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APPLICATION NUMBER:

208564Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	208564
Link to EDR	\\CDSESUB1\evsprod\NDA0208564\208564.enx
Submission Date	November 29, 2017
Submission Type	Resubmission (standard review)
Brand Name	Imvexxy®
Generic Name	Estradiol
Dosage Form and Strength	Vaginal insert; 4 mcg and 10 mcg
Route of Administration	Vaginal
Proposed Indication	Treatment of dyspareunia, a symptom of vulvar and vaginal atrophy, due to menopause
Applicant	Therapeutics MD, Inc.
OCP Review Team	LaiMing Lee, PhD; Doanh Tran, PhD
OCP Final Signatory	Doanh Tran, PhD

1. EXECUTIVE SUMMARY

Estradiol vaginal insert (referred to as TX-004HR in the study reports and in this review) was developed for the treatment of moderate to severe dyspareunia, a symptom of vulvar and vaginal atrophy (VVA) due to menopause. The original NDA was submitted on July 6, 2016 with (b) (4) proposed doses 4, 10, (b) (4) mcg. The NDA included a Phase 2 study and a Phase 3 study TXV13-01 to support efficacy of TX-004HR. The Phase 3 study was 12 weeks in duration and did not include long-term (typically 52 weeks) safety data to assess the potential for endometrial cancer that is associated with estrogen treatment. Substantial evidence of effectiveness was demonstrated by the Phase 3 study as measured by parabasal cells, superficial cells, and vaginal pH assessments, and symptomatic improvement in dyspareunia. On May 5, 2017, the Division of Bone, Reproductive and Urologic Products (DBRUP) issued a Complete Response due to lack of long-term endometrial safety data.

The Clinical Pharmacology portion of the original NDA included two relative bioavailability studies comparing TX-004HR 10 mcg and 25 mcg to Vagifem® estradiol vaginal tablet 10 and 25 mcg in studies ESTR-1K-499-12 and ESTR-1K-500-12, respectively. In addition, a PK-substudy TXV14-01 using TX-004HR 4, 10, and 25 mcg was conducted as part of the phase 3 study. The Clinical Pharmacology review team concluded that the original NDA was acceptable, and included a review of the phase 3 study and the 3 Clinical Pharmacology studies (DARRTS April 7, 2017).

The Applicant is currently seeking approval of two doses 4 and 10 mcg. The proposed dosing instruction states (b) (4) and that the use include the lowest effective dose and for the shortest duration, and dosage adjustment should be guided by clinical response. The current Clinical Pharmacology review focuses on the newly submitted cross-study comparison of systemic estrogen exposure from the proposed vaginal insert TX-004HR, estradiol oral tablets (ANDA 40-275), and Premarin® oral conjugated estrogen tablets (NDA 04-782).

1.1 Recommendations

The Office of Clinical Pharmacology Division of Clinical Pharmacology-3 has reviewed the information contained in current NDA 208564 and recommends approval from a Clinical Pharmacology perspective. The long-term endometrial safety of unopposed (without a progestin) estrogen exposure has not been demonstrated for this product; however, the Applicant has proposed to conduct long-term post-marketing epidemiological study to assess this risk, and the label will include the standard estrogen class labeling.

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

Cross-Study Comparison of Estrogen Exposure

In the Complete Response Letter dated May 5, 2017, DBRUP stated that there was lack of long-term endometrial safety data. The Applicant needed to quantify and characterize the general safety of the drug over a reasonable duration consistent with its intended chronic use. Such extended duration of exposure is needed to adequately characterize the pattern of drug-related adverse reactions over time or to detect adverse reactions that may occur only with a longer duration of treatment.

To address this CR safety issue, the Applicant proposed to rely on published literature via the 505(b)(2) pathway to support long-term safety for the proposed estradiol vaginal inserts 4 and 10 mcg.

The following comparison of systemic exposure is via a cross-study comparison of the estradiol vaginal insert TX-004HR, estradiol oral tablets (ANDA 40-275), and Premarin® oral conjugated estrogen tablets (NDA 04-782). Pharmacokinetic data from approval packages and approved labels were used for the systemic estrogen comparison with sources noted in the table footnotes.

Estradiol

Compared to TX-004HR 10 mcg, systemic estradiol exposure is 6.6-fold higher in AUC0-24, 6.6-fold higher in Cavg, and 5.4-fold higher in Cmax with estradiol oral tablets, 2 mg (Table 1).

Table 1. Estradiol Pharmacokinetics of Estrogen Products

	$AUC_{0\text{-}72hr}$ (pg.hr/mL)	$AUC_{0\text{-}24hr}$ (pg.hr/mL)	Cavg $(0\text{-}72hr)^1$ (pg/mL)	Cavg $(0\text{-}24hr)^2$ (pg/mL)	Cmax (pg/mL)
Estradiol oral tablets, 2 mg[a]	1620.6	914.5[3]	22.5	38.1	59.4
Premarin® oral tablets, 2 x 0.3 mg[b]		na			na
TX-004HR vaginal insert, 10 mcg[c]		138.2		5.8	10.9
TX-004HR vaginal insert, 4 mcg[c]		91.7		3.8	6.5

[a] ANDA 40-275; baseline-adjusted values; $AUC_{0\text{-}72}$, not $AUC_{0\text{-}24}$, was available in the review.

[b] NDA 04-782; Premarin does not include estradiol; therefore, estradiol PK data were not available for comparison

[c] NDA 208564; PK Sub-Study TXV14-01; baseline-unadjusted values on Day 1

[1] calculated from AUC0-72

[2] calculated from AUC0-24

[3] AUC0-24 determined by subtracting AUC24-72 from AUC0-72; AUC24-72 was determined by using PK data from ANDA 40-275 and trapezoidal rule

Unconjugated Estrone

Compared to TX-004HR 10 mcg, systemic unconjugated estrone exposure is 12.3- and 11.6-fold higher in AUC0-24, 12.3- and 11.6-fold higher in Cavg, and 17.5- and 3.5-fold higher in Cmax with estradiol oral tablets, 2 mg and Premarin® 2 x 0.3 mg oral tablets, respectively (Table 2).

Table 2. Unconjugated Estrone Pharmacokinetics of Estrogen Products

	AUC0-72hr (pg.hr/mL)	AUC0-24hr (pg.hr/mL)	Cavg (0-72hr)[1] (pg/mL)	Cavg (0-24hr)[2] (pg/mL)	Cmax (pg/mL)
Estradiol oral tablets, 2 mg[a]	8686.6	5679.0[3]	12.6	236.6	411.0
Premarin® oral tablets, 2 x 0.3 mg[b]		5390		224.6	82
TX-004HR vaginal insert, 10 mcg[c]		462.7		19.3	23.5
TX-004HR vaginal insert, 4 mcg[c]		290.2		12.1	15.8

[a] ANDA 40-275; baseline-adjusted values; AUC0-72, not AUC0-24, was available in the review.

[b] NDA 04-782; baseline-unadjusted; AUC not duration specified

[c] NDA 208564; PK Sub-Study TXV14-01; baseline-unadjusted values on Day 1

[1] calculated from AUC0-72

[2] calculated from AUC0-24

[3] AUC0-24 determined by subtracting AUC24-72 from AUC0-72; AUC24-72 was determined by using PK data from ANDA 40-275 and trapezoidal rule

Conjugated Estrone

The incurred samples reanalysis (ISR) for estrone conjugates in PK sub-study TXV14-01 failed; therefore, comparison of systemic estrone conjugates between the proposed product TX-004HR and estradiol oral tablets and Premarin is not possible.

Table 3. Estrone Conjugates Pharmacokinetics of Estrogen Products

	AUC0-72hr (pg.hr/mL)	AUC0-24hr (pg.hr/mL)	Cavg (0-72hr)[1] (pg/mL)	Cavg (0-24hr)[2] (pg/mL)	Cmax (pg/mL)
Estradiol oral tablets, 2 mg[a]	524.9	404.1[3]	7.3	16.8	51.4

Premarin® oral tablets, 0.625 mg[b]		na			na
TX-004HR vaginal insert, 10 mcg[c]		na		na	na
TX-004HR vaginal insert, 4 mcg[c]		na		na	na

[a] ANDA 40-275; baseline-adjusted values; AUC_{0-72}, not AUC_{0-24}, was available in the review.

[b] NDA 04-782; not available

[c] NDA 208564; PK Sub-Study TXV14-01; baseline-unadjusted values on Day 1; conjugate values not reliable due to failed ISR acceptance criteria

[1] calculated from AUC0-72

[2] calculated from AUC0-24

[3] AUC0-24 determined by subtracting AUC24-72 from AUC0-72; AUC24-72 was determined by using PK data from ANDA 40-275 and trapezoidal rule

Overall Conclusions:

- A review of the analytical methods for each study was not possible and is therefore a limitation of this cross-study review. The fold changes are suggestive of higher estrogen exposure from estradiol oral tablets and Premarin oral conjugated estrogens; however, a direct, quantitative comparison to the proposed estradiol vaginal insert is not feasible due to the cross-study nature of comparison.
- A cross-study comparison showed that systemic estradiol exposure from estradiol oral tablet, 2 mg was 5.4- to 6.6-fold higher compared to TX-0004HR 10 mcg.
- A cross-study comparison showed that systemic estrone and estrone conjugate exposure from estradiol oral tablet, 2 mg and Premarin, 2 x 0.3 mg oral tablets was generally higher compared to TX-0004HR 10 mcg.
- TX-004HR 4 mcg has a lower systemic exposure than TX-004HR 10 mcg; therefore, systemic estrogen exposure from the 4 mcg dose is also lower than estradiol oral tablet, 2 mg and Premarin, 2 x 0.3 mg oral tablets.
- In relative bioavailability study ESTR-1K-499-12, the Applicant evaluated the systemic estrogen exposure of TX-004HR, 10 mcg and Vagifem estradiol vaginal tablet, 10 mcg. The results showed that baseline-unadjusted estradiol exposure was higher (2.0-fold for AUC0-24 and 1.5-fold for Cmax) with Vagifem, 10 mcg, compared to TX-004HR, 10 mcg. Baseline-unadjusted estrone was higher (1.2-fold for AUC0-24 and 1.1-fold for Cmax) with Vagifem 10 mcg, compared to TX-004HR, 10 mcg. For details on the study design and results, see the Clinical Pharmacology review of original NDA 208564 in DARRTS April 7, 2017.

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Office of Clinical Pharmacology Review

NDA Number	208564
Link to EDR	\\CDSESUB1\evsprod\NDA208564\208564.enx
Submission Date	July 7, 2016
Submission Type	Standard review
Brand Name	Pending review by DMEPA
Generic Name	Estradiol
Dosage Form and Strength	Vaginal inserts; 4, 10, (b) (4) mcg estradiol
Route of Administration	Intravaginal
Proposed Indication	Treatment of moderate to severe vulvar and vaginal atrophy (dyspareunia) due to menopause
Applicant	TherapeuticsMD
Associated IND	118,439
OCP Review Team	LaiMing Lee, PhD, Li Li, PhD, Doanh Tran, PhD

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1. EXECUTIVE SUMMARY

Estradiol vaginal insert (the drug product is referred to as TX-004HR in the study reports and in this review) was developed for the treatment of moderate to severe dyspareunia, a symptom of vulvar and vaginal atrophy (VVA) due to menopause. The Applicant conducted a Phase 2 study and a Phase 3 study to support efficacy of TX-004HR. The Phase 3 study was 12 weeks in duration and did not include long-term (typically 52 weeks) safety data to assess the potential for endometrial cancer that is associated with estrogen treatment.

1.1 Recommendations

The Office of Clinical Pharmacology Division of Clinical Pharmacology-3 has reviewed the information contained in NDA 208564 and recommend approval from a Clinical Pharmacology perspective. The long-term endometrial safety of unopposed (without a progestin) estrogen exposure has not been demonstrated for this product. Unopposed estrogen use is associated with endometrial carcinoma and is a factor in the safety determination for any estrogen product indicated for VVA. The key issues with specific recommendations/comments are summarized in the table below:

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Substantial evidence of effectiveness was demonstrated by the Phase 3 study as measured by parabasal cells, superficial cells, and vaginal pH assessments, and symptomatic improvement in dyspareunia.
General dosing instructions	The Applicant is seeking approval of (b) (4) doses – 4, 10, (b) (4) mcg. The proposed dosing instruction does not specify a dose. It states (b) (4) and that the use include lowest effective dose and for the shortest duration. If approved, the starting dose should be clearly specified as 4 mcg. All three proposed doses were evaluated in the Phase 3 study and were shown to be effective; the 4 mcg is the lowest effective dose identified in the study.
Dosing in patient subgroups (intrinsic and extrinsic factors)	None
Labeling	The review team has specific content and formatting change recommendations.
Bridge between the to-be-marketed and clinical trial formulations	The formulation for the clinical and commercial products is the same, but the products differ in the manufacturer. The Clinical product was manufactured by (b) (4). The commercial product will be manufactured by Catalent Pharm Solutions. At the time of NDA filing, the Office of Pharmaceutical

	Quality (OPQ) and Biopharmaceutic review teams stated that the manufacturing at the two different sites are the same, the comparative dissolution data alone is sufficient to support the manufacturing site change, and a bioequivalent study is not required to bridge the clinical and the commercial products. Please refer to review by OPQ for details.

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

TX-004HR refers to estradiol vaginal insert. The product is referred to as a vaginal capsule by the Applicant in the NDA submission. Endogenous estrogens, including estradiol, are produced in women for the development and maintenance of the female reproductive system and secondary characteristics. TX-004HR was developed for the treatment of dyspareunia due to menopause.

The Phase 3 study included PK assessment for a subset of patients on Days 1, 14, and 84 (4 days after last dose of drug or placebo) for assessment of estradiol, estrone, and estrone conjugates. Blood samples were draw at 0, (pre-dose), 2, 4, 6, 10, and 24 hrs post-dose on Days 1 and 14 and single blood sample on Day 84. Data for estrone conjugates are not presented in this review due to assay reliability concerns.

Absorption: Median (CV%) time to peak concentrations of estradiol for 4, 10, and 25 mcg doses was 5.0 (31), 4.6 (17), and 2.7 (76) hrs, respectively, following 14 days of once daily vaginal administration of TX-004HR. Refer to section 3.2 for exposure (Cmax and AUC) data for TX-004HR 4, 10, and 25 mcg. The pharmacokinetic profiles of mean (SD) of baseline-unadjusted estradiol and estrone serum concentrations following TX-004HR 4, 10, and 25 mcg, and placebo administered vaginally once daily for 2 weeks in postmenopausal women are presented in Figures 1 and 2 below.

Figure 1. Mean (SE) Pharmacokinetic Profile of Baseline-Unadjusted Serum Estradiol on Day 14 Following Once Daily Vaginal Administration of TX-004HR 4 mcg (N=17), 10 mcg (N=18), and 25 mcg (N=18), and placebo (N=16) in Postmenopausal Women.

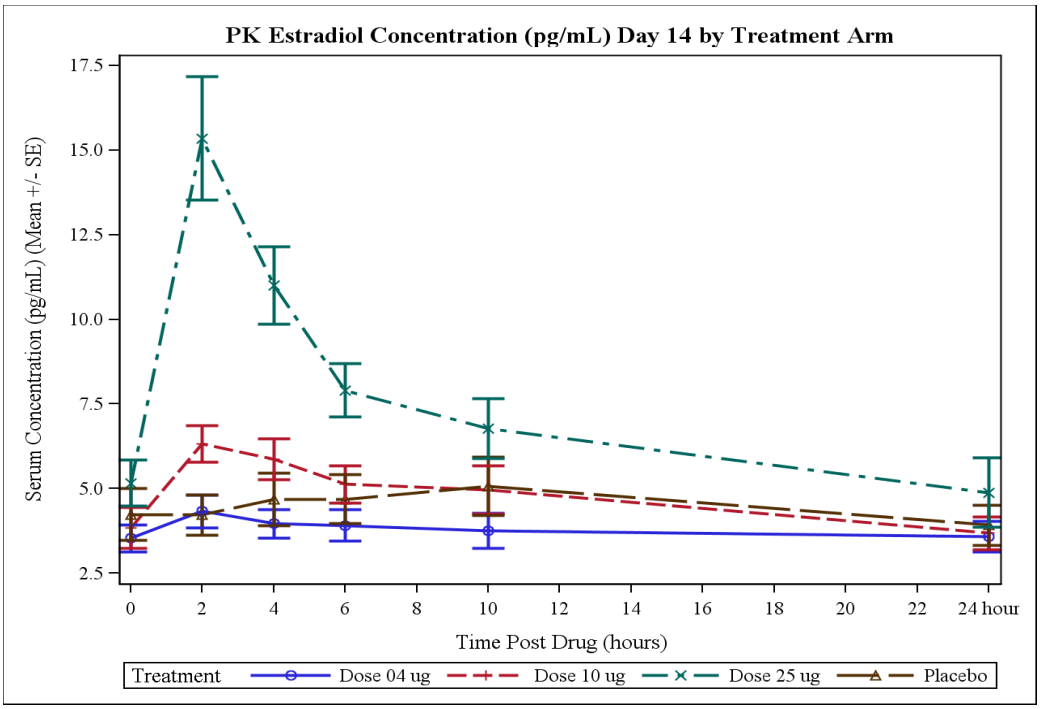
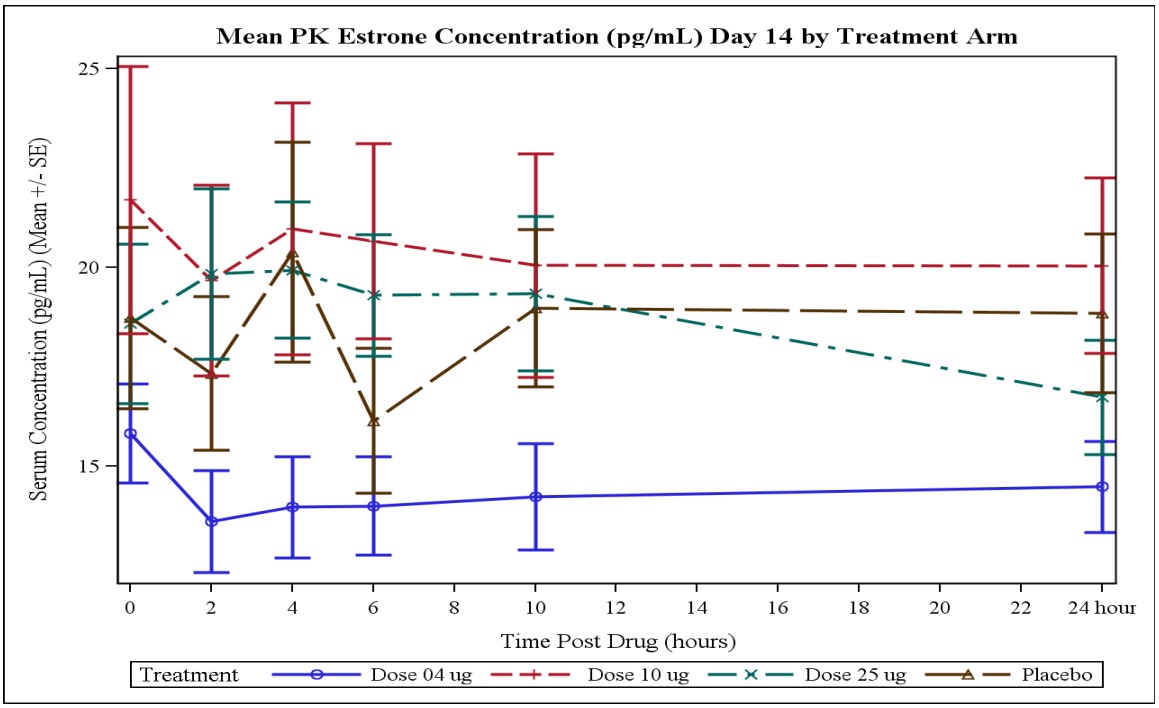


Figure 2. Mean (SE) Pharmacokinetic Profile of Baseline-Unadjusted Serum Estrone on Day 14 Following Once Daily Vaginal Administration of TX-004HR 4 mcg (N=17), 10 mcg (N=18), and 25 mcg (N=18), and placebo (N=16) in Postmenopausal Women.



Distribution: Estradiol circulates in the blood and is highly bound to sex hormone binding globulins (SHBG) and albumin.

Metabolism: In vitro and in vivo studies have shown that estrogens, including estradiol, are subject to metabolism by CYP3A4. Estradiol is reversibly converted to estrone; both can be converted to estriol. Estriol is the major urinary metabolite. Estradiol undergoes conjugation to sulfate and glucuronide conjugates in the liver. In postmenopausal women, majority of endogenous estrogens exist as estrone conjugates.

Excretion: Estradiol, estrone, estriol, and estrogen conjugates (sulfates and glucuronides) are excreted in the urine.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The proposed dose was not clearly specified and states "lowest effective dose." TX-004HR should be administered intravaginally once daily for 14 days, then twice weekly thereafter for maintenance. Generally, therapy should be started at the 4 mcg dose and for the shortest duration consistent with treatment goals and risk. Dose adjustment guided by clinical response is recommended.

2.2.2 Therapeutic individualization

Doses should be adjusted per individual response. No other dose adjustments are recommended.

2.3 Outstanding Issues

None

2.4 Summary of Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling elements be included in the final labeling insert:

- Highlights: Dosage and Administration should be updated to recommend a specific starting dose.
- FPI: Section 2.1, Dosage and Administration should be updated to recommend specific starting dose.
- FPI: Section 12.3, Pharmacokinetics section should include PK profiles of estradiol.
- [redacted] (b) (4)
- FPI: Section 12.3, Pharmacokinetics section should include table with PK parameters for estradiol and estrone on Days 1 and 14 following multiple doses of TX004-HR and placebo.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

TX-004HR, estradiol vaginal insert, was developed for the treatment of dyspareunia due to menopause. Estradiol is a critical estrogen for the development and maintenance of the female reproductive system and secondary characteristics. Estradiol is approved and available in several different formulations including vaginal inserts (Vagifem®), vaginal rings (Femring®), and vaginal cream (Estrace®) for the treatment of VVA in postmenopausal women.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Estradiol is the primary source of estrogens in premenopausal women. Estradiol is converted to estrone and estriol. Estradiol is the most potent endogenous estrogen responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. The primary source of endogenous estrogens is produced in the ovaries of adult women. In postmenopausal women, estrone and estrone sulfate (a conjugate) are the most abundant as estrogen production in the ovaries decline.
Active Moieties	Estradiol (and active metabolite estrone)
General Information	
Maximum Tolerated Dose	25 mcg (highest dose evaluated in the clinical program)
Dose Proportionality	Not established. Baseline-adjusted estradiol systemic exposure (Cmax and AUC_{0-24}) increase was more than dose proportional with 25 mcg dose, relative to 4 and 10 mcg. However, data was highly variable.
Bioanalysis	Gas chromatography/tandem mass spectrometry (GC-MS/MS) methods were used to measure serum and plasma concentration of estradiol, estrone, and estrone conjugates (sulfate, glucuronide). The bioanalytical methods were adequately validated except for the assay for estrone conjugates used in study TXV14-01 PK Sub-Study, where the assay failed the incurred sample reanalysis.
Drug interaction	Estradiol is metabolized by CYP3A4; therefore, inhibitors and inducers of CYP3A4 enzymes may affect estradiol metabolism. No drug interaction studies were conducted with TX-004HR. Class labeling would apply to this product.
Absorption	
Tmax (median)	For estradiol: 4, 4, and 2 hrs for TX-004HR 4, 10, and 25 mcg, respectively. For estrone: 6, 6, and 2 hrs for TX-004HR 4, 10, and 25 mcg, respectively.

Systemic exposure (mean (SD))	PK parameters following 14 daily doses of TX-004HR: Baseline-adjusted serum estradiol Cmax: 1.3 (1.1), 3.0 (1.7), 12.1 (7.3) pg/mL for TX-004HR 4, 10, and 25 mcg, respectively. Baseline-adjusted serum estradiol AUC_{0-24}: 3.3 (16), 5.7 (29), and 84.6 (63) pg*hr /mL for TX-004HR 4, 10, and 25 mcg, respectively. Baseline-adjusted serum estrone Cmax: 0.7 (3.5), 3.7 (8.8), and 5.6 (4.8) pg/mL for TX-004HR 4, 10, and 25 mcg, respectively. Baseline-adjusted serum estrone AUC_{0-24}: NA*, NA*, and 27.0 (115) pg*hr /mL for TX-004HR 4, 10, and 25 mcg, respectively.
Distribution	
Protein binding	Estradiol is highly bound to sex hormone binding globulin (SHBG) and albumin.
Elimination	
Metabolism	Estradiol is metabolized by CYP1A2, 3A4, and 2C9 via hydroxylation. It is converted to estrone.
Excretion	Estradiol and estrone is then conjugated to sulfate and glucuronides before urinary excretion.

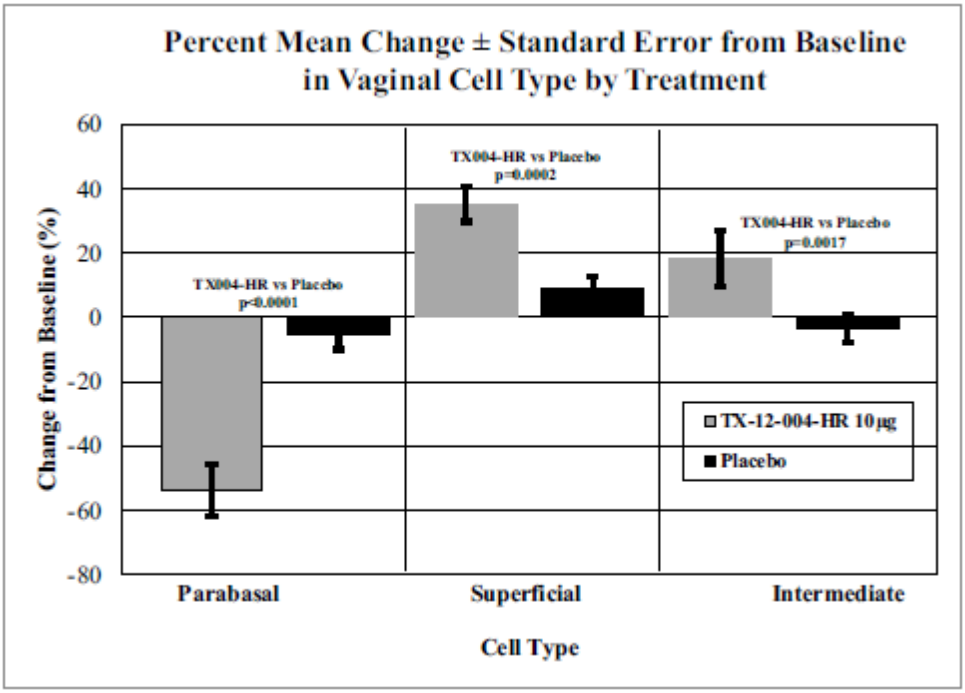
NA*- calculated values were negative.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

Phase 2 study TXV13-01, a pilot safety and efficacy study, provided the supportive evidence for the proposed dose and dosing regimen. It was a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of estradiol vaginal insert 10 mcg in treating postmenopausal women with moderate to severe VVA following 14 days of once daily treatment. Summary of efficacy results are shown in Figure 3.

Figure 3. Mean (SE) percent change from baseline in vaginal cells.



Source: Sponsor's study report TXV13-01 figure 3.

The Applicant demonstrated statistically significant efficacy in the following three critical endpoints (% change from baseline to Day 15) following 14 days of once daily vaginal administration of TX-0040HR 10 mcg, compared to placebo:

- Decrease in parabasal cells (54% vs. 5%; p-value < 0.0001)
- Increase in superficial cells (35% vs. 9%; p-value = 0.0002)
- Decrease in vaginal pH (0.97 pH unit vs. 0.34 pH unit; p-value = 0.0002)

Though there was no statistically significant improvement in clinically relevant most bothersome symptom (MBS), there was a numerical difference (~0.18 to 0.3 units) in the reduction of severity symptoms of vaginal dryness, irritation, and dyspareunia with 14 days treatment of TX-004HR 10 mcg, compared to placebo. The lack of statistically significant symptomatic changes is possible considering the short duration of therapy.

Based upon the efficacy results (objective cellular and pH data) from the pilot study TXV13-01 with TX-004HR 10 mcg, the Applicant decided to carry forward the 10 dose into Phase 3.

Based on the PK results from a relative bioavailability study (ESTR-1K-500-12) showing that Cmax and AUC of estradiol and estrone from TX-004HR 25 mcg was lower than the approved Vagifem 25 mcg, the Applicant included their higher 25 mcg dose into the Phase 3 study. The Applicant also incorporated a lower dose (4 mcg) in the Phase 3 study to identify a lower effective dose and to minimize systemic estrogen exposure. The Applicant selected the 4 mcg

dose based on the existing dose proportionality with the 10 and 25 mcg doses evaluated in the comparative bioavailability studies.

Phase 3 study TXV14-01 provided the pivotal evidence to support efficacy for the once daily for 14 days, then twice weekly thereafter vaginal administration of estradiol vaginal insert 4, 10, and 25 mcg. The primary efficacy endpoints were (1) mean change from baseline to Week 12 in percentage of vaginal superficial cells compared to placebo, (2) mean change from baseline to Week 12 in percentage of parabasal cells compared to placebo, (3) mean change in vaginal pH compared to placebo, and (4) mean change in the severity of the most bothersome symptom (dyspareunia) associated with VVA compared to placebo. Refer to section 3.3.2 for a summary of the efficacy results.

The primary endpoints assessed by the Applicant is consistent with those described in the *FDA's Draft Guidance for Industry: Estrogen and Estrogen/Progestin Drug Products to Treat Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms – Recommendations for Clinical Evaluation* (January 2003).

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen is appropriate for the patient population of postmenopausal women with VVA.

Efficacy

The proposed dosing regimen was assessed in Phase 3 study TXV14-01. The applicant evaluated the effect of TX-004HR vaginal insert 4, 10, 25 mcg and placebo in a 12-week, randomized, double-blind, placebo-controlled, multi-center study in postmenopausal women with moderate to severe dyspareunia (pain with sexual activity), a VVA symptom, due to menopause. The four co-primary endpoints were change from baseline to Week 12 in the following variables: (1) percentage of vaginal parabasal cells, (2) percentage of vaginal superficial cells, (3) vaginal pH, and (4) severity of most bothersome symptom of dyspareunia.

The percentage of parabasal cells decreased by 41%, 44%, 46% and 6% following 12 weeks of treatment with TX004-HR 4, 10, and 25 mcg, and placebo. Mean change from baseline to Week 12 in parabasal cells was statistically significant different for TX004-HR 4, 10, and 25 mcg, compared to placebo (Table 3.3.2.1).

Table 3.3.2-1. Primary Endpoint – **Percent of Parabasal Cells** (MITT)

	TX-004HR 4 µg	TX-004HR 10 µg	TX-004HR 25 µg	Placebo
Baseline (n)	186	188	186	187
Mean (SD)	52.3 (39.2)	51.3 (38.0)	53.5 (38.3)	52.0 (39.2)
Week 12 (n)	170	171	174	172
Mean (SD)	12.0 (22.3)	7.8 (18.5)	6.6 (16.6)	45.2 (40.3)
Change from Baseline				
Mean (SD)	-41.1 (41.6)	-43.8 (37.8)	-46.2 (40.0)	-6.3 (29.8)
LS Mean (SE)	-40.6 (1.75)	-44.1 (1.75)	-45.5 (1.74)	-6.7 (1.75)
Diff. from Placebo (95% CI)	-33.90 (-38.76, -29.04)	-37.34 (-42.19, -32.48)	-38.82 (-43.67, -33.97)	
MMRM P-value vs placebo	<0.0001	<0.0001	<0.0001	

MITT – modified intent-to-treat; SD – standard deviation; Min – minimum; Max- maximum; LS – least square; SE – standard error; MMRM - Mixed Model Repeated Measures
Source: Table 24 in Clinical Report and Biostat Reviewer's analysis.

The percentage of superficial cells increased by 18%, 17%, 24% and 6% following 12 weeks of treatment with TX004-HR 4, 10, and 25 mcg, and placebo. Mean change from baseline to Week 12 in superficial cells was statistically significant different for TX004-HR 4, 10, and 25 mcg, compared to placebo (Table 3.3.2.2).

Table 3.3.2-2. Primary Endpoint – **Percent of Superficial Cells** (MITT)

	TX-004HR 4 µg	TX-004HR 10 µg	TX-004HR 25 µg	Placebo
Baseline (n)	186	188	186	187
Mean (SD)	1.3 (1.24)	1.2 (1.23)	1.3 (1.16)	1.3 (1.31)
Week 12 (n)	170	171	174	172
Mean (SD)	18.7 (19.54)	18.5 (19.95)	24.9 (24.23)	7.0 (14.70)
Change from Baseline				
Mean (SD)	17.5 (19.33)	17.3 (19.76)	23.6 (24.17)	5.7 (14.27)
LS Mean (SE)	17.50 (1.54)	16.72 (1.54)	23.20 (1.53)	5.63 (1.54)
Diff. from Placebo (95% CI)	11.87 (7.60, 16.14)	11.09 (6.82, 15.36)	17.57 (13.32, 21.82)	
MMRM P-value vs placebo	<0.0001	<0.0001	<0.0001	

MITT – modified intent-to-treat; SD – standard deviation; Min – minimum; Max- maximum; LS – least square; SE – standard error; MMRM - Mixed Model Repeated Measures
Source: Table 25 in Clinical Report and Biostat Reviewer's analysis.

Vaginal pH decreased by 1.3, 1.4, 1.4, and 0.3 pH units following 12 weeks of treatment with TX004-HR 4, 10, and 25 mcg, and placebo. Mean change from baseline to Week 12 in vaginal pH was statistically significant different for TX004-HR 4, 10, and 25 mcg, compared to placebo (Table 3.3.2.3).

Table 3.3.2-3. Primary Endpoint –**Vaginal pH** (MITT)

	TX-004HR 4 µg	TX-004HR 10 µg	TX-004HR 25 µg	Placebo
Baseline (n)	186	188	186	187
Mean (SD)	6.34 (0.87)	6.27 (0.83)	6.33 (0.91)	6.33 (1.04)
Week 12 (n)	170	171	174	174
Mean (SD)	5.03 (0.96)	4.86 (0.74)	4.98 (0.87)	6.07 (1.37)
Change from Baseline (n)				
Mean (SD)	-1.33 (1.111)	-1.41 (1.027)	-1.37 (1.143)	-0.26 (1.048)
LS Mean (SE)	-1.32 (0.07)	-1.42 (0.07)	-1.34 (0.07)	-0.28 (0.07)
Diff. from Placebo (95% CI)	-1.03 (-1.22, -0.85)	-1.14 (-1.32, -0.95)	-1.06 (-1.24, -0.88)	
MMRM P-value vs placebo	<0.0001	<0.0001	<0.0001	

MITT – modified intent-to-treat; SD – standard deviation; Min – minimum; Max- maximum; LS – least square; SE – standard error; MMRM - Mixed Model Repeated Measures
Source: Table 26 in Clinical Report and Biostat Reviewer's analysis

MBS of dyspareunia decreased by 1.5, 1.6, 1.7, and 1.3 units following 12 weeks of treatment with TX004-HR 4, 10, and 25 mcg, and placebo. Mean change from baseline to Week 12 in vaginal pH was statistically significant different for TX004-HR 4, 10, and 25 mcg, compared to placebo (Table 3.3.2.4).

Table 3.3.2-4. Primary Endpoint – Severity of MBS of **Dyspareunia** (MITT, LOCF)

	TX-004HR 4 µg	TX-004HR 10 µg	TX-004HR 25 µg	Placebo
Baseline (n)	186	188	186	187
Mean (SD)	2.7 (0.48)	2.6 (0.48)	2.7 (0.44)	2.7 (0.46)
Week 12 (n)	178	176	174	183
Mean (SD)	1.18 (0.97)	1.03 (0.95)	1.03 (0.97)	1.45 (1.03)
Change from Baseline				
LS Mean (s.e.)	-1.50 (0.07)	-1.64 (0.07)	-1.67 (0.07)	-1.25 (0.07)
Diff. from Placebo (95% CI)	-0.25 (-0.44, -0.05)	-0.38 (-0.58, -0.19)	-0.42 (-0.62, -0.22)	
GLM P-value vs placebo	0.0156	0.0002	<0.0001	

[1] Difference from placebo = TX-004HR (Week 12 mean – baseline mean) – Placebo (Week 12 mean – baseline mean)
[2] ANCOVA: Treatment as the main factor and baseline value as the covariate.
Source: Table 14.2.1.4X in Clinical Information Amendment and Reviewer's analysis.

Due to patients in all groups with either no reported sexual activity at Week 12 (N=68, 9.1% of women) or missing data at Week 12 (N=52, 7.0%), the Biostat reviewer requested that the sponsor conduct a sensitivity analysis to evaluate severity of dyspareunia using Mantel-Haenszel test. According to the Biostat review, for analysis using all available data for patients with at least one post-baseline measurement LOCF, a statistically significant reduction in the severity of dyspareunia, change from Baseline to Week 12, was found only in two higher (10 and 25 mcg) doses of TX-004HR compared to placebo. Based on the sensitivity analysis, the 4 mcg dose was not statistically significant, compared to placebo with a p-value of 0.0501 (Table 3.3.2-5).

Table 3.3.2-5 MBS of Dyspareunia: Change in Severity from Baseline to Week 12 (MITT Population)

Change in Severity	TX-004HR 4 µg	TX-004HR 10 µg	TX-004HR 25 µg	Placebo
MITT Population	178	176	174	183
3 less pain	34 (18.3)	35 (18.6)	34 (18.3)	26 (13.9)
2 less pain	54 (29.0)	61 (32.4)	76 (40.9)	49 (26.2)
1 less pain	58 (31.2)	56 (29.8)	41 (22.0)	58 (31.0)
0 no change	28 (15.1)	24 (12.8)	23 (12.4)	48 (25.7)
1 more pain	4 (2.2)	0 (0)	0 (0)	2 (1.1)
P-Value LOCF[2]	0.0501	0.0014	0.0001	

Source: Table 14.2.1.4X in Clinical Information Amendment and Biostat Review Table 8

Safety

Phase 1 study ESTR-1K-499-12 compared single dose bioavailability of TX-004HR 10 mcg (Test) developed by TherapeuticsMD to Vagifem® (estradiol vaginal inserts) 10 mcg (Reference) in healthy postmenopausal women. Estradiol Cmax and AUC from TX-004HR 10 mcg were lower, compared to Vagifem 10 mcg. For baseline-unadjusted estradiol plasma concentrations, the Test/Reference ratio for Cmax was 67% (90% CI: 58% to 77%) and for AUC was 51% (45% to 58%). For baseline-adjusted estradiol, the T/R ratios and 90% confidence intervals for Cmax and AUC were 72% (57% to 90%) and 38% (29% to 49%), respectively. Tmax was earlier by up to 4 hrs for TX-004HR, compared to Vagifem.

Phase 1 study ESTR-1K-500-12 compared bioavailability of TX-004HR 25 mcg (Test) to Vagifem® (estradiol vaginal inserts) 25 mcg (Reference) in healthy postmenopausal women. For baseline-unadjusted estradiol plasma concentrations, the Test/Reference ratio (T/R) for Cmax was 56% (47% to 66%; 90% CI) and for AUC was 40% (34% to 47%). For baseline-adjusted estradiol, the T/R ratios and 90% confidence intervals for Cmax and AUC were 54% (44% to 66%) and 31% (24% to 39%), respectively. Tmax was earlier by 2 hrs for TX-004HR, compared to Vagifem.

Based upon the two relative bioavailability studies ESTR-1K-499-12 and ESTR-1K-500-12, estradiol and estrone systemic exposures in postmenopausal women were lower with TX004-HR 10 and 25 mcg, compared to Vagifem 10 and 25 mcg, respectively.

Phase 3 study showed that drug-related adverse events occurring in >3% of any treatment arm in the safety population were headache, vaginal discharge, and vulvovaginal pruritus. These AES were similar for all three treatment groups and placebo. For headache, 3.7%, 2.6%, 1.6%, and 3.1% of patients experienced headache in the 4 mcg, 10 mcg, and 25 mcg TX-004 HR, and placebo groups, respectively. For vaginal discharge, 2.6%, 3.1%, 2.1%, and 6.3% of patients experienced vaginal discharge in the 4 mcg, 10 mcg, and 25 mcg TX-004 HR, and placebo groups, respectively. For vulvovaginal pruritus, 1%, 1.6%, 2.6%, and 4.2% of patients

experienced vulvovaginal pruritus in the 4 mcg, 10 mcg, and 25 mcg TX-004 HR, and placebo groups, respectively.

Serious adverse events for the safety population are summarized in the following table.

System Organ Class Preferred Term	TX-004HR 4 µg (N=191)	TX-004HR 10 µg (N=191)	TX-004HR 25 µg (N=190)	Placebo (N=192)	Total Subjects (N=764)
Subjects with at least one SAE	0 (0)	3 (1.6)	4 (2.1)	1 (0.5)	8 (1)
Cardiac disorders					
Atrial fibrillation[a]	0 (0)	0 (0)	1 (0.5)	0 (0)	1 (0.1)
Sinus node dysfunction	0 (0)	1 (0.5)	0 (0)	0 (0)	1 (0.1)
Gastrointestinal disorders					
Appendicitis	0 (0)	0 (0)	1 (0.5)	0 (0)	1 (0.1)
Infections and infestations					
Endophthalmitis	0 (0)	0 (0)	1 (0.5)	0 (0)	1 (0.1)
Injury, poisoning and procedural complications					
Ankle fracture	0 (0)	1 (0.5)	0 (0)	0 (0)	1 (0.1)
Musculoskeletal and connective tissue disorders					
Arthralgia	0 (0)	1 (0.5)	0 (0)	0 (0)	1 (0.1)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)					
Malignant melanoma	0 (0)	1 (0.5)	0 (0)	0 (0)	1 (0.1)
Nervous system disorders					
Cervical myelopathy	0 (0)	0 (0)	0 (0)	1 (0.5)	1 (0.1)
Respiratory, thoracic and mediastinal disorders					
Chronic obstructive pulmonary disease	0 (0)	0 (0)	1 (0.5)	0 (0)	1 (0.1)

Source: Sponsor's study report TXV14-01 table 78.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

No. The proposed target population is postmenopausal women with moderate to severe dyspareunia, a symptom of VVA, due to menopause, which is the same as the population enrolled in the Phase 3 studies. There are no intrinsic factors that would alter the proposed dosing regimen.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

There is no potential for drug-food interaction as the drug product is proposed for vaginal administration. However, there is a potential for local drug-drug interaction (DDI) in the vaginal space (e.g., the presence of active/inactive ingredients from a second vaginally applied product may alter the release and absorption potential of estradiol). The Applicant did not conduct drug interaction studies with commonly use vaginal products such as antifungals. This type of assessment is important in determining if an interaction between estradiol/inactive ingredients from TX-004HR and active/inactive ingredients from other vaginal products within the vaginal space can affect systemic estrogen exposure and potentially impacting safety. The sponsor was not requested to address DDI during the IND phase, but was requested to address this potential interaction in the 74-day filing letter.

On October 10, 2016, the Applicant stated that they acknowledge concomitant use of TX-004HR and vaginal antifungals can increase or decrease estrogen exposure. The Applicant believes their draft label is consistent with other estrogen products (class labeling) with higher systemic exposure and includes ketoconazole and itraconazole (CYP3A4 inhibitors) that may increase estrogen exposure. The rationale provided by the Applicant addresses the potential metabolic interaction between estradiol and CYP3A4 inhibitors.

The Applicant did not address how physical interactions with excipients from TX-004HR vaginal insert and other vaginal products can affect estrogen bioavailability. From a safety perspective, the proposed product is an immediate release product that has demonstrated low systemic exposure and clearance within 24 hrs; therefore a PMR will not be requested.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

Estradiol, estrone and estrone conjugate concentrations for TXV14-01 PK Sub-Study were determined in serum using two validated gas chromatography /tandem mass spectrometry (GC/MS/MS) methods at [redacted] (b) (4) The details of analytical methods are described in Table 4.1-1. For method validation results of other Phase 1 PK studies, refer to the individual study reports.

Table 4.1-1: Overview of Bioanalytical Method for TXV14-01 PK Sub-Study

Analyte(s) of interest	**Estradiol (E2) & Estrone(E1)**	**Estrone Conjugates**
Method No.	TM.1381	TM.1472
Methodology	GC-MS/MS	GC-MS/MS
Biological matrix	Serum	Serum
Extraction method	Solid phase	Solid phase separation of conjugates and hydrolysis to estrone
Calibration curve range	E2: 2.00-500 pg/mL E1: 5.00-1000 pg/mL	25.00-5000 pg/mL
Internal standard	d4-estradiol & d4-estrone	d4-estrone sulfate
Bioanalytical Report No.	8831.082015	8831.082015
Validation Report No.	8267.063014	8752.111514
Inter-run accuracy for each QC	E2: LQC (6.52 pg/mL): -3.37% bias MQC (51.1 pg/mL): 0.00% bias HQC (441 pg/mL): 1.36% bias E1: LQC (17.1 pg/mL): 3.51% bias MQC (120 pg/mL): 2.50% bias HQC (865 pg/mL): 4.62% bias	LQC (50.0 pg/mL): -3.60% bias MQC (625 pg/mL): -1.76% bias HQC (4000 pg/mL): 1.50% bias
Inter-run precision for each QC	E2: LQC (6.52 pg/mL): 8.46% CV MQC (51.1 pg/mL): 3.51% CV HQC (441 pg/mL): 4.92% CV E1: LQC (17.1 pg/mL): 6.78% CV MQC (120 pg/mL): 5.37% CV HQC (865 pg/mL): 6.82% CV	LQC (50.0 pg/mL): 5.31% CV MQC (625 pg/mL): 2.59% CV HQC (4000 pg/mL): 4.63% CV
Long-term stability	368 days at -20°C & -80°C	512 days at -20°C & -80°C
Freeze-thaw stability	Demonstrated for 2 cycles for E2 and 5 cycles for E1 at -20ºC	Demonstrated for 4 cycles at -20ºC
Incurred sample reanalysis (ISR)	E2: 83.8% passed E1: 82.1% passed % analyzed: ~12%	33.7% passed % analyzed: ~12%

CV = LQC = low quality control, MQC = mid quality control, HQC = high quality control

Reviewer's Comments:

- Time from first sample drawn to last sample analyzed including ISR was 428 days. Therefore, samples were analyzed within the demonstrated long term stability time.
- It should be noted that the 3 Phase 1 PK studies included in the submission determined the concentrations of estrone sulfate, the largest component of potential estrone conjugates. TXV14-01 PK sub-study quantified the concentrations of total conjugated estrone which includes both glucuronides and sulfates.
- The ISR for estrone conjugates failed as only 33.7% meeting the acceptance criteria. The Applicant initiated an investigation to determine the cause of the ISR failure. In the March 8, 2017 response, the Applicant noted that the root cause of ISR failure for estrone conjugates could not be determined. Given the concern of problematic analytical method for estrogen conjugates, the accuracy of estrogen conjugate PK measurement is in doubt. Together with the unreliable analytical method and small contribution to the total estrogens, the Clinical Pharmacology review team (b) (4)

4.2 Individual Study Reports

- **NOTE:** The Applicant refers to their drug product as a vaginal capsule. For the purpose of this NDA review and to be consistent with current nomenclature for similar vaginal products, TX-004HR was referred to as a vaginal insert in all Individual Study Reports. Reference to vaginal capsules is made only in the Applicant's study titles.

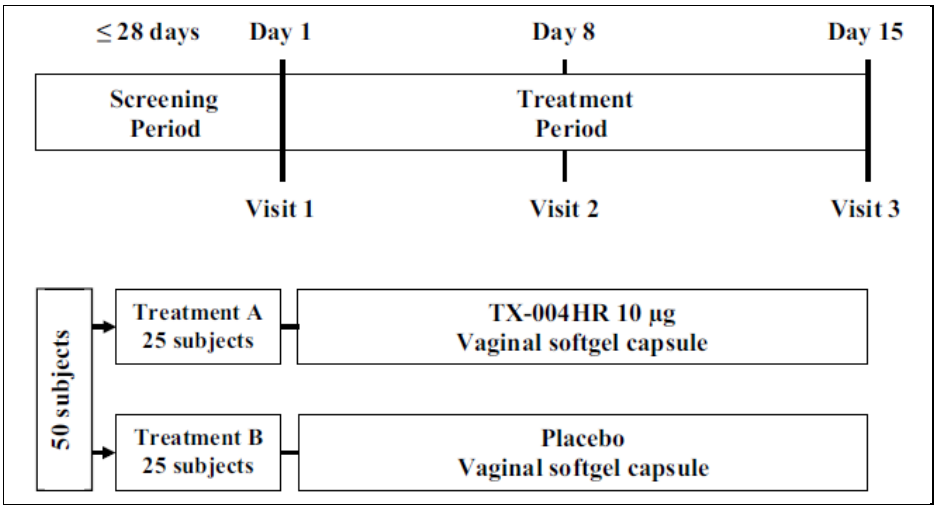
Study TXV13-01

Title: A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of TX-12-004-HR in Postmenopausal Women with Symptoms of Vulvar and Vaginal Atrophy

Objective: To evaluate the efficacy and safety of TX-004HR (estradiol vaginal insert) 10 mcg in treating moderate to severe symptoms of VVA after 14 days of treatment, and to estimate the effect size and variability of VVA endpoints.

Methods: Postmenopausal women with mean age of 62 yrs (range: 46 to 75 yrs) and mean BMI 26.8 kg/m^2 (range: 19 to 33) were randomized to one of two treatment groups: TX-004HR 10 mcg or placebo. Subjects with at least one most bothersome symptom of moderate to severe VVA, including vaginal dryness, vaginal and/or vulvar irritation/itching, pain with urination (dysuria), vaginal pain associated with sexual activity (dyspareunia) were eligible to enroll in the study. Patients self-administered the assigned product once daily, intravaginally, at approximately the same time every morning for 14 days. Fifty patients were enrolled; 48 completed the study (24 patients in TX-004HR and 24 patients in placebo). One patient discontinued from the study due to AEs (vulvovaginal discomfort and paraesthesia (tingling, tickling, pricking, numbness or burning of skin).

Study Design



Source: sponsor's figure 1, study report.

Clinical evaluations were performed at the following time points:
• Screening Period, (up to -28 days)
• Visit 1, Randomization (Day 1)
• Visit 2, Interim (Day 8)

• Visit 3, End of the treatment (Day 15)

Blood Sampling

Blood samples were collected for determination of estradiol concentrations at 0, 1.0, 3.0 and 6.0 hrs post-dosing on Day 1, Day 8 (before morning dose), and Day 15 (end of treatment, more than 24 hrs after final dose). Plasma estradiol concentrations were determined using UPLC-MS/MS method (NSF Pharmalytica method # C000-MET-213) with a quantitation range of 2 to 100 pg/mL.

Study Period: July 31, 2013 – August 12, 2015 (last patient enrolled September 5, 2013)

Study Center: Avail Clinical Research, Deland, FL

Drug Products:

Treatment A: TX-004HR 10 mcg, lot #PN0089-02
(Manufactured by (b) (4); provided by TherapeuticsMD)

Treatment B: Placebo vaginal insert; lot #PN0089-03
(Manufactured by (b) (4); provided by TherapeuticsMD)

Inclusion Criteria (major):

1. Postmenopausal woman between 40 and 75 yrs of age (at the time of Randomization)
2. Postmenopausal was defined as with at least 12 months of spontaneous amenorrhea or 12 months post bilateral oophorectomy, with or without hysterectomy (documented by an operative report or patient reported). Women $\geq$ 60 yrs of age who had a hysterectomy without bilateral oophorectomy prior to natural menopause were considered menopausal
3. Met all evaluation requirements:
   - $\leq$ 5% superficial cells on vaginal smear cytology
   - Vaginal pH > 5.0
   - Estradiol $\leq$ 50 pg/mL
   - At least one self-assessed moderate to severe symptom of VVA from the following list that was identified by the subject as being most bothersome to her:
     - Vaginal dryness
     - Vaginal pain associated with sexual activity
     - Vaginal and/or vulvar irritation/itching
     - Dysuria
     - Vaginal bleeding associated with sexual activity (absence or presence)
4. BMI less than or equal to 34 kg/m^2
5. Willing to abstain from using products (other than study medication) that contain estrogen throughout study participation

6. Generally good health based on a pre-study medical evaluation performed within 28 days prior to the initial dose of study medication. The medical evaluation findings had to include:
   a. a normal or non-clinically significant physical examination, including vital signs (sitting blood pressure, heart rate, respiratory rate and temperature)
   b. a normal or non-clinically significant pelvic examination
   c. a mammogram showing no sign of significant disease (performed within previous nine months prior to initial dose of study medication). An acceptable mammogram was defined as a mammogram in which no masses or other findings were identified that raised suspicions of malignancy. The site obtained a copy of the official report for the subject's study file and verified that the mammogram itself was available if needed for additional assessment
   d. a normal or non-clinically significant clinical breast examination. An acceptable breast examination was defined as no masses or other findings identified that raised suspicions of malignancy
   e. a normal Pap smear at Screening (ASC-US with high risk – HPV negative is acceptable)
   f. within normal limits or non-clinically significant laboratory evaluation results
   g. sitting SBP <140 mmHg and DBP <90 mmHg at Screening. Patients were permitted to take up to two antihypertensive medications.
7. Willing to abstain from sexual activity and use of vaginal douching within 24 hours prior to Screening and Visit 3 vaginal pH measurements

Exclusion Criteria (major):

1. Current hospitalization.
2. A history of thrombosis of deep veins or arteries or a thromboembolic disorder.
3. A history of coronary artery or cerebrovascular disease.
4. A history of liver or kidney dysfunction/disorder.
5. A history of gallbladder dysfunction/disorders (e.g., cholangitis, cholecystitis), unless gallbladder had been removed.
6. A history of diabetes, thyroid disease (subjects with diet-controlled diabetes, or controlled hypothyroid disease at Screening were not excluded), or any other endocrinological disease.
7. A history of estrogen-dependent neoplasia.
8. A history of atypical ductal hyperplasia of the breast.
9. A history of undiagnosed vaginal bleeding.
10. A vaginal infection requiring treatment
11. Any history of endometrial hyperplasia or uterine/endometrial, breast, or ovarian cancer.
12. Any history of other malignancy within the last five years, with the exception of basal cell (excluded if within one year) or non-invasive squamous cell (excluded if within one year) carcinoma of the skin.
13. A history of any other cardiovascular, hepatic, renal, pulmonary, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurologic, psychological, or

musculoskeletal disease or disorder that was clinically significant in the opinion of the Principal Investigator or Medical Sub-Investigator.

14. Contraindication to estrogen therapy or allergy to the use of estradiol or any components of the investigational drugs.
15. Used 15 or more cigarettes per day.
16. A history of drug and/or alcohol abuse within one year of start of study.
17. Used, within 28 days prior to Screening, or a plan to use during the study, any prescription or over-the-counter (OTC) medications (including herbal products) that would be expected to interact with estradiol therapy.
18. Used any type of vaginal preparation (including lubricants and moisturizers) within 14 days prior to Screening.
19. Used estrogen alone or estrogen/progestin within the following time periods:
    a. Vaginal hormonal products (rings, creams, gels) (three months prior to Screening).
    b. Transdermal estrogen alone or estrogen/progestin products (eight weeks prior to Screening).
    c. Oral estrogen and/or progestin therapy (eight weeks prior to Screening).
    d. Progestational implants, estrogen or estrogen/progestational injectable drug therapy (three months prior to Screening).
    e. Estrogen pellet therapy or progestational injectable drug therapy (six months prior to Screening).
    f. Percutaneous estrogen lotions/gels (eight weeks prior to Screening).
    g. Oral, topical, vaginal, patch, implantable or injectable androgen therapy (eight weeks prior to Screening).

<u>Efficacy Endpoints:</u>

- Change from baseline (screening) to Day 15 in parabasal vaginal cells
- Change from baseline (screening) to Day 15 in superficial cells
- Change from baseline (screening) to Day 15 in vaginal pH
- Change from baseline (randomization) to Day 15 in severity of most bothersome VVA symptom
- Change from baseline (randomization) to Day 15 in vaginal bleeding associated with sexual activity (presence or absence of bleeding)
- Change from baseline (randomization) to Day 15 in Investigator's assessment of vaginal mucosa

The following table is the tool used for self-assessment of VVA most bothersome symptom.

Vulvar and/or Vaginal Symptoms		Severity Score (Please select only ONE)				Most Bothersome Symptom (Please select only ONE from questions 1 to 5)
		0 = None	1 = Mild	2 = Moderate	3 = Severe	
1	Do you have vaginal dryness?	☐	☐	☐	☐	☐
2	Do you have vaginal and / or vulvar irritation /itching?	☐	☐	☐	☐	☐
3	Do you have pain, burning or stinging when you urinate?	☐	☐	☐	☐	☐
	Are you having sexual activity? ☐ NO[1] ☐ YES[2] 1- If **NO**, STOP HERE. 2- If **YES**, Please complete questions 4 and 5 below.					
4	Do you have vaginal pain associated with sexual activity?	☐	☐	☐	☐	☐
5	Do you have vaginal bleeding associated with sexual activity?	☐ NO ☐ YES				☐

Source: Sponsor's study report table 4.

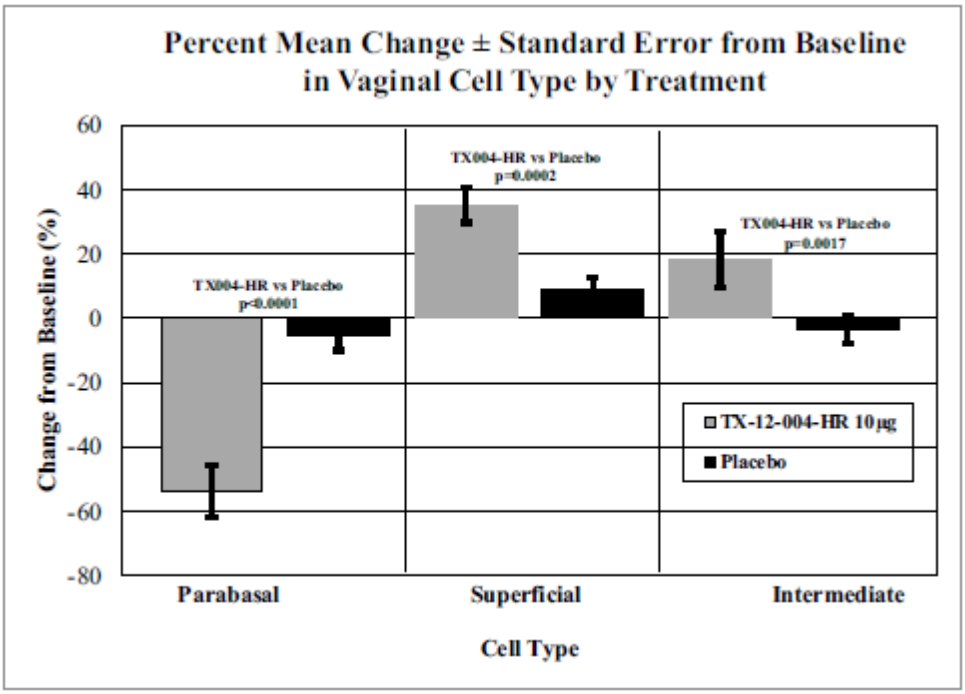
The following table summarizes the demographics and baseline estradiol and vaginal cell data in patients who completed the study.

Parameter	Category	TX-004HR (N = 24)	Placebo (N = 24)	Overall (N = 48)
Race	White	21 (87.5%)	23 (95.8%)	44 (91.7%)
	Asian	1 (4.2%)	0 (0%)	1 (2.1%)
	Black or African American	2 (8.3%)	1 (4.2%)	3 (6.3%)
Age (years)	Mean ± SD	62.4 ± 5.7	62.6 ± 7.3	62.5 ± 6.5
Weight (kg)	Mean ± SD	69.5 ± 12.0	73.4 ± 10.3	71.4 ± 11.2
Height (cm)	Mean ± SD	161.9 ± 5.0	163.5 ± 5.7	162.7 ± 5.4
BMI (kg/m^2)	Mean ± SD	26.4 ± 3.9	27.4 ± 3.4	26.9 ± 3.7
Estradiol (pg/mL)	Mean ± SD	8.38 ± 9.54	6.96 ± 7.56	7.67 ± 8.54
%Parabasal	Mean ± SD	62.5 ± 40.1	64.6 ± 38.8	63.5 ± 39.1
%Superficial	Mean ± SD	1.7 ± 2.4	0.2 ± 1.0	0.9 ± 2.0
%Intermediate	Mean ± SD	35.8 ± 38.3	35.2 ± 38.4	35.5 ± 37.9

Source: Sponsor's study report table 6.

Efficacy Results

Mean % changes in parabasal, superficial, and intermediate vaginal cells following 14 day of once daily treatment with TX004-HR 10 mcg and placebo are summarized in the following figure.



Source: Sponsor's study report figure 3.

Changes in parabasal cells, including statistical analysis, following 14 day of once daily treatment with TX004-HR and placebo are summarized in the following table.

Statistics	TX-004HR	Placebo	Difference Between Treatment Means	90% CI for Difference[a]	TX-004HR vs. Placebo P-value[b]
N	24	24	-	-	-
Baseline Mean (%)	62.5	64.6	-	-	0.8557
Day 15 Mean (%)	8.8	59.2	-	-	-
Change from Baseline Least-Squares Mean	-54.4	-4.80	-49.6	(-60.4, -38.8)	<0.0001
Mean ± SD	-53.8 ± 39.7	-5.4 ± 22.3	-	-	-
Median	-60.0	-5.0	-	-	-
Min, Max	-100.0, 0.0	-60.0, 60.0	-	-	-

[a] Confidence interval for the TX-004HR - Placebo from ANCOVA with treatment as a fixed effect and Baseline as a covariate.
[b] Change from Baseline P-value for treatment comparison from ANCOVA with treatment as a fixed effect and Baseline as a covariate. P-value < 0.05 indicates a statistically significant difference.
Source: Appendix 16.1.9

Source: Sponsor's study report table 7.

Changes in superficial cells, including statistical analysis, following 14 day of once daily treatment with TX004-HR and placebo are summarized in the following table.

Statistics	TX-004HR	Placebo	Difference Between Treatment Means	90% CI for Difference[a]	TX-004HR vs. Placebo P-value[b]
N	24	24	-	-	-
Baseline Mean (%)	1.7	0.2	-	-	0.0089
Day 15 Mean (%)	36.9	9.0	-	-	-
Change from Baseline Least-Squares Mean	35.2	8.8	26.5	(15.4, 37.6)	0.0002
Mean ± SD	35.2 ± 26.4	8.8 ± 18.7	-	-	-
Median	40.0	0.0	-	-	-
Min, Max	0.0, 80.0	0.0, 90.0	-	-	-

[a] Confidence interval for the TX-004HR - Placebo from ANOVA with treatment as a fixed effect.
[b] Change from Baseline P-value for treatment comparison from ANOVA with treatment as a fixed effect. P-value < 0.05 indicates a statistically significant difference.
Source: Appendix 16.1.9

Source: Sponsor's study report table 8.

Changes in intermediate cells, including statistical analysis, following 14 day of once daily treatment with TX004-HR and placebo are summarized in the following table.

Statistics	TX-004HR	Placebo	Difference Between Treatment Means	90% CI for Difference[a]	TX-004HR vs. Placebo P-value[b]
N	24	24	-	-	-
Baseline Mean (%)	35.8	35.2	-	-	0.9552
Day 15 Mean (%)	54.4	31.9	-	-	-
Change from Baseline Least-Squares Mean	18.7	-3.5	22.3	(11.1, 33.5)	0.0017
Mean ± SD	18.5 ± 42.7	-3.3 ± 21.6	-	-	-
Median	22.5	-5.0	-	-	-
Min, Max	-60.0, 100.0	-60.0, 20.0	-	-	-

[a] Confidence interval for the TX-004HR - Placebo from ANCOVA with treatment as a fixed effect and Baseline as a covariate.
[b] Change from Baseline P-value for treatment comparison from ANCOVA with treatment as a fixed effect and Baseline as a covariate. P-value < 0.05 indicates a statistically significant difference.
Source: Appendix 16.1.9

Source: Sponsor's study report table 9.

Changes in vaginal pH, including statistical analysis, following 14 day of once daily treatment with TX004-HR and placebo are summarized in the following table.

Statistics	TX-004HR	Placebo	Difference Between Treatment Means	90% CI for Difference[a]	TX-004HR vs. Placebo P-value[b]
N	24	24	-	-	-
Baseline Mean	6.00	6.13	-	-	0.3360
Day 15 Mean	5.08	5.73	-	-	-
Change from Baseline Least-Squares Mean	-0.97	-0.34	-0.64	(-0.90, -0.37)	0.0002
Mean ± SD	-0.917 ± 0.686	-0.396 ± 0.659	-	-	-
Median	-1.00	-0.500	-	-	-
Min, Max	-2.00, 0.500	-1.50, 0.500	-	-	-

[a] Confidence interval for the TX-004HR - Placebo from ANCOVA with treatment as a fixed effect and Baseline as a covariate.
[b] Change from Baseline P-value for treatment comparison from ANCOVA with treatment as a fixed effect and Baseline as a covariate. P-value < 0.05 indicates a statistically significant difference.
Source: Appendix 16.1.9

Source: Sponsor's study report table 10.

Changes in most bothersome symptom, including statistical analysis, following 14 day of once daily treatment with TX004-HR and placebo are summarized in the following table.

Statistics	TX-004HR 10µg	Placebo	Difference Between Treatment Means	90% CI for Difference[a]	TX-004HR 10µg vs. Placebo P-value[b]
N	23[c]	24	-	-	-
Baseline Mean	2.52	2.58	-	-	0.6792
Day 15 Mean	1.48	1.54	-	-	-
Change from Baseline Least-Squares Mean	-1.043	-1.042	-0.002	(-0.497, 0.493)	0.9951
Mean ± SD	-1.043 ± 0.928	-1.042 ± 1.08	-	-	-
Median	-1.00	-1.00	-	-	-
Min, Max	-3.00, 0.00	-3.00, 0.00	-	-	-

[a] Confidence interval for the TX-004HR – Placebo from ANOVA with treatment as a fixed effect.
[b] Change from baseline P-value for treatment comparison from ANOVA with treatment as a fixed effect. P-value < 0.05 indicates a statistically significant difference.
[c] One subject in the TX004-HR group (Subject 151) stopped sexual activity after Baseline, therefore the data was set to missing.
Source: Appendix 16.1.9

Source: Sponsor's study report table 11.

Changes in vaginal dryness, irritation, pain with sex (dyspareunia), and pain with urination (dysuria), including statistical analysis, following 14 day of once daily treatment with TX004-HR versus placebo are summarized in the following table.

Symptom	Statistical Method[a]	Least-Squares Mean		Difference Between Treatment Means	90% CI for Difference[b]	TX-004HR vs. Placebo P-value
		TX-004HR	Placebo			
Dryness	ANCOVA	-0.980	-0.729	-0.251	(-0.706, 0.204)	0.3597
Irritation	ANCOVA	-0.694	-0.514	-0.180	(-0.549, 0.189)	0.4159
Pain (Sex)	ANOVA	-0.800	-0.500	-0.300	(-1.033, 0.433)	0.4872
Pain/Burning/ Stinging (Urination)	ANCOVA	-0.391	-0.359	-0.032	(-0.263, 0.200)	0.8185

[a] ANOVA model contained a fixed effect for treatment. ANCOVA added Baseline as a covariate to the model.
[b] Confidence interval for the difference between TX-004HR and Placebo treatment least-squares means.
Source: Appendix 16.1.9

Source: Sponsor's study report table 12.

Changes in vaginal bleeding associated with sexual activity, including statistical analysis, following 14 day of once daily treatment with TX004-HR and placebo are summarized in the following table.

Treatment	N*	Baseline (Randomization) and Day 15 Summary of Vaginal Bleeding			
		Bleeding/ No Bleeding (Success)[b]	Bleeding/ Bleeding (Failure)	No Bleeding/ Bleeding (Failure)	No Bleeding/ No Bleeding (NC)
TX-004HR	10	2 (100%)	0	0	8
Placebo	10	1 (20%)	3	1	5
P-Value for TX-004HR vs. Placebo[a]		0.1429	-	-	-

*N = Total number of subjects within each treatment group who were sexually active at both Baseline and Day 15 and provided a response at both visits.
NC = No Change – not considered in the statistical comparison.
[a] P-value for treatment comparison from Fisher's Exact Test.
[b] Percent is based on the number of subjects classified as either a Success or a Failure (N=2 for TX-004HR; N=5 for Placebo)
Source: Appendix 16.1.9

Source: Sponsor's study report table 13.

Safety Findings

Adverse events following 14 days of once daily treatment with TX-004HR 10 mcg and placebo are summarized in the following table.

System Organ Class	Preferred Term	Number (%) of Subjects	
		TX-004HR (N = 24)	Placebo (N = 26)
Subjects with at Least One AE [1,2]		11 (45.8%)	3 (11.5%)
Eye Disorders	Total Eye Disorders	1 (4.2%)	0 (0.0%)
	Eye contusion	1 (4.2%)	0 (0.0%)
Investigations	Total Investigations	1 (4.2%)	0 (0.0%)
	Blood pressure increased	1 (4.2%)	0 (0.0%)
Nervous System Disorders	Total Nervous System Disorders	0 (0.0%)	1 (3.8%)
	Paraesthesia	0 (0.0%)	1 (3.8%)
Renal and urinary disorders	Total Renal and urinary disorders	1 (4.2%)	0 (0.0%)
	Nephrolithiasis	1 (4.2%)	0 (0.0%)
Reproductive system and breast disorders	Total Reproductive system and breast disorders	10 (41.7%)	3 (11.5%)
	Cervical dysplasia [1]	1 (4.2%)	0 (0.0%)
	Hot Flash	2 (8.3%)	0 (0.0%)
	Vaginal discharge	1 (4.2%)	0 (0.0%)
	Vaginal dysplasia [1,2]	3 (12.5%)	0 (0.0%)
	Vaginal haemorrhage	0 (0.0%)	2 (7.7%)
	Vulvovaginal burning sensation	1 (4.2%)	0 (0.0%)
	Vulvovaginal discomfort	0 (0.0%)	1 (3.8%)
	Vulvovaginal pain	1 (4.2%)	0 (0.0%)
	Vulvovaginal pruritus	1 (4.2%)	0 (0.0%)

Counts reflect numbers of subjects reporting one or more adverse event that map to the MedDRA system organ class/preferred term. At each level of summarization (system organ class or preferred term), subjects reporting an adverse event more than once are counted only once for that AE.

[1] Coding was corrected post database lock. Preferred term 'Cervical Dysplasia' was replaced with MedDRA preferred term of 'Vaginal dysplasia' for 'Atypical squamous cells of undetermined significance' and 'Low-grade squamous intraepithelial lesion' verbatims due to the surgical history of total hysterectomy reported for subjects 139 and 148.

[2] Vaginal dysplasia (Subject 101, ASC-US) was not reported by the investigator as an AE and was added post database lock.

Source: Sponsor's study report table 14.2.1.2.

Reviewer's Comments:

- The Applicant refers to their drug product as a vaginal capsule. For the purpose of this NDA review and to be consistent with current nomenclature for similar vaginal products, TX004-HR was referred to as a vaginal insert.
- Due to problems with assay reproducibility, the sponsor considers the estradiol concentrations obtained during the study to be invalid and therefore no PK or statistical analysis was conducted.

- At baseline, endogenous estradiol concentrations in both patient groups were similar and were within the postmenopausal range defined by the Applicant as <50 pg/mL. Postmenopausal status is defined by multiple factors including endogenous estradiol concentration which can vary from 20 to 50 pg/mL according to published literature. For the purpose of this pilot efficacy study, an endogenous estradiol concentration of < 50 pg/mL is acceptable as the inclusion criteria included other factors such as cessation of menstrual cycle for at least 12 months, hysterectomy, etc. Mean (SD) baseline estradiol concentration was 8.4 (9.5) pg/mL and 7.0 (7.6) pg/mL in the TX-004HR and Placebo groups, respectively.
- The Applicant's efficacy assessment in efficacy endpoints change from baseline in (1) parabasal cells, (2) superficial cells, (3) vaginal pH, and (4) most bothersome symptom are consistent with the Clinical Division's guidance for the proposed indication.
- The Applicant demonstrated statistically significant efficacy in the following four critical endpoints (% change from baseline to Day 15) following 14 days of once daily vaginal administration of TX-0040HR 10 mcg, compared to placebo:
    - Decrease in parabasal cells (54% vs. 5%; p-value < 0.0001)
    - Increase in superficial cells (35% vs. 9%; p-value = 0.0002)
    - Decrease in vaginal pH (0.97 pH unit vs. 0.34 pH unit; p-value = 0.0002)
- Although assessment of vaginal intermediate cells is not part of the standard assessment of vaginal cells; however there was an improvement in intermediate cells and thus provided an additional indicator for treatment benefit. In this case, there was a statistical improvement in % change from baseline of the vaginal intermediate cells.
    - Increase in intermediate cells (19% (increase with TX-004HR) vs. -4% (decrease with placebo); p-value = 0.0017)
- There was no statistically significant improvement in clinically relevant most bothersome symptom (vaginal dryness, irritation, dyspareunia, or dysuria) with 14 days treatment of TX-004HR, compared to placebo. The Applicant states that lack of symptomatic changes was not unexpected considering the short duration of therapy. This reviewer concurs with the Applicant in that improvement in VVA symptoms may be difficult to assess with only 14 days of treatment.
- Vaginal dysplasia (abnormal cells) was the most significant AE associated with TX-004HR (3 of 24 patients (13%) versus placebo (0 patients). Unopposed (without a progestin) estrogen use is associated with endometrial carcinoma; therefore, demonstration of long-term endometrial safety is imperative to the approval of any estrogen product indicated for VVA.
- Based upon the results from this pilot study with 10 mcg and the comparative bioavailability studies against Vagifem (Studies ESTR-1K-499-12 and ESTR-1K-500-12) with 10 and 25 mcg doses, the Applicant decided to carry forward both 10 and 25 mcg doses into Phase 3.

- The Applicant also included a lower dose (4 mcg) in the Phase 3 study to identify a lower dose and to minimize systemic estrogen exposure. The Applicant selected the 4 mcg dose based on the existing dose proportionality with the two doses (10 and 25 mcg) evaluated in the comparative bioavailability studies.
- This reviewer concurs with carrying the 10 and 25 mcg doses into Phase 3 based upon the objective cellular and pH data and with incorporating a lower dose.

Study ESTR-1K-499-12

Title: An open-label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, two-way crossover relative bioavailability study comparing TX12-004-HR (Estradiol Vaginal (b) (4) Capsules) 10 mcg being developed by TherapeuticsMD Inc., Florida and VAGIFEM® (Estradiol Vaginal Tablets) 10 mcg manufactured by Novo Nordisk Inc., USA in healthy, adult, human postmenopausal female subjects

Protocol No:	ESTR-1K-499-12
Phase:	1
Principal Investigator:	Dr. K. Senthilkumar, M.B.B.S, MD
Clinical Study Center:	Micro Therapeutic Research Labs Private Limited, No.29 A, "Krishna Madhuravanam", Vellakinar Pirivu, Thudiyalur, Coimbatore-641029.Tamil Nadu, India
Clinical Study Dates:	August 10, 2013– August 26, 2013
Analytical Study Facility:	Esoterix Endocrinology–Calabasas, CA4301 Lost Hills Road Calabasas Hills, CA 91301

OBJECTIVES

To compare bioavailability of TX-004HR (Estradiol Vaginal (b) (4) Inserts) 10 µg being developed by TherapeuticsMD Inc., Florida, USA and VAGIFEM (Estradiol Vaginal Tablets) 10 µg manufactured by Novo Nordisk Inc., USA in healthy postmenopausal women and to monitor the adverse events and assess safety of subjects.

STUDY DESIGN

This was a Phase 1, open-label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, two-way crossover relative bioavailability study comparing TX12-004-HR 10 µg and VAGIFEM® (Estradiol Vaginal Tablets) 10 µg manufactured by Novo Nordisk Inc., USA in 36 healthy postmenopausal women. Subjects were housed in the clinical facility for two consecutive nights for each of two dosing periods, from at least 11 hours before dosing until after the 24 hours post dose blood draw in each period. All subjects received both TX-004-HR 10 µg (Test product) and VAGIFEM® 10 µg (Reference product) during the study (one product per dosing period), with a 14-day washout period between dosing periods. Subjects were provided a standard diet and continuously monitored for their well-being and safety throughout the study. Blood samples were collected at pre-defined intervals up to 24 hours after dosing in each period for each subject.

Study Subjects

A total of 36 healthy, adult, human postmenopausal female subjects ages 42 to 63 years were enrolled in the study. All subjects were Asian with mean BMI of 25 kg/m^2. Thirty five (35) subjects completed the study.

Reviewer's Comment:

It should be noted that the study subjects were all Asian and not reflective of the target population in the US. Nonetheless, given the objective of the study is to compare the relative bioavailability of the two products, race of the subjects are not expected to have significant impact on the study outcome.

Subjects included in data analysis

Per the statistical analysis plan, if after baseline adjustment a subject had negative concentrations at all sample times that were set to zero in at least one period, the subject was excluded from the PK and statistical evaluations. Thus, Subject 30 was excluded from estradiol calculations (N = 34 subjects) and Subjects 02 and 07 were excluded from estrone calculation (N = 33 subjects). Subjects 01, 07, 09, 10, 15, 18, 19, 22, 24, 25, and 28 had levels below the LOQ prior to baseline correction in at least one period and were excluded from estrone sulfate calculations (N = 24).

MAIN INCLUSION CRITERIA

- Healthy adult human postmenopausal female volunteers between 40-65 years of age with a Body Mass Index (BMI) range between 18.50 kg/m^2 to 29.99 kg/m^2 (according to the formula of BMI = weight (kg) / $[height (m)]^2$).
- Generally healthy as documented by the ultra-sonogram, gynecological examination and breast examination. (for Period I only), physical examination, and laboratory evaluations
- Postmenopausal status confirmed by:
  - Plasma estradiol concentration <50 ng/L.
  - Plasma follicle stimulating hormone (FSH) concentration >40 IU/L.
  - No vaginal bleeding for at least 12 months [if bleeding is difficult to assess due to previous hormone replacement therapy, the Investigator will decide based on clinical history and hormone profile].
  - Or six weeks postsurgical bilateral oophorectomy with or without hysterectomy
- Within normal limits or clinically non-significant laboratory evaluation results for FSH, LH, and estradiol.
- No consumption of caffeine and/or Xanthine-containing products (e.g., coffee, tea, chocolate, caffeine-containing sodas, colas, etc.) for at least 24.00 hours prior to check-in for both dosing periods.
- Non- or ex-smoker.

MAIN EXCLUSION CRITERIA

- Breast feeding females.
- Use of hormone therapy restricted as follows:
  - Vaginal hormone therapy products for at least one week.
  - Transdermal estrogen or estrogen/progestational products for at least eight weeks.
  - Oral estrogen and/or progestational therapy for at least eight weeks.
  - Progestational implants, estrogen or estrogen/progestational injectable drug therapy for at least three months.
  - Estrogen pellet therapy or progestational injectable drug therapy for at least six months.
  - Percutaneous estrogen lotions/gels for at least four weeks.
- Uncontrolled hypertension greater than or equal to a systolic blood pressure of 140 mm Hg and/or a diastolic blood pressure of greater than or equal to 90 mm Hg.

TREATMENT

After fasting overnight for at least at least 10 hours, in the morning, either the investigational product or the Reference product was administered intravaginally to each subject under ambient temperature conditions by trained female study personnel in the presence of medically qualified personnel and Quality Assurance (QA) auditor(s). Each subject was required to remain in supine position for four hours after dosing and to refrain from strenuous activity until they were checked out of the clinic. Subjects were fasted for an additional four hours after dosing; after that, meals were provided by clinic personnel at scheduled times.

INVESTIGATIONAL PRODUCT and Mode of Administration:

Test Product: TX-004HR (Estradiol Vaginal [redacted] (b) (4) Inserts) 10 µg
Manufactured for: TherapeuticsMD Inc., Florida, USA
Manufactured by: [redacted] (b) (4)
Lot No.: PN0089-02
Manufacture date: 18 Jan 2013
Expiration date: 18 Jan 2014
Dose and Mode of Administration: TX-004HR 10 µg was administered to each subject intravaginally.

Reference Product: VAGIFEM® 10 µg (Estradiol Vaginal Tablets)
Manufactured by: Novo Nordisk A/S.
Lot No.: CE70136
Manufactured date: Not available
Expiration date: 01/2016

Dose and Mode of Administration: VAGIFEM 10 μg was administered to each subject intravaginally.

PHARMACOKINETIC EVALUATION

Blood Sampling:

A total of 13 blood samples (10 mL each) for pharmacokinetic (PK) measurements were taken at prescribed time points per the study protocol: -01.00, -00.50, 00.00, 01.00, 02.00, 04.00, 06.00, 08.00, 10.00, 12.00, 14.00, 18.00 and 24.00 hours (± 2 minutes for all sampling times except the zero hour blood draw, which could be collected within five minutes prior to dosing).

Reviewer's Note:

The time of blood collections deviated (i.e., more than ± 2 minutes of scheduled collection time) from protocol-specified times for eight subjects. Nonetheless, the deviations were all less 10 minutes and are not expected to significantly impact the study outcome.

Bioanalytical Method

Estradiol, estrone and estrone conjugate concentrations were determined in plasma and serum using a fully validated Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method for the simultaneous determination of estradiol and estrone in human plasma and for their content of estrone sulfate at Esoterix Endocrinology, Calabasas, California, USA. The detailed method is described in the Table below.

Overview of Bioanalytical Method for Study ESTR-1K-499-12

Analyte(s) of interest	**Estradiol (E2) & Estrone(E1)**	**Estrone Sulfate**
Method No.	ESO-CAL-LCMS-SOP-0013	ESO-CAL-LCMS-SOP-0011
Methodology	2D-HPLC-MS/MS	HPLC-MS/MS
Biological matrix	Plasma & Serum[a]	Plasma & Serum
Extraction method	Liquid-liquid	Protein precipitation
Calibration curve range	E2: 1.0-500 pg/mL E1: 2.5-500 pg/mL	10-1000 ng/mL
Internal standard	$^{13}C_6$-estradiol & $^{13}C_6$-estrone	d_4-estrone sulfate

Bioanalytical Report No.	102572	102572
Validation Report No.	10-ESO-CAL-080v1 & 15-ESO-CAL-019v2	06-05-106-18532 & 15-ESO-CAL-019v2
Inter-run accuracy for each QC	E2: LQC (3.00 pg/mL): -2.0% bias MQC (125 pg/mL): 1.1% bias HQC (350 pg/mL): 4.1% bias E1: LQC (7.50 pg/mL): -9.9% bias MQC (125 pg/mL): -4.7% bias HQC (350 pg/mL): -5.9% bias	LQC (25.0 ng/mL): -8.8% bias MQC (300.0 ng/mL): -9.3% bias HQC (750.0 ng/mL): -11.5% bias
Inter-run precision for each QC	E2: LQC (3.00 pg/mL): 13.8% CV MQC (125 pg/mL): 3.0% CV HQC (350 pg/mL): 2.8% CV E1: LQC (7.50 pg/mL): 9.0% CV MQC (125 pg/mL): 3.1% CV HQC (350 pg/mL): 3.6% CV	LQC (25.0 ng/mL): 5.1% CV MQC (300.0 ng/mL): 2.5% CV HQC (750 ng/mL): 4.1% CV
Long-term stability	131 days at -20°C & -80°C[b]	125 days at -20°C & -80°C[b]
Freeze-thaw stability	Demonstrated for 6 cycles for at ≤ -10ºC	Demonstrated for 6 cycles for at ≤ -10°C
Incurred sample reanalysis (ISR)	E2: 74.19% passed E1: 84.48% passed % analyzed: ~7%	80% passed % analyzed: ~7%

CV = LQC = low quality control, MQC = mid quality control, HQC = high quality control

[a] *The original method validations were performed in serum. The LC-MS/MS methods for the quantification of estrone, estradiol, and estrone sulfate were assessed for sample type effect using EDTA plasma and found to return results equivalent (± 15%) to levels quantified in serum.*

[b] *Based on the amendment submitted on December 28, 2017.*

Reviewer's Comment:

Time from first sample drawn to last sample analyzed (including ISR) was 164 days. Therefore, a portion of samples were not analyzed within the demonstrated long term stability time. Nonetheless, it may not be a concern due to the following considerations: 1) it was a short extension of about 30 days beyond the known stability window and 2) the stability data from ESTR-1K-500-12 and Study TXV14-01suggested 232- 846 days of stability for estradiol/estrone and up to 512 days of stability for estrone conjugates when stored at -20°C & -80°C. Therefore, the demonstrated long-term stability from the other two studies may cover the duration of sample storage in the current study.

Calculation for PK parameters:

The PK parameters for the estradiol, estrone, and estrone sulfate concentrations at each sampling time were evaluated by analysis of variance (ANOVA) with sequence, subjects-within-sequence, period, and treatment as factors, using a general linear models approach. The ANOVA for areas and peak concentrations were conducted on natural log (ln) transformed values. The 90% confidence intervals (CIs) on the geometric mean area and C_{max} ratios for the Test product compared to the Reference product were constructed to compare the bioavailability of the Test product to that of the Reference product. Results for both baseline adjusted and baseline unadjusted data were provided.

Baseline Adjustment: Baseline concentrations were determined at -1.0, -0.5, and zero hours for each dosing period, and baseline correction was subject and period specific. The method for baseline correction was arithmetic, with the mean of the pre-dose concentrations being subtracted from all the post-dose concentrations. If a negative concentration value resulted after baseline correction, this was set to zero. In addition, the baseline-adjusted pre-dose concentration (zero-hour) was also set to zero. The data was analyzed using both the uncorrected and baseline corrected data.

PHARMACOKINETIC RESULTS

Estradiol

- **Baseline-adjusted**

The baseline-adjusted mean plasma estradiol concentration-time profiles following vaginal administration of TX-004HR 10 µg (Test product) and VAGIFEM 10 µg (Reference product) are shown in **Figure 1.** When compared with VAGIFEM**,** TX-004HR showed a more rapid systemic absorption of estradiol with t_{max} at approximately two hours. The plasma concentrations were two- to three-fold greater than baseline at T_{max} and then returned to a measurable baseline at 6 to 10 hours after the single vaginal administration. VAGIFEM showed a broader, longer absorption profile. T_{max} was approximately 10 hours post-dose and estradiol concentrations returned to

baseline at approximately 18 hours after a single dose. Mean (± SD) C_{max} was 15.72 ± 7.92 pg /mL for TX-004HR and was 24.19 ± 11.92 pg/mL for VAGIFEM. Mean (± SD) of AUC_{0-24} was 53.01 ± 19.56 pg.hr/mL for TX-004HR 10 µg, and was 163.86 ± 72.09 pg.hr/mL for VAGIFEM **(Table 1).**

Figure 1 Linear Plot of Least-Squares Mean Plasma Estradiol – Baseline-Adjusted Concentrations versus Time (N = 34)

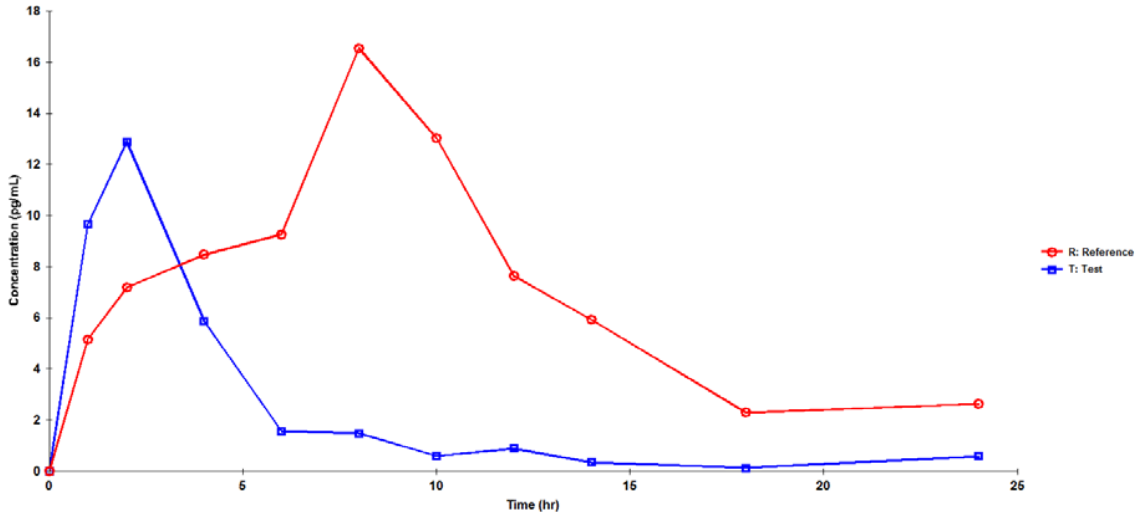


Table 1 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estradiol – Baseline-Adjusted (N = 34)

Parameter	TX-004HR	VAGIFEM
C_{max} (pg/mL)	15.72 ± 7.92	24.19 ± 11.92
AUC $_{0-24}$	53.01 ± 19.56	163.86 ± 72.09
T_{max} (hrs)	1.98 ± 1.29	10.53 ± 5.58

- **Baseline-unadjusted**

The baseline-unadjusted mean plasma estradiol concentration-time profiles following vaginal administration of TX-004HR 10 µg (Test product) and VAGIFEM 10 µg (Reference product) are shown in **Figure 2**. **Table 2** provides PK parameters of the Test product and the Reference product for estradiol without baseline adjustment. Mean (± SD) C_{max} for Test product was 21.22 ± 10.89 pg /mL, and for Reference product was 32.51 ± 22.25 pg/mL. Mean (± SD) AUC_{0-24} for Test product was 178.19 ± 150.07 pg.hr/mL, and for Reference product was 355.24 ± 452.79 pg.hr/mL.

Figure 2: Linear Plot of Least-Squares Mean Plasma Estradiol - Unadjusted Concentrations versus Time (N = 35)

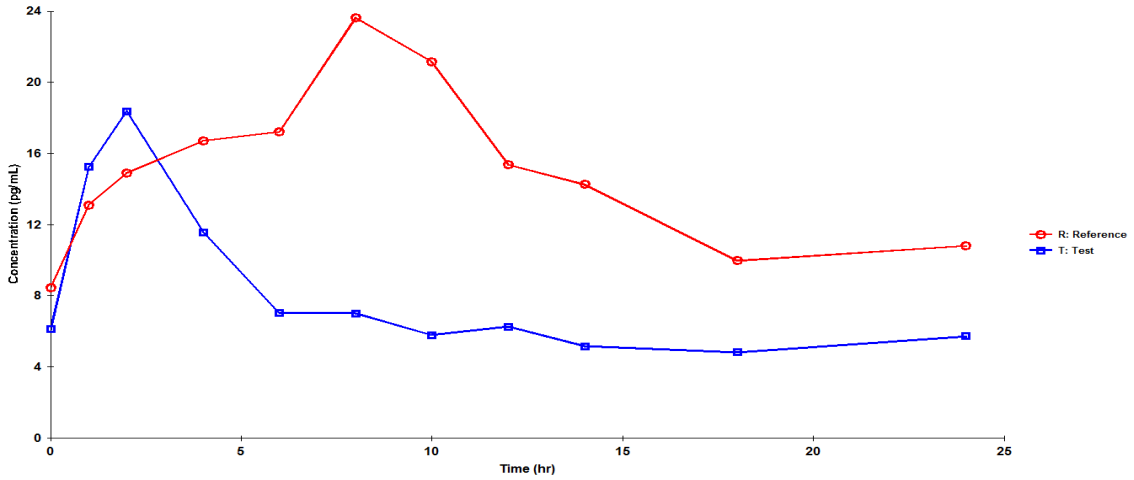


Table 2 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estradiol – Baseline-unadjusted (N = 35)

Parameter	TX-004HR	VAGIFEM
C_{max} (pg/mL)	21.22 ± 10.89	32.51 ± 22.25
AUC $_{0-24}$	178.19 ±	355.24 ± 452.79
T_{max} (hrs)	1.98 ± 1.27	10.52 ± 5.49

Estrone

- **Baseline-adjusted**

Figure 3 showed the baseline-adjusted mean plasma estrone concentration-time profiles following vaginal administration of TX-004HR 10 µg and VAGIFEM 10 µg**.** Mean (± SD) of C_{max} for TX-004HR 10 µg was 6.85 ± 6.58 pg/mL, and for VAGIFEM 10 µg was 8.83 ± 7.15 pg/mL. Mean (± SD) AUC_{0-24} for TX-004HR 10 µg was 34.71 ± 27.95 pg.hr/mL, and for VAGIFEM 10 µg was 63.00 ± 46.55 pg.hr/mL (**Table 3**).

Figure 3: Linear Plot of Least-Squares Mean Plasma Estrone – Baseline-Adjusted Concentrations versus Time (N = 33)

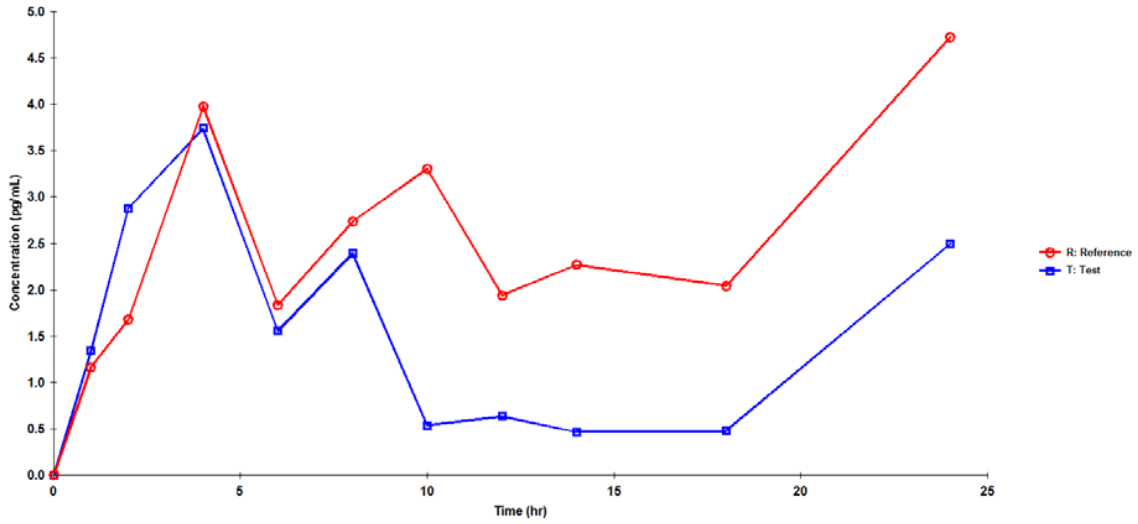


Table 3 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone – Baseline-Adjusted (N = 33)

Parameter	TX-004HR	VAGIFEM
C_{max} (pg/mL)	6.85 ± 6.58	8.83 ± 7.15
AUC_{0-24} (h*pg/mL)	34.71 ± 27.95	63.00 ± 46.55
T_{max} (hrs)	9.12 ± 8.83	11.16 ± 7.24

- **Baseline-unadjusted**

Mean (± SD) C_{max} for TX-004HR was 25.31 ± 14.66 pg /mL, and for VAGIFEM was 28.81 ± 20.01 pg/mL. Mean (± SD) AUC_{0-24} for TX-004HR was 445.12 ± 259.38 pg.hr/mL, and for VAGIFEM was 527.18 ± 372.84 pg.hr/mL (**Table 4**). The PK profiles of baseline-unadjusted estrone are shown in **Figure 4**.

Figure 4: Linear Plot of Least-Squares Mean Plasma Estrone - Unadjusted Concentrations versus Time (N = 35)

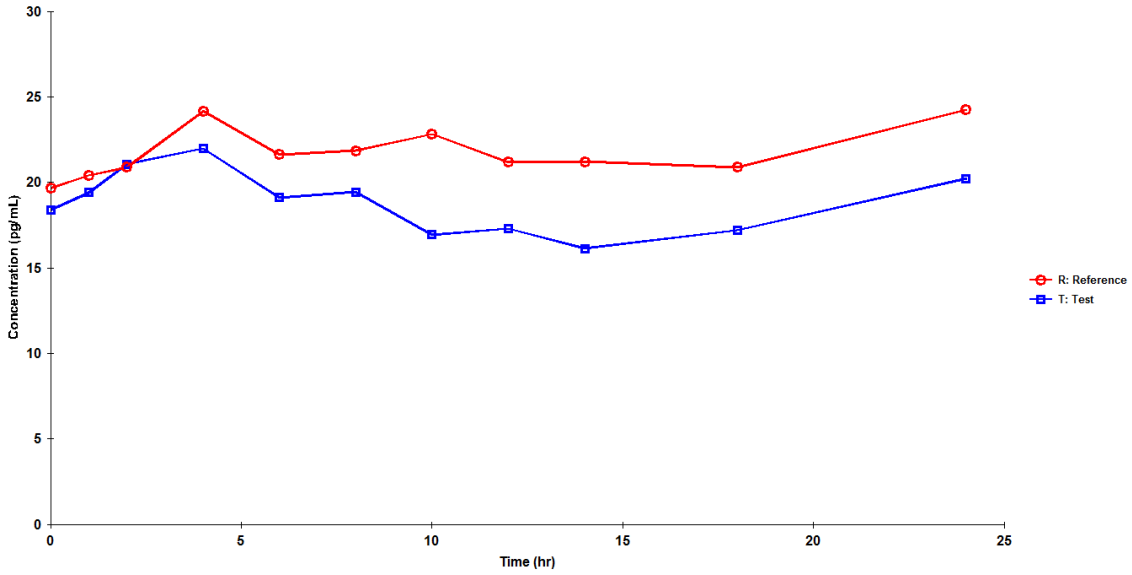


Table 4 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone – Baseline-unadjusted (N = 35)

Parameter	TX-004HR	VAGIFEM
C_{max} (pg/mL)	25.31 ± 14.66	28.81 ± 20.01
AUC $_{0-24}$	445.12 ±	527.18 ± 372.84
T_{max} (hrs)	9.32 ± 9.04	11.03 ± 7.05

Estrone Sulfate

- **Baseline-adjusted**

Figure 5 showed the baseline-adjusted estrone sulfate mean plasma concentration-time profiles following vaginal administration of TX-004HR 10 µg and VAGIFEM 10 µg. Mean (± SD) C_{max} for TX-004HR was 13.9 ± 7.0 ng/dL, and for VAGIFEM was 19.3 ± 11.4 ng/dL. Mean (± SD) AUC_{0-24} for TX-004HR was 98.0 ± 80.9 ng.hr/dL, and for VAGIFEM was 177.6 ± 166.2 ng.hr/dL (**Table 5**).

Figure 5 Linear Plot of Least-Squares Mean Plasma Estrone Sulfate – Baseline-Adjusted Concentrations versus Time (N = 24)

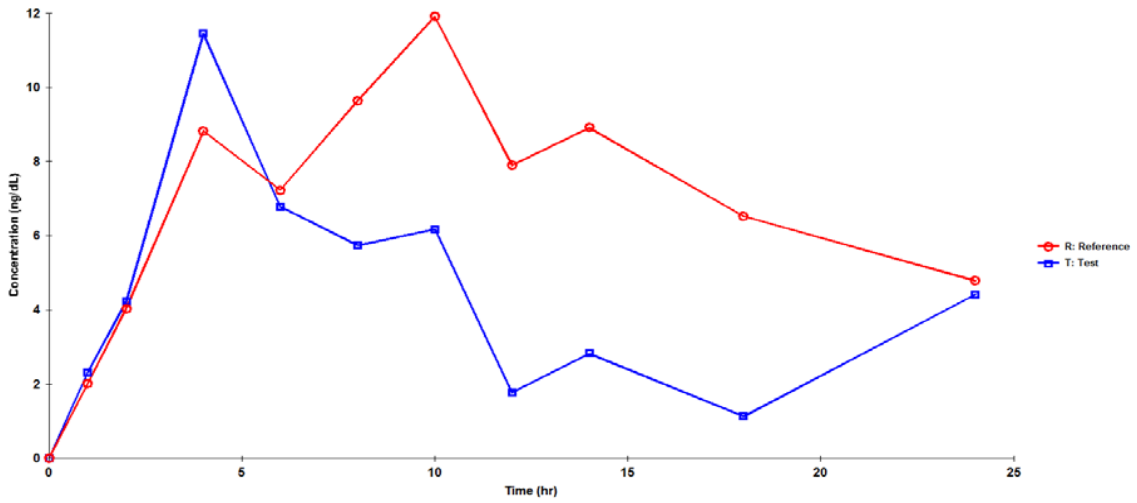


Table 5 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone Sulfate – Baseline-Adjusted (N = 24)

Parameter	TX-004HR	VAGIFEM
C_{max} (ng/dL)	13.9 ± 7.0	19.3 ± 11.4
AUC $_{0-24}$ (h*ng/dL)	98.0 ± 80.9	177.6 ± 166.2
T_{max} (hrs)	6.3 ± 4.6	10.3 ± 5.6

- **Baseline-unadjusted**

Mean (± SD) C_{max} was 25.8 ± 17.2 ng/dL for TX-004HR, and was 32.4 ± 24.4 ng/dL for VAGIFEM. Mean (± SD) AUC_{0-24} was 363.9 ± 358.7 ng.hr/dL for TX-004HR, and was 474.4 ± 475.4 ng.hr/dL for VAGIFEM (**Table 6**). The baseline-unadjusted PK profiles of estrone sulfate for TX-004HR and VAGIFEM are shown in **Figure 6**.

Figure 6: Linear Plot of Least-Squares Mean Plasma Estrone Sulfate - Unadjusted Concentrations versus Time (N = 24)

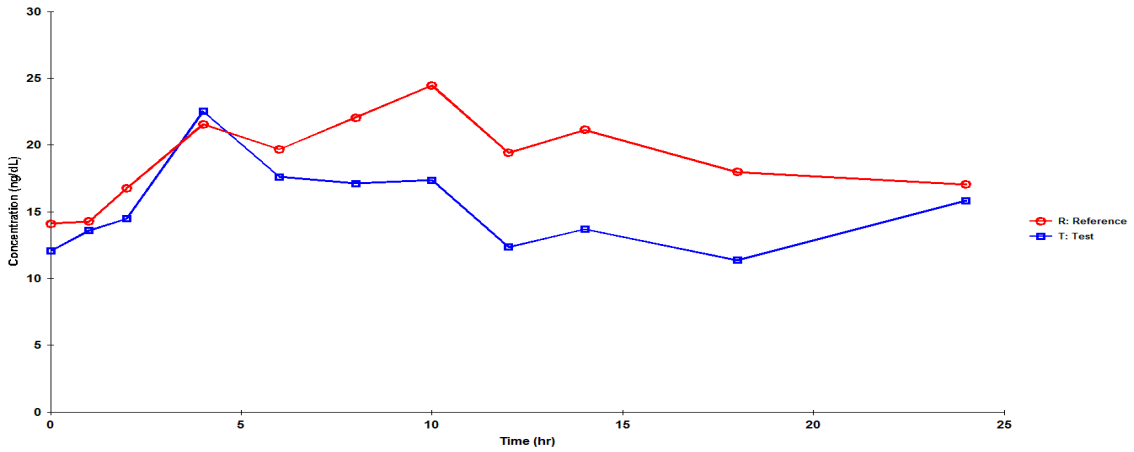


Table 6 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone Sulfate – Baseline-unadjusted (N = 24)

Parameter	TX-004HR	VAGIFEM
C_{max} (ng/dL)	25.8 ± 17.2	32.4 ± 24.4
AUC $_{0-24}$ (h*ng/dL)	363.9 ± 358.7	474.4 ± 475.4
T_{max} (hrs)	6.1 ± 4.7	10.3 ± 5.6

Statistical Results

Geometric least square means, intra-subject coefficient of variation (CV%), Test/Reference (T/R) ratios (expressed as a percentage) and 90% CI were determined for C_{max} and AUC_{0-24} for baseline adjusted and unadjusted data of estradiol, estrone and estrone sulfate. These results are summarized in **Table 7** and **Table 8**.

Based on the statistical results obtained, systemic exposures of estradiol, estrone and estrone sulfate following vaginal administration of TX-004HR 10 µg was statistically significantly lower than that of VAGIFEM 10 µg in healthy postmenopausal women.\

Table 7: Statistical Results of Test Product versus Reference Product for Baseline-Adjusted Estrogens

	PK Parameter	Geometric Least Square Mean		Intra-Subject CV%	T/R Ratio (%)	90% Confidence Interval
		Test Product	Reference Product (R)			
Estradiol (N=34)	**C_{max} (pg/mL)**	14.45	20.20	60.68	71.54**	56.82 -
	AUC $_{0-24}$	49.73	131.04	70.64	37.95**	29.21 -

Estrone (N=33)	**C_{max} (pg/mL)**	5.16	6.93	47.59	74.50**	61.69 -
	AUC_{0-24}	24.20	47.90	73.66	50.51**	38.37 -
Estrone Sulfate	**C_{max} (ng/dL)**	12.3	16.5	48.02	74.55**	59.43 -
	AUC_{0-24} (ng hr/dL)	68.5	118.4	73.87	57.87**	41.68 - 80.35

**Comparison was detected as statistically significant (P < 0.05).

Table 8: Statistical Results of Test Product versus Reference Product for Baseline-unadjusted Estrogens

	PK Parameter	**Geometric Least Square Mean**		**Intra-Subject CV%**	**T/R Ratio (%)**	**90% Confidence Interval**
		Test Product	**Reference Product (R)**			
Estradiol (N=35)	**C_{max} (pg/mL)**	19.21	28.70	36.18	66.93**	58.07 -
	AUC_{0-24}	147.96	288.08	30.87	51.36**	45.46 -
Estrone (N=35)	**C_{max} (pg/mL)**	21.99	24.44	20.53	89.98*	82.87 -
	AUC_{0-24}	388.15	446.13	17.89	87.00*	80.97 -
Estrone Sulfate	**C_{max} (ng/dL)**	21.5	24.9	21.20	86.66**	78.07 -
	AUC_{0-24} (ng hr/dL)	181.0	246.4	55.05	73.45	56.87 - 94.87

*Results of the statistical evaluation by ANOVA ($\alpha = 0.05$) for the hypothesis of equal treatment effects.

**Comparison was detected as statistically significant (P < 0.05).

SAFETY EVALUATION

No adverse events, serious adverse events, or deaths were reported during the course of the study.

Based on the review of the clinical and laboratory safety data, the study medications TX-004HR 10 μg inserts were found to be safe and well tolerated in the study subjects.

SUMMARY

TX-004HR provided a more rapid systemic absorption profile with an earlier t_{max} at approximately two hours. The plasma concentrations returned to a measurable baseline, from concentrations two- to three-fold greater than baseline, 6 to 10 hours after the single vaginal administration. This compared to a broader, longer absorption profile observed with VAGIFEM; t_{max} was approximately 10 hours and return to baseline was later, approximately 18 hours after a single dose.

C_{max} and AUC were determined as measures of systemic exposure for parent estradiol. The TX-004HR Test product provided approximately two-thirds of the C_{max} and two-fifths of the AUC exposures of the Reference VAGIFEM product. For unadjusted estradiol plasma concentrations, the Test/Reference ratio for C_{max} was 67% (90% CI: 58% to 77%) and for AUC was 51% (45% to 58%). A baseline-adjusted PK estimate for the estrogens was also provided because each is an endogenous hormone. For baseline-adjusted estradiol, the T/R ratios and 90% confidence intervals for C_{max} and AUC were 72% (57% to 90%) and 38% (29% to 49%), respectively.

Estrone and estrone sulfate concentration-time profiles were typical of metabolite formation and elimination processes. Plasma concentrations of these estrogens were slightly higher than baseline values and were reflective of limited additional systemic exposures for these oxidative and phase two metabolites. Estrone concentrations were generally only 25% above baseline values and estrone sulfate concentrations were approximately 30% above baseline values, with either treatment.

Both TX-004HR and VAGIFEM were safe and well tolerated following a single 10 µg dose in all participating subjects.

In conclusion, systemic exposures of estradiol and its metabolites estrone and estrone sulfate following vaginal administration of TX-004HR (Estradiol Vaginal (b) (4) Inserts) 10 µg was statistically significantly lower than that of VAGIFEM (Estradiol Vaginal Tablets) 10 µg in healthy, adult, postmenopausal female subjects.

Study ESTR-1K-500-12

An Open-Label, Balanced, Randomized, Two-Treatment, Two-Period, Two-Sequence, Single-Dose, Two-Way Crossover Relative Bioavailability Study Comparing TX12-004-HR (Estradiol Vaginal (b) (4) Capsules) 25 mcg of TherapeuticsMD Inc., Florida and Vagifem® (Estradiol Vaginal Tablets) 25 mcg of Novo Nordisk Inc., USA in Healthy, Adult, Human Postmenopausal Female Subjects

Protocol No:	ESTR-1K-500-12
Phase:	1
Principal Investigator:	Dr. K. Senthilkumar, M.B.B.S, MD
Clinical Study Center:	Micro Therapeutic Research Labs Private Limited, No.29 A, "Krishna Madhuravanam", Vellakinar Pirivu, Thudiyalur, Coimbatore-641029.Tamil Nadu, India
Clinical Study Dates:	September 24, 2013– October 10, 2013
Analytical Study Facility:	Micro Therapeutic Research Labs Private Limited, No.6, Kamarajar Salai, Selaiyur, East Tambaram, Chennai - 600 059, Tamil Nadu, India

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OBJECTIVES

To compare bioavailability of TX-004HR (Estradiol Vaginal (b) (4) Inserts) 25 µg being developed by TherapeuticsMD Inc., Florida, USA and VAGIFEM® (Estradiol Vaginal Tablets) 25 µg manufactured by Novo Nordisk Inc., USA in healthy, adult, human postmenopausal female subjects and to monitor the adverse events and assess safety of subjects

STUDY DESIGN

This was a Phase 1, open-label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, two-way crossover relative bioavailability study comparing TX-004-HR 25 µg and VAGIFEM® (Estradiol Vaginal Tablets) 25 µg manufactured by Novo Nordisk Inc., USA in 36 healthy postmenopausal women. Subjects were housed in the clinical facility for two consecutive nights for each of two dosing periods, from at least 11 hours before dosing until after the 24 hours post dose blood draw in each period. All subjects received both TX-004-HR 25 µg (Test product) and VAGIFEM® 25 µg (Reference product) during the study (one product per dosing period), with a 14-day washout period between dosing periods. Subjects were provided a standard diet and continuously monitored for their well-being and safety throughout the study. Blood samples were collected at pre-defined intervals up to 24 hours after dosing in each period for each subject.

Study Subjects

A total of 36 healthy, adult, human postmenopausal female subjects aged 43 to 58 years participated and completed in the study. All subjects were Asian with mean BMI of 25.61 kg/m^2.

Reviewer's Comment:
It should be noted that the study subjects were all Asian and not reflective of the target population in the US. Nonetheless, given the objective of the study is to compare the relative bioavailability of the two products, race of the subjects are not expected to have significant impact on the study outcome.

MAIN INCLUSION CRITERIA

- Healthy adult human postmenopausal female volunteers between 40-65 years of age with a Body Mass Index (BMI) range between 18.50 kg/m^2 to 29.99 kg/m^2 (according to the formula of BMI = weight (kg) / [height (m)]^2).
- Generally healthy as documented by the ultra-sonogram, gynecological examination and breast examination. (for Period I only), physical examination, and laboratory evaluations
- Postmenopausal status confirmed by:
  - Plasma estradiol concentration <50 ng/L.
  - Plasma follicle stimulating hormone (FSH) concentration >40 IU/L.
  - No vaginal bleeding for at least 12 months [if bleeding is difficult to assess due to previous hormone replacement therapy, the Investigator will decide based on clinical history and hormone profile].
  - Or six weeks postsurgical bilateral oophorectomy with or without hysterectomy
- Within normal limits or clinically non-significant laboratory evaluation results for FSH, LH, and estradiol.
- No consumption of caffeine and/or Xanthine-containing products (e.g., coffee, tea, chocolate, caffeine-containing sodas, colas, etc.) for at least 24.00 hours prior to check-in for both dosing periods.
- Non- or ex-smoker.

MAIN EXCLUSION CRITERIA

- Breast feeding females.
- Use of hormone therapy restricted as follows:
  - Vaginal hormone therapy products for at least one week.
  - Transdermal estrogen or estrogen/progestational products for at least eight weeks.
  - Oral estrogen and/or progestational therapy for at least eight weeks.
  - Progestational implants, estrogen or estrogen/progestational injectable drug therapy for at least three months.
  - Estrogen pellet therapy or progestational injectable drug therapy for at least six months.
  - Percutaneous estrogen lotions/gels for at least four weeks.
- Uncontrolled hypertension greater than or equal to a systolic blood pressure of 140 mm Hg and/or a diastolic blood pressure of greater than or equal to 90 mm Hg.

TREATMENT

After fasting overnight for at least at least 10 hours, in the morning, either the investigational product or the Reference product was administered intravaginally to each subject under ambient temperature conditions by trained female study personnel in the presence of medically qualified personnel and Quality Assurance (QA) auditor(s). Each subject was required to remain in supine position for four hours after dosing and to refrain from strenuous activity until they were checked out of the clinic. Subjects were fasted for an additional four hours after dosing; after that, meals were provided by clinic personnel at scheduled times.

INVESTIGATIONAL PRODUCT and Mode of Administration:
Test Product: TX-004HR (Estradiol Vaginal (b) (4) Inserts) 25 µg
Manufactured for: TherapeuticsMD Inc., Florida, USA
Manufactured by: (b) (4)
Lot No.: PN0089-04
Manufacture date: 31 Jul 2013
Expiration date: July 2014
Dose and Mode of Administration: TX-004HR 25 µg was administered to each subject intravaginally.

Reference Product: VAGIFEM® 25 µg (Estradiol Vaginal Tablets)
Manufactured by: Novo Nordisk A/S
Lot No.: CE70169
Manufactured date: Not available
Expiration date: December 2015
Dose and Mode of Administration: VAGIFEM® 25 µg was administered to each subject intravaginally.

PHARMACOKINETIC EVALUATION

Blood Sampling:
A total of 13 blood samples (10 mL each) for pharmacokinetic (PK) measurements were taken at prescribed time points per the study protocol: -01.00, -00.50, 00.00, 01.00, 02.00, 04.00, 06.00, 08.00, 10.00, 12.00, 14.00, 18.00 and 24.00 hours (± 2 minutes for all sampling times except the zero hour blood draw, which could be collected within five minutes prior to dosing).

Reviewer's Note:
The time of blood collections deviated from protocol-specified times for eight subjects. All deviations were within 10 minutes of scheduled time of collection. Therefore, these protocol deviations were deemed not to have any significant impact on the outcome of the study.

Bioanalytical Method

Estradiol, estrone and estrone conjugate concentrations were determined in plasma using a fully validated Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method for the simultaneous determination of estradiol and estrone in human plasma and for their content of estrone sulfate at Micro Therapy Research Labs Private Limited, Chennai, India. The detailed method is described in the Table below.

Overview of Bioanalytical Method for Study ESTR-1K-500-12

Analyte(s) of interest	**Estradiol (E2) & Estrone(E1)**	**Estrone Sulfate**
Method No.	MTR-BA-M-249-00	MTR-BA-A-002-16
Methodology	UPLC-MS/MS	UPLC-MS/MS
Biological matrix	Plasma	Plasma & Serum
Extraction method	Solid phase, derivatization, & liquid-liquid	Solid phase
Calibration curve range	E2: 1.996-703.2 pg/mL E1: 9.908-3490.6 pg/mL	20.08-5098.67 pg/mL
Internal standard	d_4-estradiol	d_4-estrone sulfate
Bioanalytical Report No.	ESTR-1K-500-12 (E2&E1)	ESTR-1K-500-12 (E1S)
Validation Report No.	MV-249-13-01	MV-266-13-01
Inter-run accuracy for each QC	E2: LQC (10.83pg/mL): 0.32% bias MQC (270.95 pg/mL): 5.52% bias HQC (537.04pg/mL): -5.47% bias E1: LQC (53.96 pg/mL): 2.29% bias MQC (1333.16 pg/mL): 7.94% bias HQC (2641.783 pg/mL): -4.49%	LQC (599.40 pg/mL): -7.55% bias MQC (2446.08 pg/mL): -6.47% bias HQC (4352.32 pg/mL): -3.72% bias
Inter-run precision for each QC	E2: LQC (10.83 pg/mL): 0.70% CV MQC (270.95 pg/mL): 5.77% CV HQC (537.04 pg/mL): 2.64% CV E1: LQC (53.96 pg/mL): 3.03% CV MQC (1333.16 pg/mL): 5.42% CV HQC (2641.78 pg/mL): 1.68% CV	LQC (599.40 pg/mL): 5.00% CV MQC (2446.08 pg/mL): 1.36% CV HQC (4352.32 pg/mL): 2.98% CV

Long-term stability	232 days at -20°C & -70°C	87 days at -20°C & -70°C
Freeze-thaw stability	Demonstrated for 4 cycles at -30 ± 20ºC and -70 ± 10ºC	Demonstrated for 4 cycles at -30 ± 20ºC and -70 ± 10ºC
Incurred sample reanalysis (ISR)	E2: 79.72% passed E1: 72.03% passed % analyzed: 15%	95.14% passed % analyzed: ~15%

CV = LQC = low quality control, MQC = mid quality control, HQC = high quality control

Reviewer's Comment:
Time from first sample drawn to last sample analyzed (including ISR) was 40 days. Therefore, the samples were analyzed within the demonstrated long term stability time.

Calculation for PK parameters:
The PK parameters for the estradiol, estrone, and estrone sulfate concentrations at each sampling time were evaluated by analysis of variance (ANOVA) with sequence, subjects-within-sequence, period, and treatment as factors, using a general linear models approach. The ANOVA for areas and peak concentrations were conducted on natural log (ln) transformed values. The 90% confidence intervals (CIs) on the geometric mean area and C_{max} ratios for the Test product compared to the Reference product were constructed to compare the bioavailability of the Test product to that of the Reference product. Results for both baseline adjusted and baseline unadjusted data were provided.

Baseline Adjustment: Baseline concentrations were determined at -1.0, -0.5, and zero hours for each dosing period, and baseline correction was subject and period specific. The method for baseline correction was arithmetic, with the mean of the pre-dose concentrations being subtracted from all the post-dose concentrations. If a negative concentration value resulted after baseline correction, this was set to zero. In addition, the baseline-adjusted pre-dose concentration (zero-hour) was also set to zero. The data was analyzed using both the uncorrected and baseline corrected data.

PHARMACOKINETIC RESULTS

Estradiol

- **Baseline-adjusted**
  The baseline-adjusted mean plasma estradiol concentration-time profiles following vaginal administration of TX-004HR 25 µg (Test product) and VAGIFEM 25 µg (Reference product) are shown in **Figure 1.** When compared with VAGIFEM**,** TX-004HR showed a more rapid systemic absorption profile with an earlier two-hour tmax and estradiol concentrations

returned to baseline in 10-14 hours. Maximum estradiol concentrations were approximately 6-9 fold greater than endogenous baseline concentrations of 5-7 pg/mL. VAGIFEM administration resulted in a broader, longer absorption profile. Mean tmax was 12 hours and estradiol concentrations did not return to baseline at the end of the 24-hour dosing interval. Mean (± SD) C_{max} was 25.33 ± 12.55 pg /mL for TX-004HR and was 51.53 ± 33.23 pg/mL for VAGIFEM. Mean (± SD) of AUC_{0-24} was 99.85 ± 57.91 pg.hr/mL for TX-004HR and was 350.5 ± 163.0 pg.hr/mL for VAGIFEM **(Table 1).**

Figure 1 Linear Plot of Least-Squares Mean Plasma Estradiol – Baseline-Adjusted Concentrations versus Time (N = 36)

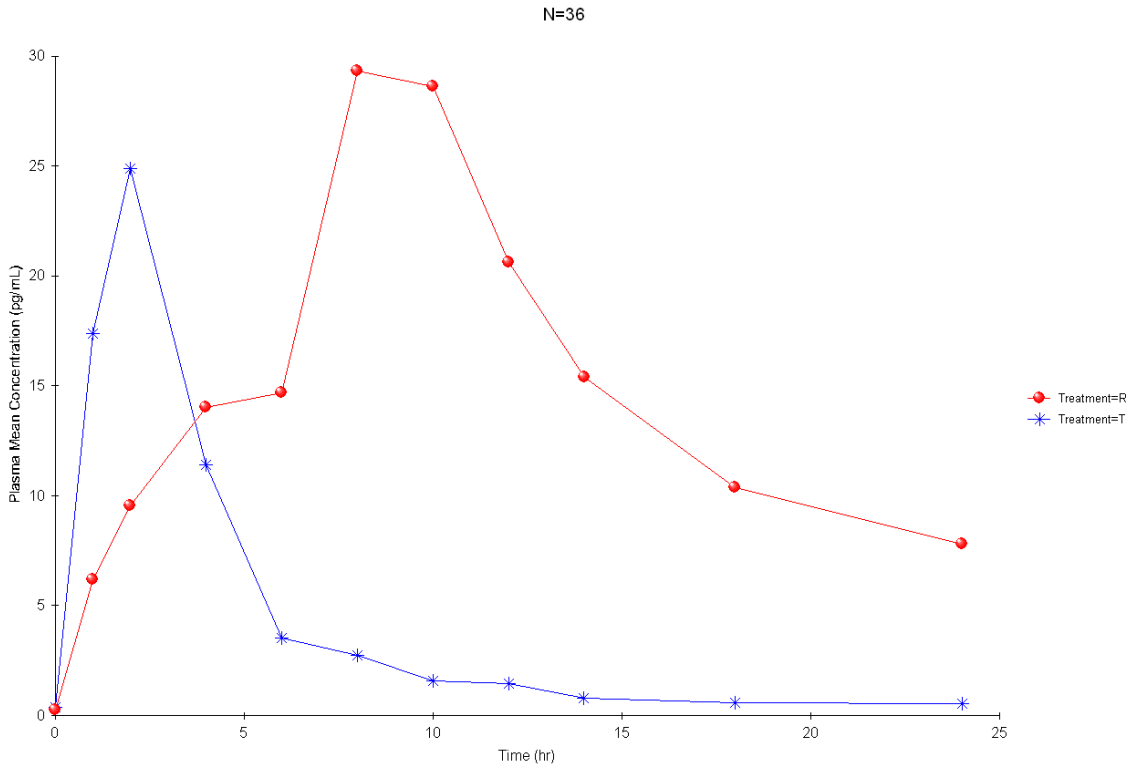


Table 1 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estradiol – Baseline-Adjusted (N = 36)

Parameter	**TX-004HR** (Test)	**VAGIFEM** (Reference)
C_{max} (pg/mL)	25.33 ± 12.55	51.53 ± 33.23
AUC_{0-24}	99.85 ± 57.91	350.5 ± 163.0
T_{max} (hrs)	1.92 ± 0.50	12.50 ± 5.66

- **Baseline-unadjusted**

The baseline-unadjusted mean plasma estradiol concentration-time profiles following vaginal administration of TX-004HR 25 µg (Test product) and VAGIFEM 25 µg (Reference product) are shown in **Figure 2**. **Table 2** provides PK parameters of the Test product and the Reference product for estradiol without baseline adjustment. Mean (± SD) of C_{max} for Test product was 31.03 ± 12.30 pg/mL, and for Reference product was 59.84 ± 34.39 pg/mL. Mean (± SD) of AUC_{0-24} for Test product was 218.7 ± 103.6 pg.hr/mL, and for Reference product was 545.4 ± 344.3 pg.hr/mL.

Figure 2: Linear Plot of Least-Squares Mean Plasma Estradiol - Unadjusted Concentrations versus Time (N = 36)

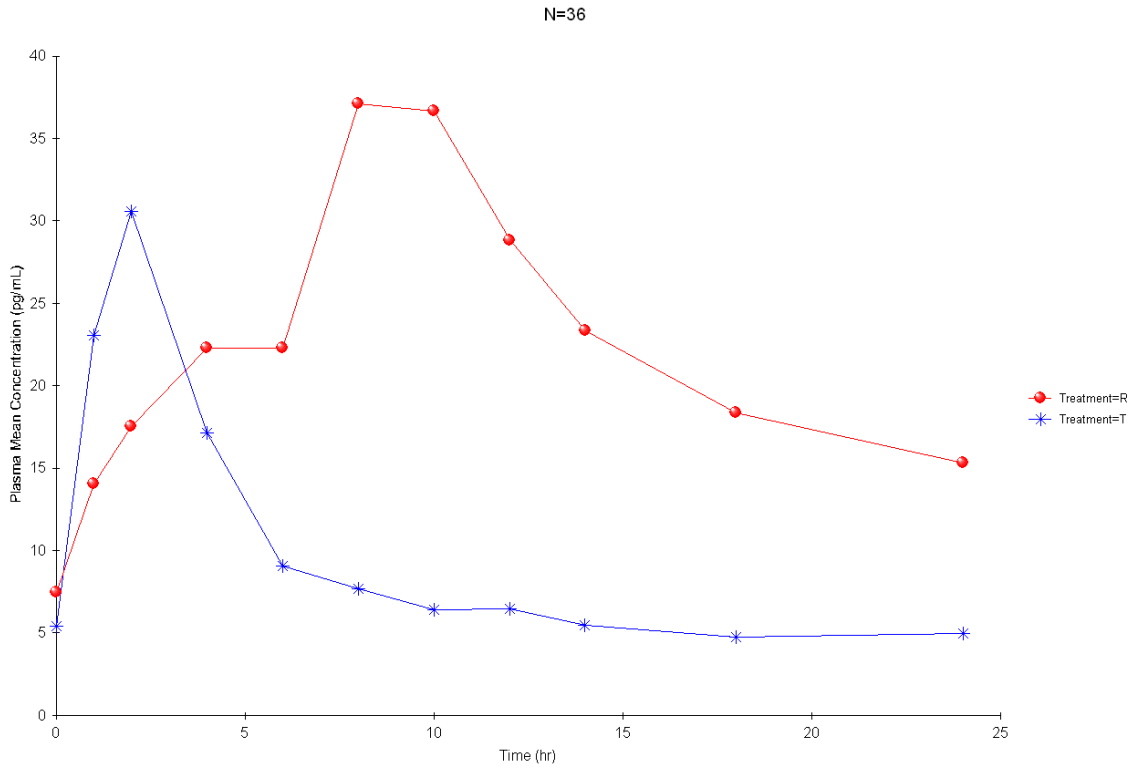


Table 2 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estradiol – Baseline-unadjusted (N = 36)

Parameter	**TX-004HR** (Test)	**VAGIFEM** (Reference)
C_{max} (pg/mL)	31.03 ± 12.30	59.84 ± 34.39
AUC_{0-24} (h*pg/mL)	218.7 ± 103.6	545.4 ± 344.3
T_{max} (hrs)	1.92 ± 0.50	12.50 ± 5.66

Estrone

- **Baseline-adjusted**

  Figure 3 showed the baseline-adjusted mean plasma estrone concentration-time profiles following vaginal administration of TX-004HR 25 µg and VAGIFEM 25 µg**.** Mean (± SD) of C_{max} was 14.19 ± 11.18 pg/mL for TX-004HR, and was 30.50 ± 31.40 pg/mL for VAGIFEM. Mean (± SD) AUC_{0-24} was 90.42 ± 85.53 pg.hr/mL for TX-004HR, and was 233.6 ± 215.5 pg.hr/mL for VAGIFEM (**Table 3**).

Figure 3: Linear Plot of Least-Squares Mean Plasma Estrone – Baseline-Adjusted Concentrations versus Time (N = 36)

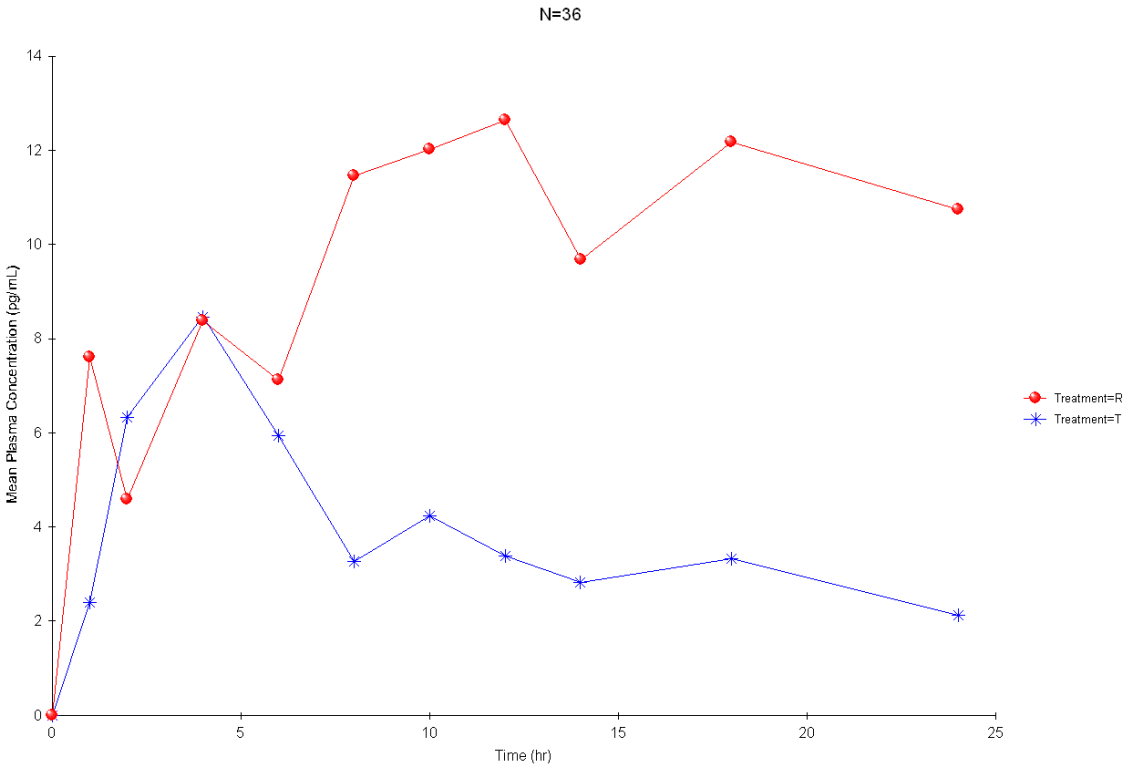


Table 3 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone – Baseline-Adjusted (N = 36)

Parameter	**TX-004HR** (Test)	**VAGIFEM** (Reference)
C_{max} (pg/mL)	14.19 ± 11.18	30.50 ± 31.40
AUC_{0-24} (h*pg/mL)	90.42 ± 85.53	233.6 ± 215.5
T_{max} (hrs)	6.34 ± 4.64	13.35 ± 6.73

- **Baseline-unadjusted**

The PK profiles of baseline-unadjusted estrone are shown in **Figure 4**. Mean (± SD) C_{max} for TX-004HR was 43.80 ± 17.40 pg /mL, and for VAGIFEM was 61.10 ± 40.24 pg/mL. Mean (± SD) AUC_{0-24} for TX-004HR was 742.7 ± 322.3 pg.hr/mL, and for VAGIFEM was 941.5 ± 576.9 pg.hr/mL (**Table 4**).

Figure 4: Linear Plot of Least-Squares Mean Plasma Estrone - Unadjusted Concentrations versus Time (N = 35)

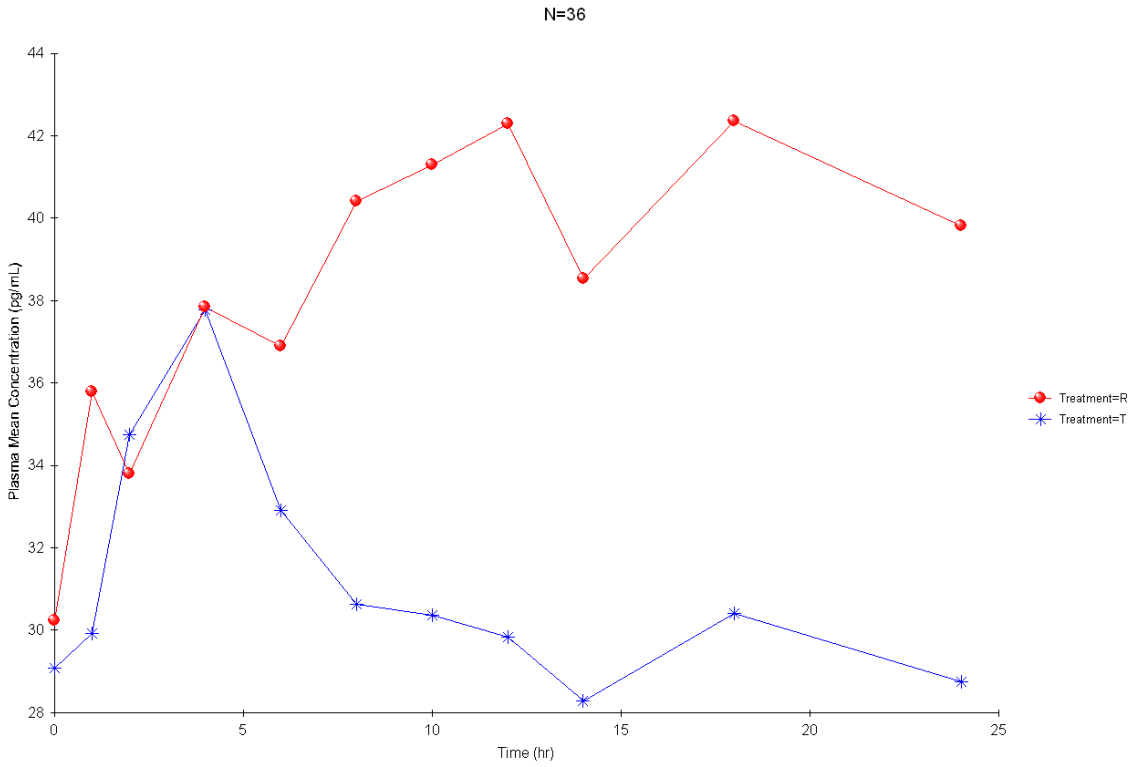


Table 4 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone – Baseline-unadjusted (N = 36)

Parameter	TX-004HR (Test)	VAGIFEM (Reference)
C_{max} (pg/mL)	43.80 ± 17.40	61.10 ± 40.24
AUC_{0-24} (h*pg/mL)	742.7 ± 322.3	941.5 ± 576.9
T_{max} (hrs)	6.17 ± 4.69	13.53 ± 6.73

Estrone Sulfate

- **Baseline-adjusted**

Figure 5 showed the baseline-adjusted estrone sulfate mean plasma concentration-time profiles following vaginal administration of TX-004HR 25 µg and VAGIFEM 25 µg. Mean (± SD) C_{max} for TX-004HR was 727.6 ± 668.3 pg/mL, and for VAGIFEM was

969.0 ± 814.8 pg/mL. Mean (± SD) AUC_{0-24} for TX-004HR was 6533 ± 6092 pg.hr/mL, and for VAGIFEM was 9343 ± 7370 pg.hr/mL (**Table 5**).

Figure 5 Linear Plot of Least-Squares Mean Plasma Estrone Sulfate – Baseline-Adjusted Concentrations versus Time (N = 36)

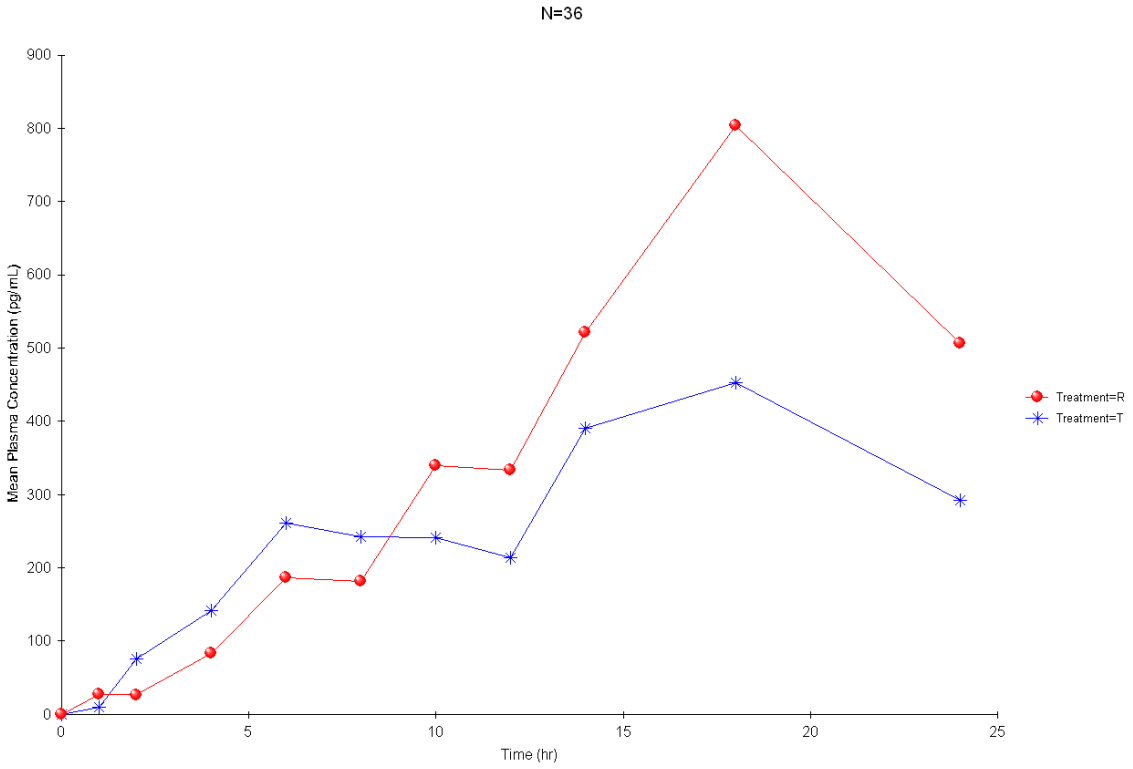


Table 5 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone Sulfate – Baseline-Adjusted (N = 36)

Parameter	**TX-004HR** (Test)	**VAGIFEM** (Reference)
C_{max} (pg/mL)	727.6 ±668.3	969.0 ± 814.8
AUC_{0-24}	6533 ± 6092	9343 ± 7370
T_{max} (hrs)	13.26 ± 6.10	16.22 ± 3.47

- **Baseline-unadjusted**

  The baseline-unadjusted PK profiles of estrone sulfate for TX-004HR and VAGIFEM are shown in **Figure 6.** Mean (± SD) C_{max} was 1210 ± 825.8 pg/mL for TX-004HR, and was 1530 ± 1200 pg/mL for VAGIFEM. Mean (± SD) AUC_{0-24} was 17500 ± 11200 pg.hr/mL for TX-004HR, and was 22500 ± 17370 pg.hr/mL for VAGIFEM (**Table 6**).

Figure 6: Linear Plot of Least-Squares Mean Plasma Estrone Sulfate - Unadjusted Concentrations versus Time (N = 36)

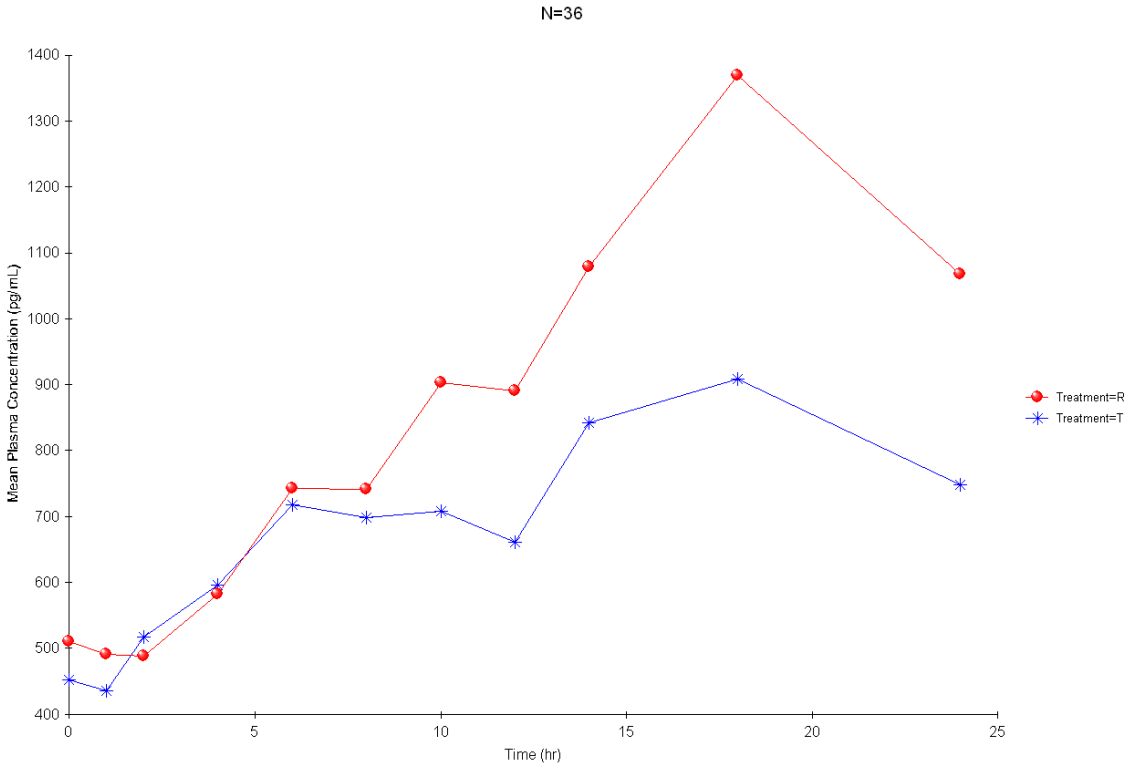


Table 6 Summary of PK Parameters (Arithmetic Mean ± Standard Deviation) of Test Product and Reference Product of Estrone Sulfate – Baseline-unadjusted (N = 36)

Parameter	**TX-004HR** (Test)	**VAGIFEM** (Reference)
C_{max} (pg/mL)	1210 ± 825.8	1530 ± 1200
AUC $_{0-24}$ (pg.hr/mL)	17500 ± 11200	22500 ± 17400
T_{max} (hrs)	12.92 ± 6.35	16.22 ±3.47

Statistical Results

Geometric least square means, intra-subject coefficient of variation (CV%), Test/Reference (T/R) ratios (expressed as a percentage) and 90% CI were determined for C_{max} and AUC_{0-24} for baseline adjusted and unadjusted data of estradiol, estrone and estrone sulfate. These results are summarized in **Table 7** and **Table 8**.

Based on the statistical results obtained, systemic exposures of estradiol, estrone and estrone sulfate following vaginal administration of TX-004HR 25 µg was statistically significantly lower than that of VAGIFEM 25 µg in healthy postmenopausal women.

Table 7: Statistical Results of Test Product versus Reference Product for Baseline-Adjusted Estrogens

	PK Parameter	Geometric Least Square Mean		Intra-Subject CV%	T/R Ratio (%)	90% Confidence Interval
		Test Product (T)	Reference Product (R)			
Estradiol (N=36)	**C_{max} (pg/mL)**	23.08	42.70	54.0	54.1	44.2 - 66.1
	AUC $_{0-24}$ (pg hr/mL)	89.21	292.1	70.4	30.5	23.7 - 39.3
Estrone (N=36)	**C_{max} (pg/mL)**	10.79	23.58	99.6	45.8	33.0 - 63.6
	AUC $_{0-24}$ (pg hr/mL)	51.25	165.47	157	31.0	19.8 - 48.4
Estrone Sulfate (N=36)	**C_{max} (pg/mL)**	490.0	730.6	58.8	67.1	53.8 - 83.6
	AUC $_{0-24}$ (pg hr/mL)	4233	7323	82.6	57.8	43.2 - 77.3

Table 8: Statistical Results of Test Product versus Reference Product for Baseline-unadjusted Estrogens

	PK Parameter	Geometric Least Square Mean		Intra-Subject CV%	T/R Ratio (%)	90% Confidence Interval
		Test Product (T)	Reference Product (R)			
Estradiol (N=36)	**C_{max} (pg/mL)**	29.07	52.06	43.3	55.8	47.4 - 65.9
	AUC $_{0-24}$ (pg hr/mL)	192.3	483.8	43.9	39.8	33.6 - 47.0
Estrone (N=36)	**C_{max} (pg/mL)**	40.73	54.25	27.51	75.1	67.4 - 83.6
	AUC $_{0-24}$ (pg hr/mL)	673.5	840.1	23.4	80.2	73.1 - 87.9
Estrone Sulfate (N=36)	**C_{max} (pg/mL)**	956.5	1190	34.3	80.4	70.4 - 91.8
	AUC $_{0-24}$ (pg hr/mL)	14310	17740	28.1	80.7	72.3 - 90.1

SAFETY EVALUATION

No adverse events, serious adverse events, or deaths were reported during course of the study. Based on the review of the clinical and laboratory safety data, the study medication TX-004HR Estradiol 25 μg inserts was found to be safe and well tolerated in the study subjects.

SUMMARY

When compared with VAGIFEM**,** TX-004HR showed a more rapid systemic absorption profile with an earlier two-hour tmax and estradiol concentrations returned to baseline in 10-14 hours. Maximum estradiol concentrations were approximately 6-9 fold greater than endogenous

baseline concentrations of 5-7 pg/mL. VAGIFEM administration resulted in a broader, longer absorption profile. Mean tmax was 12 hours and estradiol concentrations did not return to baseline at the end of the 24-hour dosing interval.

The TX-004HR Test product provided approximately one-third to two-thirds of estradiol systemic exposure of the Reference VAGIFEM product. In particular, for unadjusted estradiol plasma concentrations, the Test/Reference ratio (T/R) for C_{max} was 56% (47% to 66%; 90% CI) and for AUC was 40% (34% to 47%). For Baseline-adjusted estradiol, the T/R ratios and 90% confidence intervals for C_{max} and AUC were 54% (44% to 66%) and 31% (24% to 39%), respectively. These PK parameter ratios demonstrated that TX-004HR provides lower, more modest systemic exposure than VAGIFEM.

Estrone and estrone sulfate concentration-time profiles were typical of metabolite formation processes. They were formed and appeared later in the 24 hour sampling profile, with limited time points for evaluation of elimination processes. There were only modest increases from baseline values of estrone. Test/Reference ratios for the metabolites were similar to those observed for parent estradiol.

Both TX-004HR and VAGIFEM were safe and well tolerated following a single 25 µg dose in all participating subjects.

In conclusion, systemic exposures of estradiol and its metabolites estrone and estrone sulfate following vaginal administration of TX-004HR (Estradiol Vaginal (b) (4) Inserts) 25 µg was statistically significantly lower than that of VAGIFEM (Estradiol Vaginal Tablets) 25 µg in healthy, adult, postmenopausal female subjects.

TXV14-01 PK Sub-Study

A Phase 3, Randomized, Double-Blind, Placebo-Controlled, Multi-Center Trial to Evaluate the Safety and Efficacy of TX-004HR in Postmenopausal Women with Moderate to Severe Symptoms of Vulvar and Vaginal Atrophy

Protocol No:	TXV14-01
Phase:	3
Principal Investigator:	Sebastian Mirkin, MD
Clinical Study Center:	Therapeutics MD, 6800 Broken Sound Parkway NW, 3rd Floor Boca Raton, FL 33487
Clinical Study Dates:	October 30, 2014– October 20, 2015
Analytical Study Facility:	(b) (4)

Review Objective: To review the PK sub-study under Phase 3 study TXV14-01. This sub-study investigated the PK of estradiol and its two key metabolites following once daily vaginal administration of TX-004HR at doses of 4 µg, 10 µg, and 25 µg in a subset of subjects enrolled in the TXV14-01 trial. The data obtained from this study will be used as primary PK data for product labeling.

OBJECTIVES

Primary Objective:

- To assess the safety and efficacy of three doses of TX-004HR (4 µg, 10 µg and 25 µg) compared with placebo at 12 weeks on vaginal superficial cells, vaginal parabasal cells, vaginal pH, and the symptom of moderate to severe dyspareunia (vaginal pain associated with sexual activity) as the most bothersome symptom (MBS) associated with VVA

Secondary Objectives:

- To assess the efficacy of three doses of TX-004HR (4 µg, 10 µg and 25 µg) on vaginal superficial cells, vaginal parabasal cells, vaginal pH, and moderate to severe dyspareunia defined as the MBS compared with placebo at 2, 6, and 8 weeks;
- To assess the efficacy of three doses of TX-004HR (4 µg, 10 µg and 25 µg) compared with placebo at 2, 6, 8, and 12 weeks on vaginal dryness and on vulvar and/or vaginal itching or irritation associated with VVA;
- To assess hormone concentration of estradiol, estrone, and estrone conjugates at Screening 1A, Days 1, 14, and 84 of treatment in a subset of subjects (PK sub-study) for the 4 µg, 10 µg and 25 µg dose groups;
- To assess visual evaluation of the vaginal mucosa at 2, 6, 8, and 12 weeks; and to assess sexual function by the Female Sexual Function Index (FSFI) at 12 weeks

STUDY DESIGN

This was a Phase 3, multi-center, randomized, double-blind, placebo-controlled study design comparing three doses of TX-004HR with placebo for the treatment of moderate to severe dyspareunia due to menopause. A total of 764 postmenopausal female subjects who met study entry criteria were randomized in a 1:1:1:1 ratio to receive TX-004HR 4 µg, TX-004HR 10 µg, TX-004HR 25 µg, or placebo. The total duration of the study was approximately 20 to 22 weeks. This time included a six to eight-week Screening Period, 12 weeks on investigational product, and follow-up approximately 15 days after the last dose of investigation product.

PK Sub-Study Design

A total of 72 subjects were enrolled into the PK sub-study performed at sites with the capability to carry out PK procedures. There were 18 subjects in the 4 µg group, 19 subjects in the 10 µg group, 18 subjects in the 25 µg group, and 17 subjects in the placebo group. These subjects also participated in the main study and were included in the modified intent-to-treat (MITT) efficacy population. Blood samples were collected at Screening, on Study Day 1, Study Day 14, and Study Day 84 (approximately 4 days after the last dose). Serum samples for PK measurements were analyzed for their content of estradiol, free estrone, and total conjugated estrone (using estrone sulfate as the standard).

Selection of Doses in the Study

Based on the results of the PK studies and the Phase 2 clinical study, the 10 µg and 25 µg TX-004HR doses were selected for this study. In order to preserve dose proportionality with the 25 µg and 10 µg doses, and to identify a new lower effective dose, a 4 µg dose was also selected for investigation**.**

Study Subjects

The overall mean age of subjects was 59.1 years and ranged from 40 to 75 years. The majority of subjects were White (86.6%) and the overall mean BMI was 26.7 kg/m^2.

PK Sub-Study: A subset of 72 subjects aged from 41 to 75 years old participated in the PK sub-study. The mean age was 58.5 years and the majority of subjects were White. The overall mean BMI of the PK subjects was 28.2 kg/m^2 (**Table 1**).

Table 1 Demographic Information for Subjects in PK Sub-Study

Parameter	TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=19)	Placebo (N=17)	Total (N=72)
Age (years, Mean ± SD)	57.4 ± 7.11	58.5 ± 7.06	59.2 ± 6.37	58.9 ± 6.08	58.5 ± 6.57
Race, n (%)					
White	18 (100)	18 (94.7)	16 (88.9)	16 (94.1)	68 (94.4)
Black or African American	0 (0)	1 (5.3)	2 (11.1)	1 (5.9)	4 (5.6)

BMI (kg/m2, Mean ± SD)	28.3 ± 5.59	28.2 ± 4.51	28.9 ± 5.26	27.2 ± 5.45	28.2 ± 5.13

Abbreviation: SD – standard deviation; BMI – body mass index

MAIN INCLUSION CRITERIA

- Postmenopausal female subjects between 40 and 75 years old (at the time of Randomization) with at least:
  - 12 months of spontaneous amenorrhea (women <55 years of age with history of hysterectomy without bilateral oophorectomy prior to natural menopause must have had FSH levels > 40 mIU/mL); OR
  - 6 months of spontaneous amenorrhea with FSH levels > 40 mIU/mL; OR
  - At least 6 weeks postsurgical bilateral oophorectomy.
    - ≤ 5% superficial cells on vaginal cytological smear.
    - Vaginal pH > 5.0.
    - Moderate to severe symptom of vaginal pain associated with sexual activity considered the most bothersome vaginal symptom by the subject at Screening Visit 1A.
    - Moderate to severe symptom of vaginal pain associated with sexual activity at Screening Visit 1B.
    - Onset of moderate to severe dyspareunia in the postmenopausal years.
    - Subjects who had a Body Mass Index (BMI) less than or equal to 38 kg/m^2

EXCLUSION CRITERIA

- Use of any of the following:
  - Oral estrogen-, progestin-, androgen-, or selective estrogen receptor modulator
  - (SERM)-containing drug products within 8 weeks before Screening Visit 1A (could enter washout);
  - Transdermal hormone products within 4 weeks before Screening Visit 1A
  - Vaginal hormone products (rings, creams, gels) within 4 weeks before Screening Visit 1A;
  - Intrauterine progestins within 8 weeks before Screening Visit 1A ;
  - Progestin implants/injectables or estrogen pellets/injectables within 6 months before Screening Visit 1A;
  - Vaginal lubricants or moisturizers within 7 days before the Screening Visit 1B vaginal pH assessment
- A history or active presence of clinically important medical disease that might confound the study or be detrimental to the subject, examples include:
  - Hypersensitivity to estrogens;
  - Endometrial hyperplasia;
  - Undiagnosed vaginal bleeding;
  - Have a history of a chronic liver or kidney dysfunction/disorder (e.g., Hepatitis C or chronic renal failure);
  - Thrombophlebitis, thrombosis, or thromboembolic disorders

- Cerebrovascular accident, stroke, or transient ischemic attack;
- Myocardial infarction or ischemic heart disease;
- Malignancy or treatment for malignancy, within the previous 5 years, with the exception of basal cell carcinoma of the skin or squamous cell carcinoma of the skin.
- A history of estrogen dependent neoplasia, breast cancer, melanoma, or any gynecologic cancer, at any time, excluded the subject;
- Endocrine disease (except for controlled hypothyroidism or controlled non-insulin dependent diabetes mellitus).

- Current history of heavy smoking (more than 15 cigarettes per day) or use of e-cigarettes.
- Use of an intrauterine device within 12 weeks before Screening Visit 1A.
- Use of an investigational drug within 60 days before Screening Visit 1A.
- Any clinically important abnormalities on Screening physical exam, assessments, ECG, or laboratory tests.
- Be known to be pregnant or had a positive urine pregnancy test.
- Current use of marijuana.

Reviewer's Note:
No special inclusion or exclusion criteria for subjects in the PK sub-study.

TREATMENT
Subjects received vaginal inserts as a once daily intravaginal treatment for the first 2 weeks and a twice weekly intravaginal maintenance for the following 10 weeks. The 4 treatment arms include 4 µg, 10 µg, 25 µg and placebo. The study subjects were trained by the clinical site staff to self-administer the capsule intra-vaginally. In particular, they were instructed to find a most comfortable position and insert the capsule with the smaller end up into vaginal canal for about 2 inches.

Regarding the timing of drug administration, the capsule should be applied approximately the same hour for the first 14 days. Under the twice weekly regimen, the two drug administrations should be three to four days apart, and should not have exceeded more than twice in a seven-day period.

INVESTIGATIONAL PRODUCT

TX-004HR (estradiol vaginal (b) (4) capsule) is a small, tear-shaped, light pink (b) (4) capsule, with a cloudy fill appearance. TX-004HR is a solubilized estradiol product which is formulated with (b) (4) estradiol (b) (4) the active ingredient, in a (b) (4) capsule form intended for vaginal administration. The placebo capsules contained the excipients in TX-004HR without (b) (4) estradiol (b) (4)

TX-004HR and the matching placebo were manufactured for Therapeutics MD by (b) (4) and Company provided batch numbers, quality control analytical certificates, and other relevant regulatory documentation (**Table 2**).

Table 2 Investigational Product: Batch Numbers and Expiry Dates

Trial Product	Strength	Batch No.	Expiry Date
TX-004HR	4 µg solubilized estradiol plus excipient	PN0089-10	06/30/2016
TX-004HR	10 µg solubilized estradiol plus excipient	PN0089-08	03/31/2016
TX-004HR	25 µg solubilized estradiol plus excipient	PN0089-09	04/30/2016
Placebo	excipient (see above description)	PN0089-07	03/31/2016

PHARMACOKINETIC EVALUATION

Blood Sampling for Natural Estrogens:

- Day 1 and Day 14: Blood samples were collected pre-dose and 2, 4, 6, 10, and 24 hours post-dose.
- Screening and Day 84 (4 days after last twice weekly dose of TX-004HR): Single blood samples were collected

Blood Sampling for Sex hormone binding globulin (SHBG):

SHBG blood samples were obtained on Day 1 and Day 14 at pre-dose and on Day 84

Bioanalytical Method

Estradiol, estrone and estrone conjugates concentrations were determined in serum using two validated gas chromatography /tandem mass spectrometry (GC/MS/MS) methods at (b) (4) The details of analytical methods are described in **Table 3**:

Table 3 Overview of Bioanalytical Method for TXV14-01 PK Sub-Study

Analyte(s) of interest	Estradiol (E2) & Estrone(E1)	Estrone Conjugates
Method No.	TM.1381	TM.1472
Methodology	GC-MS/MS	GC-MS/MS
Biological matrix	Serum	Serum
Extraction method	Solid phase	Solid phase separation of conjugates and hydrolysis to estrone
Calibration curve range	E2: 2.00-500 pg/mL E1: 5.00-1000 pg/mL	25.00-5000 pg/mL
Internal standard	d4-estradiol & d4-estrone	d4-estrone sulfate

Bioanalytical Report No.	8831.082015	8831.082015
Validation Report No.	8267.063014	8752.111514
Inter-run accuracy for each QC	E2: LQC (6.52 pg/mL): -3.37% bias MQC (51.1 pg/mL): 0.00% bias HQC (441 pg/mL): 1.36% bias E1: LQC (17.1 pg/mL): 3.51% bias MQC (120 pg/mL): 2.50% bias HQC (865 pg/mL): 4.62% bias	LQC (50.0 pg/mL): -3.60% bias MQC (625 pg/mL): -1.76% bias HQC (4000 pg/mL): 1.50% bias
Inter-run precision for each QC	E2: LQC (6.52 pg/mL): 8.46% CV MQC (51.1 pg/mL): 3.51% CV HQC (441 pg/mL): 4.92% CV E1: LQC (17.1 pg/mL): 6.78% CV MQC (120 pg/mL): 5.37% CV HQC (865 pg/mL): 6.82% CV	LQC (50.0 pg/mL): 5.31% CV MQC (625 pg/mL): 2.59% CV HQC (4000 pg/mL): 4.63% CV
Long-term stability	846 days at -20°C & -80°C	512 days at -20°C & -80°C
Freeze-thaw stability	Demonstrated for 2 cycles for E2 and 5 cycles for E1 at -20ºC	Demonstrated for 4 cycles at -20ºC
Incurred sample reanalysis (ISR)	E2: 83.8% passed E1: 82.1% passed % analyzed: ~12%	33.7% passed % analyzed: ~12%

CV = LQC = low quality control, MQC = mid quality control, HQC = high quality control

Reviewer's Note and Comment:

- *Time from first sample drawn to last sample analyzed including ISR was 428 days. Therefore, samples were analyzed within the demonstrated long term stability time.*
- *It should be noted that the 3 Phase 1 PK studies included in the submission determined the concentrations of estrone sulfate, the largest component of potential estrone conjugates. The current study quantified the concentrations of total conjugated estrone which includes both glucuronides and sulfates.*

- *The ISR for estrone conjugates failed as only 33.7% of the repeat samples met the acceptance criteria. Based on the interim investigation report, stability of the ISR samples was not the cause of the ISR results. An information request (IR) was sent to the Sponsor for the final investigation report on the cause of the ISR failure. The Sponsor responded on 03/08/2017 and stated that the root cause of ISR failure for estrogen conjugates could not be determined: "The ISR failure for estrone conjugates showed a consistent 20-25% negative bias of the ISR results compared to the original analysis. These results suggested two general areas as a possible root cause: 1) stability of the samples or 2) an inaccurate assay solution. Stability of the samples was ruled out by repeat analysis of a subset of ISR samples (see Report INVRPT.000309).In order to investigate the assay solutions as a potential cause, a comparison of the peak area ratios was made. While comparing the peak area ratios, it was noted that the standard curves in the ISR run had a 20-25% negative bias compared to the analytical runs, which could have led to the bias seen in the ISR concentration results. Of note, at the time the sample analysis was completed, the reference standard had been exhausted. Since the signing of INVRPT.000309, eight experiments were carried out in November 2016.These experiments were intended to compare stored solutions that had been prepared around the time of the sample analysis from Study TXV14-01 to freshly made solutions. These experiments were inconclusive because the negative bias previously observed was not reproduced. Therefore, no assignable cause could be determined".*

Given the concern of problematic analytical method for estrogen conjugates, the accuracy of estrogen conjugate PK measurement is in doubt. (b) (4)

Calculation for PK parameters:

Because estradiol and free and conjugated estrone are present in women as endogenous compounds, the analyses determined PK parameters as "baseline-unadjusted" and "baseline-adjusted" values. Screening and Day 1 pre-dose concentrations were averaged and considered baseline for each subject, unless either measurement resulted in a non-quantifiable value. In this case, reasonable scientific judgment was utilized to estimate a baseline, endogenous estrogen concentration. This baseline value was subtracted at each time point to determine a new, baseline-adjusted concentration for subsequent PK analysis. If the baseline-adjusted concentration resulted in a negative number, the negative number was included in the PK analysis instead of being replaced by a zero as in Studies ESTR-1K-500-12, ESTR-1K-499-12, and ESTR-2036-14.

In the calculation of the unadjusted AUC_{0-24} and $C_{avg\ (0-24)}$ for each analyte and for each visit (Day 1 and Day 14), the concentration at time zero was set at 0.0 pg/mL. All other PK parameters, including all PK concentrations and Baseline concentration, are unaffected by this

rule. The Baseline was determined by averaging concentration of that analyte at screening and on Day 1 before dosing (Time=0).

PHARMACOKINETIC RESULTS

Estradiol

- **Estradiol Baseline Concentrations**

The mean baseline estradiol concentrations for each group are shown in **Table 4**. Screening 1A and Day 1 pre-dose concentrations were averaged and considered Baseline for each group. Mean Baseline values were less than or equal to 5.0 pg/mL for all groups and individual values ranged from 2.00 (the lower limit of quantification; LLOQ) to 15.70 pg/mL.

Table 4 Baseline Estradiol Concentrations

Estradiol Concentration (pg/mL)	TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
Mean (SD)	3.923 (2.433)	4.948 (3.391)	3.624 (1.697)	4.494 (2.605)
Median, CV (%)	2.79, 62.02	3.66, 68.53	3.31, 46.82	3.78, 57.97
Min, Max	2.00, 11.20	2.09, 15.70	2.00, 8.31	2.00, 10.10

- **Baseline-unadjusted Estradiol Concentrations**

The baseline-unadjusted mean serum estradiol concentration-time profiles on Day 1 and Day 14 following once daily vaginal administration of TX-004HR and placebo are shown in **Figure 1.** PK parameters including AUC_{0-24}, $C_{max)}$ and t_{max} are summarized in **Table 5.** Following vaginal administration of 4, 10 and 25 µg TX-004HR (b) (4) estradiol concentrations peaked at about 2 hours post-dose and declined to baseline levels within four to ten hours post-administration.

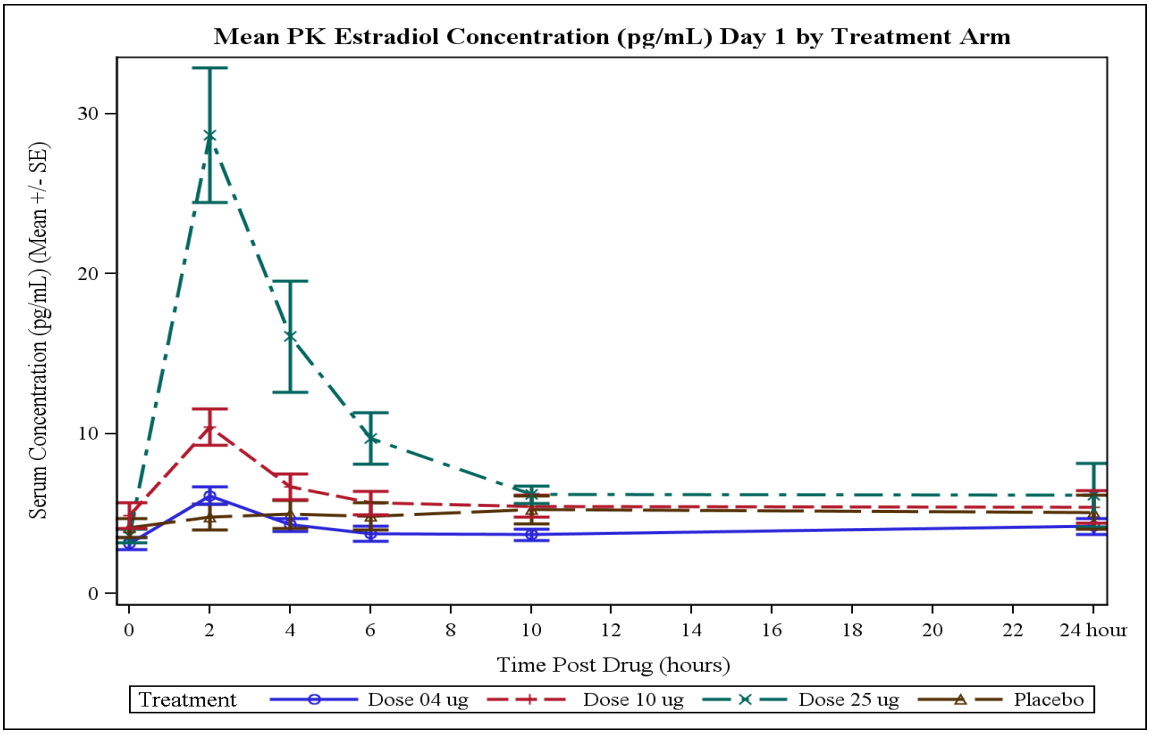
At TX-004HR 25 µg dose, baseline unadjusted C_{max} and AUC_{0-24} were both significantly higher than those of placebo on Day 1 and Day 14. At the lowest dose of TX-004HR 4 µg, systemic exposure of estradiol was minimal and comparable to baseline concentrations of 4-7 pg/mL in postmenopausal women. There were no statistical differences, between TX-004HR 4 µg and placebo, in C_{max} and AUC_{0-24} on Day 1 and Day 14. For TX-004HR 10 µg, modest increases in estradiol concentrations were observed compared to placebo. Only C_{max} on Day 1 was significantly higher than that of placebo and there was no difference as compared to placebo in AUC_{0-24}. AUC_{0-24} values were lower on Day 14 than Day 1 for all dose groups.

Reviewer's Comment

It is noted that estradiol concentrations on Day 14 were lower compared to Day 1. The decreased estradiol exposure over multiple doses is likely due to reduced estradiol

absorption, as the vaginal epithelium in postmenopausal women may become thicker with added estrogen stimulation.

Figure 1 Baseline-unadjusted Mean Estradiol Concentrations on Day 1 and Day 14 following once daily administration of TX-004HR and placebo in healthy postmenopausal women



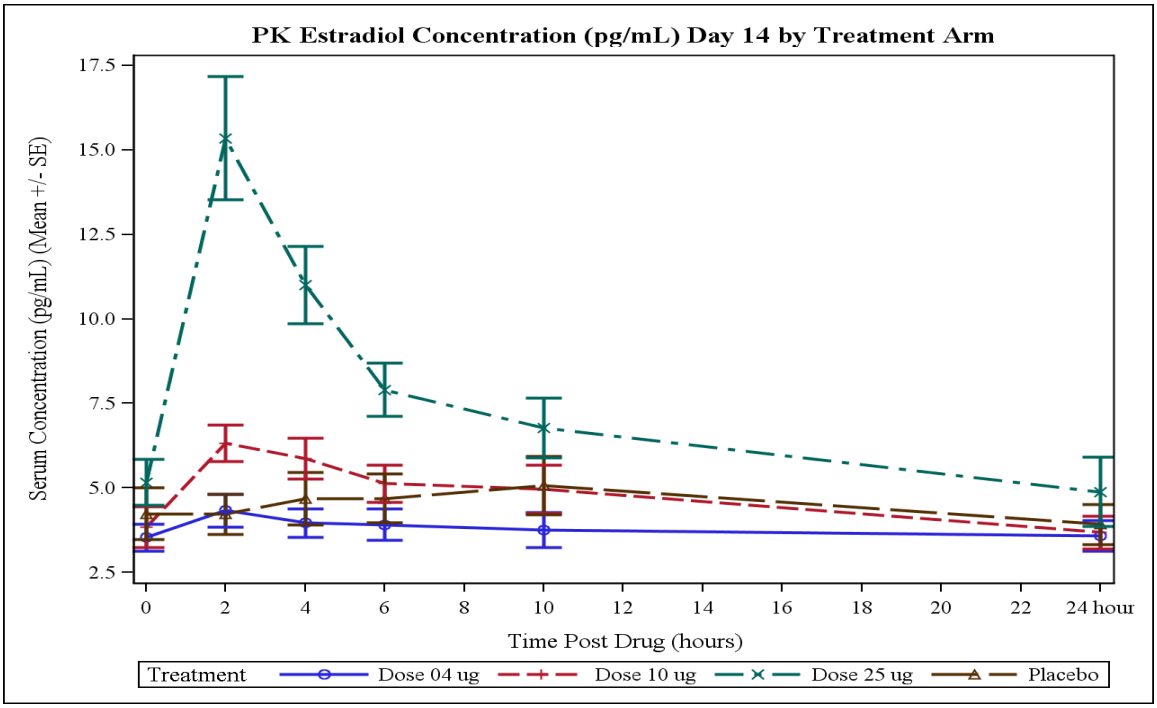


Table 5 Baseline-unadjusted Estradiol PK parameters following once daily administration of 4 µg, 10 µg, 25 µg TX-004HR and placebo in healthy postmenopausal women

Estradiol Parameter	Day	Statist	TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
C_{max} (pg/mL)	Day 01	Mean (SD)	6.484(2.131)	10.94(4.997)	29.8(17.51)	6.55(4.851)
		Median, CV	6.43,32.86	9.53,45.67	23.8,58.75	4.63,74.07
		Pairwise test*	0.9586	0.0116	< 0.0001	--
	Day 14	Mean (SD)	4.826(2.315)	7.337(2.363)	15.67(7.606)	5.484(3.43)
		Median, CV	4.86,47.96	7.6,32.21	14.6,48.54	4.74,62.55
		Pairwise test*	0.5174	0.0702	< 0.0001	--
AUC_{0-24} (h*pg/mL)	Day 01	Mean (SD)	91.66(37.86)	138.2(75.22)	217.4(99.02)	116.6(77.3)
		Median, CV	83.4,41.3	120,54.43	181,45.55	81.9,66.27
		Pairwise test*	0.2292	0.4028	0.0021	--
	Day 14	Mean (SD)	87.22(42.77)	110.1(54.57)	171.6(80.13)	104.2(66.39)
		Median, CV	67.2,49.04	102,49.55	144,46.71	82.6,63.73
		Pairwise test*	0.3829	0.7724	0.0108	--
T_{max} (hrs)	Day 01	Median, CV	2,134	2,132	2,156	10,78
	Day 14	Median, CV	4,95	4,64	2,73	6,42

* pairwise test to placebo

- **Baseline-adjusted Estradiol Concentrations**
  As shown in **Table 6**, baseline-adjusted estradiol systemic exposure was highest with TX-004HR 25 µg and lowest with TX-004HR 4 µg. Although there was a dose-related increase in AUC and C_{max} among the three dose levels, the quantitative assessment in dose proportionality is quite difficult because of the highly variable estradiol concentrations and minimal exposure after baseline correction at 4 µg dose. Compared to the placebo group, the baseline-adjusted AUC_{0-24} and C_{max} were significantly higher for TX-004HR 25 µg on Day 1 and on Day 14. There was no statistical difference in baseline-adjusted AUC_{0-24} and C_{max} between TX-004HR 4 µg and placebo on either day. For TX-004HR 10 µg, only baseline-adjusted C_{max}, but not $AUC_{0-24,}$ was significantly higher compared to placebo on Day 1 and Day 14. Estradiol concentrations were lower on Day 14 compared to Day 1.

Table 6 Baseline-adjusted Estradiol PK parameters following once daily administration of 4 µg, 10 µg, 25 µg TX-004HR and placebo in healthy postmenopausal women

Estradiol Parameter	Day	Statist	TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)

C_{max} (pg/mL)	Day 01	Mean (SD)	2.562(2.173)	5.994(4.441)	26.18(18.19)	2.056(3.479)
		Median, CV	2.33,84.81	5.14,74.08	19.6,69.47	0.895,169.2
		Pairwise test*	0.6074	0.0059	<0.0001	--
	Day 14	Mean (SD)	1.332(1.077)	2.984(1.734)	12.05(7.321)	0.99(1.815)
		Median, CV	1.1,80.88	2.89,58.1	10.8,60.78	0.73,183.4
		Pairwise test*	0.5088	0.0022	<0.0001	--
AUC_{0-24} (h*pg/mL)	Day 01	Mean (SD)	-0.42(46.26)	19.45(22.77)	130.4(111.9)	8.793(32.28)
		Median, CV	7.87,-11100	19.8,117	75.8,85.85	3.01,367.2
		Pairwise test*	0.5018	0.2564	0.0001	--
	Day 14	Mean (SD)	3.348(16.25)	5.662(29.25)	84.58(62.7)	-3.686(30.69)
		Median, CV	1.45,485.3	7.16,516.6	53.8,74.12	-3.65,-832.6
		Pairwise test*	0.4098	0.3629	<0.0001	--

- **Estradiol Concentrations on Day 84**
  Estradiol concentrations at Day 84 were similar to Baseline levels for the TX-004HR 4 µg (4.25 vs 3.92 pg/mL), 10 µg (4.79 vs 4.95 pg/mL, respectively) and placebo (4.36 vs 4.49 pg/mL) groups. For TX-004HR 25 µg group, the Day 84 concentration was 6.65 pg/mL vs 3.62 pg/mL at Baseline. Considering baseline concentration in postmenopausal women ranges between 4-7 pg/ml, the current data indicate no significant estradiol accumulation at Day 84.

  Reviewer's comments:
  C_{max} and AUC values for 25 µg dose on Day 1 in the current study seem to be consistent with the values observed from single-dose PK study ESTR-1K-500-12 and ESTR-2036-14.

Estrone

- **Estrone Baseline Concentrations**
  Estrone is a primary downstream metabolite of estradiol. In the current study, mean baseline estrone concentrations for all groups ranged from 15.3 to 20.3 pg/mL. The mean baseline serum estrone concentrations for each group are shown in **Table 7**.

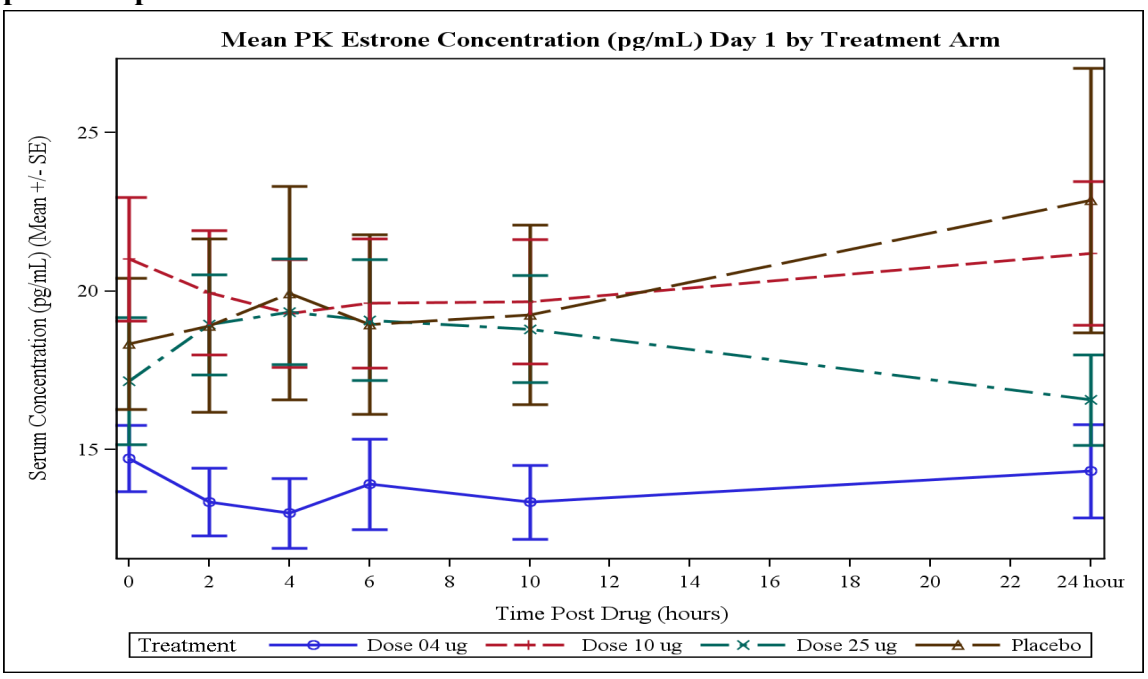
Table 7 Baseline Estrone Concentrations

Estrone Concentration (pg/mL)	**TX-004HR 4 µg (N=18)**	**TX-004HR 10 µg (N=19)**	**TX-004HR 25 µg (N=18)**	**Placebo (N=17)**
Mean (SD)	15.33 (4.803)	20.34 (8.567)	16.73 (7.794)	19.37 (8.778)
Median, CV (%)	15.30, 31.33	19.20, 42.12	15.20, 46.58	17.50, 45.33
Min, Max	8.51, 27.40	9.61, 42.60	8.03, 34.50	6.51, 37.00

- **Baseline-unadjusted Estrone Concentrations**

  Figure 2 shows the mean serum estrone concentration-time profiles following once daily vaginal administration TX-004HR (b) (4) on Day 1 and Day 14**.** PK parameters including AUC_{0-24}, C_{max}, and t_{max} are summarized in **Table 8.** For TX-004HR 10 µg and 25 µg groups, estrone concentrations were similar to the placebo group with no differences noted at either day. Interestingly, TX-004HR 4 µg group showed lower estrone concentrations compared to placebo, likely due to low baseline values for this dose group.

Figure 2: Baseline-unadjusted Mean Estrone Concentrations on Day 1 and Day 14 following once daily administration of TX-004HR and placebo in healthy postmenopausal women



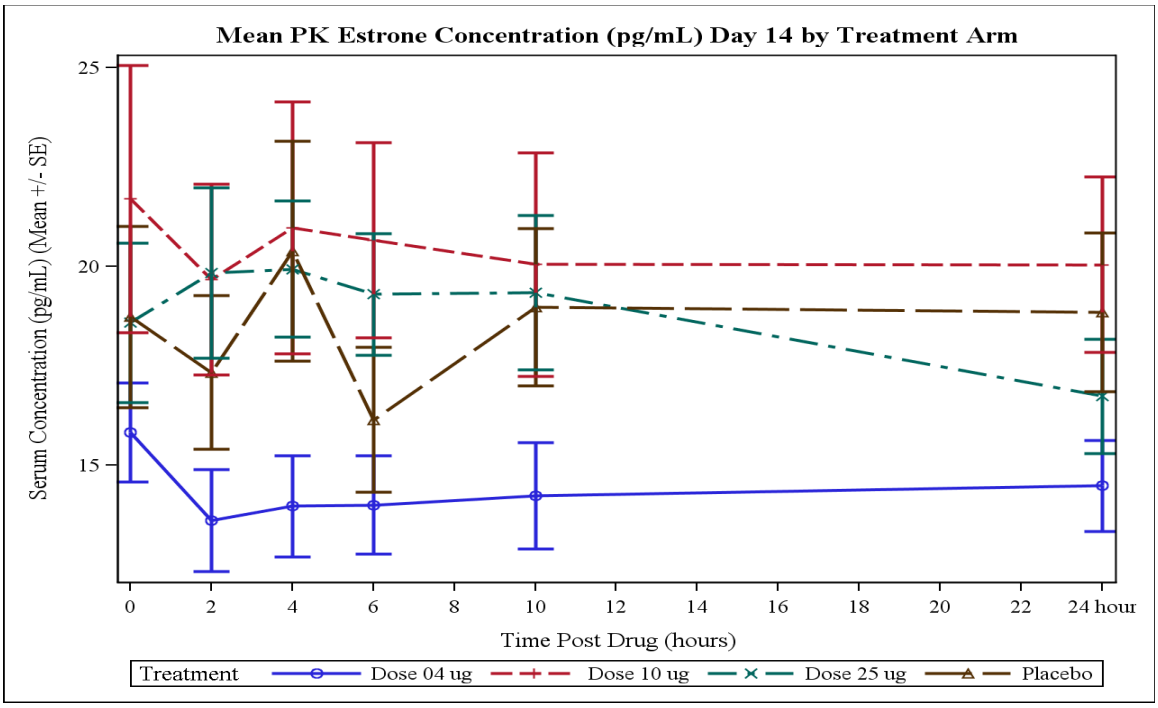


Table 8 Baseline-unadjusted Estrone PK parameters following once daily administration of 4 µg, 10 µg, 25 µg TX-004HR and placebo in healthy postmenopausal women

Estrone Parameter	Day	Statistic	TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
C_{max} (pg/mL)	Day 01	Mean (SD)	15.75 (6.068)	23.52 (9.871)	21.85 (7.733)	25.69 (18.43)
		Median, CV	15.3, 38.54	21.2, 41.98	20.8, 35.39	19.4, 71.74
		Pairwise test*	0.0373	0.6567	0.4223	--
	Day 14	Mean (SD)	15.97 (5.499)	23.93 (13.45)	22.37 (8.947)	22.81 (10.89)
		Median, CV	14.9, 34.43	21.1, 56.21	20.3, 39.99	22, 47.73
		Pairwise test*	0.0275	0.7878	0.8979	--
AUC_{0-24} (h*pg/mL)	Day 01	Mean (SD)	290.2 (123.7)	462.7 (195.6)	419.1 (147.9)	467.9 (278.8)
		Median, CV	284, 42.61	419, 42.28	390, 35.28	360, 59.59
		Pairwise test*	0.0193	0.9487	0.5190	--
	Day 14	Mean (SD)	326.6 (114.1)	464.1 (243.9)	428.7 (161.7)	426.8 (180.7)
		Median, CV	310, 34.94	400, 52.56	396, 37.73	418, 42.34
		Pairwise test*	0.0621	0.6117	0.9738	--
T_{max} (hrs)	Day 01	Median, CV	10,66	6,82	6,82	10,78
	Day 14	Median, CV	6,82	6,85	4,109	10,76

- **Baseline-adjusted Estrone Concentrations**

There were no significant differences in baseline-adjusted C_{max} and AUC_{0-24} values for TX-004HR (at all dose levels) compared to placebo on either Day 1 or on Day 14. Negative values of baseline-adjusted AUC_{0-24} values in TX-004HR 4 and 10 µg groups indicate negligible increase in estrone systemic exposure following TX-004HR administration at these two doses (**Table 9**).

Table 9 Baseline-adjusted Estrone PK parameters following once daily administration of 4 µg, 10 µg, 25 µg TX-004HR and placebo in healthy postmenopausal women

			TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
Estrone Parameter	**Day**	**Statist**				
C_{max} (pg/mL)	Day 01	Mean (SD)	0.42(3.045)	3.178(2.987)	5.115(4.776)	6.327(12.81)
		Median, CV	-0.235,724.5	3.4,93.99	5,93.37	1.6,202.4
		Pairwise test*	0.0659	0.3046	0.71	--
	Day 14	Mean (SD)	0.65(3.488)	3.655(8.792)	5.638(4.806)	3.441(5.686)
		Median, CV	-0.05,539.3	2.2,240.6	5.58,85.25	1.8,165.2
		Pairwise test*	0.0938	0.933	0.2249	--
AUC_{0-24} (h*pg/mL)	Day 01	Mean (SD)	-64.12 (81.83)	-25.4 (63.84)	17.46 (89.57)	3.101 (120.8)
		Median, CV	-58.2, -127.6	-15.9, -251.4	20.1, 513.1	-38.6, 3894
		Pairwise test*	0.0612	0.3751	0.6910	--
	Day 14	Mean (SD)	-41.25 (78.04)	-22.56 (141.8)	27.03 (115.3)	-38.01 (91.14)
		Median, CV	-50.5, -189.2	-49.1, -628.4	15.6, 426.6	-25.1, -239.8
		Pairwise test*	0.9120	0.7058	0.0742	--

- **Estrone Concentrations on Day 84**
  Mean estrone concentrations at Day 84, compared to Baseline, were 16.5 vs 15.3 pg/mL for TX-004HR 4 µg group, 21.0 vs 20.3 pg/mL for TX-004HR 10 µg group, 19.5 vs 16.7 pg/mL for TX-004HR 25 µg group and 17.9 vs 19.4 pg/mL for the placebo group.

Estrone Conjugates

The chromatographic assay for estrone conjugates is designed to quantify all conjugated estrone in the serum. The standard curve is prepared with estrone sulfate which is the largest component (per Sponsor, 90% of the circulating conjugates) of potential estrone conjugates. Since the assay provides a quantitative value for total conjugated estrone, the following tables use the terminology "estrone conjugates" to identify the analytes measured.

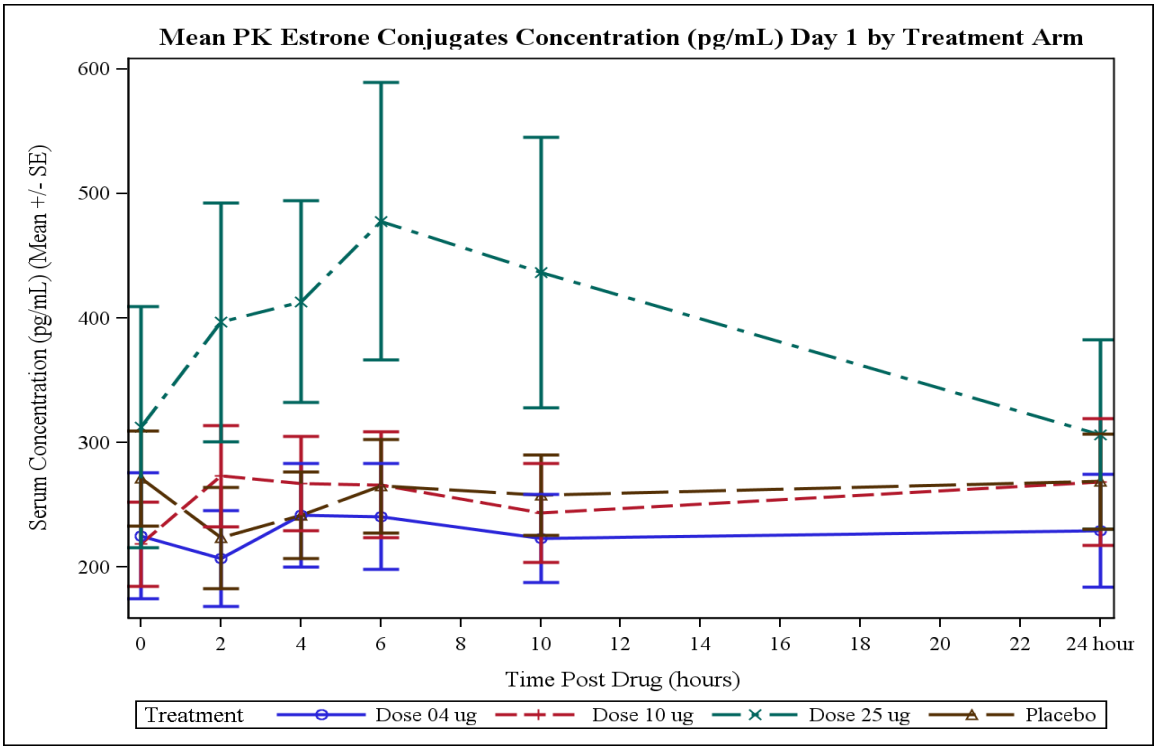
- **Estrone conjugates Baseline Concentrations**
  Mean baseline estrone conjugates serum concentrations are shown in **Table 10**.

Table 10 Baseline Estrone Conjugates Concentrations

Estrone Conjugate Concentration (pg/mL)	TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
N	18	19	18	16
Mean (SD)	237.7 (180.8)	239.2 (174.2)	343.4 (421.1)	275.9 (152.6)
Median, CV (%)	183, 76.04	179, 72.86	176, 122.6	293, 55.3
Min, Max	25.0, 672	59.3, 670	62.6, 1460	67.2, 627

- **Baseline-unadjusted Estrone Conjugates Concentrations**
  Baseline-unadjusted mean serum estrone conjugates concentration-time profiles on Day 1 and Day 14 following once daily vaginal administration TX-004HR (b) (4) are shown in **Figure 3**. Baseline-unadjusted PK parameters for estrone conjugates are summarized in **Table 11**. TX-004HR 10 µg and 25 µg dose groups showed higher estrone conjugates concentrations compared to placebo group. Nonetheless, there were no statistical differences in C_{max} and AUC_{0-24} values between the TX-004HR groups compared to placebo on either day.

Figure 3 Baseline-unadjusted Mean Estrone Conjugates Concentrations on Day 1 and Day 14



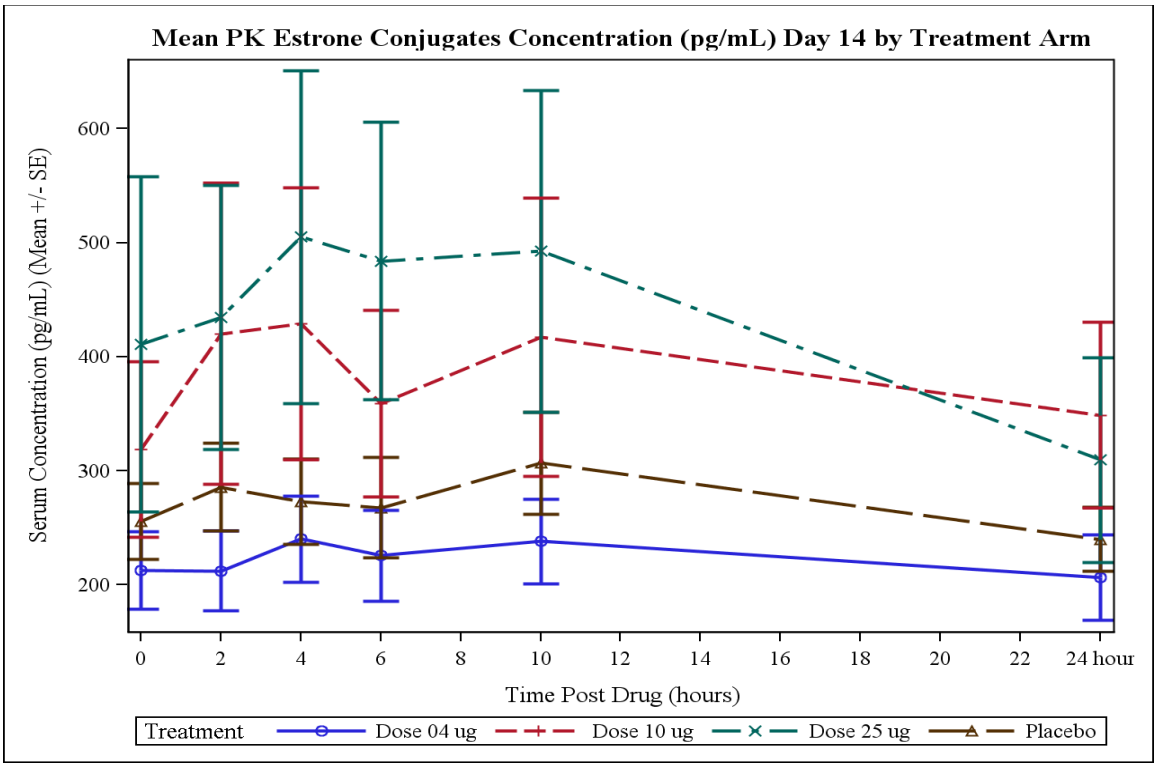


Table 11 Baseline-unadjusted Estrone Conjugate PK parameters following once daily administration of 4 µg, 10 µg, 25 µg TX-004HR and placebo in healthy postmenopausal women

			TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
Parameter	**Day**	**Statist**				
C_{max} (pg/mL)	Day 01	Mean (SD)	273.1 (196.4)	329.4 (226.6)	542.1 (475.5)	309.8 (146.1)
		Median, CV	196, 71.89	247, 68.79	436, 87.72	281, 47.15
		Pairwise test*	0.5369	0.7629	0.0625	--
	Day 14	Mean (SD)	289 (183.8)	511.7 (568.8)	579.5 (610.1)	343.6 (182.2)
		Median, CV	313, 63.6	309, 111.1	371, 105.3	330, 53.02
		Pairwise test*	0.3902	0.2533	0.1356	--
AUC_{0-24} (h*pg/mL)	Day 01	Mean (SD)	5078 (3798)	5932 (4210)	9126 (9186)	5638 (3151)
		Median, CV	4010, 74.81	4530, 70.97	6870, 100.7	4870, 55.9
		Pairwise test*	0.639	0.8157	0.1472	--
	Day 14	Mean (SD)	5173 (3383)	8978 (9811)	9930 (11710)	6275 (3398)
		Median, CV	5810, 65.4	4590, 109.3	6330, 117.9	5890, 54.14
		Pairwise test*	0.3503	0.2898	0.2246	--
T_{max} (hrs)	Day 01	Median, CV	6,79.49	6,100.95	6,48.48	10,74.27
	Day 14	Median, CV	8.4(7.79)	9(8.6)	5.9(2.87)	8.1(6.76)

- **Baseline-adjusted Estrone Conjugates Concentrations**
  Table 12 showed baseline-adjusted PK parameters for estrone conjugates following TX-004HR administration. When adjusting for baseline, mean C_{max} values for TX-004HR 10 µg and 25 µg were significantly higher than placebo on Day 1. But on Day 14, there were no significant differences noted. For baseline adjusted AUC_{0-24}, the only significant difference noted was for TX-004HR 10 µg compared to placebo on Day 1. For TX-004HR 4µg group, AUC values are mostly negative, demonstrating noise around Baseline values, rather than increases in the concentration-time profile in response to TX-004HR dosing.

Table 12 Baseline-adjusted Estrone Conjugates PK parameters following once daily administration of 4 µg, 10 µg, 25 µg TX-004HR and placebo in healthy postmenopausal women

Parameter	Day		TX-004HR 4 µg (N=18)	TX-004HR 10 µg (N=19)	TX-004HR 25 µg (N=18)	Placebo (N=17)
C_{max} (pg/mL)	Day 01	Mean (SD)	35.41(89.09)	90.24(65.2)	198.6(301.5)	27.12(49.69)
		Median, CV	15.3,251.6	73,72.25	177,151.8	36.7,183.2
		Pairwise test*	0.7444	0.0033	0.0318	--
	Day 14	Mean (SD)	48.16(132.6)	277.8(493.6)	236.1(372.4)	67.05(121.8)
		Median, CV	35,275.4	121,177.7	192,157.8	39,181.7
		Pairwise test*	0.6735	0.1065	0.0928	--
AUC_{0-24} (h*pg/mL)	Day 01	Mean (SD)	-510.1 (2123)	192.1 (821)	883.8 (7001)	-807.7 (1127)
		Median, CV	-322, -416.3	287, 427.3	1420, 792.2	-437, -139.5
		Pairwise test*	0.6199	0.0047	0.3473	--
	Day 14	Mean (SD)	-606.2 (2897)	3364 (7934)	1688 (7209)	-189 (2157)
		Median, CV	-65.1, -477.8	419, 235.9	2300, 427	-368, -1141
		Pairwise test*	0.6439	0.0928	0.3244	--

Reviewer's Note and Comment:

One subject in the TX-004HR 10 µg group (Subject 498-013) and one subject in the TX-004HR 25 µg group (473-002) had concentrations in the ng/mL range rather than the pg/mL range of all other subjects. The Sponsor could not find a cause for the extraordinarily high concentrations and believed that the bioanalytical samples were validly assayed. Therefore, the Sponsor included this Subject's data in the tables and PK calculations, which led to some of the higher results and the large variability (400% ~700% CV) on estrone conjugate concentrations for these two dose group.
Given the failed ISR for estrone conjugate as indicated in the earlier section, the large data variability on estrone conjugate exposures may also relate to unreliable bioanalytical method.

Other PK studies including ESTR-2036-14, ESTR-1K-499-12 and ESTR-1K-500-12 all determined estrone sulfate concentrations. Estrone conjugates consist of glucuronide and sulfate metabolites. Considering that estrone sulfate is the predominant conjugate (per Sponsor, 90%), whether determining estrone sulfate concentrations or total conjugated estrone concentrations may not make much difference in terms of numerical values. Therefore, it is reasonable to compare estrone conjugates concentrations determined in the current study with estrone sulfate concentrations obtained from other PK studies. As shown in the Table below, the baseline unadjusted C_{max} and AUCs are comparable across the studies. However, the baseline-corrected AUC of estrone conjugates in the current study were 10-fold lower than the AUC values of estrone sulfate. The discrepancy may provide additional support that the current analytical method for estrone conjugates was not reliable.

Cross-study comparison with Study ESTR-2036-14 and ESTR-1K-500-12 at 25 µg dose

	Study No.	**Current Study Day 1**	**ESTR-2036-14**	**ESTR-1K-500-12**
	Analytes	**Estrone Conjugate**	**Estrone Sulfate**	**Estrone Sulfate**
No Baseline adjustment	C_{max} (pg/mL)	542.1 (475.5)	857.1 (927.1)	1210 (825.8)
	AUC_{0-24} (h*pg/mL)	9126 (9186)	11020 (7414)	17500 (11200)
Baseline-adjusted	C_{max} (pg/mL)	198.6(301.5)	512.1 (985.1)	727.6 (668.3)
	AUC_{0-24} (h*pg/mL)	883.8 (7001)	3908 (8006)	6533 (6092)

- **Estrone Conjugates Concentrations on Day 84**
  Mean estrone conjugates concentrations at Day 84, compared to baseline, were 237 vs 237, 222 vs 239, 500 vs 343, and 250 vs 276 pg/mL for TX-004HR 4 µg, 10 µg, 15 µg, and placebo, respectively.

Sex Hormone Binding Globulin (SHBG)

SHBG values at week 2 and week 12 were comparable to the values at baseline for all treatment groups (**Table 13**)

Table 13 SHBG levels at Baseline, Week 2, and Week 12

	TX-004HR 4 µg (N=18)	**TX-004HR 10 µg (N=19)**	**TX-004HR 25 µg (N=18)**	**Placebo (N=17)**	**Total Subjects (N=72)**

Baseline SHBG (nmol/L) (N)	18	19	18	17	72
Mean (SD)	67.1 (35.65)	74.8 (45.39)	63.3 (34.43)	67.8 (28.47)	68.4 (36.21)
Week 2 SHBG (nmol/L) (N)	17	19	18	17	71
Mean (SD)	68.5 (43.29)	76.7 (48.11)	65.5 (36.21)	65.7 (33.43)	69.3 (40.24)
Week 12 SHBG (nmol/L) (N)	17	19	18	16	70
Mean (SD)	67.5 (44.84)	79.2 (50.44)	62.7 (35.56)	79.4 (64.63)	72.2 (49.03)

SUMMARY

The baseline-unadjusted arithmetic means of estradiol, estrone, and estrone conjugates PK parameters (Mean ± SD) following multiple doses of placebo, TX-004HR 4 µg, 10 µg and 25 µg are summarized in **Table 14**.

Table 14 Arithmetic Means of Estradiol (E2), Estrone (E1), and Estrone Conjugate (E1C) PK Parameters (Mean ± SD) Following Multiple Doses of Placebo, 4 µg, 10 µg and 25 µg TX-004HR – Unadjusted for Baseline

		E2		**E1**		**E1C**	
		AUC_{0-24} (pg·h/mL)	**C_{max} (pg/mL)**	**AUC_{0-24} (pg·h/mL)**	**C_{max} (pg/mL)**	**AUC_{0-24} (pg·h/mL)**	**C_{max} (pg/mL)**
Placebo	Day 1 (N=17)	116.6 ± 77.3	6.6 ± 4.8	467.9 ± 278.8	25.7 ± 18.4	5638 ± 3151	309.8 ± 146.1
	Day 14 (N=17)	104.2 ± 66.4	5.5 ± 3.4	426.8 ± 180.7	22.8 ± 10.9	6275 ± 3398	343.6 ± 182.2
TX004HR 4 µg	Day 1 (N=18)	91.7 ± 37.9	6.5 ± 2.1	290.2 ± 123.7	15.8 ± 6.1	5078 ± 3798	273.1 ± 196.4
	Day 14 (N=17)	87.2 ± 42.8	4.8 ± 2.3	326.6 ± 114.1	16.0 ± 5.5	5173 ± 3383	289 ± 183.8
TX004HR 10 µg	Day 1 (N=19)	138.2 ±75.2	10.9 ± 5.0	462.7 ±195.6	23.5± 9.9	5932 ±4210	329.4 ±226.6
	Day 14 (N=18)	110.1 ±54.6	7.3 ±2.4	464.1±243.9	23.9±13.4	8978 ±9811	511.7 ±568.8
TX004HR 25 µg	Day 1 (N=18)	217.4 ±99.0	29.8 ±17.5	419.1 ±147.9	21.8 ±7.7	9126 ±9186	542.1 ±475.5
	Day 14 (N=18)	171.6 ±80.1	15.7 ±7.6	428.7 (161.7)	22.4 ±9.0	9930 ±11710	579.5 ±610.1

Estradiol

At the lowest dose of TX-004HR 4 µg, there was minimal systemic absorption of estradiol and estradiol concentrations were comparable to baseline concentrations of 4-7 pg/mL in postmenopausal women at all time points. Estradiol concentrations at Day 84 (4 days after last TX-004HR dose) were also similar to Baseline. For subjects in TX-004HR 10 µg group, estradiol concentrations peaked at about 2 hours post-dose and dropped to the baseline range at ~6 hours post-dose. Baseline-adjusted C_{max}, but not $AUC_{0-24,}$ was significantly higher compared to placebo on Day 1 and Day 14. Estradiol concentrations at Day 84 were similar to Baseline. Highest estradiol exposures were seen with the highest dose of TX-004HR 25 µg. The baseline-adjusted AUC0-24 and Cmax were both statistical significantly higher than placebo on Day 1 and at Day 14. Additionally, there was no evidence of accumulation at Day 84.

Although there was a dose-related increase in estradiol exposure among the three dose groups, a quantitative assessment in dose proportionality is quite difficult due to highly variable estradiol serum concentrations and minimal exposure after baseline correction at 4 µg dose. It should be noted that AUC0-24 values on Day 14 were lower or close to the values on Day 1. This is likely due to decreased absorption of estradiol, as vaginal epithelium in postmenopausal women may become thicker with continuous estrogen stimulation.

Estrone and Estrone Conjugates

Similar to the trend observed with estradiol, lowest dose of TX-004HR 4 µg did not lead to a significant increase in serum concentrations of the two primary metabolites, estrone and estrone conjugates. Serum concentrations of both metabolites were consistent with normal postmenopausal female concentrations. It is likely that absorption of estradiol was not sufficient enough to result in an increase in the systemic exposure of these downstream, sequential metabolites. For TX-004HR 10 and 25 µg dose groups, estrone exposures as measured by C_{max} and AUC_{0-24} were both similar to the placebo group with no statistical differences noted at either Day 1 or Day 14.µ It should be noted that the PK data for estrone conjugates may not be reliable due to ISR failure.

SHBG

There was no change in SHBG concentrations over 14 days of TX-004HR treatment.

Study ESTR-2036-14 (Not Reviewed)

Study ESTR-2036-14 entitled "An Open-Label, Single-Arm, Single-Period, Single-Dose, Bioavailability Study To Evaluate TX-12-004-HR (Estradiol Vaginal (b) (4) Capsules) Test Formulation Capsules 25 mcg Of TherapeuticsMD Inc., Florida In Healthy, Adult, Human Postmenopausal Female Subjects Of Previous Study" was submitted to the NDA but was not reviewed.

The Applicant stated that study ESTR-2036-14 was conducted to assess the effect of normal activity on the bioavailability of the proposed estradiol vaginal capsule. The sixteen subjects enrolled in study ESTR-2036-14 (seated or ambulatory for 4 hrs post-dose) were a subset of the 36 subjects enrolled in study ESTR-1K-500-12 (supine for 4 hrs post-dose). The Applicant concluded that estrogen concentrations were similar regardless of whether subjects were ambulatory or supine for 4 hrs, and that the activity level or body position does not affect estradiol absorption. It was unclear from the study report how many of the subjects were seated, ambulatory (i.e., upright and walking), or a combination of seated and ambulatory. The Applicant made the above conclusion without presenting statistical analysis of estrogen concentrations to compare body position or activity level.

In the 74-day filing letter, the Applicant was requested to clarify the positioning of the subjects and to submit the statistical analysis comparing body position/activity level vs supine position. In the IR response dated October 10, 2016, the Applicant noted that the records for Study ESTR-2036-14 did not collect whether the 16 subjects were seated, ambulatory, or a combination of seated or ambulatory, only that they did not recline. From this reviewer's perspective, activity level for a subject seated is different from being ambulatory; therefore, subjects from study ESTR-2036-14 should not be combined into one seated/ambulatory group. Without data on the exact positioning of each subject, it not possible to accurately assess how activity or body positioning can affect estradiol exposure from Study ESTR-2036-14.

4.3 Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling elements be included in the final labeling insert:

- Highlights: Dosage and Administration should be updated to recommend a specific starting dose.
- FPI: Section 2.1, Dosage and Administration should be updated to recommend specific starting dose.
- FPI: Section 12.3, Pharmacokinetics section should include PK profiles of product (estradiol concentration vs time) on Day 14.
- [redacted] (b) (4)
- FPI: Section 12.3, Pharmacokinetics section should include table with PK parameters AUC0-24 and Cmax (not Cavg as proposed by the Applicant as this is essentially the same as AUC) for estradiol and estrone on Days 1 and 14 following multiple doses of TX004-HR and placebo.

Arithmetic Mean (SD) of Baseline-Unadjusted Serum Estradiol (E2) and Estrone (E1) Pharmacokinetic Parameters on Days 1 and 14 Following Multiple Doses of TX-004HR and Placebo in Postmenopausal Women.

		E2		E1	
		AUC_{0-24} (pg·h/mL)	**C_{max} (pg/mL)**	**AUC_{0-24} (pg·h/mL)**	**C_{max} (pg/mL)**
Placebo	Day 1 (N=17)	116.6 (77.3)	6.6 (4.8)	467.9 (278.8)	25.7 (18.4)
	Day 14 (N=17)	104.2 (66.4)	5.5 (3.4)	426.8 (180.7)	22.8 (10.9)
TRADENAME 4 mcg	Day 1 (N=18)	91.7 (37.9)	6.5 (2.1)	290.2 (123.7)	15.8 (6.1)
	Day 14 (N=17)	87.2 (42.8)	4.8 (2.3)	326.6 (114.1)	16.0 (5.5)
TRADENAME 10 mcg	Day 1 (N=19)	138.2 (75.2)	10.9 (5.0)	462.7 (195.6)	23.5 (9.9)
	Day 14 (N=18)	110.1 (54.6)	7.3 (2.4)	464.1 (243.9)	23.9 (13.4)
TRADENAME 25 mcg	Day 1 (N=18)	217.4 (99.0)	29.8 (17.5)	419.1 (147.9)	21.8 (7.7)
	Day 14 (N=18)	171.6 (80.1)	15.7 (7.6)	428.7 (161.7)	22.4 (9.0)

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