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Review

17β-Estradiol and natural progesterone for menopausal hormone therapy: REPLENISH phase 3 study design of a combination capsule and evidence review

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ABSTRACT

Several formulations combining estrogens and progestins for hormone therapy (HT) have been approved worldwide for the treatment of menopausal symptoms, yet recent data indicate a decline in their use and an increase in compounded bioidentical HT. Up to now, no single product combining natural 17β-estradiol and progesterone has been approved by the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA). A phase 3 trial (REPLENISH) is underway to study a novel oral formulation of solubilized 17β-estradiol and natural progesterone combined in a single gelatin capsule (TX-001HR; TherapeuticsMD, Inc, Boca Raton, FL) for treating vasomotor symptoms (VMS) in postmenopausal women. The REPLENISH trial evaluates the efficacy and safety of TX-001HR (4 doses) versus placebo for the reduction of moderate to severe VMS frequency and severity at 4 and 12 weeks and evaluates the endometrial safety of the combinations at 1 year. TX-001HR contains hormones that are molecularly identical to endogenous estradiol and progesterone and is intended as an option for women who prefer bioidentical hormones; further, it does not contain peanut oil, a common allergen. The constituents of TX-001HR, in a pharmacokinetic report, showed similar bioavailability and safety compared with reference estradiol tablets and micronized progesterone capsules administered together. Published data suggest a safer profile of estradiol and natural progesterone compared with HT containing conjugated equine estrogens and progestins. This report summarizes the methodology of the REPLENISH trial and reviews the evidence suggesting clinical differences between HT containing progesterone or progestins, and estradiol or conjugated equine estrogens.

Contents

Abbreviations: CBHT, compounded bioidentical hormone therapy; CEE, conjugated equine estrogens; EMA, European Medicines Agency; FDA, Food and Drug Administration; HDL-C, high-density lipoprotein cholesterol; HT, hormone therapy; KEEPS, Kronos Early Estrogen Prevention Study; LDL-C, low-density lipoprotein cholesterol; MPA, medroxyprogesterone acetate; NETA, norethisterone acetate; PEPI, Postmenopausal Estrogen/Progestin Interventions study; VMS, vasomotor symptoms; WHI, Women's Health Initiative.

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1. Introduction

Several formulations of hormone therapy (HT) containing estrogens and progestins have been approved by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of menopausal symptoms. The primary indication for HT is the relief of moderate to severe vasomotor symptoms (VMS) [1]. The most effective treatment for hot flushes is HT consisting of estrogens with or without progestogens [2]. However, publication of data showing possible harm in women of a mean age of 63 that were treated for more than 5 years with conjugated equine estrogens (CEE) and medroxyprogesterone acetate (MPA) from the Women's Health Initiative (WHI) in 2002 [3] deterred many women from initiating or continuing prescribed HT [4–7]. An increase in the use of compounded bioidentical hormone therapy (CBHT) [7–9] has occurred in the United States, since this publication, indicating that women appear to be concerned with the hormones contained in FDA-approved HT. Using a combination of cross-sectional Internet survey data, US Census Bureau statistics, and PHAST 2.0 prescription data, a recent US study estimated that CBHT may account for 28% to 68% of all HT prescriptions and may be used by 1 to 2.5 million women aged ≥40 years annually, accounting for $1 to $2 billion in health care spending every year [10].

Women with a uterus take a progestogen with exogenous estrogen to prevent uterine stimulation and possible endometrial cancer [1,11]. Progestogens such as micronized progesterone have been shown to inhibit endometrial hyperplasia related to unopposed estrogen stimulation [12]. Although FDA-approved separate tablet/capsule combinations of estrogen and progesterone monotherapies are available for menopausal symptoms, no single tablet or capsule product combining the natural hormones 17β-estradiol and progesterone has been approved by the FDA. 17β-estradiol and progesterone combinations that do not have regulatory agency approval are available through compounding pharmacies, but have variable purity and potency and lack efficacy and safety data. This has resulted in medical societies [1,8,13] and the FDA [14] cautioning against the use of CBHT.

REPLENISH is a phase 3 trial studying a novel oral formulation of solubilized 17β-estradiol and natural progesterone combined using SYMBODA™ technology in a single gelatin capsule (TX-001HR; TherapeuticsMD, Inc, Boca Raton, FL) for the treatment of VMS in postmenopausal women. TX-001HR capsules contain hormones that are molecularly identical to endogenous estradiol and progesterone, without peanut oil, a common allergen [15]. This formulation is intended to provide a therapeutic option for women who prefer "natural" hormones. Until now, it has been difficult to effectively combine progesterone and estradiol together in a single capsule [15]. One reason may be that effective absorption of oral progesterone is difficult to achieve, although studies have clarified that absorption is influenced by the vehicle used and progesterone particle size [16].

The estradiol and progesterone of the single capsule (TX-001HR) have bioavailability similar to their respective reference estradiol tablets and micronized progesterone capsules administered together, as shown in a preliminary report [15]. This product, if approved, will be the first FDA/EMA-approved HT to combine 17β-estradiol and progesterone in a single oral dosage form and will be the first oral 17β-estradiol/progesterone combination that is available without peanut oil. The purpose of this report is to detail the study methods of the REPLENISH trial of TX-001HR and to review the relevant literature on the benefits of estradiol and progesterone present in this combination capsule.

2. Replenish study

The purpose of the REPLENISH trial is to determine whether different doses of TX-001HR are effective at reducing the frequency and severity of moderate to severe menopause related VMS versus placebo at 4 and 12 weeks, and to evaluate endometrial safety after 12 months of continuous use of this combination.

2.1. Study population

Eligible participants are healthy postmenopausal women ($N = 1750$) with a uterus who are seeking treatment for menopause-related VMS and fulfill additional inclusion and exclusion criteria (Table 1). During the screening period, all women will complete diaries for 14 consecutive days to assess the frequency and severity of VMS. The 12-week VMS substudy will include women who report ≥7 moderate to severe hot flushes per day, or ≥50 per week, for at least 14 days during screening.

2.2. Study design

The REPLENISH trial (NCT01942668; www.clinicaltrials.gov) is a phase 3, prospective, randomized, double-blind, placebo-controlled, parallel-group, 12-month, multicenter trial (80 sites in the United States) evaluating the safety and efficacy of a 17β-estradiol-natural progesterone combination capsule in postmenopausal women. Approximately 4000 women will be screened for study eligibility to enroll 1750 women who meet the inclusion and exclusion criteria (Table 1).

At baseline (week 0), 1750 eligible women will be randomly assigned to self-administer orally at bedtime 1 of 4 doses of TX-001HR (estradiol/progesterone: 1.0 mg/100 mg, 0.5 mg/100 mg, 0.5 mg/50 mg, or 0.25 mg/50 mg) or placebo for 12 months. Participants in the 12-week VMS substudy ($n = 750$) will be randomized equally within each study site to each active treatment group

Table 1
Main inclusion and exclusion criteria in the REPLENISH study.

Inclusion criteria	Exclusion Criteria
• Women • Aged 40 to 65 years old • Intact uterus • Postmenopausal (serum estradiol, ≤50 pg/mL) • Generally healthy per pre-specified criteria • BMI ≤34 kg/m^2 • Seeking treatment for menopause-related VMS • Willing to abstain from non-study hormone products • Use of no more than 2 antihypertensive drugs Negative screening mammogram; normal breast exam and endometrial biopsy	• Contraindications to hormone use • Heavy smoker (≥15 cigarettes/day) • History of endometrial hyperplasia or of undiagnosed vaginal bleeding • History of melanoma or of breast, uterine, or ovarian cancer • History of clinically significant, relevant physical or mental illness, including but not limited to thromboembolic disorder or other vascular disease, clotting or malabsorption disorder, estrogen-dependent neoplasia, or chronic kidney or liver disease Recent use of a CYP3A4 inhibitor, certain hormones, or an IUD
Additional Criteria for the VMS substudy 14-day diary showing ≥7 moderate to severe hot flushes per day or ≥50 per week during screening	Use of medication in past 28 days that may affect VMS prior to screening

BMI = body mass index; IUD = intrauterine device; VMS = vasomotor symptoms.

(n = 150 per group) or the placebo group (n = 150). Non-substudy participants will be randomized 1:1:1:1 to the 4 active treatment groups only. Randomization at each site was achieved using a reproducible, computer-generated block randomization schedule. All study staff and study participants will be blinded throughout the study. The blind may only be broken in emergency situations to protect subject safety.

2.3. Study endpoints

The 4 co-primary efficacy endpoints (evaluated in the VMS substudy) are as follows: mean change in frequency of moderate to severe VMS from baseline to week 4 and to week 12 for each active treatment versus placebo, and mean change in severity of moderate to severe VMS from baseline to mild, moderate and severe VMS at week 4 and week 12 for each active treatment versus placebo. Rate of improvement in VMS frequency and severity from baseline will be assessed using a 7-point scale, ranging from 'very much improved' to 'very much worse'. Weekly frequency of hot flushes will be defined by the number of moderate and severe hot flushes over 7 days. The weekly severity score will be calculated by adding (the number of mild hot flushes for 7 days × 1) + (number of moderate hot flushes for 7 days × 2) + (number of severe hot flushes for 7 days × 3), divided by the total number of hot flushes over 7 days.

The primary safety endpoint (evaluated in the overall population) will be the incidence of endometrial hyperplasia at 12 months. Each endometrial biopsy will be evaluated by 3 pathologists. The study meets FDA and EMA requirements for evaluation of endometrial safety as outlined by their respective guidelines [17,18].

Several pre-specified secondary endpoints will also be analyzed in the VMS substudy and in the total population (Table 2). During the study, women will record in a daily diary the severity and frequency of hot flushes and endometrial bleeding or spotting. Follow-up visits will take place at weeks 4, 8, and 12; and at months 6, 9, and 12 (Fig. 1). At weeks 4, 8, and 12, VMS substudy participants will be asked to rate the improvement in VMS from baseline. The Menopause-Specific Quality of Life Questionnaire (MENQOL) and the Medical Outcomes Study (MOS)-Sleep questionnaire will be completed at baseline, at week 12, and at months 6 and 12. At each visit, vital signs, adverse events, and concomitant drug use will be recorded; daily diaries and unused study medication will be collected; and new medication will be dispensed. Adverse events will be assessed for severity and relationship to study medication in the 5 treatment groups over 12 months.

Table 2
Secondary endpoints.

VMS substudy	Total Population
• Mean change from baseline to week 12 (calculated **each** week) in ○ Frequency and severity of moderate to severe VMS; and of mild, moderate, and severe VMS ○ Per-person rate of reduction in the frequency and severity of VMS ○ Rate of women with 50% and with 75% decreases in moderate to severe VMS Percentage of responders at weeks 4, 8, and 12	• Rates of amenorrhea • Number of days with bleeding and spotting • MENQOL scores • MOS-Sleep scores

MENQOL = Menopause-Specific Quality of Life **Questionnaire; MOS = Medical Outcomes Study;** VMS = vasomotor symptoms.

2.4. Statistical analysis

Sample size is based on the combination therapy achieving a ≤1% endometrial hyperplasia incidence rate after 12 months of therapy with a one-sided 95% upper confidence limit of ≤4%. More than 250 subjects per active group are anticipated to have an end-of-study biopsy. The VMS sub-study sample size is based on the expected changes in average weekly frequency and severity of vasomotor symptoms from baseline to weeks 4 and 12. A VMS substudy sample size of 150 women per treatment group, accounting for up to 20% of the subjects per group to be ineligible for the primary analysis, will provide at least 90% power to test the primary hypotheses of the VMS substudy.

For endometrial hyperplasia, an observed incidence rate of 1% or less with an upper one-sided 95% confidence limit of ≤4% will be considered an acceptably low incidence. Confidence intervals (95%, 2-sided) will be calculated for pairwise differences between groups for endometrial hyperplasia incidence. The incidence of hyperplasia was calculated as $I = A/B$, where I is the incidence at year 1, A is the number of women with biopsies positive for endometrial hyperplasia during the study, and B is the number of women with biopsies at year 1, plus all women with positive biopsies before year 1.

Mean changes from baseline in frequency and severity of vasomotor symptoms will be assessed for the four co-primary endpoints; the mean of the active treatment group will be compared with placebo using an analysis of covariance (ANCOVA) adjusting for the baseline. Statistical significance will be declared if $P < 0.05$ for each dose comparison of each of the 4 co-primary endpoints.

To account for the multiple comparisons, procedural testing will first examine the highest dose (estradiol 1 mg/progesterone 100 mg) for the co-primary endpoints. If the two p-values for the co-primary endpoints are significant ($P \leq 0.05$), then the hypothesis testing will continue on to the next lower dose (estradiol 0.5 mg/progesterone 100 mg) for each of the co-primary endpoints,

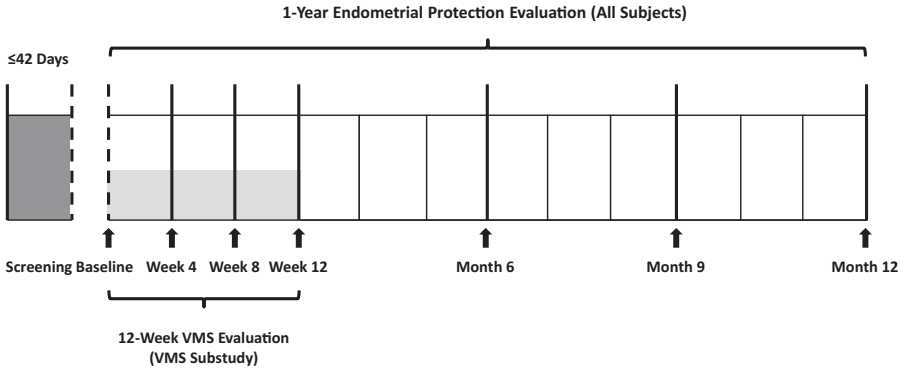

Fig. 1. The REPLENISH Trial Timeline. VMS = vasomotor symptoms.

as described above. The hypothesis testing will be stopped if at any point the testing yields a non-significant result.

3. Review of reported differences between estrogens and progestogens

As discussed above, TX-001HR contains estradiol and progesterone combined in a single capsule. This formulation is expected to offer both efficacy and safety for treating menopausal symptoms in women with a uterus, as suggested by preliminary data on the bioequivalence of the new capsule formulation to separate approved estradiol and approved progesterone products [15]. Published data suggests that this hormone formulation may represent a safer alternative than existing HT regimens. The following review of the literature supports the use of natural estrogen combined with natural progesterone over other combinations of estrogens and synthetic progestins.

3.1. Tolerability of progesterone formulations

Studies have shown that HT containing estrogen plus progesterone is better tolerated than HT containing MPA in terms of spotting/bleeding, and quality of life. In a randomized 9-month study by Ryan and Rosner of women taking CEE plus either micronized progesterone ($n = 89$) or MPA ($n = 93$), the progesterone group experienced fewer days of bleeding (4.3 vs 6.2 days; $P = 0.001$) and less blood flow (0.9 vs 1.4 on a 1–4 scale; $P < 0.001$) than the MPA group [19]. This better bleeding profile observed with progesterone may be related to the effect of progestogens on several angiogenic factors in the glandular endometrium. In vitro studies in Ishikawa (endometrial epithelial) cells demonstrated that progestins, but not progesterone, may alter the balance between angiogenic promoters and inhibitors [20]. These alterations with progestins could induce a unique pro-angiogenic activity in the endometrial capillary plexus, with consequent aberrant vasculogenesis, which may result in irregular endometrial bleeding [20].

In a cross-sectional study of 176 women who had previously switched from HT containing MPA to HT containing micronized progesterone, 71% had switched because of the better side effect profile, 35% because they believed the long-term risks would be fewer, and 23% because of intolerance to MPA [21]. When evaluated at 1 to 6 months after switching, the women experienced significantly better quality of life, including less depression and anxiety, than with MPA (both $P < 0.001$) [21]. Patient satisfaction questionnaires also indicated that women preferred micronized progesterone over their previous regimen for better symptom control and fewer adverse effects ($P < 0.001$) [21]. In the study by Ryan and Rosner of CEE with either progesterone or MPA, results on the Women's Health Questionnaire showed a significant group-by-visit interaction indicating better quality of life in the progesterone group in the cognitive difficulties domain ($P = 0.015$) [19].

Sleep was significantly improved after 6 months of CEE plus micronized progesterone but not with CEE plus MPA in a randomized study of 21 postmenopausal women tested in a sleep laboratory [22]. Specifically, the progesterone group (but not MPA) had significant improvements in sleep efficiency due to decreases in time spent awake, although subjective ratings did not differ between groups [22]. In addition, it should be acknowledged that progesterone can induce sleepiness when given in high doses [23–25].

3.2. Comparison of progestogen effects on the breast

The impact of HT on the breast is a significant concern. While both CEE and estradiol stimulate breast cancer cell proliferation [26], it is the progestogen component that likely has the greatest influence on breast cancer risk with HT. In follow-up studies of the WHI trial, CEE alone reduced the risk of breast cancer (hazard ratio [HR] 0.77; 95% CI, 0.62–0.95) [27], whereas CEE plus MPA increased the risk of breast cancer [3].

The type of progestogen can also influence the incidence of breast cancer. Observational studies have reported that oral estrogens plus micronized progesterone has less effect on increasing breast cancer risk than oral estrogens with various synthetic progestins (Table 3) [28–30]. A more detailed analysis of the E3N study showed estrogens plus dydrogesterone significantly increased lobular breast cancer and that estrogens plus other progestins significantly increased ductal, lobular, pure lobular and mixed ductal/lobular cancer, but that estrogens plus progesterone did not increase any of these breast cancer subtypes [31].

In addition, differences in mammographic breast density and abnormalities have been reported between progestogens. Mammographic breast density and breast cancer cell proliferation significantly increased in studies of postmenopausal women receiving CEE/MPA but these parameters did not increase with administration of transdermal estradiol with oral micronized progesterone [32,33]. The progestin drospirenone (DRSP) has been shown to significantly increase breast density when used in combination with estrogen in perimenopausal women [34].

In vitro and *in vivo* studies have shown that MPA alone or with estrogens (estradiol or CEE) stimulates proliferation, while progesterone showed a lesser effect on proliferation [35–40]. Studies in postmenopausal monkeys randomized to estradiol plus MPA or micronized progesterone found greater increases in proliferation with MPA than with progesterone, including lobular proliferation (194% versus 58%) and ductal proliferation (544% versus 75%), as

Table 3
Breast cancer risk with hormone therapy by type of Progestogen in observational studies.

Study (duration of HT)	Estrogen + progesterone risk estimate (95% CI)	Estrogen + synthetic progestins risk estimate (95% CI)
Fournier et al. [30]		
Mean duration 2.8 yr*	RR 0.9 (0.7–1.2)	RR 1.4 (1.2–1.7)
<2 yr†	RR 0.9 (0.6–1.4)	RR 1.6 (1.3–2.0)
2–4 yr†	RR 0.7 (0.4–1.2)	RR 1.4 (1.0–1.8)
≥4 yr†	RR 1.2 (0.7–2.0)	RR 1.2 (0.8–1.7)
Fournier et al. [31]		
<2 yr	RR 0.71 (0.44–1.14)	RR 1.36 (1.07–1.72)
2–4 yr	RR 0.95 (0.67–1.36)	RR 1.59 (1.30–1.94)
4–6 yr	RR 1.26 (0.87–1.82)	RR 1.79 (1.44–2.23)
≥6 yr	RR 1.22 (0.89–1.67)	RR 1.95 (1.62–2.35)
Cordina-Duverger et al. [28]		
Any use	OR 0.80 (0.44–1.43)	OR 1.72 (1.11–2.65) (any synthetic) OR 1.57 (0.99–2.49) (P4 derivatives)
<4 yr	OR 0.69 (0.29–1.68)	OR 1.17 (0.48–2.86) (any synthetic) OR 1.02 (0.40–2.58) (P4 derivatives)
≥4 yr	OR 0.79 (0.37–1.71)	OR 2.07 (1.26–3.39) (any synthetic) OR 1.92 (95% CI, 1.13–3.27) (P4 derivatives)

E = estrogen; HT = hormone therapy; P4 = progesterone.
* Transdermal or oral estrogens.
† Transdermal estrogens only; incomplete data reported for oral estrogens.

well as unfavorable gene expression profiles with MPA leading to cellular proliferation [41,42].

A study using breast cancer cells, demonstrated that cell invasive behavior was significantly increased with the addition of MPA, progesterone, nestorone, and DRSP when compared with a control, with MPA having the highest and DRSP having the lowest invasion index [43]. However, when combined with estradiol, invasion indexes were significantly reduced with progesterone, DRSP, and nestorone, but not with MPA when compared with estradiol alone, although the indexes were still significantly higher compared with the control [43]. A study reporting the effects of different progestins on the apoptosis:proliferation ratio of MCF-7 breast cancer cells, demonstrated that MPA, norethisterone acetate (NETA), and dienogest when alone or combined with estradiol stimulated proliferation of the cells, while estradiol combined with dihydrodydrogesterone induced apoptosis [37]. Progesterone alone induced apoptosis in the breast cancer cells but when combined with estradiol no proliferation or apoptosis was observed [37].

A study of ovariectomized mice treated with estradiol combined with various doses of progesterone or MPA, reported that MPA induced proliferative activity in the mammary gland and antiproliferative activity in the uterus at the same dose, whereas progesterone showed antiproliferative uterine activity at doses lower than those required for significant proliferative activity in the mammary gland. These results suggest that there is a safety window between uterine activity and proliferative mammary gland effects for progesterone but not for MPA [39].

3.3. Comparison of progestogen effects on the cardiovascular system

The addition of progesterone to estrogen therapy maintained the favorable impact of estrogen alone on lipid profiles, while the addition of MPA did not. Among 875 women of the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial randomly assigned to HT treatments, those receiving CEE plus micronized progesterone had mean increases in high-density lipoprotein cholesterol (HDL-C) equivalent to that with CEE alone, and both values were significantly higher than with CEE plus MPA given cyclically or continuously [12]. No significant differences among groups were found for low-density lipoprotein cholesterol (LDL-C) or triglycerides [12]. Similarly, in a randomized study of micronized progesterone versus norethisterone acetate (NETA) both administered without estrogen ($n = 40$ each), progesterone had no effect on HDL-C, whereas NETA provoked a significant decrease in HDL-C ($P < 0.001$) [44]. In a study comparing the effects of intranasal estradiol with vaginal micronized progesterone and oral estradiol with DRSP in early postmenopausal women, both combinations lowered total cholesterol, non-HDL cholesterol, and LDL cholesterol [45]. However, estradiol plus DRSP lowered HDL cholesterol, and only estradiol plus progesterone lowered triglycerides [45].

Progesterone and progestins also differ in their effects on the vasculature. Rosano et al. studied 18 women with coronary artery disease in a randomized trial and found a significant increase in treadmill exercise duration until 1-mm ST segment depression among those taking estradiol alone ($P < 0.001$); subsequent addition of intravaginal progesterone further increased exercise time ($P < 0.001$), whereas addition of MPA did not enhance the estrogen benefit [46]. A rabbit model of myocardial ischemia and reperfusion showed that the addition of MPA attenuated the cardioprotective benefits of CEE on infarct size [47]. Progesterone stimulated nitric oxide synthesis and inhibited adhesion of platelets to endothelial cells in rat endothelial cell cultures, whereas MPA inhibited nitric oxide synthesis and increased platelet adhesion [48]. Progesterone and the progestins NETA and chlormadinone acetate (CMA) each produced a relaxation of precontracted rat thoracic aorta, while dienogest (DNG) had no effect [49]. Likewise, progesterone and CMA decreased the contractile response to phenylephrine ($P < 0.05$), but the effects of NETA and DNG were not significant [49]. Finally, progesterone, but not MPA, inhibited the expression of vascular cell adhesion molecule-1 in TNF-α-induced human umbilical vein endothelial cells [50].

When taken with oral or transdermal estrogens, no significant association of venous thromboembolism (VTE) with

Table 4
Venous thromboembolism risk with current hormone therapy use by type of progestogens.

Study	Risk estimate (95% CI)			
	Micronized progesterone	Pregnane derivatives	Norpregnane derivatives	Nortestosterone derivatives
E3N French cohort [51]	HR 0.9 (0.6–1.5)	HR 1.3 (0.9–2.0)	HR 1.8 (1.2–2.7)	HR 1.4 (0.7–2.4)
ESTHER study [52]	OR 0.7 (0.3–1.9)	OR 0.9 (0.4–2.3)	OR 3.9 (1.5–10.0)	NR

CI—confidence intervals; HR—hazards ratios; NR—not reported; OD—odd ratios.

concomitant micronized progesterone, pregnane derivatives, or nortestosterone derivatives was found; however, norpregnane derivatives were associated with an increased VTE risk, in the E3N French cohort (Table 4) [51]. Similar results had been previously reported for the ESTHER study in which micronized progesterone and pregnane derivatives did not increase risk for VTE, while norpregnane derivatives increased VTE risk (Table 4) [52].

3.4. Comparison of progestogen effects on diabetes

In the French E3N study, the incidence of diabetes was significantly lower in women who used HT compared with women who never used HT (HR 0.82, 95% CI, 0.72–0.93) [53]. When different progestogens were analyzed, transdermal estrogens with progesterone (HR 0.67, 95% CI, 0.54–0.84) and oral estrogens with NETA (HR 0.44, 95% CI, 0.26–0.75) or cyproterone acetate (HR 0.44, 95% CI, 0.23–0.85) were the only formulations that significantly lowered diabetes risk (oral estrogens with progesterone could not be analyzed because of too few women in that group) [53].

3.5. Comparison of estrogen effects on cardiovascular system

Recent studies highlight the advantages of estradiol over CEE. Estradiol has been shown to have beneficial effects on the cardiovascular system when taken early in menopause. The Early versus Late Intervention Trial with Estradiol (ELITE) was a double-blinded, placebo-controlled trial of healthy postmenopausal women ($N = 643$) without cardiovascular disease who were randomized by time since menopause (<6 years, n = 271 or >10 years, $n = 372$) to take oral estradiol daily with vaginal progesterone gel 10 days per month [54]. The rate of progression of carotid artery intima media thickness in women <6 years from menopause was significantly lower than that in women who were >10 years from menopause (P-value for interaction = 0.007) [54].

Other studies show differences between estradiol and CEE on cardiovascular parameters. In the Kronos Early Estrogen Prevention Study (KEEPS) 4-year trial of 116 menopausal women randomized to oral CEE or transdermal estradiol, each with micronized progesterone, or placebo, significantly higher triglyceride levels and C-reactive protein were found in the CEE group compared with estradiol (both $P \leq 0.01$), possibly related in part to dose and route of administration [55]. No significant differences were found between estrogen groups for endothelial function as measured by the reactive hyperemia index [55].

Several observational and experimental studies indicate more favorable cardiovascular effects with estradiol than with CEE. In an observational study of oral HT users, CEE was associated with a significantly higher risk of incident venous thrombosis (OR, 2.08; 95% CI, 1.02–4.27), significantly higher activated protein C resistance (OR 1.68; 95% CI, 1.24–2.28), and a nonsignificant elevation in myocardial infarction risk (OR, 1.87; 95% CI, 0.91–3.84) when compared with estradiol use [56].

The hemostatic profile of women taking CEE was shown to be more prothrombotic than that of women using oral estradiol, including significantly higher thrombin generation peak value and decreased total protein S ($P = 0.001$ and $P \leq 0.001$, respectively) [57]. In an oophorectomized pig model, both estradiol and CEE reduced aggregation of platelets, but only estradiol increased platelet secretion of nitric oxide, and platelets from estradiol-treated animals caused relaxation of coronary arteries [58]. On the other hand, in an ovariectomized rat model of inflammation induced after 3 weeks of HT, CEE administration prevented the inflammatory response while estradiol did not have any effects on inflammation [59].

4. Summary and conclusions

The REPLENISH trial is a phase 3, randomized, placebo-controlled study designed to evaluate the safety and efficacy of TX-001HR, which combines solubilized 17β-estradiol plus natural progesterone for the treatment of menopause-related moderate to severe VMS. It is anticipated that the combination of estradiol and progesterone will have a favorable risk-benefit profile. If approved, TX-001HR would become the first FDA/EMA-approved HT that combines 17β-estradiol with progesterone in a single dosage form. Such a regimen could provide a newer, and possibly safer, alternative to existing synthetic HT regimens and unregulated and unapproved CBHT for menopausal women experiencing VMS.

Conflict of interest statement

Dr. Archer has received research funds from AbbVie, Bayer Healthcare, Endoceutics, Merck (previously Schering Plough), TherapeuticsMD, Actavis; consultant fees from AbbVie Laboratories, Agile Therapeutics, Bayer Healthcare, CHEMO, Endoceutics, Pfizer, Shionogi, Teva Women's Healthcare, TherapeuticsMD; honoraria for lecturing from Ascend Therapeutics, Bayer Healthcare, Merck, and Pfizer; and has stock options with Agile Therapeutics. Dr. Mirkin, Ms. Amadio, and Dr. Bernick are employees of TherapeuticsMD. Dr. Pickar was formerly an employee of Wyeth Research; has received consultant fees from Wyeth/Pfizer, Besins Healthcare, Shionogi Inc., Metagenics, and TherapeuticsMD; and has stock options with TherapeuticsMD. TherapeuticsMD sponsored the REPLENISH study and provided support for manuscript preparation to Precise Publications, LLC.

Contributors

Dr. Mirkin, Ms. Amadio, and Dr. Bernick participated in study design, statistical analysis planning, data monitoring, and manuscript preparation and approval. Dr. Pickar participated in study design and manuscript preparation and approval. Dr. Archer participated in study design, patient recruitment and treatment, study site coordination, and manuscript preparation and approval.

Laura Ninger, ELS and Kathleen Ohleth, PhD participated in medical writing support.

Author/contributor	Nature of contribution	Conflict of interests
Sebastian Mirkin, MD	Study design, statistical analysis plan, data monitoring, manuscript preparation and approval	Employee of TherapeuticsMD
Julia M. Amadio	Study design, statistical analysis plan, data monitoring, manuscript preparation and approval	Employee of TherapeuticsMD
Brian A. Bernick, MD	Study design, statistical analysis plan, data monitoring, manuscript preparation and approval	Employee of TherapeuticsMD
James H. Pickar, MD	Study design, manuscript preparation and approval	Formerly an employee of Wyeth Research; has received consultant fees from Wyeth/Pfizer, Besins Healthcare, Shionogi Inc., Metagenics, and TherapeuticsMD; and has stock options with TherapeuticsMD
David F. Archer, MD	Study design, patient recruitment and treatment, study site coordination, manuscript preparation and approval	Has received research funds from AbbVie, Bayer Healthcare, Endoceutics, Merck (previously Schering Plough), TherapeuticsMD, Actavis; consultant fees from AbbVie Laboratories, Agile Therapeutics, Bayer Healthcare, CHEMO, Endoceutics, Pfizer, Shionogi, Teva Women's Healthcare, TherapeuticsMD; honoraria for lecturing from Ascend Therapeutics, Bayer Healthcare, Merck, and Pfizer; and has stock options with Agile Therapeutics. Bayer Healthcare, Merck
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Competing interests

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