Protocol for Vestibulodynia: UPDATe

Vestibulodynia: **U**nderstanding **P**athophysiology and **D**etermining **A**ppropriate **T**r**e**atments

Compiled by:
Vestibulodynia UPDATe Research Team
Version 21 February 2024

Distributed by:
Andrea Nackley
Duke University
3 Genome Court
Durham, NC 27710

NCT03844412

Study Sponsor:	National Institute of Child Health & Human Development National Institutes of Health
Project Officer:	Jennie Conroy, MD
Principal Investigator:	Andrea Nackley, PhD (Duke University)
Co-Investigators:	Andrea Rapkin, MD (UCLA) Erin Carey, MD (UNC) Elizabeth Geller, MD (UNC)
Clinical Site Team:	Haley Cutting, Physician Assistant (UCLA) Jasmyn Akaninwor, Study Coordinator (UCLA) Debbie Farb, Research Nurse, UNC
Protocol Development Team:	Andrea Nackley, PhD (Duke University) Andrea Rapkin, MD (UCLA) Erin Carey, MD (UNC) Elizabeth Geller, MD (UNC) Christopher Stirling, MA (Duke) Andrey Bortsov, MD, PhD (Duke) Sandra O'Buckley, MS (Duke) Nicole Willis, BS (Duke)
Coordinating Center:	**Clinical Coordinating Center** Andrea Nackley, PhD **Data Coordinating Center** Christopher Stirling, MA **Statistical Coordinating Center** Sheng Luo, PhD Pearl Zakroysky, MPH Peter Merrill, PhD Duke Clinical Research Institute Durham, NC
Central Compounding Center:	Jennifer Burch 6224 Fayetteville Road Durham, NC 27713
Pacifica Compounding Pharmacy:	Jeffrey Barris 23560 S. Madison Suite 112 Torrance, CA 90505
Substrate Services Core:	Mary-Beth Joshi, MPH Duke University Durham, NC
Sequencing Core:	Zhao Lai, PhD Greehey Children's Cancer Research Institute University of Texas San Antonio, TX

1.1 List of Abbreviations

Abbreviation	Definition
AE	Adverse Event
BPSFS-F	Brief Profile Sexual Function and Satisfaction-Female
CC	Coordinating Center
CNS	Central Nervous System
COPC	Chronic Overlapping Pain Conditions
DCRI	Duke Clinical Research Institute
DSMB	Data Safety Monitoring Board
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
eICF	Electronic Informed Consent Form
FMS	Fibromyalgia Syndrome
HC	Healthy Controls
IBS	Irritable Bowel Syndrome
IDS	Investigational Drug Section
IRB	Institutional Review Board
ITT	Intent-to-Treat
MAOI	Monoamine Oxidase Inhibitor
miRNA	microRNA
NRS	Numeric Rating Score
PILL	Pennebaker Index of Limbic Languidness
PPT	Pressure Pain Threshold
PROMIS	Patient-Reported Outcomes Measurement Information System
PSS	Perceived Stress Scale
QST	Quantitative Sensory Testing
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SCL-27	Symptom Checklist-27
SF12v2	Short Form 12 version 2
SF-MPQ	Short Form- McGill Pain Questionnaire
sIRB	single Institutional Review Board
SNRI	Selective Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
TCA	Tricyclic Antidepressant
TMD	Temporomandibular Disorder
UCLA	University of California, Los Angeles
UNC	University of North Carolina at Chapel Hill
UP	Unanticipated Problem
VBD	Vestibulodynia
VBD-c	Vestibulodynia central
VBD-p	Vestibulodynia peripheral

TABLE OF CONTENTS

2 EXECUTIVE SUMMARY

Title	**Vestibulodynia UPDATe**: **V**estibulodynia: **U**nderstanding **P**athophysiology and **D**etermining **A**ppropriate **T**r**e**atments
Indication	Three continuous months of insertional entryway dyspareunia and/or pain to touch/tampon insertion indicated by self-report as well as pain score of "3" or greater on the tampon test during screening.
Locations	Duke University, UNC, UCLA
Brief Rationale	Vestibulodynia (VBD) is a chronic pelvic pain condition that affects 1 in 6 reproductive aged women, yet remains ineffectively treated by standard trial-and-error approaches. Our group has identified two distinct VBD subtypes that may benefit from different types of treatment: VBD peripheral (VBD-p) subtype characterized by localized pain specific to the vulvar vestibule, and VBD central (VBD-c) subtype characterized by pain at both vaginal and remote body regions. Preliminary data further demonstrate that VBD-p and VBD-c subtypes differ with respect to patient reported outcomes (e.g., physical and mental health), production of cytokines (intracellular proteins that regulate the activity of pain nerves and inflammatory processes), and expression of microRNAs (small non-coding RNA molecules that regulate gene expression). Women with VBD-p exhibit normal psychological profiles; balanced circulating pro- and anti-inflammatory cytokines; and dysregulation in microRNAs that regulate the expression of genes in estrogen pathways. In contrast, women with VBD-c report decreased functional status and increased somatization; increased pro- but not anti-inflammatory cytokines; and dysregulation in microRNAs that regulate the expression of genes relevant to muscle, nerve, and immune cell function.
Study Design	Two-center, randomized, double-blind, placebo-controlled 2x2 factorial study enrolling 400 women to participate for 24-week duration.
Treatment	Participants will receive one of four treatments for 16 weeks: • 5% lidocaine/0.5mg/ml 0.07% estradiol compound vaginal cream + placebo pill • placebo vaginal cream + nortriptyline pill • 5% lidocaine/0.5mg/ml 0.07% estradiol compound vaginal cream + nortriptyline pill • placebo vaginal cream + placebo pill
Aims & Endpoints	Among women with VBD-p and VBD-c, determine the efficacy of peripheral, central, and combined treatments in: **Aim 1. Alleviating pain** <u>Primary Endpoints:</u> Ia. pain score during tampon test at 16 weeks Ib. self-reported pain *via* SF-MPQ at 16 weeks <u>Secondary Endpoints:</u> IIa. experimental pain *via* local and remote PPTs at 16 weeks IIb. COPC pain levels *via* COPC follow-up survey at 16 weeks IIc. Pain scores and PPTs at 8 weeks and 24 weeks **Aim 2. Improving patient reported outcomes** <u>Primary Endpoints:</u> Ia. self-reported physical/mental health *via* SF12v2 at 16 weeks Ib. sexual health *via* PROMIS at 16 weeks <u>Secondary Endpoints:</u> IIa. mood *via* SCL-27 at 16 weeks IIb. somatic awareness *via* PILL at 16 weeks

IIc. perceived stress *via* PSS at 16 weeks
IId. sleep *via* sleep scale at 16 weeks
IIe. Patient reported outcomes at 8 weeks and 24 weeks

Aim 3. Normalizing cytokine and microRNA expression
Primary Endpoints:
Ia. cytokine levels *via* mesoscale discovery assays at 16 weeks
Ib. microRNA levels *via* sequencing read at 16 weeks
Secondary Endpoints:
IIa. cytokine and microRNA levels at 8 weeks and 24 weeks
IIb. Aim 3 will also identify cytokine and microRNA biomarkers at baseline that predict treatment response at 8, 16, and 24 weeks

3 INTRODUCTION

3.1 Overall Scientific Premise

Vestibulodynia (VBD) is the most common cause of sexual pain, affecting 16% of reproductive aged women in the United States.[1] The pain is chronic, lasting over 3 months, and compromises psychological functioning, interpersonal relations, and daily activity.[2] As further evidence of its public health significance, VBD and related vulvar pain conditions cost the US economy over $70 billion annually.[3] Despite its high prevalence and significant economic burden, VBD remains ineffectively treated due to its unclear etiology and heterogeneous clinical presentation. Some women present with localized provoked pain restricted to the vaginal vestibule, while others present with vestibular pain and generalized pain at other genital sites. Still others present with VBD and chronic overlapping pain conditions (COPCs) such as irritable bowel syndrome (IBS; 35%),[4] temporomandibular disorder (TMD; 78%),[5] and fibromyalgia syndrome (FMS; 17%).[4] In the absence of data to guide treatment approaches for VBD patients with diverse symptomology, many different therapies are used on a trial-and-error basis which can delay effective care.[6] Thus, it is urgent that we unravel the clinical and pathophysiological complexities of VBD in order to effectively resolve the pain and its devastating consequences.

Our group has identified two distinct VBD subtypes that may benefit from different types of treatment: 1) VBD peripheral (VBD-p) subtype is characterized by localized pain specific to the vaginal vestibule, and 2) VBD central (VBD-c) subtype is characterized by pain at vestibular and remote body regions.[5, 7] Women with VBD-p exhibit normal psychological profiles; balance in circulating pro- and anti-inflammatory cytokines; and dysregulation in microRNAs that regulate the expression of genes in estrogen pathways. In contrast, women with VBD-c report decreased functional status and increased somatization; lack compensatory increases in anti-inflammatory cytokines; and have dysregulation in microRNAs that regulate the expression of genes relevant to muscle, nerve, and immune cell function. These data suggest that VBD-p and VBD-c have unique etiologies (localized pain with peripheral neurosensory disruption versus widespread pain with a central sensory contribution) and may, therefore, respond differently to peripheral and centrally-targeted treatments.

Local anesthetics and estrogen are among the most commonly prescribed peripheral treatments[6,8,9] and tricyclic antidepressants are the most commonly prescribed centrally-targeted treatment.[8] However, as concluded in a recent Cochrane systematic review, interpretation of treatment success remains difficult due to the limitations of published studies, including poor patient selection, lack of adequate controls, and limited follow-up data.[10] Thus, there is a critical need for randomized controlled trials to evaluate the efficacy of these peripheral and centrally-targeted treatments, especially among women with different VBD subtypes. In response to RFA-HD- 18-480, we propose new multidisciplinary studies that will identify effective strategies for the management of two distinct VBD subtypes, while advancing our understanding of their different pathophysiologic mechanisms. We will compare the efficacy of peripheral, central, and combined treatments in alleviating objectively-measured pain and improving patient reported outcomes for women with VBD. In addition, we will evaluate cytokine and microRNA biomarkers that help determine underlying pathophysiology and predict treatment response. We will apply a randomized, double-blinded, placebo-controlled factorial study design to evaluate the analgesic efficacy of: 1) peripheral treatment with a 5% lidocaine/0.5 mg/ml 0.07% estradiol compound cream, 2) centrally-targeted treatment with the tricyclic nortriptyline 10 mg strength, or 3) combined peripheral and central treatment in women with newly diagnosed VBD. Women will be recruited from two study sites and complete a 4-month treatment phase, with outcome measures and biomarkers assessed at four time points (0, 2, 4, and 6 months). We hypothesize that women with VBD-p will exhibit increased levels of inflammatory mediators in the vaginal vestibule and preferentially respond to peripheral lidocaine/estradiol treatment, while women with VBD-c will exhibit increased levels of inflammatory mediators in blood and preferentially respond to centrally-targeted or combined treatment.

Our findings will 1) advance our knowledge of the pathophysiologic mechanisms underlying VBD-p and VBD-c, 2) determine the efficacy of peripheral, central, and combined therapies in alleviating pain in women with VBD-p and VBD-c, and 3) identify biomarkers that predict treatment response.

3.2 Scientific Premise for Aim 1

Compare the efficacy of peripheral, central, and combined treatments in alleviating pain among women with VBD-p and VBD-c

1) Women with VBD-p and VBD-c exhibit unique pain profiles. In general, VBD is associated with heightened pain responses that may result from dysregulations in processing that occur in the peripheral, central nervous system (CNS), or both.[11] Women with VBD exhibit increased levels of pro-inflammatory cytokines that bind to receptors on pain-sensing nociceptors to increase their activity and on immune cells to increase the synthesis of additional inflammatory mediators.[12-16] This self-perpetuating process of *peripheral sensitization* results in reduced pain thresholds in the affected tissues. Sustained peripheral inflammation can increase central responses to peripheral stimuli.[12, 13, 17, 18] This *central sensitization* causes women to exhibit reduced pressure pain thresholds at regions remote from the original painful site.[17-19] To distinguish VBD subtypes that may be peripherally- versus centrally-mediated, we enrolled women with localized VBD (VBD-p; N=33), VBD+COPCs (VBD-c; N=28), and healthy controls (HC; N=22).[7] As shown in Figure 1, we found that gentle pressure applied to the vaginal vestibule elicits increased pain ratings in both VBD subtypes, with the highest pain ratings in women with VBD-c. Using a modified Wegner Digital Algometer to measure vestibular pain in a standardized manner, we found that women with VBD-c exhibited the lowest vulvovaginal pain thresholds compared to those with VBD-p and HC. Similarly, women with VBD-c exhibited reduced pain thresholds at remote body sites, including the trapezius muscles.

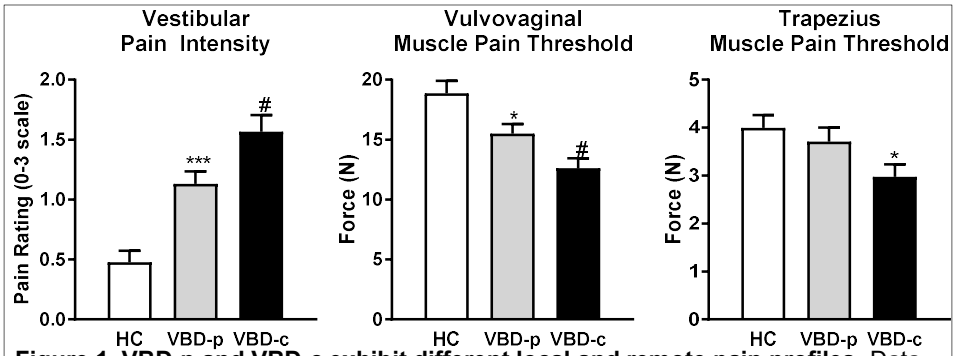


Figure 1. VBD-p and VBD-c exhibit different local and remote pain profiles. Data are Mean ± SEM. *p<0.05, ***p<0.001 vs HC. #p<0.05 vs HC and VBD-p.

2) Women with VBD-p and VBD-c exhibit differences in self-reported pain characteristics. In the same cohort, we found that women with VBD-c, but not VBD-p, had greater levels of affective, aching, tender and stabbing pain versus HC (Table 1).[7] More women with VBD-c reported a history of unprovoked genital pain, a hallmark of central sensitization.[20-23] We also found that more women with VBD-p had early onset (primary) VBD with first vaginal penetration compared to VBD-c, in contrast to some studies observing a positive correlation between VBD onset and pain severity.[24-26]

Table 1. Self-reported Pain Among Women with VBD-p and VBD-c

Phenotype	Questionnaire	HC	VBD-p	VBD-c	Overall P
Affective Pain	MPQ	11.67 (0.41)	12.50 (0.62)	14.78 (0.8)***	0.001
Aching Pain	MPQ	1.14 (0.10)	1.23 (0.10)	2.06 (0.17)###	<0.0001
Tender Pain	MPQ	1.10 (0.06)	1.37 (0.13)	1.61 (0.16)**	0.003
Stabbing Pain	MPQ	1.00 (0.00)	1.13 (0.10)	1.39 (0.20)*	0.038
Unprovoked Genital Pain	GYN	0%	20%	30%	
Pain with 1st Intercourse	GYN	N/A	67.5%	58.6%	
Pain with 1st Tampon	GYN	N/A	51.3%	27.9%	

McGill Pain Questionnaire (MPQ), Gynecological Health Survey (GYN). MPQ data are Mean (SEM). *p<0.05, **p<0.01, ***p<0.001 vs HC. ###p<0.001 vs HC and VBD-p. GYN data are % women/ group.

Thus, qualitative and quantitative assessments of pain in the vaginal vestibule and remote body regions together with self-reported measures of perceived pain traits can distinguish women with VBD-p and VBD-c, and may be used to differentiate underlying mechanisms and indicate different pain management strategies.

3) VBD-p may be more effectively managed by peripheral therapy, while VBD-c may be more effectively managed by centrally-targeted or combined therapies. Treatment options for VBD include 1) non-medical interventions such as pelvic floor physical therapy and cognitive behavioral therapy, 2) topical medications (e.g., analgesic and hormone creams) applied to the vaginal vestibule, 3) centrally-targeted medications (e.g., tricyclics) taken orally, and 4) surgery.[6, 10, 27] Surgical procedures such as vestibulectomy are high-risk and used as a last resort. Physical and psychological therapies are low-risk with published evidence, however are almost always used in combination with peripheral and/or centrally-targeted medications that have mixed results for successful pain management. A recent Cochrane review emphasized the need for randomized controlled trials to verify the efficacy of these commonly-prescribed medications, especially amongst different VBD subtypes.[10]

Local anesthetics (e.g., lidocaine) and topical estrogen are among the most commonly prescribed peripheral treatments.[6, 8, 9] Topical 5% lidocaine for 7 weeks has been shown to decrease dyspareunia in women with VBD,[28] likely due to its ability to block the activity of sodium channels on peripheral nociceptors and prevent the transmission of pain to the CNS.[29, 30] Topical estrogen for 3 months has been shown to reduce peripheral pain and inflammation[31] as well as improve sexual function for premenopausal women with vulvar pain.[32] Estrogen plays a critical role in trophic support and neuroplasticity of vaginal tissues, with deficient estrogen levels resulting in atrophy and increased innervation of pain-sensing nerve fibers.[33, 34] Lidocaine and estrogen are often compounded into a combined cream that can produce a synergistic analgesic effect.[9] Thus, combined topical lidocaine and estrogen may effectively alleviate pain in women with VBD-p.

Tricyclic antidepressants (e.g., nortriptyline) are the most commonly prescribed centrally-targeted treatment for the management of vulvar pain,[8] despite limited evidence. An open trial of nortriptyline for 2 months found that 6/7 women had a complete or partial reduction in chronic pelvic pain.[35] In a case study, nortriptyline completely alleviated vaginal pain in a woman for whom topical lidocaine, estrogen, and other peripheral treatments failed.[36] While the anti-epileptic gabapentin has also been commonly prescribed for vaginal pain, recent results from a randomized controlled trial found that gabapentin was ineffective in reducing pain among women with VBD.[37] Nortriptyline has been well studied for the treatment of other neuropathic pain disorders and evidence-based guidelines support its use as a first-line medication.[38] Nortriptyline produces analgesia *via* multiple molecular mechanisms in the CNS, including 1) inhibiting reuptake of norepinephrine to promote descending inhibition,[39] 2) blocking sodium channels to inhibit the activity of nociceptive neurons,[40] and 3) inhibiting the release of pro-inflammatory cytokines from glia so as to reduce neuroinflammation.[41] Together, these findings suggest that nortriptyline, alone or together with peripheral treatments, may effectively alleviate pain in women with VBD-c.

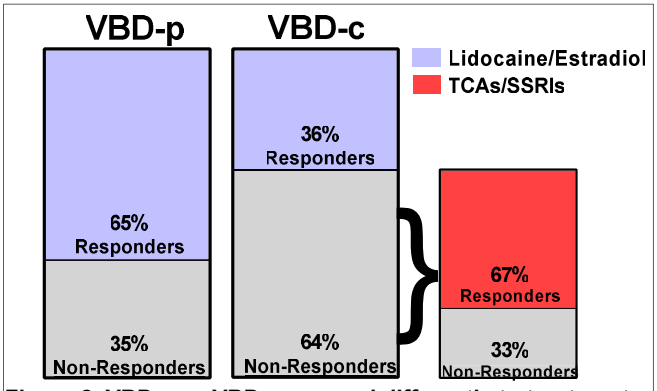


Figure 2. VBD-p vs VBD-c respond differently to treatment

To determine effectiveness of peripheral versus centrally-targeted treatments in women with VBD-p and VBD-c, we measured self-reported pain (on a scale of 0-10) in response to a Q-tip applied to the 6 o'clock site of the vaginal vestibule prior to treatment and again at the follow-up visit 3-6 months following treatment. All 47 women with VBD (28 with VBD-p and 19 with VBD-c) were first treated with topical lidocaine and/or estrogen cream. Women who did not respond to topical treatments,

were sequentially treated with TCAs or selective serotonin reuptake inhibitors (SSRIs). We found that topical lidocaine and/or estrogen cream alleviated pain in 65% of women with VBD-p, but only 36% of women with VBD-c (Figure 2). Furthermore, centrally-acting TCAs or SSRIs alleviated pain in 67% of women with VBD-c that failed to respond to peripheral treatments. *These preliminary data demonstrate that women with VBD-p preferentially respond to peripheral lidocaine/estradiol, while those with VBD-c preferentially respond to centrally-targeted treatments such as nortriptyline.*

3.3 Scientific Premise for Aim 2

Compare the efficacy of peripheral, central, and combined treatments in improving patient reported outcomes in women with VBD-p and VBD-c.

1) Women with VBD-p and VBD-c exhibit differences in self-reported physical, mental, and sexual health. VBD has severe consequences on all aspects of health. Women with VBD report lower sexual desire, arousal, and frequency of intercourse than pain-free controls.[42, 43] They also report more difficulty reaching orgasm, more negative attitudes toward sexuality, and more sexual distress. VBD is also associated with increased prevalence of stress and mood disorders.[27, 44] In our VBD cohort, we found that women with VBD-c perceived lower general and physical health status relative to HC, while women with VBD-p perceived lower mental health status relative to HC (Table 2).[7] Additionally, women with VBD-c reported more headaches, more somatization, and greater impact of pain on daily activity. Consistent with these findings, Zolnoun et al. found that women with VBD and TMD reported more somatization, anxiety, psychological distress, and intercourse-related pain.[5] *While it is uncertain whether all of these perceived health traits precede or follow the development of VBD,[45, 46] their characterization may inform treatment approaches and outcomes.*

Table 2. Self-reported Physical and Mental Health Among Women with VBD-p and VBD-c

Phenotype	Questionnaire	HC	VBD-p	VBD-c	Overall P
General Health	SF12v2	4.51 (0.11)	4.31 (0.15)	4.01 (0.15)*	0.012
Physical Health	SF12v2	55.8 (1.12)	57.6 (0.85)	50.5 (1.26)##	0.003
Mental Health	SF12v2	51.8 (1.87)	45.0 (1.96)*	46.4 (2.20)	0.069
Somatization	PILL	89.8 (4.15)	98.4 (3.60)	115.6 (4.61)##	0.0002
Headache Types	CPSQ	1.37 (0.19)	1.93 (0.19)	2.37 (0.23)**	0.009
Impact of Pain on Daily Activity	CPSQ	0.30 (0.20)	0.81 (0.37)	2.05 (0.64)*	0.028

Short Form 12 version 2 (SF12v2), Pennebaker Index of Limbic Languidness (PILL), Comprehensive Pain and Symptom Questionnaire (CPSQ). Data=Mean(SEM). *p<0.05, **p<0.01 vs HC. ##p<0.01 vs HC and VBD-p.

3.4 Scientific Premise for Aim 3

Determine cytokine and microRNA biomarkers that predict treatment response among women with VBD-p and VBD-c.

1) Women with VBD-p and VBD-c exhibit unique cytokine expression profiles. A large body of work suggests a role for cytokines, in the pathophysiology of VBD. Cytokines are secreted by immune and other cells in the periphery and by neurons and glia in the CNS.[47] In an acute setting, cytokines promote wound healing,[48] but sustained elevations promote tissue damage and pain.[47] Pro-inflammatory cytokines activate and sensitize nociceptors via direct receptor-mediated actions or via recruitment of additional mediators.[49, 50] In this manner, cytokines play an essential role in peripheral and central sensitization (as described above). Elevated pro-inflammatory cytokine levels are also associated with pain intensity,[51-55] perceived stress,[56] and mood disorders (e.g., depression)[57, 58] that often accompany chronic pain.

Women with VBD display signs of mucosal inflammation[11] accompanied by enhanced production of the pro-inflammatory cytokines interleukin 1β (IL-1β),[59-61] interleukin 8 (IL-8),[60, 62] and interleukin 17 (IL-17)[63] in the vaginal vestibule and/or in circulating blood. A study by Gerber et al. further demonstrated that increased levels of IL-1β were accompanied by reduced levels of interleukin 1 receptor antagonist (IL-1ra),[61] an anti-inflammatory cytokine that negatively regulates the expression of IL-1β and IL-8.[64-66] To determine inflammatory profiles in VBD-p and VBD-c subtypes, we used a multiplex assay to measure circulating cytokines in blood.[7] Compared to HC, women with VBD-p had increased levels of both IL-8 and IL-1ra (Figure 3). In contrast, women with VBD-c had increased levels of IL-8 with no increase in IL-1ra, indicating a pro-/anti-inflammatory imbalance. There have been no studies of these changes in vaginal tissues. We performed a pilot study to demonstrate our ability to measure cytokine protein in vaginal lavage samples. Results show that IL-8 is detectable by multiplex in 7 HC vaginal lavage samples (Figure 3). *These results establish the utility of cytokines as diagnostic biomarkers for functional VBD-p and VBD-c groups.*

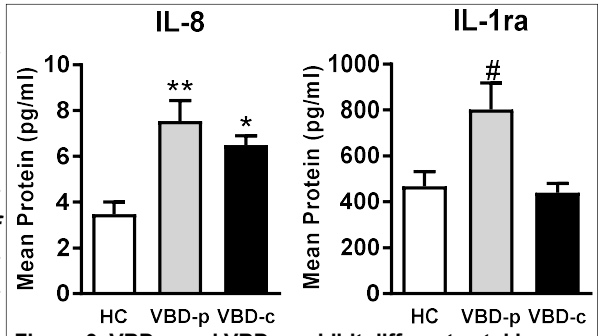


Figure 3. VBD-p and VBD-c exhibit different cytokine profiles. Data are Mean ± SEM. *p<0.05, **p<0.01 vs HC.

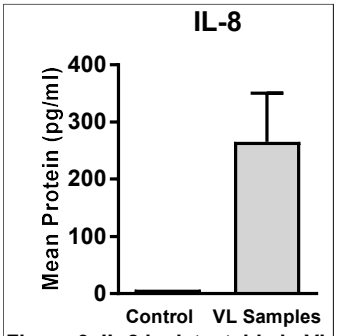


Figure 3. IL-8 is detectable in VL samples. Data are Mean ± SEM.

2) Women with VBD-p and VBD-c exhibit unique microRNA expression profiles. MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate expression of ≥ 1/3 of all human genes by degrading messenger RNA (mRNA) transcripts or by inhibiting mRNA translation.[67] miRNAs regulate molecular pathways linked to pain, inflammation and immune response.[68-76] Differential expression of circulating miRNAs has been reported in chronic pain conditions.[72] A recent preclinical study on neuropathic pain reported a positive correlation between pain thresholds and miRNA levels in plasma and cerebrospinal fluid.[69] A clinical study of regional pain syndrome identified 18 miRNAs differentially expressed in blood.[72] Thus, miRNA expression profiles may also be informative about the pathophysiology VBD. We conducted a study to measure miRNAs in women with VBD; profiling >750 miRNAs in blood from our VBD cohort using OpenArray cards.[7] Compared to HC, women with VBD-p exhibited a downregulation of 4 and upregulation of 2 miRNAs (Table 3). Three of these miRNAs (miR-449b, miR-133b, and miR-200b) are dysregulated in endometriosis and bladder pain syndrome.[77, 78] Women with VBD-c exhibited a downregulation of 3 and upregulation of 4 miRNAs. These miRNAs have been associated with cancer,[79-84] infectious disorders,[85] inflammatory disorders,[86] and orofacial pain.[87] Four miRNAs were dysregulated in both subtypes. Dysregulation of miR-520f and miR-520D may explain the increased IL-8 levels in women with both VBD-p and VBD-c, as the miR-520 family regulates IL-8.[88]

Table 3. miRNAs dysregulated in VBD-p and VBD-c

VBD-p		
miRNA	**Fold Change**	**P-value**
miR-449b	-330.14	0.017
miR-34b	-239.45	0.003
miR-645	-117.87	0.038
miR-503	-92.20	0.037
miR-200b	94.52	0.048
miR-133b	1731.74	0.014
VBD-c		
miRNA	**Fold Change**	**P-value**
miR-1825	-19012.5	0.019
miR-1288	-745.74	0.045
miR-593	-56.76	0.019
let-7f-2#	5.77	0.034
miR-512-3p	23.03	0.046
miR-125a-3p	69.59	0.041
miR-661	981.98	0.038
Both		
miRNA	**Fold Change**	**P-value**
miR-485-5p	-90.76 / -84.32	0.003 / 0.010
miR-1294	-41.47 / -60.67	0.012 / 0.014
miR-520f	-33.67 / -15.24	0.003 / 0.035
miR-520D-3p	2486.77 / 204617	0.021 / 0.002

To identify genes and pathways affected by miRNA dysregulation in our VBD cohort, we performed *in silico* pathway analysis using DIANA-miRPath v2.0.[89] In VBD-p, despite their pre-menopausal status, the estrogen and gonadotropin-releasing hormone (GnRH) pathways were blunted. This may explain why estrogen and GNRH therapy reduce pain in women with vaginal

atrophy or endometriosis.[31, 90] In VBD-c, several targets in the extracellular matrix and insulin resistance pathways were affected. The miRNA targets fibronectin and integrin promote neuropathic pain *via* upregulation of purinergic receptors on glia.[91] Further, deficiencies in the target dystroglycan cause abnormal myelination and function of sensory nerves associated with mechanical allodynia.[92] *In conclusion, circulating miRNA biomarkers indicate distinct pathophysiologic mechanisms that contribute to VBD-p and VBD-c. Assessment of miRNAs in vestibular tissues and circulating blood prior to, during, and following medical treatment may help to us better understand the factors that drive and resolve VBD.*

4 AIMS AND HYPOTHESES

1. Determine the efficacy of peripheral, central, and combined treatments in alleviating pain among women with VBD-p and VBD-c. We hypothesize that women with VBD-p will preferentially respond to peripheral treatment, while those with VBD-c will preferentially respond to central or combined treatment. To test this hypothesis, we will measure pain in response to standardized tampon insertion with a numeric rating scale and self-reported pain on the McGill questionnaire at the first baseline clinic visit (0) and at 8, 16, and 24 weeks.

2. Determine the efficacy of peripheral, central, and combined treatments in improving patient reported outcomes among women with VBD-p and VBD-c. We hypothesize that women with VBD-c will exhibit more abnormalities in perceived physical, mental, and sexual health compared to those with VBD-p. We also hypothesize that women with VBD-c will demonstrate greater improvement in perceived health with central or combined therapy; while women with VBD-p will demonstrate greater improvement with peripheral treatment. To test these hypotheses, we will measure self-reported physical /mental health with the SF12 and sexual health with the PROMIS questionnaire administered at 0, 8, 16, and 24 weeks.

3. Determine the efficacy of peripheral, central, and combined treatments in normalizing cytokine and microRNA expression profiles in vaginal and blood tissues collected from women with VBD-p and VBD-c. We hypothesize that higher levels of inflammatory mediators in peripheral vaginal samples will be associated with VBD-p, while higher levels in circulating blood will be observed in women with VBD-c. We also hypothesize that peripheral treatment will resolve abnormalities in vaginal biomarkers, while central treatment will resolve abnormalities in blood biomarkers. Finally, we hypothesize that abnormalities in vaginal cytokine and miRNA biomarkers will predict a positive response to peripheral treatment, while abnormalities in blood biomarkers will predict a positive response to central or combined treatment. To test these hypotheses, we will use multiplex assays to measure cytokines and small RNA sequencing to measure miRNAs in samples collected at 0, 8, 16, and 24 weeks.

5 BASIC STUDY DESIGN

Vestibulodynia UPDATe is a multi-center, randomized, double-blind, placebo-controlled trial designed to determine the efficacy of peripheral lidocaine/estradiol cream, centrally-targeted nortriptyline pills, and combined treatments compared to matching placebo with the primary endpoints of change in pain, self-reported health, and cytokine/microRNA measures at 16 weeks and secondary endpoints of change in these measures at 8 weeks and 24 weeks.

5.1 Feasibility Study

An initial pilot study will be conducted for the baseline (0 week) visit to optimize and standardize performance of the clinical exam, measurement of PPTs, and collection and processing of blood and vaginal samples. We anticipate consenting 5 women at each study site for this feasibility study. These volunteers will be recruited via an email blast to the UNC and UCLA students and employees, and do not have to meet inclusion/exclusion criteria. Screening of eligible study

participants will not start until a review of the baseline data has been performed and there is a determination that the sites are performing the testing accurately.

5.2 Screening

Screening will be conducted in women aged 18-50 with symptoms of VBD. Informed consent will be obtained prior to the tampon test. Participants with a pain score of ≥ "3" on a 0–10 numeric rating scale on the tampon insertion test will be eligible for enrollment. They will then complete the baseline evaluation measures and be randomized to one of four treatment arms. Women who have a pain score of 0-2 will be defined as a screen failure and will not be randomized.

5.3 Baseline Evaluation Phase and Randomization

Immediately following screening, research participants will complete all baseline assessments, including 1) completion of validated questionnaires to measure perceived pain, health, mood, and sexual function 2) vulvar exam and quantitative sensory testing to measure pressure pain thresholds at local (vaginal) and remote body sites, and 3) collection of biologic samples to measure local (vaginal) and systemic (blood) cytokines and miRNAs. The complete schedule of assessments to be obtained during the baseline visit is outlined in **Appendix A**.

Participants who fulfill all the inclusion criteria and none of the exclusion criteria will be randomly assigned on a 1:1:1:1 ratio to one of four treatment groups:

- 5% lidocaine/0.5mg/ml 0.07% estradiol compound vaginal cream + placebo pill
- placebo vaginal cream + nortriptyline pill
- 5% lidocaine/0.5mg/ml 0.07% estradiol compound vaginal cream + nortriptyline pill
- placebo vaginal cream + placebo pill

Medication assignments will be determined by the Duke Clinical Research Institute (DCRI) using randomized blocks, and then stratified by enrollment site. The DCRI will create a SAS program to generate the random sequence that will be loaded in RedCap. Participants and research personnel will be blinded to medication assignment.

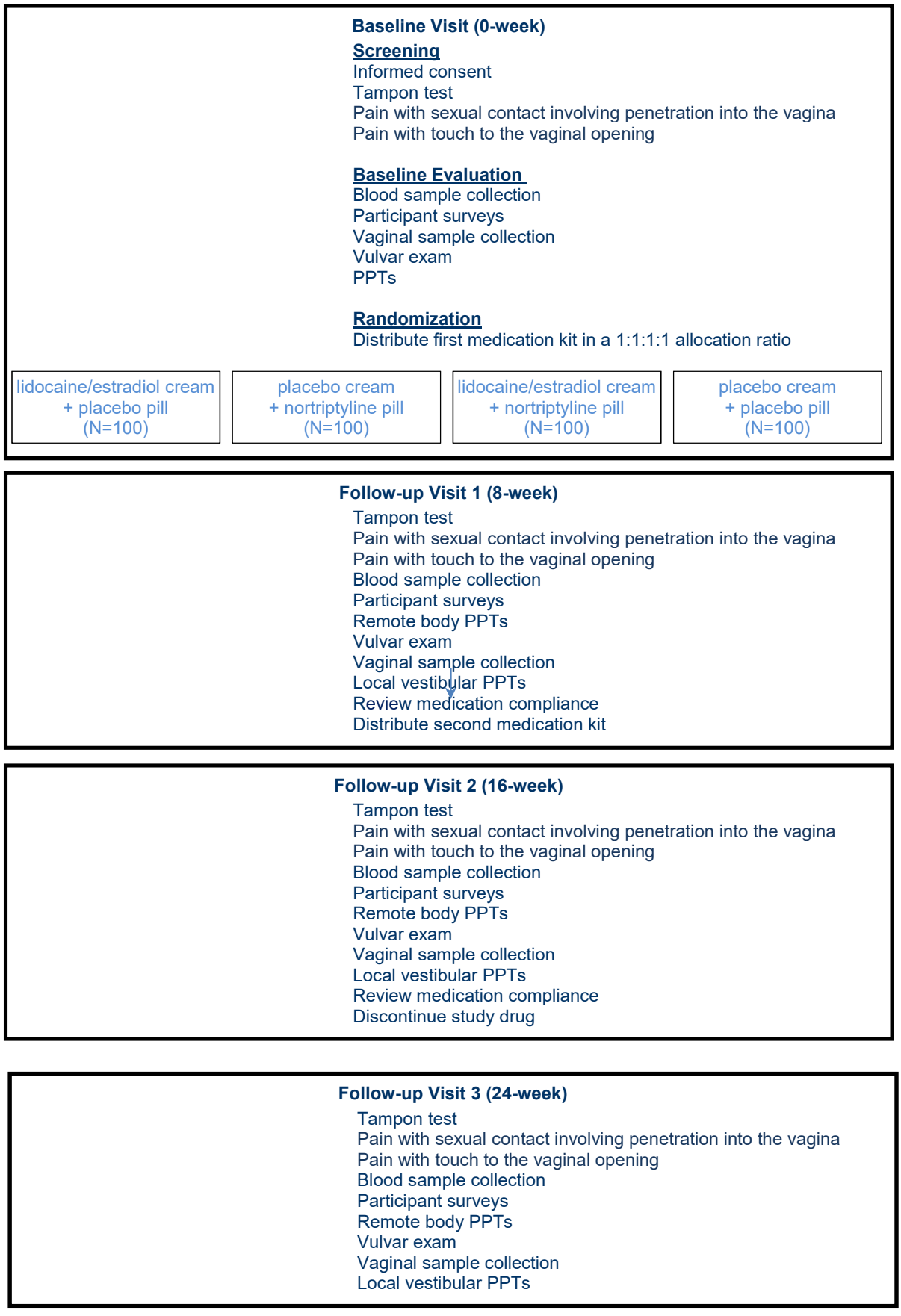
During the baseline visit, participants will be counseled on optimal study medication administration. Participants will receive a written description of the study with contact numbers of study staff to present to their usual care provider(s).

5.4 Follow-up Phase

Randomized study participants will return to the clinic for their 8-, 16-, and 24-week follow up visits. The complete schedule of assessments to be obtained during the follow-up visits is outlined in **Appendix A**.

6 STUDY FLOW DIAGRAM

The study flow diagram below outlines the study procedures and assessments in chronological order.



7 STUDY POPULATION AND ELIGIBILITY CRITERIA

7.1 Study Population

The study population is women with VBD aged 18-50 of any race or ethnicity. Based on historical patterns of recruitment, we expect the racial distribution will be 65% White, 10% Asian, 10% Hispanic, 8% African-American, and 6.5% other groups. This distribution is quite similar to that among people reporting VBD in other U.S. demographic populations.

7.2 Inclusion Criteria

1. Female
2. Age 18-50 years
3. English-literate
4. Willingness to provide informed consent
5. Meeting criteria for diagnosis of VBD based on scoring a 3 or greater on a scale of 0-10 (with 0 being no pain and 10 being the worst pain imaginable) for either the
    a. Tampon test
    b. Pain with sexual contact involving penetration into the vagina
    c. Pain with touch to the vaginal opening

7.3 Exclusion Criteria

1. Participants that will be enrolled in this study must be off medications for 30-90 days, depending on the medication dosage, frequency, duration, and half-life. All concomitant medications will need to be documented at the time of screening so the Principal Investigator and medical doctors at each participating site can determine a conservative window. Medications include, but are not limited to: topical lidocaine, estradiol, or lidocaine/estradiol to the vulvar vestibule, nortriptyline or other TCAs, pregabalin, and gabapentin.
2. Presence of active dermatologic vulvar disease or vaginal infection
3. Untreated atrophic vaginitis (participants may undergo treatment and re-evaluation for enrollment if the condition is resolved)
4. Previous vestibulectomy
5. Pregnant or planning on becoming pregnant during the study period.
6. Within the first six months of the postpartum period.
7. Currently breastfeeding/lactating, or within three months of discontinuing breastfeeding/lactation.
8. Active incarceration
9. Cancer within the past year.
10. Chemotherapy and/or radiation treatment within the past year.
11. Unstable medical condition (e.g., renal impairment, significant hematological disease, cardiovascular disease, hepatic insufficiency, neurological disorder, autoimmune disease, or respiratory illness)
12. Clear inflammatory states (e.g., morbid obesity)
13. Use of immunosuppressant medications
14. History of intolerance to nortriptyline, topical lidocaine, or topical estradiol
15. Contraindications to use of nortriptyline: current use, or use within the past 1-3 months, of MAOIs, some NDRIs, Ethylphenidate (DPH), MDPV (Methylenedioxypyrovalerone), Pipradrol (Meratran), Prolintane (Catovit/ Promotil); recent myocardial infarction, active psychotic or suicidal thoughts, narrow angle closure glaucoma
16. Contraindications to the use of lidocaine or local anesthetics
17. Contraindications to the use of topical estrogen therapy

18. Post-menopausal, defined as no menses for 12 consecutive months or surgical removal of both ovaries. (Hysterectomy is not an exclusion)
19. Have not had Botox of the pelvic floor muscles in the last 12 months, or pelvic nerve blocks in the last three months.
20. Are not currently enrolled or planning to enroll in another clinical trial during the course of this trial.
21. Are not currently receiving pelvic physical therapy

8 TREATMENT INTERVENTIONS

8.1 Interventions

Participants will be randomly assigned to one of four treatment groups: 1) peripheral treatment in the form of 5% lidocaine/0.5mg/ml 0.07% estradiol compound cream + placebo pill, 2) centrally-targeted treatment in the form of placebo cream + nortriptyline oral pill **(**up to 50 mg or highest tolerated dose), 3) combined treatment in the form of 5% lidocaine/0.5mg/ml 0.07% estradiol compound cream + nortriptyline; and 4) placebo cream + placebo pill.

Lidocaine/estradiol cream targets peripheral nerves and tissues affected in VBD; the comparison treatment will be an identical-appearing placebo Moisturel™ cream. Participants will be provided with a diagram and written instructions (see **Appendix B**), detailing how to apply the cream to the vaginal vestibule daily for weeks 1-16. Treatment with lidocaine/estradiol or placebo cream will be terminated at 16 weeks.

Nortriptyline is a centrally-acting tricyclic antidepressant that is FDA-approved for treatment of neuropathic pain. Dosing will begin with one 10 mg pill nightly for week 1, then two 10 mg pills nightly for week 2, three 10 mg pills nightly for week 3, four 10 mg pills nightly for week 4, and five for weeks 5 -16**.** In the event of side-effects without significant adverse events, participants will be advised to decrease dosage by one pill weekly until a tolerable dose is achieved. Treatment with nortriptyline or placebo pill will be tapered off over weeks 16-18, decreasing the dose by 10 mg every 4 days. Participants will be provided with a list of drugs to avoid that are known to interact with nortriptyline (see **Appendix C**).

8.2 Potential Side Effects and Risk Reduction Plan

Expected risks associated with lidocaine/estradiol compound cream include an adverse reaction to either medication or the compounded base. Lidocaine is well tolerated and safe, particularly with topical administration in small doses which significantly reduces the risk of lidocaine toxicity. The proposed daily dose is extremely small, with minimal systemic uptake when used as instructed. As with any medication, participants will be clearly instructed to use as directed. Significant over-usage would be required to approach toxicity. This is extremely rare in clinical practice. The medication will not be used in women with prior sensitivity or allergy to local anesthetics. A mild burning or tingling sensation with application is typical and resolves once the anesthetic takes effect. There is a rare risk of erythema, burning or blistering at the application site. The lidocaine will be compounded in a hydrophilic petrolatum, an inert and well-tolerated compounding cream. The second component of this compounded cream is estradiol. Like the lidocaine, women with prior sensitivity to this medication should not be enrolled, as well as women who have contraindications to topical estrogen therapy. The topical application has very low systemic uptake (0.01% per ½ gram dose), is well-tolerated and safe in reproductive-aged women. Women with potential contraindications to systemic estrogen, such as those with breast cancer, stroke or coronary artery disease, may still receive topical estrogen due to the extremely low absorption rate. In these cases, the treating physician will be contacted for permission to participate in the study, as is the protocol with clinical use of topical estrogen. Participants will be

instructed to notify the research team if they experience any adverse reaction.

Nortriptyline, a second-generation tricyclic antidepressant, with few associated risks is well-tolerated when compared to other agents in this pharmacologic class. The medication will be administered at night to minimize symptoms but it it has the effect of insomnia, participants may switch to morning administration. The maximum dose of this medication is 150 mg/day for major depression, but is efficacious at lower doses for the management of pain disorders. This medication should not be used in participants with: homicidal or suicidal ideations, in the acute recovery phase of a myocardial infarction (due to risk of arrhythmia), epilepsy, or acute angle glaucoma, Expected side effects with nortriptyline include drowsiness, difficulty sleeping (insomnia), cloudy thinking, and dry mouth. Other potential side effects include nausea, vomiting, loss of appetite, weight gain/loss, anxiety, hives/rash, delayed micturition, urinary retention, constipation, vision changes, breast swelling, decreased sexual drive, hyper/hypoglycemia, tachycardia or irregular heartbeat. Risk of serotonin syndrome is increased when participants are taking other serotonergic medications in addition to nortriptyline. Risk of long QT syndrome is increased when participants are taking citalopram or escitalopram. If the potential participant endorses either taking two or more serotonergic antidepressant medications or taking either citalopram or escitalopram, then the study MD will consult with the prescribing provider to determine if it would be safe for the potential participant to take nortriptyline in this clinical trial. Since serotonin syndrome is most likely to occur within 24 hours of starting/increasing the dosage of a serotonergic medication, the researcher will attempt to contact randomized participants within 24 hours of starting/increasing nortriptyline doses if the participant is taking other serotonergic antidepressants.

8.3 Drug Dispensing

Identical-appearing creams and pills will be packaged and distributed by Central Compounding Pharmacy to the UNC site, and by the Pacifica Compounding Pharmacy to the UCLA site. At the baseline visit, participants will receive a sufficient supply of study drug for weeks 1-8, and some (3 days) extra in the event of delay in scheduling visit 2. At the 8-week visit, participants will receive a sufficient supply of study drug for the remaining weeks 9-16, and some (3 days) extra in the event of delay in scheduling visit 3. Patients will be instructed to take the medication as required by the protocol.

8.4 Drug Storage

The study drug should be stored at controlled room temperature 15°-30°C (59°-86°F) and will be dispensed in a light-resistant container. Excessive moisture should be avoided. Study drug must be kept out of reach of children.

8.5 Drug Accountability

Participants are instructed to return all used, partly used and unused trial products (study drugs and packaging material) at each study visit. Returned trial product(s) must be stored separately from the non-allocated trial product(s) until drug accountability has been reconciled. The respective clinical investigators will be responsible for monitoring all received, used, partly used and unused trial product(s).

8.6 Drug Destruction

Used and unused study drug will be returned to the respective compounding pharmacies for destruction according to accepted pharmacy practice and both local and national guidelines. A

copy of the drug destruction procedure should be maintained in the pharmacy section of the Regulatory Binder. Study drug destruction should be documented in the comments section of the Subject Specific Drug Accountability Log.

8.7 Randomization and Blinding

Drug assignments will be determined by the DCRI using randomized blocks, and then stratified by enrollment site. The DCRI will create a SAS program to generate the random sequence that will be loaded in REDCap.

Participants and research personnel will be blinded to medication assignment. Blinding of the study, with respect to treatment groups will be preserved by the use of placebo cream and capsules that are identical in appearance to the active study drugs. Investigators may be asked at the end of the trial if he/she had obtained any information that may have led to the potential unblinding of treatment.

8.8 Unblinding

Randomization data are kept strictly confidential, accessible only to authorized persons (in this case a member of the DCRI team and the pharmacists), until the time of unblinding. The investigative sites will be given access to the treatment code for their participants for emergency unblinding only by calling the CC. In the rare event of necessary unblinding, the CC Medical Monitor must be contacted to discuss the case. Any suspected study drug-related event should be treated as though the patient received active therapy.

At the end of the study, the assigned treatment arm will be unblinded and disclosed to subjects individually in order to allow subjects to seek further management of vestibulodynia with their gynecologist or primary care physician.

8.9 Concomitant Medication

All research participants are not allowed to consume other topical lidocaine, estradiol, or lidocaine/estradiol medications, TCAs, MAOIs, SSRIs, SNRIs, or opiates throughout the entire course of the study. This will be confirmed by the research team at the 8-, 16-, and 24-week visits. Study team will discuss concomitant medication use at the baseline screening visit.

9 RECRUITMENT, ENROLLMENT AND SCREENING PROCEDURES

9.1 Common Recruitment Procedures

Recruitment will occur via proven paths in clinics attended by members of the research team. At UNC this includes the Hillsborough Medical Office Building (HMOB) in both the Divisions of Minimally Invasive Gynecology (MIGS) and Female Pelvic Medicine and Reconstructive Pelvic Surgery (FPMRS) in the Department of Obstetrics and Gynecology (Ob/Gyn), which are located on the same floor in adjacent space. Participants will be recruited directly from the MIGS chronic pelvic and vulvar pain clinics and the FPMRS clinic. Patients may be contacted by phone prior to their scheduled appointment in the clinics above. In addition to the co-investigators, there are additional providers between the two clinical divisions who specialize in the care of women with pelvic and sexual pain, who will refer potential participants for the proposed study. Additionally, the Ob/Gyn generalist division, also located in the HMOB, will be a strong referral source. At UCLA, women will be recruited from the UCLA Chronic Pelvic Pain and Vulvar Pain Program, a clinical practice focused on the assessment and management of pelvic pain syndromes.

Women identified as new patients, with appointments in the upcoming weeks/months at the UCLA Chronic Pelvic Pain and Vulvar Pain clinic, may be contacted by phone or email prior to their scheduled appointment to determine their interest as a study participant. Women will also be referred from other generalists and specialists at UCLA Westwood, Santa Monica, and Los Angeles area as well as the UCLA Student Health and Wellness Ashe Center. In addition to this, many patients self-refer or maybe referred by their primary care physician or from outside medical groups.

Site PIs will conduct a weekly teleconference to review project-wide procedures, monitor targets, discuss operational or scientific processes, and address study-wide progress. Recruitment strategies include traditional recruitment methods. Study personnel will place flyers in surrounding institutions near UNC (Duke, NC State, Wake Tech, Durham Tech, Women's Health at UNC, Hillsborough, and Raleigh) and UCLA (University of Southern California, Santa Monica Community College, California State University- Northridge, Moorpark College, Pasadena Community College, and Pomona College). A mass email blast to all UNC and UCLA students and employees is scheduled for every 3 months to coincide with the start of each quarter. Transit advertising on public buses and radio advertisements through WUNC and KPCC National Public Radio (NPR) are options that may be used if necessary. Study information will be placed online on a study-specific website, on social media sites (eg, Instagram, Facebook, Twitter), on relevant University (eg, Duke List) and Association (eg, National Vulvodynia Association) sites, and through clinicaltrials.gov. . Another tool that will be utilized to target our audience will be Research Match, https://www.researchmatch.org/. We will list our study information on this website for more publicity.

In addition, we will perform data mining of CareConnect (Epic) using UCLA Clinical and Translation Science Institute (CTSI). We will provide CDWH with the ICD-9 or ICD-10 codes that identify our population, then provide filters (eg, age < 50, seen in last 5 years at UNC, live in NC). Once CDWH identifies the list of potential participants, we will contact them by phone or mail to evaluate their interest in participating in the study.

9.2 Estimated Enrollment Period

Each site will screen for eligible participants to achieve a target of 200 randomized participants per site. We expect to randomize an average of 4-5 participants per month per study site beginning in the second quarter of funding Year 2. At that rate, the final (400th) participant will be randomized at the end of Year 5. This projected rate of randomization is readily achievable based on current patient volume at UNC and UCLA (151 and 101 new patients per year, respectively), which is expected to increase with utilization of our recruitment methods.

9.3 Screening

Screening will be conducted in women aged 18-50 with VBD. The purpose of the study, study interventions and evaluations, and the potential risks and benefits of participation, will be explained to eligible participants. If a participant agrees to participate, she will review and sign the site-specific Institutional Review Board (IRB) approved informed consent form

10 BASELINE EVALUATION AND DRUG DISPENSING (0-WEEK)

Following screening, research participants will complete all baseline procedures, including 1) completion of validated questionnaires to measure perceived pain, health, mood, and sexual function 2) a vulvar exam and quantitative sensory testing to measure pressure pain thresholds at local and remote body sites, and 3) collection of biologic samples to measure local (vaginal)

and systemic (blood) cytokines and miRNAs. A complete schedule of assessments throughout the study is outlined in **Appendix A**.

10.1 Baseline Evaluation

Baseline procedures and assessments (described in detail in **Section 15** below) will be performed in the following order:

- Participant consent
- Tampon test with 0-10 NRS
- Blood collection and processing
- Participant questionnaires:
    - Demographics
    - Social history
    - Gynecological history
    - VPAQ
    - PROMIS BPSFS-F
    - COPC inventory
    - SF12v2
    - SCL-27
    - PILL
    - PSS
    - Sleep scale
- Remote bodily PPTs (deltoid, shin, trapezius)
- Qualitative pelvic sensory testing
- Vaginal sample collection
- Local vestibular PPTs (5, 6, and 7 o'clock)

10.2 Drug Dispensing

All eligible study participants who successfully complete screening and baseline evaluations will be randomized (as detailed in **Section 8.7**) to receive one of four types of medication kits:

- 5% lidocaine/0.5mg/ml 0.07% estradiol compound vaginal cream + placebo pill
- placebo vaginal cream + nortriptyline pill
- 5% lidocaine/0.5mg/ml 0.07% estradiol compound vaginal cream + nortriptyline pill
- placebo vaginal cream + placebo pill

Identical-appearing creams and pills will be packaged and distributed by the Central Compounding Pharmacy to UNC and by the Pacifica Compounding Pharmacy to UCLA. At the baseline visit, participants will receive a sufficient supply of study drug for weeks 1-8. Patients will be instructed to take the medication as required by the protocol and complete a daily medication calendar (**Appendix D**).

11 FOLLOW-UP EVALUATIONS

11.1 Visit 2 (8-Week)

Upon arrival, participants will return any remaining study medication to the research team.

The week 8 visit procedures and assessments will be performed in the following order:

- Tampon test with 0-10 NRS
- Blood collection and processing
- Participant questionnaires:

    - VPAQ
    - PROMIS BPSFS-F
    - COPC follow-up survey
    - SF12v2
    - SCL-27
    - PILL
    - PSS
    - Sleep scale
- Remote bodily PPTs (deltoid, shin, trapezius)
- Qualitative pelvic sensory testing
- Vaginal sample collection
- Local vestibular PPTs (5, 6, and 7 o'clock)

At the end of visit 2, participants will receive a sufficient supply of study drug for weeks 9-16. Patients will be instructed to take the medication as required by the protocol and complete a daily medication diary.

11.2 Visit 3 (16-Week)

Upon arrival, participants will return any remaining study medication to the research team. Study medication will be discontinued.

The week 16 visit procedures and assessments will be performed in the following order:

- Tampon test with 0-10 NRS
- Blood collection and processing
- Participant questionnaires:
    - VPAQ
    - PROMIS BPSFS-F
    - COPC follow-up survey
    - SF12v2
    - SCL-27
    - PILL
    - PSS
    - Sleep scale
- Remote bodily PPTs (deltoid, shin, trapezius)
- Qualitative pelvic sensory testing
- Vaginal sample collection
- Local vestibular PPTs (5, 6, and 7 o'clock)

11.3 Visit 4 (24-Week)

The week 24 visit procedures and assessments will be performed in the following order:

- Tampon test with 0-10 NRS
- Blood collection and processing
- Participant questionnaires:
    - VPAQ
    - PROMIS BPSFS-F
    - COPC follow-up survey
    - SF12v2
    - SCL-27
    - PILL
    - PSS
    - Sleep scale
- Remote bodily PPTs (deltoid, shin, trapezius)

- Qualitative pelvic sensory testing
- Vaginal sample collection
- Local vestibular PPTs (5, 6, and 7 o'clock)

12 OUTCOME DETERMINATIONS

Aim 1. Determine the efficacy of peripheral, central, and combined treatments in alleviating pain among women with VBD-p and VBD-c.
Primary Endpoints:
Ia. pain score during tampon test at 16 weeks
Ib. self-reported pain *via* SF-MPQ at 16 weeks

Secondary Endpoints:
IIa. experimental pain *via* local and remote PPTs at 16 weeks
IIb. COPC pain levels *via* COPC follow-up survey at 16 weeks
IIc. Pain scores and PPTs at 8 weeks and 24 weeks

Aim 2. Determine the efficacy of peripheral, central, and combined treatments in improving patient reported outcomes among women with VBD-p and VBD-c.
Primary Endpoints:
Ia. self-reported physical/mental health *via* SF12v2 at 16 weeks
Ib. sexual health *via* PROMIS at 16 weeks

Secondary Endpoints:
IIa. mood *via* SCL-27 at 16 weeks
IIb. somatic awareness *via* PILL at 16 weeks
IIc. perceived stress *via* PSS at 16 weeks
IId. sleep *via* sleep scale at 16 weeks
IIe. Patient reported outcomes at 8 weeks and 24 weeks

Aim 3. Determine the efficacy of peripheral, central, and combined treatments in normalizing cytokine and microRNA expression profiles in vaginal and blood tissues collected from women with VBD-p and VBD-c.
Primary Endpoints:
Ia. cytokine levels *via* mesoscale discovery assays at 16 weeks
Ib. microRNA levels *via* sequencing read at 16 weeks

Secondary Endpoints:
IIa. cytokine and microRNA levels at 8 weeks and 24 weeks
IIb. Aim 3 will also identify cytokine and microRNA biomarkers at baseline that predict treatment response at 8, 16, and 24 weeks

13 METHODS TO PROMOTE ADHERENCE

Protocol training and adherence will be a major focus of the training for all Investigators, research nurses, and other essential personnel. An initial training session will take place prior to beginning recruitment in order to instruct and calibrate study personnel on all aspects of vulvar exam, QST, sample collection/processing, etc. Adherence will then be maintained by continuing to review study procedures during weekly calls and by annual follow-up training sessions (in-person at the UNC Hillsborough clinic site with a Skype connection to the UCLA clinic site). In addition, the CC will contact each site to offer per-participant feedback on adherence; will review episodes of non-adherence and reemphasize the importance of adherence; and will provide adherence reports.

14 PARTICIPANT SAFETY AND ADVERSE EVENTS

14.1 Single IRB

Our clinical trial will comply with the NIH Policy on the Use of a Single Institutional Review Board (sIRB) for Multi-Site Research (NOT-OD-16-094), with the goal to eliminate duplicative IRB review and streamline the IRB review process so that research can proceed as effectively and expeditiously as possible. The Duke University IRB will serve as the sIRB of record. UCLA and UNC will serve as participating sites and have agreed to rely on the proposed sIRB. Duke University will be responsible for maintaining records of the authorization/reliance agreements and of the communication plan. This agreement, clarifying the roles and responsibilities of the sIRB and participating sites, will be signed by all participating sites prior to initiating the study.

Communication between Duke, UCLA, UNC and the sIRB will be managed by the CC. The CC will assist with the development of IRB protocols, participant randomization, data management, safety surveillance, and medical communications.

14.2 Definitions

14.2.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence with the use of a drug in a subject whether or not considered drug or biologic related. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporarily associated with the use of a pharmaceutical product or biologic.

The study is limiting collection of AEs to focus on potential impacts of the blinded study medication. Only the following AEs will be documented in the electronic Case Report Form (eCRF):

- Swelling
- Burning
- Blistering
- Drowsiness
- Difficulty sleeping (insomnia)
- Cloudy thinking
- Dry mouth
- Nausea
- Vomiting
- Loss of appetite
- Weight gain
- Weight loss
- Anxiety
- Hives
- Rash
- Difficulty urinating
- Constipation
- Vision changes
- Breast swelling
- Decreased sexual drive

- Low blood sugar
- High blood sugar
- Increased heart rate
- Irregular heartbeat
- Suicidal thinking
- Mania
- Agitation
- Hallucinations
- Muscle tremor
- Muscle rigidity
- Seizures
- Bone pain

14.2.2 Serious Adverse Events

A serious adverse event (SAE) is an AE that results in the following outcomes:

- Death
- Life-threatening AE: Places the subject at immediate risk of death at the time of the event as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Inpatient hospitalization or prolongation of hospitalization.
- Congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition above.

This determination is based on the opinion of the investigator.

14.2.3 Assessment of Causal Relationship

A medically-qualified investigator must assess the relationship of any AE to the use of study drug, based on available information, using the following guidelines:

- **Not related:** There is no reasonable causal relationship to the investigational product and the adverse event.
- **Unlikely related:** No temporal association or the cause of the event has been identified, or the drug or biologic cannot be implicated.
- **Possibly related:** There is reasonable evidence to suggest a causal relationship between the drug and adverse event.
- **Related:** There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

14.2.4 Recording and Reporting of Adverse Events

The site investigator is responsible for monitoring the safety of participants enrolled into the study at the study sites. The investigator will probe, via discussion with the participant, for the occurrence of AEs during each study visit and record the information in the site's source documents. Participants will be instructed to report the occurrence of potential AEs between visits (on a weekly basis) using the Electronic Data Capture (EDC) system. The investigator will receive an email notification and will call the participant to gather further information about the AE(s).

The investigator will record selected AEs on the eCRF. Adverse events will be described by duration (start and stop dates), severity, outcome, and relationship to study drug. Adverse events will be reported to the Data Safety Monitoring Board (DSMB), Duke IRB and/or other agencies according to guidelines established by those entities/agencies. All AEs that are identified, whether or not listed in Section 14.2.1, will be followed by the site until resolution or stabilization. Adverse Events will be captured from the time of consent through the Month 6 visit.

14.3 Data Safety Monitoring Board

The DSMB will be composed of 4 members: a physician, a statistician, a patient representative, and a bioethicist. All DSMB operations will be managed by the DCRI.

The goal of the DSMB will be to monitor patient safety and to review the performance of the study. The DSMB will be responsible for providing recommendations regarding trial's conduct and guidance to ensure the safety and well-being of patients treated by study physicians. A separate DSMB charter will outline the operating guidelines for the committee, the protocol for evaluation of data, and data tables to be provided. The charter will be created prior to study enrollment and agreed upon during the initial meeting of the DSMB. It is anticipated that the DSMB will meet at 6-month intervals to review the accumulating data for safety.

DSMB Analyses: A DCRI Statistical Analyst and Programmer will generate the analyses required by the DSMB charter and provide these in advance of each DSMB meeting. This individual will ensure high quality and timely reports are submitted. Confidential Interim Data Reports will be prepared regularly by the DSMB statistician as specified in the Charter. The report will include recruitment and retention rates, SAEs, and other information as requested by the DSMB Chair.

15 STATISTICAL CONSIDERATIONS

15.1 VBD Subtype Designation

Women with VBD-c will be distinguished from those with VBD-p based on the presence of a COPC. The Institute of Medicine's 2011 report, Relieving Pain in America, identified 9 COPCs that predominantly affect women and frequently co-occur with VBD.[93] The ***presence of a COPC*** will be determined using the COPC survey. This is an easy-to-complete form including modules with check-box questions and a body mannequin to determine the presence of 9 COPCs: 1) FMS, 2) TMD, 3) back pain, 4) IBS, 5) chronic tension-type headache, 6) chronic migraine, and 7) chronic fatigue syndrome, 8) endometriosis, and 9) interstitial cystitis. Women with VBD and no COPCs will be designated as VBD-p, while women with VBD and ≥1 COPC will be designated as VBD-c. These criteria will be used as a primary method to distinguish between VBD-p and VBD-c in this study.

We, however, acknowledge that the criteria to distinguish VBD subtypes needs further exploration. In particular, it is possible that the optimal cutoff for VBD-c requires more than one COPC to be present. Therefore, as a secondary method to characterize VBD subtypes, we will calculate a continuous COPC 'centralness' score defined as the number of COPCs present ranging from 0 to 9. This continuous COPC score will be used to identify the optimal cutoff value between VBD-p and VBD-c in terms of treatment response.

To further characterize the two VBD subtypes, we will also measure ***generalized pain*** at 'neutral' non-pelvic sites using a quantitative sensory testing approach designed by Dr. Gracely and colleagues.[94, 95] The 3 neutral sites (deltoid muscle, shin, trapezius) are remote from the pelvic region and not used for diagnosing other pain conditions, but reflect an individual's overall

pressure pain sensitivity.[98-100] An algometer will be applied to the 3 sites, with continuously ascending pressure at a rate of 1 kg/s to a maximum of 10 kg. Participants will indicate when the evoked sensation first becomes painful. A composite pressure pain threshold (PPT) score will be compared between VBD-c and VBD-p subgroups using ANCOVA. We would expect women with VBD-p to have higher PPT scores (less pain) and women with VBD-c to have lower scores (more pain) at these sites.

15.2 Analysis of the Aim 1 Endpoints

Primary endpoints will be the net change in vaginal vestibule pain following treatment measured using the Tampon Test and the Short-Form McGill Pain Questionnaire (SF-MPQ).

- *The Tampon Test* will provide a self-reported numeric rating scale of pain with self-tampon insertion, performed by the patient and reported to the research nurse. An Original Regular Tampax tampon will be inserted by the participant into her vagina and then she will be asked to verbally rate the pain on a scale of 0-10, with 0 meaning no pain and 10 meaning the worst possible pain. The Tampon Test has good reliability, construct validity, and responsiveness and is a recommended outcome measure for VBD clinical trials.[96]

- *The SF-MPQ* measures perceived sensory qualities of pain using 11 describers and affective qualities related to pain using 5 describers. Responses on 4-point scales are summed to compute scores for each section.[97] SF-MPQ subscales have been successfully used to quantify treatment responses in trials for VBD and vulvodynia.[98-100]

Secondary endpoints include changes in pressure pain thresholds (PPTs) measured at the

- *Vaginal Vestibule PPT* will be determined using a digital vestibular algometer applied to 3 externally-accessed sites (2 and 10 o'clock on the upper vestibule and 6 o'clock on the lower vestibule) beginning at 1N and increasing until the participant's first sensation of pain or a maximum applied pressure of 10N.[102]

- *Levator Muscle Complex PPTs* will be determined in a similar manner by applying the algometer internally to the right, midline, and left puborectalis levator muscles sites (5, 6, and 7 o'clock) just lateral to the perineum.

- *Remote Bodily PPTs* will be determined in a similar manner by applying the algometer to 3 'neutral' non-pelvic body sites (deltoid, shin, and trapezius), right and left, and calculating a composite score.

Data Analysis. The two primary statistical questions for Aim 1 are: 1a) Is peripheral treatment with lidocaine/estradiol compound cream, or central treatment with nortriptyline, or a combination of both treatments more effective for either VBD subtype than placebo? 1b) Is peripheral treatment more effective than central treatment in VBD-p, and is central treatment more effective than peripheral treatment in VBD-c?

To answer questions 1a and 1b, we will fit mixed model repeated measures (MMRM) models which account for missing data as an integral part of the analyses under the assumption of ignorable missing. To answer question 1a, the MMRM model includes longitudinal pain score (0-10 NRS on Tampon Test) as a continuous outcome variable; includes fixed effect terms for treatment (nortriptyline, lidocaine/estradiol, and their interaction), visit, treatment visit interaction, center, and fixed effect covariates vector including age, VBD subtype (binary variable where VBD-c is coded as 0 and VBD-p coded as 1), race (three levels "white", "black" and "other" race, coded into two binary dummy variables with European American serving as a reference level).

Overall treatment effect of nortriptyline, lidocaine/estradiol, and their interaction will be evaluated using an F-test. If significant ($p < 0.05$), treatment differences (each of 3 treatment groups minus placebo) in the Least-Squares means (LS means), their 2-sided 95% Cis, and associated p-

values will be estimated from the MMRM model. P-values will be compared with the significance threshold Bonferroni-corrected for 3 tests (i.e. alpha 0.0166).

To answer question 1b, we will fit a MMRM model analogous to 1a, with additional terms of VBD subtype visit interaction, treatment VBD subtype interaction, and treatment visit VBD subtype interaction. The significance of the additional interaction terms will be evaluated using a F-test comparing the full model in 1b and the reduced model in 1a. Significant ($p < 0.05$) F-test will suggest that the effect of either nortriptyline or lidocaine/estradiol cream, or both, differ by VBD subtype. In this case, we will compare the LS means of the treatment differences in each treatment-VBD-subtype subgroup, after appropriate Bonferroni correction.

If the results for question 1a or 1b are statistically significant, the following primary outcomes will be analyzed using the same modeling strategies (models in 1a and 1b above): % decrease in pain score (tampon test) from baseline to the 16-week time point and % decrease in perceived pain on the SF-MPQ from baseline to the 16-week time point. Secondary outcomes include change in mucosal, vulvovaginal muscle, and remote bodily PPTs from baseline to the 16-week time point. Secondary outcomes also include change in pain scores and PPTs from baseline to the 8-week time point and change in pain scores and PPTs from the 16-week to the 24-week time point.

If the result for question 1b is non-significant, we will use the continuous COPC score in model 1b instead of a dichotomous VBD classification to compare treatment success for VBD-p and VBD-c. In addition, we use a binary treatment outcome (achievement of 30% pain reduction) to identify the optimal cutoff of the continuous COPC score in each treatment group.

Secondary analyses of muscle pain based on PPTs will also determine: a) the proportion of women with VBD that have a pelvic muscle pain component; b) differences in the distribution of muscle pain in VBD-p and VBD-c groups; and c) presence of muscle pain to predict treatment outcome. The latter will be tested by including the muscle pain variable (continuous or dichotomous) as part of the fixed effect term in model Ia.

Expected Outcomes. 1) For women with VBD-p, peripheral lidocaine/estradiol treatment will be more effective than placebo or nortriptyline in reducing numeric pain ratings on the tampon test, perceived pain on the SF-MPQ, and pelvic PPTs on the algometer test at week 8 and week 16. **2)** For women with VBD-c, centrally-targeted nortriptyline treatment will be more effective than placebo or lidocaine/estradiol in reducing numeric pain ratings on the tampon test, perceived pain on the SF-MPQ, and pelvic/remote bodily PPTs on the algometer test at week 16. **3)** For women with VBD-c, combined peripheral and centrally-targeted treatments will be most effective in reducing pain on primary and secondary outcome measures at week 16. **4)** The two VBD subtypes will exhibit differential responses to treatment withdrawal at week 16, such that at the week 24 visit women with VBD-c will exhibit increases in pain after treatment withdrawal compared to women with VBD-p.

15.3 Analysis of the Aim 2 Endpoints

Primary endpoints include the net change in perceived sexual health measured using the Patient-Reported Outcomes Measurement Information System (PROMIS) and perceived physical and mental health measured using the SF-12 Health Survey (SF-12).

- The *PROMIS* is a 96-item form developed by the NIH that measures 11 domains of biopsychosocial function and includes an assessment of sexual function measures (e.g., desire, frequency, fear, and pain) related to sexual intercourse.[115]

- The *SF-12* assesses 6 domains: global health, physical functioning, physical roles, emotional functioning, emotional roles and pain interference using an algorithm[118] based on answers to 12 physical and mental health-related questions.

Secondary endpoints include net changes in perceived mood and health using:

- Symptom Check List 27 (*SCL-27)*, which measures a broad range of psychological symptoms (e.g., anxiety and depression) and has high reliability and strong correlations to pain.[121,122]

- Pennebaker Index of Limbic Languidness (*PILL),* which is used to create a summary score of somatic symptoms (e.g., itchy eyes, dizziness). Symptom frequency is recorded on a five-point Likert scale ranging from "never" to "more than once a week".[108]

- Perceived stress scale (PSS), which is a 10-item scale that measures the impact of personal stress on thoughts and feelings.

- Sleep Scale, which is a 12-item scale that measures amount of sleep and ease/difficulty of initiating and maintaining sleep

Data Analysis. The two primary statistical questions for Aim 2 are: 2a) At baseline, do women with VBD-c exhibit worse perceived physical, mental, or sexual health than women with VBD-p? 2b) Considering physical, mental, or sexual health, is peripheral treatment with lidocaine/estradiol cream more effective than nortriptyline in VBD-p, and central treatment with nortriptyline more effective than lidocaine/estradiol cream in VBD-c?

To answer question 2a, we will fit three linear regression models with the dependent continuous variable being a variable for each of the 3 patient reported outcomes (SF12 physical health score, SF12 mental health score, and PROMIS sexual health score). VBD subtype will be the predictor of interest. Covariates will include age (continuous variable), race (coded as in model Ia), study site. Regression coefficient for VBD subtype will be tested for statistical significance using Bonferroni-corrected threshold alpha 0.0166 (corrected for 3 tests corresponding to 3 outcomes). Regression coefficient significance will suggest outcomes differ by VBD subtype.

To answer question 2b, three MMRM models analogous to model 2b will be fit, where Y is a continuous variable representing the primary outcomes measured at 16 weeks. Similar to 2b, we will jointly evaluate the significance of additional interaction terms using an F-test ("overall test"). Significant F-test will suggest that the effect of either nortriptyline or lidocaine/estradiol cream, or both, differ by VBD subtype. In this case, we will compare the LS means of the treatment differences in each treatment-VBD-subtype subgroup, after appropriate Bonferroni correction. Secondary outcomes will also include change in SCL-27 mood score, PILL somatization score, and PROMIS biopsychosocial trait scores from baseline to 16 weeks following treatment. Secondary outcomes also include change in patient-reported outcomes from baseline to the 8-week time point and change in pain scores and PPTs from the 16-week to the 24-week time point.

Expected Outcomes. 1) For women with VBD-p, peripheral lidocaine/estradiol treatment will be more effective than placebo or nortriptyline in improving perceived physical, mental, and sexual health at 8 and 16 weeks. **2)** For women with VBD-c, centrally-targeted nortriptyline treatment will be more effective than placebo or lidocaine/estradiol in improving perceived physical, mental, and sexual health at 16 weeks. **3)** For women with VBD-c, combined peripheral and centrally-targeted treatments will be most effective in improving health at 16 weeks. **4)** The two VBD subtypes will exhibit differential responses to treatment withdrawal, such that women with VBD-c report decreases in perceived health from 16 to 24 weeks compared to women with VBD-p.

15.4 Analysis of the Aim 3 Endpoints

Cytokine Expression levels will be measured in vaginal lavage samples and plasma isolated from whole blood. We will use a standard Human MesoScale Discovery multiplex kit to measure 36 inflammatory mediators (4 tier-1 and 32 tier-2).

Tier-1 mediators include the pro-inflammatory cytokines IL-1β,[59-61] IL-8,[7,60,62] and IL-17[63] and anti-inflammatory cytokine IL-1ra.[7,61] These cytokines were selected based on previous associations with VBD case status and symptom severity.

Tier-2 mediators include cytokines (e.g., interleukin 6; IL-6), chemokines (e.g., monocyte chemoattractant protein 1; MCP1), and growth factors (e.g., vascular endothelial growth factor; VEGF) that are involved in peripheral and central sensitization, inflammation, and chronic pain.[128]

miRNA Expression levels will be measured in vaginal swab samples and blood. We will isolate total RNA and then ship the RNA to UT Health Genome Sequencing Facility (GSF), directed by Dr. Zhao Lai. The GSF core will perform small RNA library preparation and sequencing.

Data Analysis. The two primary statistical questions for Aim 3 are: 3a) At baseline, is there an inverse association between VBD 'centralness' score (determined by the number of COPC sites) and the levels of Tier-1 cytokines in vaginal samples; and a positive association between VBD 'centralness' score and the levels of Tier-1 cytokines in plasma samples? 3b) Do biomarkers in vaginal fluid predict response to peripheral treatment and biomarkers in blood predict response to central treatment?

To answer question 3a, we will fit a linear regression model where cytokine level (skewed variables will be transformed (e.g., log, box-cox) to approximate normal distribution) will be a continuous dependent variable and VBD 'centralness' score, will be the main predictor of interest. Covariates will include age and race. Significance of regression coefficient associated with the 'centralness' score will be evaluated using an F-test and significance threshold (alpha) Bonferroni-corrected for the number of cytokines assessed (4 cytokines corresponding to 4 statistical tests). Subgroup means, mean differences and 95% confidence intervals will be output.

To answer question 3b, we will fit a MMRM model analogous to 1a, with additional terms of treatment cytokine (in blood and vaginal samples) interaction, visit cytokine interaction, and treatment visit cytokine interaction. Significance of additional interaction terms will be evaluated using an F-test. Significant ($p < 0.05$) F-test will suggest that treatment effects by nortriptyline or lidocaine/estradiol cream or both, are modified by cytokines.

Secondary analyses for Aim 3 will include the association of Tier 2 biomarkers (cytokines and miRNAs) with VBD subtype and interaction with treatment response. These analyses will be analogous to those specified for questions 3a and 3b, with p-value thresholds kept at 0.05 due to exploratory nature of these analyses. In our secondary analyses we will also evaluate the change of biomarker concentrations (blood and vaginal samples) over time (at 0, 8, 16, and 24 weeks) in each treatment group and VBD subtype. We hypothesize that these biomarkers may serve as intermediate phenotypes for treatment success in VBD patient subgroups.

Expected Outcomes. 1) Women with VBD-p will have higher levels of inflammatory mediators in peripheral vaginal samples, while women with VBD-c will have higher levels of inflammatory mediators in circulating blood measured at the baseline visit (0 weeks). **2)** Abnormalities in baseline vaginal cytokine and miRNA biomarkers will predict a positive response to peripheral lidocaine/estradiol treatment, while abnormalities in blood biomarkers will predict a positive response to centrally-targeted nortriptyline or combined treatment. **3)** Peripheral treatment will resolve abnormalities in vaginal biomarkers evaluated at 8, 16, and 24 weeks, while central treatment will resolve abnormalities in blood biomarkers evaluated at 16 weeks.

All analyses will be performed using the Intent-to-Treat (ITT) population. The ITT population will include all randomized participants. Participants will be grouped according to their randomized allocation, regardless of whether the allocated therapy was administered or switched.

Any deviations from the statistical analyses described above, due to unforeseen circumstances, will be documented in the Statistical Analysis Plan (SAP).

15.5 Analysis of Safety Data and Statistical Monitoring Plan

Interim data analysis for efficacy and futility will not be conducted due to relatively small size and short duration of this clinical trial. Safety data, summarized at the treatment level, will be assessed approximately every 6 months by the DSMB. Adverse Events will be summarized by treatment group and will include incidence by term, severity, relationship to study medication as well as overall incidence rates (regardless of severity and relationship to study medication).

15.6 Sample Size and Power

We are planning to enroll 400 women with VBD, with an estimated attrition rate of 20%. Of note, Reed et al. found that vulvar pain measured on a 0-10 numeric scale has standard deviation 2.0.[104] Salaffi et al[105] noted that 2 points on a 0-10 NRS correspond to a clinically meaningful difference in pain severity. Using these results and assuming the within-subject correlation of 0.5, at a significance level of 0.05, the proposed sample size will provide sufficient power of at least 0.80 to detect differences as small as 0.82 on a 0-10 scale for the overall treatment main effects (3 arms) vs. placebo. To test each individual treatment effect vs. placebo, at a significance level of 0.0167 (Bonferroni-corrected for 3 arms), the proposed sample size can detect differences as small as 0.98 on a 0-10 scale. At a significance level of 0.05, the proposed sample size can detect differences as small as 1.35 and 1.20 on a 0-10 scale for the interaction effect of treatment and each VBD-subtype, given possible splits of 40/60, 50/50, or 60/40 (of note, VBD-p vs VBD-c frequencies were 40/60 at UNC and 50/50 at UCLA clinics in previous studies).

To calculate power for research question IIIa, we used data from our previous study of biomarkers in VBD patients.[7] Conservatively assuming that VBD-c patients represent 60% (192/320) of the sample and VBD-p patients represent 40% (128/320) of the sample, and adjusting the significance threshold for 4 tier 1 cytokine tests, we will be able to detect mean differences of at least 0.4 SD with the power of 0.84 or greater using a t-test. For IL-8, 0.4 SD translates to approximately 2 pg/mL, and for IL-1ra 0.4 SD translates to 240 pg/mL of the minimal detectable difference. Of note, the mean differences between controls and VBD patients for IL-8 and IL-1ra in our previous study were 4 pg/mL and 300 pg/mL, respectively. Thus, we have sufficient power to detect plausible differences in cytokine levels between the VBD subgroups.

16 DATA MANAGEMENT PROCEDURES

16.1 Overview of Data Management

The CC will have primary responsibility for data management, including the development of data collection systems, data monitoring processes, and data storage and back-up. State-of-the-art technology will be used for the management of the network's data.

eCRF: The CC team will develop modules, designed in Redcap, that will capture patient responses to questions having to do with their VBD symptoms. The Redcap instruments will include an enrollment and demographics form; forms for recording relevant history, Pain symptoms, vulvar exam results, laboratory results, baseline biomarker levels, and other baseline presenting characteristics; follow-up forms for use during regular follow-up visits; forms to track the participant's clinical course over time; and others that track all related information for Patients that meet the trial criteria.

System: The data will be collected in a validated, IRB approved Redcap survey environment. The CC team of skilled data managers and programmers will design and produce a tailored network system that provides operational efficiency and meaningful reporting of metrics.

Data Management Process: The Redcap survey environment will be used for data entry and simple reports. All data will be entered into Redcap by personnel at the clinical sites. Any out-of-range values and missing key variables will be flagged and addressed in real-time at the site during data entry. When a query is generated on a particular variable, a flag is raised in a database field; the system tracks the queries and produces reports of outstanding queries. Queries can also be generated from manual or statistical review of the data forms.

The CC will create reports to identify trends in the data that may require additional clarification and training. These reports will be available to the sites and to the study leadership as we work with the sites to correct negative trends and eliminate future data errors. The CC will perform internal database quality-control checks during the study to identify systematic deviations requiring corrections.

Data Quality Control: A three-step approach to data quality control will be implemented.

1. Training: Prior to the start of enrollment, the Investigators and Nurse Practitioners will be trained on the clinical protocol and data collection procedures. Recent site surveys indicate that most Coordinators are very familiar with the EDC system, so training is typically targeted to a specific protocol. For Coordinators new to REDCap, the CC will provide training with hands-on database interaction, demonstration of key REDCap system functionality, and practice exercises. Personnel at the clinical sites will enter the data mandated by the protocol into REDCap. All CRFs will be completed according to the current Good Clinical Practice (GCP) guidelines. The CC will conduct follow-up training and training for new study personnel as needed.

2. Monitoring: A CC monitor will visit sites during the enrollment period to ensure that data collection is being handled properly, to provide in-service training, and to address questions from site investigators and coordinators. Additional details will be outlined in the Clinical Monitoring Plan.

3. Managing data: A series of computerized data validation checks will be programmed by the CC to check for missing data, inconsistencies in the data or data that is out of range. After the data have been exported from the EDC system to SAS for statistical summarization and data analysis, further cross-checking of the data will be performed by the CC and queries issued through REDCap for any discrepancies.

16.2 Data Security

Access to databases will be controlled centrally by the CC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage. Database and web servers will be secured by a firewall and through controlled physical access. Database back-up will be performed daily using standard procedures in place at the CC. All disk drives that provide network services, and all user computers, will be protected using virus-scanning software.

16.3 Publication Policy

Dissemination of preliminary information can adversely affect the objectivity of study data. For this reason, Investigators will be prohibited from performing subset analyses at any point prior to the conclusion of the study, and any data, other than safety data, cannot be used for publication or reporting outside of this study until the study is completed or discontinued by the DSMB or NICHD.

17 STUDY ADMINISTRATION

17.1 Data and Safety Monitoring Board

As noted above, the DSMB will be composed of 4 members: a physician, a statistician, a patient representative, and a bioethicist. All DSMB operations will be managed by the DCRI.

DSMB Charter and Meetings. The DCRI Statistical Team will develop a DSMB Charter that details specific data tables to be provided and procedures to be followed. The DSMB Charter will also describe the overall mission, responsibilities, and meeting schedule of the board. The DCRI statistical team will be responsible for DSMB charter review and approval. The draft charter will be submitted for review and approval by NICHD, the DSMB chairperson, and study Investigators prior to study enrollment. After the charter has been finalized, any changes to the plan will be documented in the minutes of the DSMB meeting. At the conclusion of each DSMB meeting, the DSMB Chair will make a recommendation to the study team about continuation of the trial. It is expected that the DSMB will meet every 6-months. If safety issues arise, additional conference calls can be requested by the DSMB chairman. DCRI statistical team will coordinate and convene all DSMB meetings.

17.2 Coordinating Center

Dr. Nackley will function as the Clinical CC, Dr. Stirling will function as the Data Management CC, and the DCRI will function as the Statistical CC for this trial.

17.3 Core Laboratories

17.3.1 Central Compounding Pharmacy and Pacifica Compounding Pharmacy

The investigational product (IP) and placebo capsules to be dispensed at UNC will be prepared by Central Compounding Pharmacy, directed by Jennifer Burch, and shipped to study personnel at the UNC Hillsborough clinic.

The IP and placebo capsules to be dispensed at UCLA will be prepared by Pacifica Pharmacy, directed by Jeffrey Barris.

17.3.2 Substrate Services Core

The Substrate Services Core, directed by Mary-Beth Joshi, will provide quality control and a state-of-the-art repository for study samples. Precise sample inventory will be facilitated through a 2D Barcode system. This system includes the sample tubes themselves, which have a 2D barcode with 14-digit alpha-numeric code etched on the bottom of the sample tubes. A scanner will be used to ensure accurate reading of barcodes, and the data will be downloaded into the LabVantage Laboratory Information Management System (LIMS) for electronic inventory.

17.3.3 Sequencing Core

Isolated RNA will be shipped from the Substrate Services Core to the University of Texas Health Genome Sequencing Facility (GSF), directed by Dr. Zhao Lai. The GSF core will perform small RNA library preparation and sequencing.

18 ETHICAL AND REGULATORY CONSIDERATIONS

18.1 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol to ensure adherence to Good Clinical Practice, as described in the following documents:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

By signing the protocol, the investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice to which it conforms.

18.2 Institutional Review Board

Before implementing this study, the protocol, the proposed informed consent form and other information available to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). A signed and dated statement that the protocol and informed consent have been approved by the IRB must be provided to the CC before study initiation. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

18.3 Informed Consent Procedures

18.3.1 Informed Consent

The Investigator or designee must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard non-technical language. If a patient agrees to participate in the Vestibulodynia: Update study, they will review and sign the IRB-approved electronic Informed Consent Form (eICF). No patient can enter the study before her informed consent has been obtained.

18.3.2 Confidentiality and HIPAA Requirements

We will obtain the same data forms from all participants, including vulvar examination/assessment, self-reports of pain and function via questionnaire, and collection of biologic specimens (local vaginal swabs for vaginal miRNA, vaginal lavage with aspiration for vaginal protein expression, and venipuncture for whole blood miRNA and protein). Methods for collecting and storing data will be fully described in the informed consent procedure and signed eICFs.

Vulvar examination/assessment will be administered by the investigators or study personnel who have completed the ethical training for protection of human participants in research. The same study personnel will also administer questionnaires. Only the investigators for this study will have access to all the individually identifiable private information about human participants. The clinical sites will not be providing any direct patient identifiers when entering data into the database and that those identifiers will be kept securely stored at the site. Each participant will be assigned a

unique number at the time of screening that will represent their study identifier.

18.3.3 Protections of Human Subjects

All subject interactions, from initial discussion about the study to the actual study visit, will take place in a private setting (examination/assessment rooms) behind closed doors. Drs. Geller, Carey, and Rapkin may be involved as “passive” recruiters, providing information and written materials to patients who may be eligible to participate, but a more detailed description of the study and the process of obtaining informed consent will the responsibility of another member of the research team in order to avoid the possibility or perception of coercion. All subject data will be stored centrally under the purview of a designated IT specialist.

18.3.4 Summary of the Risks and Benefits

Risks.
Anticipated risks specific to this study are outlined in **Section 14.2** above.

Benefits.
Topical lidocaine and estradiol are used to manage pelvic pain disorders such as VBD, but are prescribed in the form of two separate creams. Compounding lidocaine and estradiol in the same cream would improve ease of use, promote more uniform distribution and absorption, and likely have added analgesic benefit. Additionally, the hydrophilic petrolatum is a nonirritating base that may be less likely to cause irritation compared to the typical ointment or jelly form of lidocaine. This compound improves ease of use and compliance. Nortriptyline is a tricyclic antidepressant medication that is FDA-approved to treat chronic neuropathic pain disorders (e.g., post-herpetic neuralgia). Nortriptyline is also a hypothesized treatment for VBD that is frequently prescribed to VBD participants and regularly used in the management of female pain syndromes such as VBD. When combined, the lidocaine/estradiol cream and nortriptyline therapies are theorized to have a synergistic effect. It is hoped that these interventions will provide a safe and effective therapeutic option for painful VBD. However, during the consent process, it will be stated that participants may experience no therapeutic benefit, but that their participation may inform additional research that helps others in the future. This research study has been designed to minimize the risks to participants. The risks in this study are reasonable in relation to the importance of knowledge gained as a result of this work.

19 REFERENCES

1. Harlow BL and Stewart EG. A population-based assessment of chronic unexplained vulvar pain: have we underestimated the prevalence of vulvodynia? *Journal of the American Medical Women's Association (1972)*. 2003;58:82-8.
2. Lev-Sagie A and Witkin SS. Recent advances in understanding provoked vestibulodynia. *F1000Research*. 2016;5:2581.
3. Xie Y, Shi L, Xiong X, Wu E, Veasley C and Dade C. Economic burden and quality of life of vulvodynia in the United States. *Current medical research and opinion*. 2012;28:601-8.
4. Williams RE, Hartmann KE, Sandler RS, Miller WC and Steege JF. Prevalence and characteristics of irritable bowel syndrome among women with chronic pelvic pain. *Obstetrics and gynecology*. 2004;104:452-8.
5. Zolnoun DA, Rohl J, Moore CG, Perinetti-Liebert C, Lamvu GM and Maixner W. Overlap between orofacial pain and vulvar vestibulitis syndrome. *The Clinical journal of pain*. 2008;24:187-91.
6. Goldstein AT, Pukall CF, Brown C, Bergeron S, Stein A and Kellogg-Spadt S. Vulvodynia: Assessment and Treatment. *The journal of sexual medicine*. 2016;13:572-90.
7. Ciszek BP, Khan AA, Dang H, Slade GD, Smith S, Bair E, Maixner W, Zolnoun D and Nackley AG. MicroRNA expression profiles differentiate chronic pain condition subtypes. *Transl Res*. 2015;166:706-720 e11.
8. Updike GM and Wiesenfeld HC. Insight into the treatment of vulvar pain: a survey of clinicians. *Am J Obstet Gynecol*. 2005;193:1404-9.
9. Corbett SH, Cuddeback G, Lewis J, As-Sanie S and Zolnoun D. Trends in pharmacy compounding for women's health in North Carolina: focus on vulvodynia. *Southern medical journal*. 2014;107:433-6.
10. De Andres J, Sanchis-Lopez N, Asensio-Samper JM, Fabregat-Cid G, Villanueva-Perez VL, Monsalve Dolz V and Minguez A. Vulvodynia--An Evidence-Based Literature Review and Proposed Treatment Algorithm. *Pain practice : the official journal of World Institute of Pain*. 2016;16:204-36.
11. Akopians AL and Rapkin AJ. Vulvodynia: The Role of Inflammation in the Etiology of Localized Provoked Pain of the Vulvar Vestibule (Vestibulodynia). *Seminars in reproductive medicine*. 2015;33:239-45.
12. Bohm-Starke N, Hilliges M, Falconer C and Rylander E. Increased intraepithelial innervation in women with vulvar vestibulitis syndrome. *Gynecologic and obstetric investigation*. 1998;46:256-60.
13. Bohm-Starke N, Hilliges M, Falconer C and Rylander E. Neurochemical characterization of the vestibular nerves in women with vulvar vestibulitis syndrome. *Gynecologic and obstetric investigation*. 1999;48:270-5.
14. Tympanidis P, Casula MA, Yiangou Y, Terenghi G, Dowd P and Anand P. Increased vanilloid receptor VR1 innervation in vulvodynia. *European journal of pain*. 2004;8:129-33.
15. Tympanidis P, Terenghi G and Dowd P. Increased innervation of the vulval vestibule in patients with vulvodynia. *The British journal of dermatology*. 2003;148:1021-7.
16. Warner TF, Tomic S and Chang CK. Neuroendocrine cell-axonal complexes in the minor vestibular gland. *J Reprod Med*. 1996;41:397-402.
17. Giesecke T, Gracely RH, Grant MA, Nachemson A, Petzke F, Williams DA and Clauw DJ. Evidence of augmented central pain processing in idiopathic chronic low back pain. *Arthritis and rheumatism*. 2004;50:613-23.
18. Gracely RH, Lynch SA and Bennett GJ. Painful neuropathy: altered central processing maintained dynamically by peripheral input. *Pain*. 1992;51:175-94.
19. Gracely RH, Grant MA and Giesecke T. Evoked pain measures in fibromyalgia. *Best Pract Res Clin Rheumatol*. 2003;17:593-609.
20. Reed BD, Plegue MA, Williams DA and Sen A. Presence of Spontaneous Pain and Comorbid Pain Conditions Identifies Vulvodynia Subgroups. *Journal of lower genital tract disease*. 2016;20:57-63.

21. Reed BD, Sen A, Harlow SD, Haefner HK and Gracely RH. Multimodal Vulvar and Peripheral Sensitivity Among Women With Vulvodynia: A Case-Control Study. *Journal of lower genital tract disease*. 2017;21:78-84.
22. Hampson JP, Reed BD, Clauw DJ, Bhavsar R, Gracely RH, Haefner HK and Harris RE. Augmented central pain processing in vulvodynia. *J Pain*. 2013;14:579-89.
23. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain*. 2011;152:S2-15.
24. Goetsch MF, Morgan TK, Korcheva VB, Li H, Peters D and Leclair CM. Histologic and receptor analysis of primary and secondary vestibulodynia and controls: a prospective study. *Am J Obstet Gynecol*. 2010;202:614.e1-8.
25. Bornstein J, Maman M and Abramovici H. "Primary" versus "secondary" vulvar vestibulitis: one disease, two variants. *Am J Obstet Gynecol*. 2001;184:28-31.
26. Granot M, Friedman M, Yarnitsky D, Tamir A and Zimmer EZ. Primary and secondary vulvar vestibulitis syndrome: systemic pain perception and psychophysical characteristics. *Am J Obstet Gynecol*. 2004;191:138-42.
27. Carey ET, Till SR and As-Sanie S. Pharmacological Management of Chronic Pelvic Pain in Women. *Drugs*. 2017;77:285-301.
28. Zolnoun DA, Hartmann KE and Steege JF. Overnight 5% lidocaine ointment for treatment of vulvar vestibulitis. *Obstetrics and gynecology*. 2003;102:84-7.
29. Butterworth JFt and Strichartz GR. Molecular mechanisms of local anesthesia: a review. *Anesthesiology*. 1990;72:711-34.
30. Devor M. Sodium channels and mechanisms of neuropathic pain. *J Pain*. 2006;7:S3-s12.
31. Bazin S, Lefebvre J, Fortier M, Brisson J, Brouillette F, Bujold E and Bouchard C. [Evaluation of an estrogen vaginal cream for the treatment of dyspareunia: a double-blind randomized trial]. *Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC*. 2011;33:838-43.
32. Gardella B, Iacobone AD, Porru D, Musacchi V, Dominoni M, Tinelli C, Spinillo A and Nappi RE. Effect of local estrogen therapy (LET) on urinary and sexual symptoms in premenopausal women with interstitial cystitis/bladder pain syndrome (IC/BPS). *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology*. 2015;31:828-32.
33. Bhattacherjee A, Rumi MA, Staecker H and Smith PG. Bone morphogenetic protein 4 mediates estrogen-regulated sensory axon plasticity in the adult female reproductive tract. *J Neurosci*. 2013;33:1050-61a.
34. Ting AY, Blacklock AD and Smith PG. Estrogen regulates vaginal sensory and autonomic nerve density in the rat. *Biology of reproduction*. 2004;71:1397-404.
35. Walker EA, Roy-Byrne PP, Katon WJ and Jemelka R. An open trial of nortriptyline in women with chronic pelvic pain. *International journal of psychiatry in medicine*. 1991;21:245-52.
36. Stolar AG and Stewart JT. Nortriptyline for depression and vulvodynia. *The American journal of psychiatry*. 2002;159:316-7.
37. Brown CS, Bachmann GA, Wan J and Foster DC. Gabapentin for the Treatment of Vulvodynia: A Randomized Controlled Trial. *Obstet Gynecol*. 2018;131:1000-1007.
38. Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpaa ML, Kent JL, Krane EJ, Lebel AA, Levy RM, Mackey SC, Mayer J, Miaskowski C, Raja SN, Rice AS, Schmader KE, Stacey B, Stanos S, Treede RD, Turk DC, Walco GA and Wells CD. Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin Proc*. 2010;85:S3-14.
39. Millan MJ. Descending control of pain. *Prog Neurobiol*. 2002;66:355-474.
40. Dick IE, Brochu RM, Purohit Y, Kaczorowski GJ, Martin WJ and Priest BT. Sodium channel blockade may contribute to the analgesic efficacy of antidepressants. *J Pain*. 2007;8:315-24.
41. Obuchowicz E, Kowalski J, Labuzek K, Krysiak R, Pendzich J and Herman ZS. Amitriptyline and nortriptyline inhibit interleukin-1 release by rat mixed glial and microglial cell cultures. *Int J Neuropsychopharmacol*. 2006;9:27-35.
42. Desrochers G, Bergeron S, Landry T and Jodoin M. Do psychosexual factors play a role in the etiology of provoked vestibulodynia? A critical review. *Journal of sex & marital therapy*. 2008;34:198-226.

43. van Lankveld JJ, Granot M, Weijmar Schultz WC, Binik YM, Wesselmann U, Pukall CF, Bohm-Starke N and Achtrari C. Women's sexual pain disorders. *The journal of sexual medicine*. 2010;7:615-31.
44. Chisari C and Chilcot J. The experience of pain severity and pain interference in vulvodynia patients: The role of cognitive-behavioural factors, psychological distress and fatigue. *Journal of psychosomatic research*. 2017;93:83-89.
45. Zolnoun D, Hartmann K, Lamvu G, As-Sanie S, Maixner W and Steege J. A conceptual model for the pathophysiology of vulvar vestibulitis syndrome. *Obstetrical & gynecological survey*. 2006;61:395-401; quiz 423.
46. Talley SJ, Bytzer P, Hammer J, Young L, Jones M and Horowitz M. Psychological distress is linked to gastrointestinal symptoms in diabetes mellitus. *The American journal of gastroenterology*. 2001;96:1033-8.
47. Miller RJ, Jung H, Bhangoo SK and White FA. Cytokine and chemokine regulation of sensory neuron function. *Handb Exp Pharmacol*. 2009:417-49.
48. Dinarello CA, Kluger MJ, Oppenheim JJ and Powanda MC. *The physiological and pathological effects of cytokines*. New York: Liss/John Wiley & Sons Inc; 1990.
49. Kress MaS, C. Neuroimmunology and Pain: Peripheral Effects of Proinflammatory Cytokines. In: K. a. H. Brune, H.O., ed. *Hyperalgesia: Molecular Mechanisms and Clinical Implications, Progress in Pain Research Management* Seattle: IASP Press; 2004(30): 57-65.
50. Hartung JE, Ciszek BP and Nackley AG. beta2- and beta3-adrenergic receptors drive COMT-dependent pain by increasing production of nitric oxide and cytokines. *Pain*. 2014;155:1346-55.
51. Gur A, Karakoc M, Nas K, Remzi, Cevik, Denli A and Sarac J. Cytokines and depression in cases with fibromyalgia. *J Rheumatol*. 2002;29:358-61.
52. Kopp S. The influence of neuropeptides, serotonin, and interleukin 1beta on temporomandibular joint pain and inflammation. *J Oral Maxillofac Surg*. 1998;56:189-91.
53. Ogura N, Satoh K, Akutsu M, Tobe M, Kuyama K, Kuboyama N, Sakamaki H, Kujiraoka H and Kondoh T. MCP-1 production in temporomandibular joint inflammation. *J Dent Res*. 2010;89:1117-22.
54. Shafer DM, Assael L, White LB and Rossomando EF. Tumor necrosis factor-alpha as a biochemical marker of pain and outcome in temporomandibular joints with internal derangements. *J Oral Maxillofac Surg*. 1994;52:786-91; discussion 791-2.
55. Takahashi T, Kondoh T, Fukuda M, Yamazaki Y, Toyosaki T and Suzuki R. Proinflammatory cytokines detectable in synovial fluids from patients with temporomandibular disorders. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85:135-41.
56. Maes M, Song C, Lin A, De Jongh R, Van Gastel A, Kenis G, Bosmans E, De Meester I, Benoy I, Neels H, Demedts P, Janca A, Scharpe S and Smith RS. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine*. 1998;10:313-8.
57. Maes M. Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry*. 1995;19:11-38.
58. Maes M. Major depression and activation of the inflammatory response system. *Adv Exp Med Biol*. 1999;461:25-46.
59. Foster DC and Hasday JD. Elevated tissue levels of interleukin-1 beta and tumor necrosis factor-alpha in vulvar vestibulitis. *Obstet Gynecol*. 1997;89:291-6.
60. Foster DC, Piekarz KH, Murant TI, LaPoint R, Haidaris CG and Phipps RP. Enhanced synthesis of proinflammatory cytokines by vulvar vestibular fibroblasts: implications for vulvar vestibulitis. *Am J Obstet Gynecol*. 2007;196:346.e1-8.
61. Gerber S, Bongiovanni AM, Ledger WJ and Witkin SS. Defective regulation of the proinflammatory immune response in women with vulvar vestibulitis syndrome. *Am J Obstet Gynecol*. 2002;186:696-700.
62. Baker DA, Peresleni T and Kocis C. Inflammatory Markers in Vestibulodynia [4]. *Obstet Gynecol*. 2016;127 Suppl 1:1s-2s.
63. Ventolini G, Gygax SE, Adelson ME and Cool DR. Vulvodynia and fungal association: a preliminary report. *Medical hypotheses*. 2013;81:228-30.

64. Davis AJ and Perkins MN. The involvement of bradykinin B1 and B2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat. *Br J Pharmacol*. 1994;113:63-8.
65. Schwarz YA, Amin RS, Stark JM, Trapnell BC and Wilmott RW. Interleukin-1 receptor antagonist inhibits interleukin-8 expression in A549 respiratory epithelial cells infected in vitro with a replication-deficient recombinant adenovirus vector. *Am J Respir Cell Mol Biol*. 1999;21:388-94.
66. Gabay C, Lamacchia C and Palmer G. IL-1 pathways in inflammation and human diseases. *Nature reviews Rheumatology*. 2010;6:232-41.
67. Friedman JM and Jones PA. MicroRNAs: critical mediators of differentiation, development and disease. *Swiss Med Wkly*. 2009;139:466-72.
68. Ajit SK. Circulating microRNAs as biomarkers, therapeutic targets, and signaling molecules. *Sensors (Basel)*. 2012;12:3359-69.
69. Brandenburger T, Castoldi M, Brendel M, Grievink H, Schlosser L, Werdehausen R, Bauer I and Hermanns H. Expression of spinal cord microRNAs in a rat model of chronic neuropathic pain. *Neurosci Lett*. 2012;506:281-6.
70. Chattopadhyay M, Zhou Z, Hao S, Mata M and Fink DJ. Reduction of voltage gated sodium channel protein in DRG by vector mediated miRNA reduces pain in rats with painful diabetic neuropathy. *Mol Pain*. 2012;8:17.
71. Kusuda R, Cadetti F, Ravanelli MI, Sousa TA, Zanon S, De Lucca FL and Lucas G. Differential expression of microRNAs in mouse pain models. *Mol Pain*. 2011;7:17.
72. Orlova IA, Alexander GM, Qureshi RA, Sacan A, Graziano A, Barrett JE, Schwartzman RJ and Ajit SK. MicroRNA modulation in complex regional pain syndrome. *Journal of translational medicine*. 2011;9:195.
73. Pauley KM, Cha S and Chan EK. MicroRNA in autoimmunity and autoimmune diseases. *J Autoimmun*. 2009;32:189-94.
74. Saba R, Gushue S, Huzarewich RL, Manguiat K, Medina S, Robertson C and Booth SA. MicroRNA 146a (miR-146a) is over-expressed during prion disease and modulates the innate immune response and the microglial activation state. *PLoS One*. 2012;7:e30832.
75. Saba R, Storchel PH, Aksoy-Aksel A, Kepura F, Lippi G, Plant TD and Schratt GM. Dopamine-regulated microRNA MiR-181a controls GluA2 surface expression in hippocampal neurons. *Mol Cell Biol*. 2012;32:619-32.
76. Willemen HL, Huo XJ, Mao-Ying QL, Zijlstra J, Heijnen CJ and Kavelaars A. MicroRNA-124 as a novel treatment for persistent hyperalgesia. *J Neuroinflammation*. 2012;9:143.
77. Gheinani AH, Burkhard FC and Monastyrskaya K. Deciphering microRNA code in pain and inflammation: lessons from bladder pain syndrome. *Cellular and molecular life sciences : CMLS*. 2013;70:3773-89.
78. Nothnick WB. MicroRNAs and Endometriosis: Distinguishing Drivers from Passengers in Disease Pathogenesis. *Seminars in reproductive medicine*. 2017;35:173-180.
79. Haj-Ahmad TA, Abdalla MA and Haj-Ahmad Y. Potential Urinary miRNA Biomarker Candidates for the Accurate Detection of Prostate Cancer among Benign Prostatic Hyperplasia Patients. *Journal of Cancer*. 2014;5:182-91.
80. Ito T, Sato F, Kan T, Cheng Y, David S, Agarwal R, Paun BC, Jin Z, Olaru AV, Hamilton JP, Selaru FM, Yang J, Matsumura N, Shimizu K, Abraham JM, Shimada Y, Mori Y and Meltzer SJ. Polo-like kinase 1 regulates cell proliferation and is targeted by miR-593* in esophageal cancer. *International journal of cancer Journal international du cancer*. 2011;129:2134-46.
81. Shigehara K, Yokomuro S, Ishibashi O, Mizuguchi Y, Arima Y, Kawahigashi Y, Kanda T, Akagi I, Tajiri T, Yoshida H, Takizawa T and Uchida E. Real-time PCR-based analysis of the human bile microRNAome identifies miR-9 as a potential diagnostic biomarker for biliary tract cancer. *PloS one*. 2011;6:e23584.
82. Pereira PM, Marques JP, Soares AR, Carreto L and Santos MA. MicroRNA expression variability in human cervical tissues. *PloS one*. 2010;5:e11780.
83. Ninio-Many L, Grossman H, Levi M, Zilber S, Tsarfaty I, Shomron N, Tuvar A, Chuderland D, Stemmer SM, Ben-Aharon I and Shalgi R. MicroRNA miR-125a-3p modulates molecular pathway of motility and migration in prostate cancer cells. *Oncoscience*. 2014;1:250-61.
84. Vetter G, Saumet A, Moes M, Vallar L, Le Bechec A, Laurini C, Sabbah M, Arar K, Theillet C, Lecellier CH and Friederich E. miR-661 expression in SNAI1-induced epithelial to

mesenchymal transition contributes to breast cancer cell invasion by targeting Nectin-1 and StarD10 messengers. *Oncogene*. 2010;29:4436-48.
85. Lam WY, Yeung AC, Ngai KL, Li MS, To KF, Tsui SK and Chan PK. Effect of avian influenza A H5N1 infection on the expression of microRNA-141 in human respiratory epithelial cells. *BMC microbiology*. 2013;13:104.
86. Hewagama A, Gorelik G, Patel D, Liyanarachchi P, McCune WJ, Somers E, Gonzalez-Rivera T, Michigan Lupus C, Strickland F and Richardson B. Overexpression of X-linked genes in T cells from women with lupus. *Journal of autoimmunity*. 2013;41:60-71.
87. Dong Y, Li P, Ni Y, Zhao J and Liu Z. Decreased microRNA-125a-3p contributes to upregulation of p38 MAPK in rat trigeminal ganglions with orofacial inflammatory pain. *PloS one*. 2014;9:e111594.
88. Keklikoglou I, Koerner C, Schmidt C, Zhang JD, Heckmann D, Shavinskaya A, Allgayer H, Guckel B, Fehm T, Schneeweiss A, Sahin O, Wiemann S and Tschulena U. MicroRNA-520/373 family functions as a tumor suppressor in estrogen receptor negative breast cancer by targeting NF-kappaB and TGF-beta signaling pathways. *Oncogene*. 2012;31:4150-63.
89. Vlachos IS, Kostoulas N, Vergoulis T, Georgakilas G, Reczko M, Maragkakis M, Paraskevopoulou MD, Prionidis K, Dalamagas T and Hatzigeorgiou AG. DIANA miRPath v.2.0: investigating the combinatorial effect of microRNAs in pathways. *Nucleic acids research*. 2012;40:W498-504.
90. Practice Committee of the American Society for Reproductive M. Treatment of pelvic pain associated with endometriosis. *Fertility and sterility*. 2006;86:S18-27.
91. Tsuda M, Toyomitsu E, Komatsu T, Masuda T, Kunifusa E, Nasu-Tada K, Koizumi S, Yamamoto K, Ando J and Inoue K. Fibronectin/integrin system is involved in P2X(4) receptor upregulation in the spinal cord and neuropathic pain after nerve injury. *Glia*. 2008;56:579-85.
92. Saito F, Moore SA, Barresi R, Henry MD, Messing A, Ross-Barta SE, Cohn RD, Williamson RA, Sluka KA, Sherman DL, Brophy PJ, Schmelzer JD, Low PA, Wrabetz L, Feltri ML and Campbell KP. Unique role of dystroglycan in peripheral nerve myelination, nodal structure, and sodium channel stabilization. *Neuron*. 2003;38:747-58.
93. Institute of Medicine Committee on Advancing Pain Research C and Education. The National Academies Collection: Reports funded by National Institutes of Health *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research* Washington (DC): National Academies Press (US)

National Academy of Sciences.; 2011.
94. Atzeni F, Salaffi F, Bazzichi L, Gracely RH, Carignola R, Torta R, Gorla R, Marsico A, Ceccherelli F, Cazzola M, Buskila D, Spath M, Di Franco M, Biasi G, Cassisi G, Stisi S, Casale R, Altomonte L, Arioli G, Alciati A, Leardini G, Marinangeli F, Giamberardino MA and Sarzi-Puttini P. The evaluation of the fibromyalgia patients. *Reumatismo*. 2008;60 Suppl 1:36-49.
95. Turk DC, Dworkin RH, Allen RR, Bellamy N, Brandenburg N, Carr DB, Cleeland C, Dionne R, Farrar JT, Galer BS, Hewitt DJ, Jadad AR, Katz NP, Kramer LD, Manning DC, McCormick CG, McDermott MP, McGrath P, Quessy S, Rappaport BA, Robinson JP, Royal MA, Simon L, Stauffer JW, Stein W, Tollett J and Witter J. Core outcome domains for chronic pain clinical trials: IMMPACT recommendations. *Pain*. 2003;106:337-45.
96. Foster DC, Kotok MB, Huang LS, Watts A, Oakes D, Howard FM, Stodgell CJ and Dworkin RH. The tampon test for vulvodynia treatment outcomes research: reliability, construct validity, and responsiveness. *Obstet Gynecol*. 2009;113:825-32.
97. Slade GD, Conrad MS, Diatchenko L, Rashid NU, Zhong S, Smith S, Rhodes J, Medvedev A, Makarov S, Maixner W and Nackley AG. Cytokine biomarkers and chronic pain: association of genes, transcription, and circulating proteins with temporomandibular disorders and widespread palpation tenderness. *Pain*. 2011;152:2802-12.
98. Murina F, Bianco V, Radici G, Felice R, Di Martino M and Nicolini U. Transcutaneous electrical nerve stimulation to treat vestibulodynia: a randomised controlled trial. *BJOG : an international journal of obstetrics and gynaecology*. 2008;115:1165-70.
99. McDonald JS and Rapkin AJ. Multilevel local anesthetic nerve blockade for the treatment of generalized vulvodynia: a pilot study. *The journal of sexual medicine*. 2012;9:2919-26.

100. Meltzer-Brody SE, Zolnoun D, Steege JF, Rinaldi KL and Leserman J. Open-label trial of lamotrigine focusing on efficacy in vulvodynia. *J Reprod Med*. 2009;54:171-8.
101. Cyr MP, Bourbonnais D, Pinard A, Dubois O and Morin M. Reliability and Convergent Validity of the Algometer for Vestibular Pain Assessment in Women with Provoked Vestibulodynia. *Pain medicine (Malden, Mass)*. 2016;17:1220-8.
102. Zolnoun D, Bair E, Essick G, Gracely R, Goyal V and Maixner W. Reliability and reproducibility of novel methodology for assessment of pressure pain sensitivity in pelvis. *The journal of pain : official journal of the American Pain Society*. 2012;13:910-20.
103. Giesecke J, Reed BD, Haefner HK, Giesecke T, Clauw DJ and Gracely RH. Quantitative sensory testing in vulvodynia patients and increased peripheral pressure pain sensitivity. *Obstet Gynecol*. 2004;104:126-33.
104. Reed BD, Caron AM, Gorenflo DW and Haefner HK. Treatment of vulvodynia with tricyclic antidepressants: efficacy and associated factors. *Journal of lower genital tract disease*. 2006;10:245-51.
105. Salaffi F, Stancati A, Silvestri CA, Ciapetti A and Grassi W. Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. *Eur J Pain*. 2004;8:283-91.
106. Chelimsky-Fallick C, Stevenson LW, Lem V and Whipp BJ. Excessive oxygen deficit during low-level exercise in heart failure. *The American journal of cardiology*. 1995;76:799-802.
107. Sietsema KE, Ben-Dov I, Zhang YY, Sullivan C and Wasserman K. Dynamics of oxygen uptake for submaximal exercise and recovery in patients with chronic heart failure. *Chest*. 1994;105:1693-700.
108. Harris RC, Edwards RH, Hultman E, Nordesjo LO, Nylind B and Sahlin K. The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. *Pflugers Arch*. 1976;367:137-42.
109. Lucas C, Stevenson LW, Johnson W, Hartley H, Hamilton MA, Walden J, Lem V and Eagen-Bengsten E. The 6-min walk and peak oxygen consumption in advanced heart failure: aerobic capacity and survival. *American heart journal*. 1999;138:618-24.

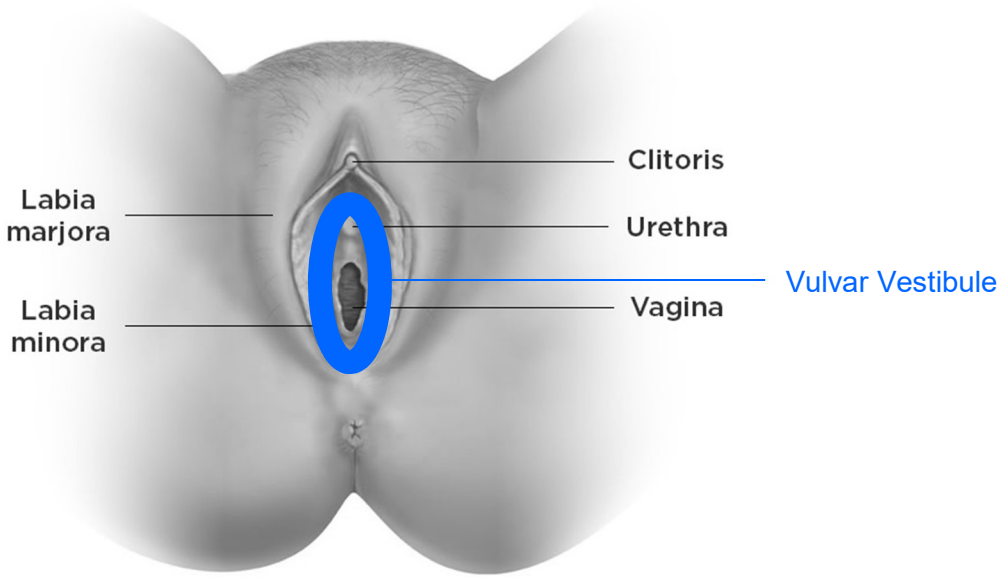
20 APPENDICES

Appendix A. Schedule of Assessments

Assessment	Screening (0 week)	Baseline Visit (0 week)	Treatment Visit (8 week)*	Treatment Visit (16 week)**	Post-treatment (24 week)***
Patient Consent	X^{FS}				
Tampon Test	X^{FS}		X	X	X
Pain with sexual contact involving penetration into the vagina	X		X	X	X
Pain with touch to the vaginal opening	X		X	X	X
Blood Collection		X^{FS}	X	X	X
Demographics		X^{FS}			
Social History		X^{FS}			
Gynecologic History		X^{FS}			
VPAQ		X^{FS}	X	X	X
PROMIS BPSFS-Female		X^{FS}	X	X	X
COPC Inventory		X^{FS}			
COPC Follow-Up			X	X	X
SF-12v2		X^{FS}	X	X	X
SCL-27		X^{FS}	X	X	X
PILL		X^{FS}	X	X	X
PSS		X^{FS}	X	X	X
Sleep Scale		X^{FS}	X	X	X
Vaginal Sample Collection		X^{FS}	X	X	X
Pelvic Sensory Testing		X^{FS}	X	X	X
Vaginal Vestibule PPTs		X^{FS}	X	X	X
Perineal Muscle PPTs		X^{FS}	X	X	X
Levator Muscle PPTs		X^{FS}	X	X	X
Remote Bodily PPTs		X^{FS}	X	X	X
Medication Compliance			X	X	

FS = assessments collected for the feasibility study
X = assessments collected for the full study
*8 weeks (56 days) following randomization, +/- 5 days
**16 weeks (112 days) following randomization, +/- 5 days
***24 weeks (168 days) following randomization, +/- 5 days

Appendix B. Lidocaine/Estradiol Application Handout



Appendix C. Nortriptyline Medication Handout

Severe Drug-drug Interactions with Nortriptyline

Drug interactions may change how your medications work or increase your risk for serious side effects. This document does not contain all possible drug interactions, but it highlights the most serious. Keep a list of all the products you use (including prescription/nonprescription drugs and herbal products) and share it with the research team.

Taking MAO inhibitors with this medication may cause a serious (possibly fatal) drug interaction. Avoid taking MAO inhibitors for two weeks before and after nortriptyline use:

- Isocarboxazid
- Linezolid
- Methylene blue
- Moclobemide
- Phenelzine
- Procarbazine
- Rasagiline
- Safinamide
- Selegiline
- Tranylcypromine

The risk of serotonin syndrome/toxicity increases if you are also taking other drugs that increase serotonin and may be more likely to occur when you start or increase the drug dose:

- MDMA/"ecstasy"
- St. John's wort
- Antidepressants- specifically SSRIs and SNRIs
- Other tricyclic antidepressants- amitriptyline, desipramine, imipramine
- Topiramate, cyclobenzaprine

Avoid combination of drugs that affect the heart rhythm, specifically causing QT prolongation on EKG:

- Amiodarone
- Cisapride
- Dofetilide
- Sotalol
- Pimozide
- Procainamide
- Quinidine
- Macrolide antibiotics- i.e. erythromycin

Other drugs to avoid while taking nortriptyline:

- Arbutamine
- Disulfiram
- Anticholinergic drugs such as benztropine, belladonna
- Cimetidine
- Blood thinners such as warfarin
- Thyroid supplements
- Certain drugs for high blood pressure- clonidine, guanabenz
- Terbinafine

Tell your research team if you are taking other products that cause drowsiness, including alcohol, marijuana, antihistamines, drugs for sleep or anxiety (such as alprazolam, diazepam, zolpidem), muscle relaxants, or narcotic pain relievers.

Check the labels on all your medicines (such as allergy or cough-and-cold products) because they may contain decongestants or ingredients that cause drowsiness.

Appendix D. Medication Calendar

Medication Calendar

Please complete the survey below.

Thank you!

Please select the date you started your study medication.	_____
Can you please confirm the day of the week you started your medication?	◯ Sunday ◯ Monday ◯ Tuesday ◯ Wednesday ◯ Thursday ◯ Friday ◯ Saturday

The questions below will ask about your daily use of the prescribed medication for the first two months following your initial study visit. If, for example, begin your medication on a Wednesday, you will need to complete your responses into Wednesday of Week 9.

	Cream Applied: No	Cream Applied: Yes	Pills taken: 0	Pills taken: 1	Pills taken: 2	Pills taken: 3	Pills taken: 4	Pills taken: 5	Pills taken: 6
Week 1: Sunday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week 1: Monday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week 1: Tuesday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week1: Wednesday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week1: Thursday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week1: Friday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week1: Saturday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Sunday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Monday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Tuesday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Wednesday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Thursday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Friday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week2: Saturday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week3: Sunday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week3: Monday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week3: Tuesday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week3: Wednesday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week3: Thursday	☐	☐	☐	☐	☐	☐	☐	☐	☐
Week3: Friday	☐	☐	☐	☐	☐	☐	☐	☐	☐