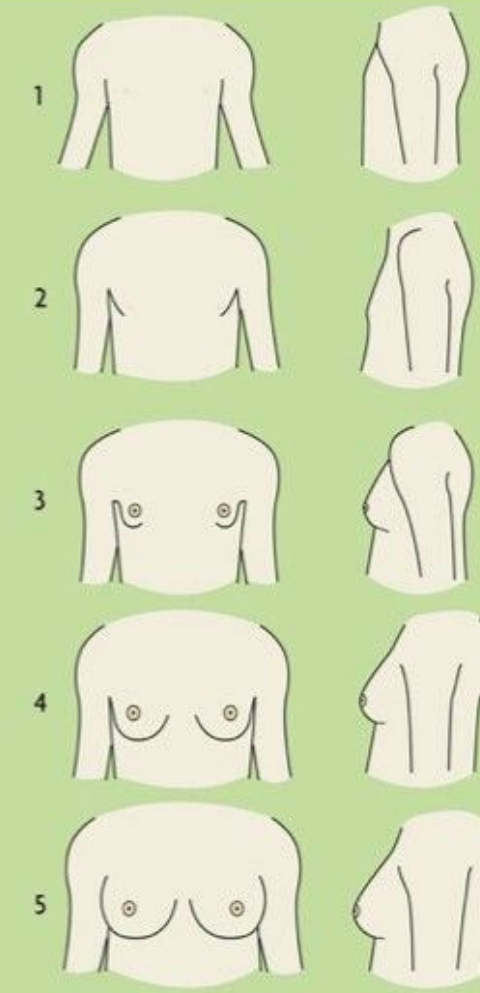
BUDGET (ONLY FOR PROGYNOVA SUBCOMPONENT OF THE GUP PROJECT)

Vot Number	Details	Total
27000	Progynova (tablets) for 12 patients assigned to the Progynova arm based on the planned intervention schedule (cost per unit: RM 21.90 per Progynova box containing 28 Progynova tablets) Hence, per patient, 38.75 Progynova boxes are required.	38.75 boxes x RM 21.90 X 12 patients = RM 9997.50
Grand Total		RM 9997.50

APPENDIX A: Pictorial details on the Tanner Staging for Breast and Pubic Hair Development



Breasts

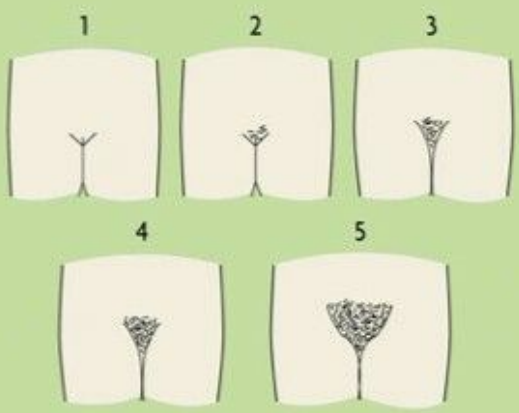
Stage 1: No breast development.

Stage 2: The first sign of breast development has appeared. This stage is sometimes referred to as the breast budding stage. Some palpable breast tissue under the nipple, the flat area of the nipple (areola) may be somewhat enlarged.

Stage 3: The breast is more distinct although there is no separation between contours of the two breasts.

Stage 4: The breast is further enlarged and there is greater contour distinction. The nipple including the areola forms a secondary mound on the breast.

Stage 5: Size may vary in the mature stage. The breast is fully developed. The contours are distinct and the areola has receded into the general contour of the breast.



Pubic Hair

Stage 1: No pubic hair.

Stage 2: There is a small amount of long pubic hair chiefly along the vaginal lips.

Stage 3: The hair is darker, coarser, and curlier and spreads sparsely over the skin around the vaginal lips.

Stage 4: The hair is now adult in type, but the area covered is smaller than in most adults. There is no pubic hair on the inside of the thighs.

Stage 5: The hair is adult in type, distributed as an inverse triangle. There may be hair on the inside of the thighs.

APPENDIX I

PATIENT INFORMATION SHEET

Research Title:

Comparison of Hormone Replacement Therapy Regimes for Pubertal Induction in Adolescents and Young Women with Turner Syndrome Introduction

Introduction:
You are invited to participate in a research study. Before participating in this study, it is crucial that you thoroughly read and understand the information provided in this sheet. However, before you take part or agree to continue in this research study, the study will be verbally explained to you, and you will be allowed to ask questions. After you are adequately satisfied that you understand this study and you wish to take part or continue to participate in this study, you must sign this informed consent form. You will be given a copy of these patient information sheet and consent forms to take home with you.

Purpose of Study:
Pubertal delay or absence is a common finding in Turner Syndrome patients. Therefore, pubertal induction using hormone replacement therapy (HRT) is required in individuals with Turner Syndrome. However, the best HRT regime for the induction of puberty in Turner Sydrome patients is still unknown for certain. Hence, this study aims to determine whether oral estradiol valerate (Progynova) or Transdermal formulation of 17β Estradiol (Oestrogel) is the better HRT regime for inducing puberty in Turner Syndrome patients.

What will the study involve?
You / Your child will undergo a pubertal induction treatment. You / Your child will be randomly allocated to either Progynova (and a dummy gel) or Oestrogel (and a dummy tablet) intervention arm based on the intervention sequences that we have created. You / Your child will be then followed up for every three monthly for the span of two years (24 months). During the follow-up visits, your compliance with the allotted interventions will be evaluated and a series of clinical examinations and radiological assessments to evaluate breast development, withdrawal bleeding status, time to first withdrawal uterine bleeding from the first day of intervention and bone mineral profile using DEXA scan will be performed.

For this study, we will be evaluating whether Progynova or Oestrogel will have a better pubertal induction efficacy as evidenced from speedier breast development, withdrawal bleeding occurrence, shorter time to the first withdrawal bleeding occurrence and better bone mineral profile that will be assessed at several periodic follow-up time points.

Risks:
The interventions (Progynova or Oestrogel) pose several risks to the study participants. Risks of oestrogen replacement therapy with Progynova or Oestrogel may involve, but are not restricted to heart attacks, clot development in the blood vessels, stroke, gallstone formation, liver disease, breast cancer, womb cancer and fibroid tumours (non-cancerous growth). Among the side effects associated with Progynova or Oestrogel are increased body fat, bloating, water retention (excess fluid) in the body, bleeding from the womb, low mood, headaches, worsening of migraine and impaired blood sugar level. However, these risks are small (around 1-10% in

post-menopausal women undergoing hormone replacement therapy) and can be much smaller in young Turner Syndrome patients (none so far in our experience).

Should you / your child experience one of the side effects above, we will promptly give appropriate treatments to mitigate such side effects. If the side effects are serious, the interventions will be promptly and completely halted. You/your child will be then withdrawn from the trial.

Benefits:
You / Your child may experience normal pubertal development. You / Your child may also experience other HRT-associated benefits such as a general sense of well-being, increased energy, reduced hot flushes and/or night sweats, reduced bone loss, enhanced memory and cognitive ability, reduction in sleep problems (insomnia) and possible reductions in future risks of dementia and heart diseases.

Do you have to take part?
Participation in this study is absolutely voluntary. Your / your child's medical care is not affected if you / your child decide(s) not to participate in this study. You / Your child will have the usual standard of care according to the day-care protocol.

If you agree to participate, you will be asked to sign the "Informed Consent Form". You will be given a copy of the informed consent form and this Patient Information Sheet. Should you decide to participate, you are still free to withdraw from the study at any time without giving a reason or penalty. If you decide to cease participating in this study, you must inform your study investigator and no new data will be collected from your child. The researcher may also remove your child from the study for various reasons. In this event, your child will not lose his/her rights as a patient and will still receive the usual standard of care.

Data & Confidentiality:
Participant's confidentiality will be maintained throughout the investigation. The personal data will be anonymized. Hence your identity will be kept confidential. Data collected and entered into the Case Report Form and the Electronic Data Capture System remain the property of UKM. In the event of any publication regarding this study, your identity will remain confidential.

By signing the Informed Consent form attached, you (or your legally acceptable representative, if relevant) are authorising such access to your study records.

Payment and compensation:
You do not have to pay, nor will you be paid to participate in this study. You do have to pay for the usual hospital charges.

Whom can I ask about the study?
If you have any questions about this study or your rights, please contact

Principal Investigators: Profesor Madya Dr Ani Amelia Dato' Zainuddin
Jabatan Obstetrik dan Ginekologi
Pusat Perubatan Universiti Kebangsaan Malaysia
No. Telefon : 03-9145 5950

Co-investigator	Profesor Dr Nur Azurah Abdul Ghani Jabatan Obstetrik dan Ginekologi Pusat Perubatan Universiti Kebangsaan Malaysia No. Telefon : 03-9145 6485

Signatures

To be entered into this study, you or a legal representative must sign and date the signature page [APPENDIX II]

APPENDIX II

Patient/Subject Information and Consent Form
(Signature Page)

Research Title: Bayesian Pragmatic Trial for Pubertal Induction in Turner Syndrome: Transformation Initiative For Efficient Clinical TriAl Design Advancement in RarE Diseases (TRIFECTA-DARED framework)

Researcher's Name: Professor Dr Ani Amelia Dato Zainuddin / Prof. Dr Nur Azurah Abdul Ghani

To become a part of this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Patient Information and Consent Form, including any information regarding the risk in this study and I, have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
- I may freely choose to withdraw from this study at any time without reason or without repercussion.
- I understand that my anonymity will be ensured during research write-up
- I have received a copy of this Patient Information and Consent Form to keep for myself.

Patient Name (Print or type) | Patient Initials and Number

Patient I.C No. (New)

Signature of Patient or Legal Representative
(Add time if applicable) | Date (dd/MM/yy)

Name of Individual
Conducting Consent Discussion (Print or Type)

Signature of Individual
Conducting Consent Discussion | Date (dd/MM/yy)

Name & Signature of Witness | Date (dd/MM/yy)

Note: i) All subject/patients who are involved in this study will not be covered by insuranc

APPENDIX III

Research Ethics Committee UKM
(RECUKM)

20, AVENUE APPIA – CH-1211 GENEVA 27 – SWITZERLAND – HTTP://WWW.WHO.INT/ETHICS/REVIEW-COMMITTEE

Informed Assent Form Template for Children/Minors

UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia

An Informed Assent Form does not replace a consent form signed by parents or guardians. The assent is in addition to the consent and signals the child's willing cooperation in the study.

Informed Assent Form for Turner Syndrome Children Aged Between 11-17 years old who attend Pediatric and Adolescent Gynaecology Clinic and who we are inviting to participate in research "Bayesian Pragmatic Trial for Pubertal Induction in Turner Syndrome: TRansformation Initiative For Efficient Clinical TriAl Design Advancement in RarE Diseases (TRIFECTA-DARED framework")

Principal Investigator: Professor Dr Ani Amelia Zainuddin
Location: Universiti Kebangsaan Malaysia
Sponsor: Universiti Kebangsaan Malaysia
Project Title: Bayesian Pragmatic Trial for Pubertal Induction in Turner Syndrome: TRansformation Initiative For Efficient Clinical TriAl Design Advancement in RarE Diseases (TRIFECTA-DARED framework)

This Informed Assent Form has two parts:

- **Information Sheet (gives you information about the study)**
- **Certificate of Assent (this is where you sign if you agree to participate)**

You will be given a copy of the full Informed Assent Form

Part I: Information Sheet
Introduction

My name is ____and my job is to research and test two types of hormone replacement therapy (Oestrogel and Progynova) to see which works best to stimulate normal development of breast and womb in children with Turner Syndrome and we think this research could help tell us that.

I am going to give you information and invite you to be part of a research study. You can choose whether or not you want to participate. We have discussed this research with your parent(s)/guardian and they know that we are also asking you for your agreement. If you are going to participate in the research, your parent(s)/guardian also have to agree. But if you do not wish to take part in the research, you do not have to, even if your parents have agreed.

You may discuss anything in this form with your parents or friends, or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately.

There may be some words you don't understand or things that you want me to explain more about because you are interested or concerned. Please ask me to stop at any time, and I will take the time to explain.

Purpose: Why are you doing this research?
We want to find better ways to improve the pubertal development in Turner Syndrome children. We have two types of medications for the delivery of the hormone estrogen. We thus want to know which one is better in inducing normal development of the breast and womb in children with Turner Syndrome. We need to test it to determine if it is better.

Choice of participants: Why are you asking me?

We are testing these two types of types of medications for hormone replacement on participants who are at your age - between 12 and 17 years old - who have Turner Syndrome. We want to know which medication is better in terms of its efficacy for stimulating puberty in children with Turner Syndrome.

Participation is voluntary: Do I have to do this?
You don't have to be in this research if you don't want to be. It's up to you. If you decide not to be in the research, it is okay and nothing changes. This is still your clinic, everything stays the same as before. Even if you say "yes" now, you can change your mind later and it is still okay.

If anything changes and we want you to stay in the research study even if you want to stop, we will talk to you first.

I have checked with the child, and they understand that participation is voluntary __(initial)

Information on the Trial Drug [Name of Drug]: What is this drug and what do you know about it?

The types of medications that we are testing in this research are Progynova (in pill form) and Oestrogel (in gel form). Both medications have been in pre-menopausal adults to alleviate pre-menopausal signs and symptoms. However, we now want to test the medications on teenagers and young women with Turner Syndrome to induce normal development of the breast and uterus. This research is called a "phase 2" trial.

Both medications have several side effects. Possible side effects include changes in mood, headaches, feeling bloated, gaining weight, swelling in the legs or hands, soreness in the breasts, and some bleeding from the womb. In rare cases, hormone treatment can cause more serious side effects such as blood clots, stroke, heart problems, liver problems, or breast and womb cancer. These risks are much more common in older women who use hormone therapy for many years. In young patients with Turner Syndrome, these serious side effects are extremely rare, and none have been reported in our experience so far.

Procedures: What is going to happen to me?

We are going to test the medications by giving some of the children in the research Progynova and others are going to receive Oestrogel. You will be randomly allocated to receive either Progynova or Oestrogel. By doing the research like this, we can compare which of the types of hormone replacement therapy is better without being influenced by what we think or hope the research will show.

If you decide that you want to do this, several things will happen:

1. You will come to the clinic with your parents and your eligibility (your suitability) for trial participation will be evaluated against certain criteria.

2. If you are eligible to take part in this study, the doctor will ask about your history, will take blood samples, examine your breasts and will perform an ultrasound scan to measure your uterus.

3. You will then receive either Progynova or Oestrogel and you will have to take them for two years (24 months)

4. You also need to come to the clinic every three months to check if you are taking the medicine correctly, check your breast development and ultrasound scan to measure your uterus. And to check if you have side effects, so we can help.

Altogether, you will come to the clinic 7 times over 2 years (24 months). At the end of 2 years, the research will be completed.

I have checked with the child and they understand the procedures _____(initial)

Risks: Is this bad or dangerous for me?

Both Progynova and Oestrogel are considered safe. They have already been tested on pre-menopausal adults. There has been nothing that has worried us at all. If anything unusual happens to you, however, we need to know and you should feel free to call us anytime with your concerns or questions. Another way for us to know how you are is by having you come to the clinic every 3 months for a check-up. If you get sick or have concerns or questions in-between the scheduled visits to the clinic, you should inform me or the staff nurse. You don't have to wait for a scheduled visit.

Discomforts: Will it hurt?

There are a few other things I would like you to know.

I do not anticipate any significant discomfort when taking the study medications. This is because the medications being used — Progynova, which is a tablet, and Oestrogel, which is applied as a gel to the skin — are both given in ways that are generally easy and painless. You will not be getting any injections as part of this treatment.

However, as with any medication, there is a chance that you might experience some mild side effects. If anything feels unusual, uncomfortable, or if you're worried about how you feel after using the medicine, please tell your parents or let me know as soon as possible.

We don't expect that taking part in the study will interfere much with your daily life. You may have to come to the clinic from time to time, but we will try to make those visits as short and easy as possible. If you ever feel unsure, upset, or uncomfortable about anything, it's okay to speak up.

I have checked with the child and they understand the risks and discomforts ____(initial)

Benefits: Is there anything good that happens to me?
Yes, there may be some good things that happen if you take part in this study. By being in this study, you (or your child) may start puberty in a way that is more like how it happens naturally. This means the body may begin to develop in ways that include breast development, the start of periods, and the growth of the womb. These are important parts of growing up and becoming more like other girls your age.

The hormone treatment may also help you feel better in other ways. It might help with:

- Having more energy
- Sleeping better
- Thinking more clearly
- Feeling happier
- Building stronger bones
  Starting puberty at the right time may also reduce the chances of having health problems later in life, such as heart disease or dementia when you're older.
  Even if you don't get all of these benefits, the information we learn from this study could help doctors take better care of other girls with Turner Syndrome in the future.

I have checked with the child and they understand the benefits_____ (initial)

You will **not have to pay** to take part in this study, and you will **not be paid** for participating. Whilst you are in the trial, the initial blood tests, the ultrasound scans, visits to the doctor and the medicine (hormones) will be given to you free of charge.

However, you will still need to cover the **usual hospital charges** that are not related to the research. These are the same fees you would normally pay if you were not in the study.

Confidentiality: Is everybody going to know about this?

We will not tell other people that you are in this research and we won't share information about you with anyone who does not work in the research study. After the research is over, you and your parents will be told which of the two injections you received and the results.

Information about you that will be collected from the research will be kept away and no one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except [name who will have access to the information, such as research sponsors and your doctor.

Compensation: What happens if I get hurt?

If you become sick during the research, we will look after you. We have given your parents information about what to do if you are hurt or get sick during the research.

Sharing the Findings: Will you tell me the results?

When we are finished the research, I will sit down with you and your parent and I will tell you about what we learnt. Afterwards, we will be telling more people, scientists and others, about the research and what we found. We will do this by writing and sharing reports and by going to meetings with people who are interested in the work we do.

Right to Refuse or Withdraw: Can I choose not to be in the research? Can I change my mind?

You do not have to be in this research. No one will be mad or disappointed with you if you say no. It is your choice. You can think about it and tell us later if you want. You can say "yes" now and change your mind later and it will still be okay.

Who to Contact: Who can I talk to or ask questions to?

You can ask me questions now or later. I have written a number and address where you can reach us, or, if you are nearby, you can come and see us. If you want to talk to someone else that you know, like your personal doctor or relatives, that's okay too.

If you choose to be part of this research, I will also give you a copy of this paper to keep for yourself. You can ask your parents to look after it if you want.
You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART 2: Certificate of Assent

I understand that this research is about **helping girls with Turner Syndrome start puberty** using special hormone treatments. In this study, I will get one of two types of medicine for estrogen replacement — either a pill called Progynova or a skin gel called Oestrogel. I won't choose which one I get; it will be decided by chance (like flipping a coin). The treatment will continue for two years, and I will come to the hospital every three months for check-ups.

At each visit, the doctor will check how I'm doing, whether I'm taking the medicine correctly, and how my body is developing — like whether my breasts are growing or if my periods have started.

I have read this information (or had the information read to me), I have had my questions answered, and I know that I can ask questions later if I have them.

I agree to take part in the research.

OR

I do not wish to take part in the research and I have <u>not</u> signed the assent below._____(initialled by child/minor)

<u>Only if child assents:</u>

Print name of child _____

Signature of child: _____

Date:_____
day/month/year

If illiterate:
A literate witness must sign (if possible, this person should be selected by the participant, not be a parent, and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness (not a parent)_____ AND Thumb print of participant

Signature of witness _____

Date _____
Day/month/year

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Print name of researcher_____

Signature of researcher_____

Date_____
Day/month/year

Statement by the researcher/person taking consent
I have accurately read out the information sheet to the potential participant, and to the best of my ability, made sure that the child understands that the following will be done:
1.
2.
3.
I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this assent form has been provided to the participant.

Print Name of Researcher/person taking the assent_____

Signature of Researcher /person taking the assent _____

Date _____
Day/month/year

Copy provided to the participant _____(initialed by researcher/assistant)

Parent/Guardian has signed an informed consent ___Yes ___No _____(initialed by researcher/assistant)

APPENDIX IV

CASE REPORT FORM

Research title: Bayesian Pragmatic Trial for Pubertal Induction in Turner Syndrome: Transformation Initiative For Efficient Clinical TriAl Design Advancement in RarE Diseases (TRIFECTA-DARED framework)

Research Investigators: Dr Muhammad Irfan Abdul Jalal, Associate Prof Dr Ani Amelia Zainuddin, Professor Dato' Dr A Rahman A Jamal, Professor Dr Nur Azurah Abdul Ghani, Dr Esther Loh Sweet Yi.

Hospital Sticker

Subject ID ☐☐☐☐☐ **Date:** ☐☐ / ☐☐ / ☐☐
Day Month Year

Randomisation ID:

A. SUBJECT'S DETAIL

Subject's name: _____

Age : ☐ years ☐ months

Registration No (RN) : _____

Telephone no : Handphone: _____

Home : _____

Date of birth : _____ / _____ / _____

Date of consent: _____ / _____ / _____

Ethnicity : ☐ Malay ☐ Chinese ☐ Indian

Others (please specify): _____

B. FAMILY HISTORY OF ILLNESS

Has any of the family members having any significant disease in their history of illness.

First degree	**Yes**	**No**
Father	☐	☐
Mother	☐	☐
Siblings	☐	☐

Others: _____

If Yes, please specify the types of diseases and age at diagnosis: _____

_____

_____

C. REVIEW OF MEDICAL HISTORY / MEDICATIONS CHECKLIST

Relevant medical history (Y/N)

Diabetes : ☐

Hypertension : ☐

Hyperlipidemia : ☐

Hypothyroidism : ☐

Autoimmune Disease : ☐

Cancer : ☐

Osteoporosis : ☐

Others : _____

_____

_____

Medications (please specify the name, indications, dosage and administration frequency)

Name	Reasons	Doses	Frequency of administration	Duration been on this medication

Has subject ever experienced any allergy or adverse event from any medications?

Yes * ☐ No ☐

*****If **yes,** please specify the type of medication: _____

D. CLINICAL DATA

Date of first physical examination:

i) Anthropometric measurement

1. Weight at recruitment : kg
2. Height at recruitment : cm
3. Body mass index (BMI) at recruitment : kg/m^2
4. Systolic Blood Pressure (SBP): mmHg
5. Diastolic Blood Pressure (DBP): mmHg

ii) Turner Syndrome's profile

1. Karyotype :
2. Breakpoint locations :
3. Cell line ratios (for mosaicism):
4. Confirmed with the genetic report :
5. Type of genetic test :
6. Date of diagnosis (genetic test) :
7. Age at diagnosis :
7. Height at diagnosis : cm
8. Weight at diagnosis : kg
9. Father's height : cm
10. Father's weight : kg
11. Mother's height : cm
12. Mother's weight : kg

13. Menstrual status (Y/N)* (if yes, give date of 1st menstruation) :

14. Puberty status (Y/N) :

15. Has gonadectomy been done? Y/N
If have been performed, please answer questions no 16 & 17

16. Left gonad histopathology (describe) :

17. Right gonad histopathology (describe) :

18. Inspection of External genitalia (describe) :

19. Genital surgery (Y/N) (if yes, what was the indication and what surgery was performed and when - year) :

20. Tanner Stage (Breast) :

21. Tanner Stage (Pubic Hair) :

22. Presence of axillary hair: Y / N

iii) USS Uterine profile (baseline) date of baseline ultrasound scan :

Ultrasonographer's name:

i) Uterine length : cm 2 readings each

ii) Uterine AP diameter: cm 2 readings each

APPENDIX V

ADVERSE EVENTS TRACKING LOG

No.	Date reported	Adverse event description	Start date	End date	Ongoing (Yes or No)	Outcome[1]	Severity / grade[2]	Serious (Yes or No)	AE treatment[3]	Expected (Yes or No)	Intervention Attribution / Relatedness[4]

Scales:

Outcome[1]	**Severity / grade[2]**	**AE treatment[3]**	**Intervention Attribution / Relatedness[4]**
0- Fatal	0- Mild	0- None	0- Definite
1- Not recovered / Not resolved	1- Moderate	1- Medication(s)	1- Probable
2- Recovered w/sequelae	2- Severe	2- Medication TX	2- Possible
3- Recovered w/o sequelae	3- Life-threatening		3- Unrelated
4- Recovering / Resolving	4- Death / Fatal		4- Not applicable (did not receive intervention)

Verified by (Prior to data entry):
Signature:
Name:
Date

APPENDIX VI

BASELINE AND FOLLOW UP MEASUREMENTS

Visit No	1	2	3	4	5
Scheduled Time	Day 1	Month 1	Month 7	Month 13	Month 19
Visit Windows	Baseline (Consent) Visit	1 month after Visit 1 ± 1 week	6 Months after Visit 2 ± 1 week	6 Months after Visit 3 ± 1 week	6 Months After Visit 4 ± 1 week
Clinical Parameters					
Systolic Blood Pressure (SBP) (mmHg)					
Diastolic Blood Pressure (DBP) (mmHg)					

Pubertal Development					
Tanner Stage (Breast Development)					
Withdrawal bleeding status (Y/N) if yes, put in date of forst withdrawal bleeding					
Time to the 1st withdrawal bleeding (only fill in when withdrawal bleeding 1st occurs)					
Laboratory Parameters					
Serum FSH Level (U/L)					

Need only 2 readings at the most at baseline					
Serum LH Level (U/L) Need only 1 reading at baseline					
Serum Prolactin Level (U/L) Need only 1 reading at baseline					
Serum Estradiol Level (pg/mL) Need only 1 reading at baseline					
USS Uterine					

Uterine length (cm)					
Uterine AP Diameter (cm)					
Endometrial thickening (hyperplasia) (Yes/No) if yes, ET size (mm)					
Quality of Life Psychometrics (Questionnaires)					
WHOQOL-BREF (General QOL)					
Breast-Q (breast satisfaction score)					

Safety Profile (based on adverse event tracking log)					

APPENDIX VI

WITHDRAWAL BLEEDING MONITORING LOG (adapted from Zhang et al. 2023)

Subject ID No																															
Month / Year																															
Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
Bleeding																															
No of pads / tampons used																															
Grading (for withdrawal bleeding)																															

The participant has to mark either X = red vaginal bleeding; o = brown discharge. For withdrawal bleeding grading (Wise et al. 2011; Mejia et al. 2016; Wesselink et al. 2016) (33-35): 1 = no bleeding; 2 = spotting, 3 = light bleeding (≤10 pads/tampons per menses); 4 = moderate bleeding (11-20 pads/tampons per menses); 5 = heavy bleeding (20-30 pads/tampons per menses); 6 = very heavy bleeding (>30 pads/tampons per menses). Spotting is defined as bloody discharge that requires not more than a panty liner (Zhang et al. 2023) (83).