

# Visual improvements in vaginal mucosa correlate with symptoms of VVA: data from a double-blind, placebo-controlled trial

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## Abstract

**Objective:** To evaluate the response of the vaginal mucosa with TX-004HR and its correlation with vulvar and vaginal atrophy (VVA) symptoms, and whether visual examination is a useful measure for assessing VVA.

**Methods:** REJOICE was a 12-week, phase 3, multicenter, randomized, double-blind, placebo-controlled study of a vaginal, muco-adhesive, 17 $\beta$ -estradiol softgel capsule (TX-004HR 4, 10, and 25  $\mu$ g) in postmenopausal women with VVA and moderate-to-severe dyspareunia. Treatments were self-administered vaginally once per day for 2 weeks, then twice per week for 10 weeks. The vagina was visually examined at baseline and at weeks 2, 6, 8, and 12; changes were evaluated using a 4-item scale for vaginal color, vaginal epithelial integrity, vaginal epithelial surface thickness, and vaginal secretions.

**Results:** Significant improvements were observed with all three TX-004HR doses versus placebo in vaginal color (least square mean score changes of  $-0.96$  to  $-1.06$  for TX-004HR doses vs  $-0.60$  for placebo at week 12), epithelial integrity ( $-0.97$  to  $-1.07$  vs  $-0.60$ ), epithelial surface thickness ( $-0.94$  to  $-1.03$  vs  $-0.61$ ), and secretions ( $-1.01$  to  $-1.06$  vs  $-0.64$ ) ( $P < 0.001$  for all comparisons at all time points). Both Pearson's correlations and logistic regression receiver-operating characteristic curve analyses significantly correlated the sum of the individual visual assessment scores with dyspareunia ( $P < 0.0001$ ) and vaginal dryness ( $P < 0.0001$ ) at 12 weeks.

**Conclusions:** Greater improvements in the vaginal mucosa of postmenopausal women with VVA and moderate-to-severe dyspareunia were observed with TX-004HR versus placebo, and vaginal mucosa assessment scores correlated with vaginal symptoms of dyspareunia and dryness. Visual vaginal assessment by healthcare professionals is a useful measure for diagnosing VVA and assessing response to treatment.

**Key Words:** Dyspareunia – Estradiol – Estrogen therapy – Menopause – Vaginal atrophy – Visual examination.

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**D**epletion of estrogens during menopause can lead to symptoms of vulvar and vaginal atrophy (VVA) in women. VVA is a component of the genitourinary syndrome of menopause (GSM), and collectively includes changes to the labia majora/minora, clitoris, vestibule/introitus, vagina, urethra, and bladder.<sup>1,2</sup> A review of surveys reported up to two-thirds of women have physical evidence of VVA,<sup>3</sup> and approximately 50% of postmenopausal women have VVA symptoms.<sup>4</sup> These symptoms may include vaginal dryness, irritation, itching, dysuria, and/or pain or bleeding with sexual activity.<sup>5,6</sup> Symptoms of VVA are not limited to sexually active women,<sup>5</sup> can range from mild to debilitating,<sup>4</sup> and can negatively affect quality of life.<sup>3,7</sup> Unfortunately, VVA is unlikely to improve over time without treatment,<sup>8</sup> and if treatment is discontinued, the signs and symptoms of VVA tend to recur.

Clinically, a variety of objective and subjective tools are available to practitioners to determine the extent of VVA in postmenopausal women, based mostly on patient history and/or physical examination.<sup>9-12</sup> The most common objective measures used to assess VVA include measuring vaginal pH<sup>13</sup> and vaginal maturation index (VMI).<sup>14</sup> A vaginal pH  $\leq 4.5$  is considered normal, whereas a pH  $\geq 4.6$  is supportive of VVA, as long as the patient does not have bacterial vaginosis.<sup>14,15</sup> Superficial cells above 15% on a vaginal smear is considered normal, whereas  $\leq 5\%$  is typical for postmenopausal women with VVA.<sup>14</sup> Whereas VMI is the standard for VVA confirmation, it is generally not used in clinical practice,<sup>14</sup> because vaginal cytological smears need to be collected and sent for analysis.

Symptoms of VVA can be subjectively assessed using patient questionnaires evaluating the severity of symptoms, such as dyspareunia, vaginal dryness, soreness, irritation, or discharge. These are reflected in quality-of-life measures. VVA can also be objectively measured with a visual examination of the vagina using assessment tools such as the Vaginal Atrophy Index (VAI),<sup>16</sup> the Genital Health Clinical Evaluation (GHCE),<sup>17</sup> the Vaginal Physical Examination Scale,<sup>18</sup> the Vaginal Health Index (VHI),<sup>19</sup> or the Global Atrophy Score.<sup>20</sup> However, no clear consensus has been reached as to which tool is best when visually evaluating VVA. An accepted method of visually assessing the vagina of postmenopausal women could aid clinicians in opening dialog about VVA with their patients, accurately diagnosing VVA, and ultimately determining their patients' optimal treatment and response to treatment.

TherapeuticsMD Inc (Boca Raton, FL) has developed a vaginal, muco-adhesive, softgel capsule that contains solubilized 17 $\beta$ -estradiol (TX-004HR) designed for the treatment of postmenopausal VVA and moderate-to-severe dyspareunia. Each softgel capsule is self-administered without an applicator for ease of use, and rapidly dissolves leading to the release of the micronized estradiol with minimal messy vaginal leakage. The REJOICE trial has recently demonstrated that TX-004HR (4, 10, and 25  $\mu\text{g}$ ) is safe and effective for the treatment of vaginal changes and various self-reported

symptoms of VVA in postmenopausal women.<sup>21</sup> TX-004HR provided significant improvements in the percentage of vaginal superficial and parabasal cells, vaginal pH, and severity of dyspareunia compared with placebo as early as week 2, with improvements maintained through week 12.<sup>21</sup> Treatment with TX-004HR also significantly improved symptomatic vaginal dryness and vulvar and/or vaginal itching/irritation compared with placebo.<sup>21</sup> Moreover, these improvements were achieved with negligible to very low estrogen absorption.<sup>22</sup>

This study reports the effects of TX-004HR (4, 10, and 25  $\mu\text{g}$ ) versus placebo on the vaginal mucosa of postmenopausal women using a visual examination evaluating vaginal color, vaginal epithelial integrity, vaginal epithelial surface thickness, and vaginal secretions. Also reported are correlations between the vaginal visual assessment scores and symptoms of vaginal dryness and dyspareunia. It is proposed that the visual examination may be a meaningful and useful tool for documenting VVA, potentially predicting VVA symptoms, and evaluating women's response to treatment.

## METHODS

### Study design and population

The REJOICE trial was a 12-week, double-blind, randomized, placebo-controlled, phase 3 trial conducted at 89 sites in Canada and the United States (NCT02253173) between October 2014 and October 2015. Women eligible for the study were randomized to either TX-004HR 4, 10, or 25  $\mu\text{g}$  softgel capsules or matching placebo. One capsule was inserted vaginally daily for 2 weeks, and then once every 3 to 4 days for 10 weeks (twice a week). The study was conducted in accordance to the Declaration of Helsinki and complied with the ethical principles of Good Clinical Practice. An independent institutional review board (IRB) approved the study protocol and informed consent form. All participants gave written informed consent before any study-related activities.

Inclusion and exclusion criteria of the REJOICE trial have been previously described in detail.<sup>21</sup> Briefly, postmenopausal women (40-75 years of age) were eligible if they had  $\leq 5\%$  superficial cells on vaginal cytological smear, vaginal pH  $> 5.0$ , menopausal onset of moderate-to-severe dyspareunia as self-reported most bothersome symptom (MBS), body mass index  $\leq 38 \text{ kg/m}^2$ , and were currently sexually active (with vaginal penetration). Women could not participate in the study if they had a history or active presence of clinically important medical disease that might confound the study or be detrimental to their health. Additionally, study participants could not have used oral, transdermal, vaginal, intrauterine, implants/injectables drugs that contained either estrogens, progestins, androgens, or selective estrogen receptor modulators (SERMs) before the study (a washout period between 4 weeks and 6 months was required depending on the specific medication used). Use of prescription and nonprescription medications and remedies known to treat VVA, including vaginal lubricants and moisturizers, was not allowed during the study.

The secondary endpoint of visually evaluating the vaginal epithelium was performed during the gynecological examination at screening and at weeks 2, 6, 8, and 12. A 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe) was used to assess parameters of vaginal color, vaginal epithelial integrity, vaginal epithelial surface thickness, and vaginal secretions (Table 1). VVA symptoms, including dyspareunia, vaginal dryness, and vaginal itching and/or irritation, were also self-assessed using a 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe). The visual examination assessment tool was consistent with vaginal parameters used in the GHCE,<sup>17</sup> the Vaginal Physical Examination Scale,<sup>18</sup> and the VHI.<sup>19</sup> The 4-item visual examination worksheet can be found in the Supplemental Digital Content 1 (<http://links.lww.com/MENO/A224>).

**Statistical analysis**

The intent-to-treat (ITT) population included all women who were randomized and received at least one dose of treatment (N = 764). The modified intent-to-treat (MITT) population (n = 747), which was used for the primary efficacy analyses, included all women in the ITT population who had baseline values for all co-primary efficacy variables (parabasal and superficial cells, vaginal pH, dyspareunia severity) and with at least one postbaseline value for any of the co-primary variables.

Change from baseline in the visual examination of the vaginal epithelium for each TX-004HR dose, as prespecified, was compared with placebo at weeks 2, 6, 8, and 12 using a mixed-effects repeated measures (MMRM) model with the MITT population. The MMRM analysis used participant and intercept as random effects, and treatment group and visit as fixed effects, with an interaction term for visit and treatment, and baseline values as a covariate.

Correlations between the total sum of the visual examination individual scores and the severity of dyspareunia and dryness at week 12 were evaluated by Pearson’s *r* in the entire ITT population, independent of treatment groups. Receiver-operating characteristic (ROC) curves based on logistic regression models were used to determine whether total visual assessment scores had good sensitivity and specificity to detect moderate-to-severe dryness or dyspareunia at week 12. The c-statistic was used to assess the significance of the ROC curve. Sensitivity and specificity, and visual summary score threshold were determined by the standard method of precisely where the line with slope = -1 intersects the tangent line to the ROC curve.

**RESULTS**

**Participant disposition and demographics**

Of the 2,183 women screened, 764 were enrolled and randomized to TX-004HR 4 µg (n = 191), 10 µg (n = 191), 25 µg (n = 190), or placebo (n = 192). Of these, 92% (704/764) of the ITT participants and 94% (703/747) of the MITT participants completed the study. Reasons for discontinuation within the MITT population were similar between groups and

**TABLE 1. Vaginal mucosa assessment scale**

Assessment criteria	Severity			
	No atrophy (0)	Mild (1)	Moderate (2)	Severe (3)
Vaginal color	Pink	Lighter in color	Pale in color	Transparent, either no color or inflamed
Vaginal epithelial integrity	Normal	Vaginal surface bleeds with scraping	Vaginal surface bleeds with light contact	Vaginal surface has petechiae before contact and bleeds with light contact
Vaginal epithelial surface thickness	Rugation and elasticity of vault	Poor rugation with some elasticity noted of vaginal vault	Smooth, some elasticity of vaginal vault	Smooth, no elasticity, constriction of the upper one third of vagina or loss of vaginal tone (cystocele and rectocele)
Vaginal secretions	Normal clear secretions noted on vaginal walls	Superficial coating of secretions, difficulty with speculum insertion	Scant not covering the entire vaginal vault, may need lubrication with speculum insertion to prevent pain	None, inflamed, ulceration noted, need lubrication with speculum insertion to prevent pain

**TABLE 2.** Participant demographic and baseline characteristics (MITT population)

Characteristic	TX-004HR 4 µg (n = 186)	TX-004HR 10 µg (n = 188)	TX-004HR 25 µg (n = 186)	Placebo (n = 187)
<b>Participants</b>				
Age, yrs	59.8 ± 6.0	58.6 ± 6.3	58.8 ± 6.2	59.4 ± 6.0
Race, n (%)				
White	162 (87.1)	165 (87.8)	161 (86.6)	160 (85.6)
Black or African American	20 (10.8)	21 (11.2)	24 (12.9)	21 (11.2)
Asian	3 (1.6)	2 (1.1)	1 (0.5)	1 (0.5)
BMI, kg/m <sup>2</sup>	26.6 ± 4.9	26.8 ± 4.7	26.8 ± 4.8	26.6 ± 4.6
Natural menopause, n (%)	111 (59.7)	114 (60.6)	118 (63.4)	124 (66.3)
Hysterectomized, n (%)	87 (46.8)	86 (45.7)	85 (45.7)	73 (39.0)
Years since menopause, yrs	14.2 ± 8.9	14.3 ± 9.4	13.8 ± 9.4	13.9 ± 9.4
<b>Baseline characteristics</b>				
Superficial cells, %	1.3 ± 1.2	1.2 ± 1.2	1.3 ± 1.2	1.3 ± 1.3
Parabasal cells, %	52.3 ± 39.2	51.3 ± 38.0	53.5 ± 38.3	52.0 ± 39.2
Vaginal pH	6.3 ± 0.9	6.3 ± 0.8	6.3 ± 0.9	6.3 ± 1.0
Dyspareunia, severity score	2.7 ± 0.5	2.6 ± 0.5	2.7 ± 0.4	2.7 ± 0.5
Dryness, severity score	2.3 ± 0.7	2.4 ± 0.7	2.4 ± 0.7	2.4 ± 0.7
<b>Vaginal color</b>				
Mean ± SD	1.8 ± 0.6	1.8 ± 0.6	1.8 ± 0.6	1.8 ± 0.6
Moderate-to-severe, n (%)	139 (74.7)	133 (70.7)	131 (70.4)	130 (69.5)
<b>Vaginal epithelial integrity</b>				
Mean ± SD	1.6 ± 0.9	1.4 ± 0.9	1.5 ± 0.8	1.5 ± 0.8
Moderate-to-severe, %	101 (54.3)	98 (52.1)	93 (50.0)	95 (50.8)
<b>Vaginal epithelial surface thickness</b>				
Mean ± SD	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.6	1.9 ± 0.6
Moderate-to-severe, %	146 (78.5)	136 (72.3)	144 (77.4)	145 (77.5)
<b>Vaginal secretions</b>				
Mean ± SD	1.7 ± 0.7	1.7 ± 0.7	1.7 ± 0.6	1.8 ± 0.6
Moderate-to-severe, %	128 (68.8)	126 (67.0)	125 (67.2)	137 (73.3)

Data are represented as mean ± SD unless stated otherwise.  
MITT, modified intent-to-treat.

included adverse events (n = 9), lack of efficacy (n = 4), investigator decision (n = 2), lost to follow up (n = 9), protocol violation (n = 2), and consent withdrawal (n = 18).

Demographic and baseline characteristics of the MITT population were similar among groups (Table 2). The majority of the women were white, and had a mean age of 59 years and a mean BMI of 27 kg/m<sup>2</sup>.

Baseline vaginal characteristics and visual examination scores are shown in Table 2. Most of the women (50%-79%) had the visual inspection parameters rated as moderate-to-severe at baseline. At baseline, all women had moderate-to-severe dyspareunia with a mean severity score of 2.7; 93.4% reported moderate-to-severe dryness with a mean severity score of 2.4.

### Vaginal assessment

Vaginal color, vaginal epithelial integrity, vaginal epithelial surface thickness, and vaginal secretions significantly improved from baseline at all time points for all TX-004HR groups compared with placebo ( $P < 0.0001$  for all, except  $P < 0.001$  for vaginal secretions at weeks 2 and 6 for TX-004HR 4 µg; Table 3). Specifically at 12 weeks, severity scores significantly decreased by -0.96 to -1.06 for the TX-004HR doses compared with -0.60 for the placebo for vaginal color, by -0.97 to -1.07 vs -0.60 for epithelial integrity, by -0.94 to -1.03 vs -0.61 for epithelial surface thickness, and by -1.01 to -1.06 vs -0.64 for vaginal secretions. Significant improvements in all four visual inspection parameters were noted as early as 2 weeks and were

maintained throughout the 12 weeks of the study with all three doses of TX-004HR. No dose-specific effects were observed.

Significantly more women in the TX-004HR groups than in the placebo group had a none-to-mild severity rating by week 2, which was maintained through week 12 for vaginal color (week 2: TX-004HR 74%-80% vs placebo 57%;  $P \leq 0.0001$  for each dose; week 12: 79%-84% vs 65%;  $P < 0.0001$  for each dose), vaginal epithelial integrity (week 2: 87%-80% vs 72%;  $P < 0.001$  for each dose; week 12: 83%-89% vs 71%;  $P \leq 0.0001$  for each dose), vaginal epithelial surface thickness (week 2: 70%-74% vs 50%;  $P < 0.0001$  for each dose; week 12: 78%-83% vs 55%;  $P \leq 0.0001$  for each dose), and vaginal secretions (week 2: 78%-82% vs 61%;  $P < 0.001$  for each dose; week 12: 80%-83% vs 61%;  $P < 0.0001$  for each dose).

Changes in severity scores at 12 weeks from baseline were similar across each visual assessment item (Fig. 1A-D). Approximately 63% to 71% women treated with TX-004HR had a reduction (improvement) in their visual examination severity scores (vs 47%-55% with placebo), whereas 20% to 27% had no improvement (vs 28% to 42% with placebo). Less than 5% of women receiving TX-004HR had an increase in their severity scores at week 12 compared with less than 12% receiving placebo.

### Sensitivity and specificity of visual examination score

A simple linear correlation was observed between the total sum of the individual visual examination score and severity of dyspareunia ( $r = 0.31$ ,  $P < 0.0001$ ), and the severity of vaginal

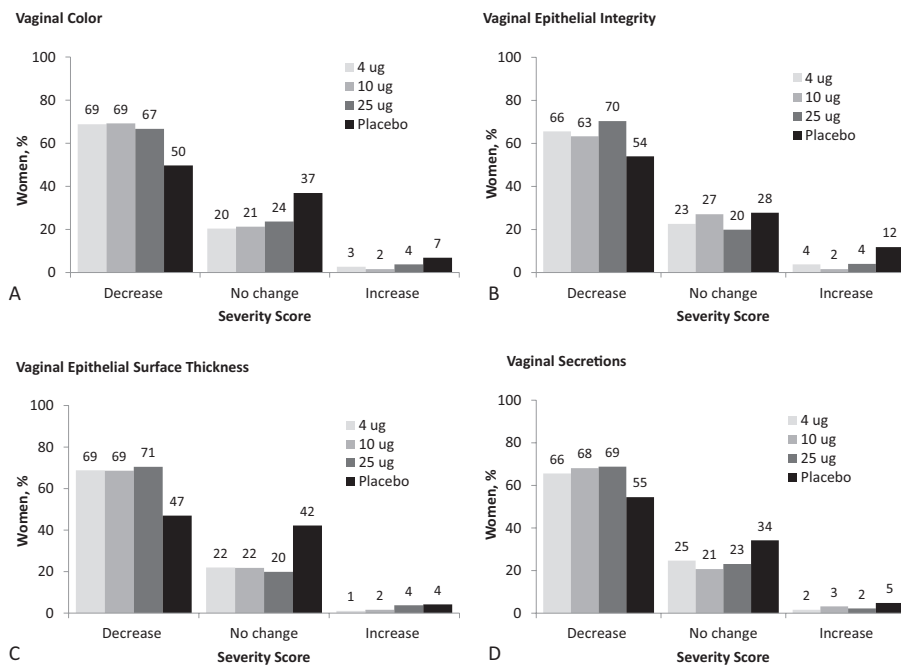
**TABLE 3.** Change from baseline to weeks 2, 6, 8, and week 12 in vaginal parameters in the modified intent-to-treat (MITT) population

Vaginal parameters	TX-004HR 4 µg (n = 186)	TX-004HR 10 µg (n = 188)	TX-004HR 25 µg (n = 186)	Placebo (n = 187)
<b>Vaginal color</b>				
Baseline score, mean ± SD	1.8 ± 0.61	1.7 ± 0.59	1.8 ± 0.60	1.7 ± 0.64
LS mean (SE)	n	n	n	n
Wk 2	185	187	184	186
Wk 6	172	170	173	176
Wk 8	164	165	166	167
Wk 12	171	173	175	175
	-0.69 (0.05) <sup>a</sup>	-0.77 (0.05) <sup>a</sup>	-0.78 (0.05) <sup>a</sup>	-0.40 (0.05)
	-0.82 (0.05) <sup>a</sup>	-0.93 (0.05) <sup>a</sup>	-0.89 (0.05) <sup>a</sup>	-0.50 (0.05)
	-0.98 (0.05) <sup>a</sup>	-1.04 (0.05) <sup>a</sup>	-0.99 (0.05) <sup>a</sup>	-0.50 (0.05)
	-0.97 (0.05) <sup>a</sup>	-1.06 (0.05) <sup>a</sup>	-0.96 (0.05) <sup>a</sup>	-0.60 (0.05)
<b>Vaginal epithelial integrity</b>				
Baseline score, mean ± SD	1.6 ± 0.84	1.4 ± 0.83	1.5 ± 0.77	1.5 ± 0.84
LS mean (SE)	n	n	n	n
Wk 2	185	187	184	186
Wk 6	172	170	173	176
Wk 8	164	165	166	167
Wk 12	171	173	175	175
	-0.85 (0.05) <sup>a</sup>	-0.87 (0.05) <sup>a</sup>	-0.93 (0.05) <sup>a</sup>	-0.53 (0.05)
	-0.97 (0.05) <sup>a</sup>	-1.02 (0.05) <sup>a</sup>	-1.01 (0.05) <sup>a</sup>	-0.61 (0.05)
	-1.03 (0.05) <sup>a</sup>	-1.08 (0.05) <sup>a</sup>	-1.08 (0.05) <sup>a</sup>	-0.66 (0.05)
	-0.97 (0.05) <sup>a</sup>	-1.07 (0.05) <sup>a</sup>	-1.01 (0.05) <sup>a</sup>	-0.60 (0.05)
<b>Vaginal epithelial surface thickness</b>				
Baseline score, mean ± SD	1.9 ± 0.67	1.8 ± 0.63	1.9 ± 0.59	1.9 ± 0.65
LS mean (SE)	n	n	n	n
Wk 2	185	187	184	186
Wk 6	172	170	173	176
Wk 8	164	165	166	167
Wk 12	171	173	175	175
	-0.76 (0.05) <sup>a</sup>	-0.76 (0.05) <sup>a</sup>	-0.76 (0.05) <sup>a</sup>	-0.40 (0.05)
	-0.85 (0.05) <sup>a</sup>	-0.93 (0.05) <sup>a</sup>	-0.90 (0.05) <sup>a</sup>	-0.53 (0.05)
	-0.96 (0.05) <sup>a</sup>	-1.04 (0.05) <sup>a</sup>	-0.99 (0.05) <sup>a</sup>	-0.59 (0.05)
	-0.98 (0.05) <sup>a</sup>	-1.03 (0.05) <sup>a</sup>	-0.94 (0.05) <sup>a</sup>	-0.61 (0.05)
<b>Vaginal secretions</b>				
Baseline score, mean ± SD	1.8 ± 0.68	1.7 ± 0.66	1.7 ± 0.63	1.8 ± 0.63
LS mean (SE)	n	n	n	n
Wk 2	185	187	184	186
Wk 6	172	170	173	176
Wk 8	164	165	166	167
Wk 12	171	173	175	175
	-0.79 (0.05) <sup>b</sup>	-0.83 (0.05) <sup>a</sup>	-0.86 (0.05) <sup>a</sup>	-0.54 (0.05)
	-0.90 (0.05) <sup>b</sup>	-0.95 (0.05) <sup>a</sup>	-0.97 (0.05) <sup>a</sup>	-0.60 (0.05)
	-1.00 (0.05) <sup>a</sup>	-1.04 (0.05) <sup>a</sup>	-1.06 (0.05) <sup>a</sup>	-0.63 (0.05)
	-1.01 (0.05) <sup>a</sup>	-1.06 (0.05) <sup>a</sup>	-1.04 (0.05) <sup>a</sup>	-0.64 (0.05)

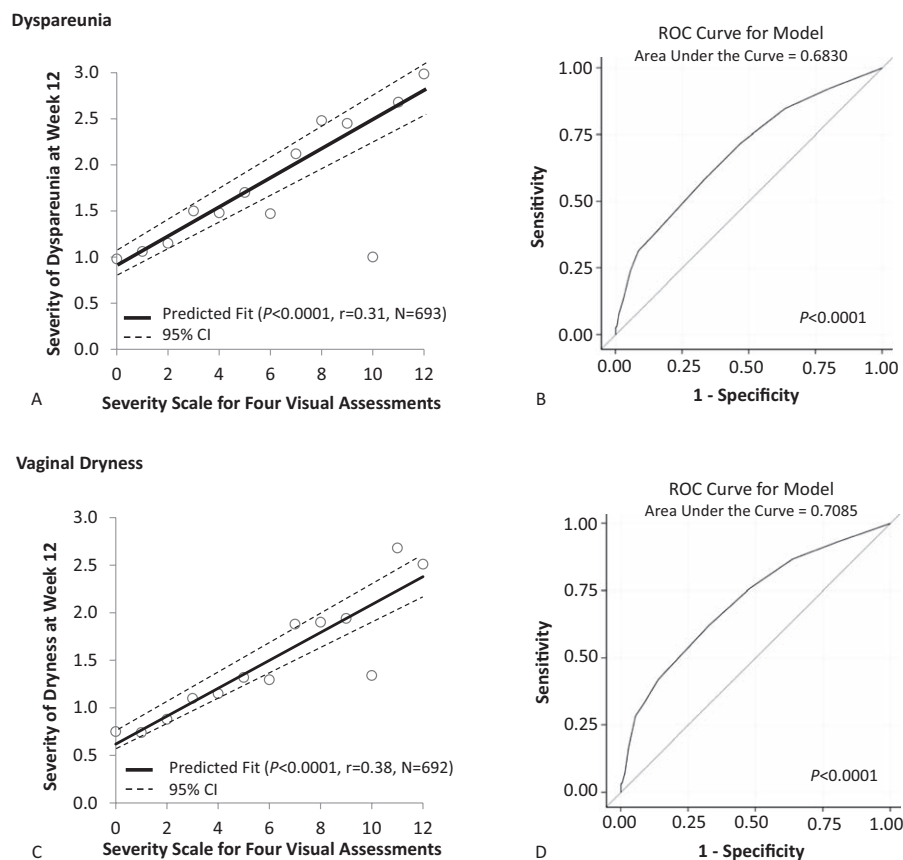
Data are mean ± SD unless otherwise noted.  
 LS, least square; SD, standard deviation; SE, standard error.  
<sup>a</sup>*P* < 0.0001 versus placebo; <sup>b</sup>*P* < 0.001.

dryness (*r* = 0.38, *P* < 0.0001) at 12 weeks (Fig. 2A and C) when all participants were analyzed independent of treatment. Furthermore, ROC analyses for both dyspareunia and vaginal dryness showed a significant visual score threshold.

For dyspareunia, ROC analysis revealed an area under the curve (AUC, c-statistic) of 0.68 (*P* < 0.0001; Fig. 2B) with a significant odds ratio (OR) of 1.34 (95% confidence interval [CI] 1.24-1.44, *P* < 0.0001). Using an ROC threshold of ≥5,



**FIG. 1.** Change in severity of visual assessments from baseline to week 12 in (A) vaginal color, (B) vaginal epithelial integrity, (C) vaginal epithelial thickness, (D) vaginal secretions in MITT population. MITT, modified intent-to-treat.



**FIG. 2.** Pearson's correlations and logistic regression ROC curves between the sum of the four individual visual assessment scores and dyspareunia (A and B) or vaginal dryness (C and D) at week 12 in the ITT population. ITT, intention to treat; ROC, receiver-operating characteristic.

sensitivity and specificity levels were 58% and 67%, respectively. For vaginal dryness, the AUC was 0.71 ( $P < 0.0001$ ; Fig. 2D) with a significant OR of 1.39 (95% CI 1.29-1.49,  $P < 0.0001$ ). Sensitivity and specificity levels with an ROC threshold  $\geq 5$  were 62% and 67%, respectively. A visual summary score of  $\geq 5$  can correspond to many different combinations including a women with a mild score in three of the four components and a moderate score in the fourth component.

## DISCUSSION

Visual examination of the vaginal epithelium of postmenopausal women with VVA and moderate-to-severe dyspareunia showed that treatment with TX-004HR clinically and significantly improved vaginal color, vaginal epithelial integrity, vaginal epithelial surface thickness, and vaginal secretions as early as 2 weeks, and maintained those improvements through 12 weeks compared with placebo. Approximately two-thirds of women receiving TX-004HR had improvements in their severity scores for each assessment. Moreover, the total sum of the visual assessments was moderately correlated with dyspareunia and dryness severity scores at 12 weeks.

Interestingly, women treated with placebo also showed some improvements in their scores at week 2, whereas women treated with TX-004HR showed continued improvements

through 12 weeks of treatment; such continued improvements were not observed to the same extent with the placebo. Three possible explanations for the improvements observed with the placebo include the potential lubricating effect of the excipient Miglyol, a fractionated coconut oil contained in all softgel capsules, improved appearance based on vaginal lubrication caused by increased sexual activity,<sup>23</sup> and/or bias on the part of the physicians performing the examinations as they may anticipate improvement. Nevertheless, TX-004HR still significantly improved evaluated signs and symptoms of VVA better than placebo.

Correlations between objective and subjective measures have been previously evaluated for VVA. For example, a vaginal pH  $> 6.0$  measured at the midvagina was shown to correlate with elevated levels ( $\geq 20\%$ ) of parabasal cells.<sup>13</sup> Visual assessments of vaginal atrophy using the Vaginal Physical Examination Scale also significantly correlated with vaginal pH and the maturation index score.<sup>18</sup> Similar correlations were observed using the visual assessment scoring in the phase 2 pilot trial of TX-004HR, which showed significant correlations with the change from baseline to day 15 for percentage of vaginal cells and vaginal pH ( $r = 0.35$ ,  $P = 0.02$ ) with the sum of the four visual assessments<sup>24</sup>; however, these correlations have limited utility in the clinical setting, because measurements of vaginal pH and cells are not often performed.

Beyond simple correlations, the ROC curve analyses also showed that visual assessment summary scores  $\geq 5$  had good sensitivity and specificity to detect moderate-to-severe dryness or dyspareunia. Because visual inspection of the vagina with the 4-point assessment tool showed moderate positive correlation with dyspareunia and vaginal dryness in this study, this tool may help healthcare professionals diagnose VVA and assess its treatment, and provide a vehicle for healthcare professionals to initiate discussion with their patients about a sensitive topic. Several large-scale studies have shown that it is difficult for patients to discuss vulvovaginal health openly with their healthcare professionals because they are either embarrassed, uninformed about VVA and its treatments, or believe that the topic is not appropriate for discussion.<sup>4</sup> Therefore, of the 50% of postmenopausal women who have symptoms of VVA, far fewer seek treatment.<sup>4</sup>

Visual examination of the vagina may help practitioners identify women at risk of dyspareunia and vaginal dryness, and allow them to proactively engage women in conversations about VVA symptoms such as dyspareunia and dryness, and discuss available treatment options. Whereas various assessment tools, such as the VAI,<sup>16</sup> the GHCE,<sup>17</sup> the Vaginal Physical Examination Scale,<sup>18</sup> the VHI,<sup>19</sup> or the Global Atrophy Score,<sup>20</sup> have been described, consensus in the healthcare community on the best tool to assess VVA has not been reached, possibly because patients and clinicians have both been shown to be reluctant to address this issue.<sup>25,26</sup> Furthermore, this topic has not been studied extensively or systematically, contributing to the lack of consensus on a method used to evaluate and describe VVA. This is in contrast to other tools that have been adopted by the majority of healthcare professionals to assess other disorders such as obstructive sleep apnea, sexual dysfunction, and pelvic relaxation and/or prolapse.<sup>27-30</sup> Although the vaginal mucosa assessment scale reported in this study has not been previously validated, the statistically significant results obtained from the data of 89 different sites across the United States and Canada suggest that the scale may yield consistent and reproducible scores. This visual scale, which is both consistent and reproducible, may be of utility in research to reduce development costs, ease regulatory approval, and be used as a practical tool in clinical settings.

One limitation of this analysis was that VVA measurement using the vaginal mucosa assessment scale was not a primary endpoint of the study, but was included as a secondary endpoint to determine whether visual examinations could be used to determine treatment effects and their correlation with VVA symptoms. Furthermore, the correlations observed in this study may not be applicable to all populations as all women had moderate-to-severe dyspareunia and the majority were white, and in good health. Studies evaluating this vaginal mucosa assessment scale in postmenopausal women with symptoms other than dyspareunia would be useful in validating the scale.

## CONCLUSIONS

Improvements observed in the vaginal mucosa assessment scores support the robust efficacy of TX-004HR (4, 10, and

25  $\mu$ g) observed in the treatment of VVA and moderate-to-severe dyspareunia in postmenopausal women. Overall, visual assessments of the vagina performed by experienced healthcare professionals appear to be a useful measure to diagnose VVA and assess response to treatment. In an era of increasing dependence on objective measures of clinical changes, even for VVA, knowing that an experienced clinician's judgment correlates strongly with objective measures is reassuring and can be self-empowering.

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