Longitudinal changes in women's pelvic health and sexual function after pelvic radiation

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Protocol

I. Objectives

Purpose: The purpose of this study is to evaluate changes in physical measures of pelvic health and patient-reported outcomes of sexual function, intimate relationship and quality of life over time in women receiving radiation for pelvic cancer.

Primary Objectives:

1) To evaluate changes in objective physical exam findings from baseline to the end of radiation and through two years follow-up for women with pelvic cancer.
2) To examine changes in sexual, relational and quality of life outcomes from baseline to the end of radiation and through one year follow-up for women with pelvic cancer using the patient reported outcome measures.
3) To compare physical exam and patient-reported outcome trajectories over time.

Primary Hypotheses:

1) We hypothesize that women with pelvic cancer treated with radiation experience clinically significant morbidity of pelvic health that does not return to baseline two years post treatment
2) We hypothesize that women with pelvic cancer treated with radiation experience negative effects on sexual function, intimate relationships and quality of life that does not return to baseline two years post treatment
3) We hypothesize that both pelvic health and patient-reported outcomes will be affected negatively immediately after treatment, and that patient-reported outcomes are less likely to improve over time as compared to pelvic health.

Exploratory Objectives:

1) To determine whether women who experience vaginal toxicity have a distinct microbiome compared to those who do not (1) at baseline (pre-treatment) or (2) over the course of treatment and recovery (longitudinal).
2) To explore potential mechanisms of microbiome-associated development of vaginal toxicity through correlation with deconvolved immune cell abundances and host gene expression.
3) Examine associations between the vaginal microbiome and tumor radiation response and recurrence in women with gynecologic cancer

Exploratory Hypotheses:

1) We hypothesize that low LPR (pre-treatment, baseline) will correlate with post-treatment vaginal dryness.
2) We hypothesize that LPR will negatively correlate with the abundance of macrophages.

3) We hypothesize that LPR will negatively correlate aggregated expression of standard inflammasome markers: ASC, CASP 1, GSDMA, GSDMB, GSDMC, GSDMD, GSDME, GSDMF, HMGB1, NLRC4, NLRP 3.
4) LPR will negatively correlate with tumor radiation response and recurrence.

II. Background and Rationale

Radiation therapy leads to vaginal toxicity in many women. As a result of treatment, women with gynecologic cancers have high incidence of dyspareunia, vaginal dryness, and sexual dysfunction (Jensen & Froeding, 2015). Approximately 65% of survivors have reported symptoms of vaginal atrophy and 72% have reported dyspareunia (Amsterdam & Krychman, 2006). Radiation therapy is frequently used as standard adjuvant therapy for many women with gynecologic cancers. Pelvic radiation is associated with dyspareunia, vaginal dryness, decreased elasticity, and narrowing and shortening of the vagina (Vaz et al., 2011). Women treated with radiation therapy have more side effects related to sexual functioning than patients treated with surgery, including higher rates of vaginal shortening and dyspareunia (Noronha, Mello de Figueiredo, Rossi de Figueiredo Franco, Candido, & Silva-Filho, 2013). The radiation related edema and inflammation that develops during treatment can progress over the following one to two years to vaginal necrosis and fibrosis (Hofsjo et al., 2017). The vascular supply to the vaginal epithelium is also affected by radiation and may result in tissue hypoxia leading to a fragile epithelium which can lead to thinning of the vaginal wall, atrophy, decreased elasticity, vaginal narrowing, and worsened vaginal shortening. Severe vaginal stenosis may result in some cases (Jensen & Froeding, 2015). In addition to the direct effects of radiation on the vaginal tissue, surgical or radiation induced menopause can contribute to genitourinary symptoms including dyspareunia, vaginal atrophy, dysuria, urinary frequency, and recurrent urinary tract infections that result from a decrease in estrogen (Van Le & McCormack, 2016).

Post-radiation vaginal changes are understudied. While vaginal changes are known to occur and have long-term effects on the patient, there has been little research done on the timing of these changes after radiation or on prevention and management of symptoms. A recent large prospective study of women with cervical cancer treated with definitive chemoradiation and brachytherapy demonstrated a 3.6% rate of vaginal morbidity at 2 years follow-up based on the Common Terminology Criteria for Adverse Events, version 3 (CTCAE). However 89% of women had grade 1 or higher vaginal symptoms and 29% had grade 2 or higher vaginal symptoms based on the CTCAE that developed within 6 months of radiation therapy. (Kirchheiner et al., 2014) This same study also showed that vaginal dryness and dyspareunia increased as a result of treatment and did not return to baseline (Kirchheiner et al., 2016). The use of a vaginal dilator two to three times per week starting about one month after radiation therapy has been recommended to prevent vaginal stenosis (Bakker et al., 2014). However, vaginal dilators are not proven to be effective and there is no strong evidence to support that use prevents stenosis or improves sexual function. Compliance with vaginal dilators is also reported to be low amongst women with gynecologic cancers treated with radiation therapy (Bakker et al., 2015; Friedman, Abdallah, Schluchter, Panneerselvam, & Kunos, 2011; Miles & Johnson, 2010). Knowledge of the time course that vaginal changes and symptoms develop

may be important in determining more optimal methods of prevention and/or treatment of a complication. A prospective longitudinal study of vaginal length and diameter was done in women with locally advanced cervical cancer which demonstrated decreased measurements but no patient reported symptoms were obtained.

Post-radiation vaginal changes affect sexual wellbeing. Most colorectal cancer survivors remain sexually active (Lange et al., 2009), but surgery or radiation can have lasting effects on sexual wellbeing. Treatments may cause dyspareunia, vaginal dryness, vaginal stenosis and disruption of autonomic nerve and vascular tracks impairing lubrication, engorgement and orgasm (Breukink & Donovan, 2013; Den Oudsten et al., 2012). One third of colorectal cancers occur in the rectum. A comprehensive review of sexual function, incontinence and wellbeing in women treated for rectal cancer showed approximately 1/3 of women aged 50-70 years have a lack of sexual desire and 60% report sexual problems (Panjari et al., 2012). Bladder and bowel incontinence were ongoing concerns for many women. Often radiation can be a greater risk factor than surgery for sexual dysfunction (Incrocci & Jensen, 2013). In a study of women with rectal cancer randomized to surgery (total mesorectal excision) with or without pre-operative radiation, most reported increases in sexual dysfunction, dyspareunia, and vaginal dryness associated with radiation and presence of a stoma (Lange et al., 2009). In addition, vaginal stenosis and sexual dysfunction have been retrospectively reported in woman undergoing treatment for anal cancer, in which high doses of radiation are often utilized for definitive treatment without surgery (Mirabeau-Beale et al., 2015). Dosimetric radiation data for this retrospective cohort were limited, but suggested that higher radiation dose was associated with increased risk of vaginal stenosis.

Sexual wellbeing is measured as a patient reported outcome. Patient reported outcomes after pelvic radiation therapy relevant to sexual function include not only vaginal symptoms, but other symptoms and outcomes that affect women's sexual wellbeing. There is evidence to suggest impacts of treatment on women's sexual distress, sexual communication with their partner, relationship satisfaction, and health-related quality of life (Kirchheiner et al., 2015; Mirabeau-Beale & Viswanathan, 2014; Westin et al., 2016). On the other hand, despite evidence of need from patients, providers often feel uncomfortable discussing sexual function with patients and partners (Krouwel et al., 2015). Therefore it is important to understand how vaginal changes and patient-reported sexual wellbeing outcomes change over time in order to inform provider communication and intervention.

The vaginal microbiome is associated with sexual health. The human microbiome consists of the collection of microorganisms within and on the body that interact with and affect with the host organism (Ursell, Metcalf, Parfrey, & Knight, 2012). Vaginal atrophy, changes in mucosal integrity, hormonal dysregulation, and vaginal pH have all been associated with differences into the microbiome (Chase, Goulder, Zenhausern, Monk, & Herbst-Kralovetz, 2015). To date no study has assessed the vaginal microbiome in association with progression of vaginal changes after pelvic radiation. Assessment of these changes will help improve our understanding of the vaginal response to radiation treatment and allow us to potentially identify risk factors and biomarkers that could predict treatment toxicity, with the goal of guiding preventative and

personalized therapeutic interventions to reduce short- and long-term toxicity (Chase et al., 2015).

Vaginal microbiome and tumor response to treatment and recurrence. The gut, tumoral, and cervicovaginal microbiome are areas of current interest in examining gynecologic cancer patients' development of disease and response to therapy. The vaginal microbiome has been implicated in the risk of Human Papillomavirus persistence and development of cervical cancer. Recent studies have shown increased diversity of vaginal microbiota and reduced abundance of Lactobacillus spp. contribute to HPV acquisition, persistence, and development of cervical cancer. The vaginal microbiome has also been studied in relation to radiation treatment in gynecologic malignancies. Examination of the cervicovaginal microbiome before and after RT has identified distinct differences in bacterial composition. In a study of women with gynecological cancer undergoing RT compared to healthy controls, there was reduced *Lactobacillus* and *Bifidobacterium* and a higher abundance of rare anaerobic species of the *Lacnospiraceae*, *Sneathia, Prevotella, Peptoniphilus*, and *Fusobacterium* families, previously linked to inflammation and opportunistic bacterial infections. Advancing our understanding of how the gut and female reproductive tract microbiota impacts radiation toxicities and treatment efficacy has significant potential for modulation in the future.

Acquiring additional details regarding patient's oncologic outcomes in the GEORGIA Trial will allow us to utilize the vaginal microbiome data to elucidate correlations between radiation response and recurrence. By understanding vaginal microbiota composition in responders to therapy we may be able to modulate the microbiota to enhance the efficacy of current treatments in the future. The vaginal microbiome could not only be used as a tool to predict response to initial therapy, but also risk of recurrence, sites of recurrence, as well as response to 2nd and 3rd line therapies

RNA-seq is a cost-effective method to analyze both human and microbial transcripts. The RNA that is sequenced from a standard biosample such as a swab is a snapshot of the expression of many different cells. In a vaginal swab, typically 90% are human and the rest various types of microbes including bacteria, fungi, and viruses (Human Microbiome Project, 2012); all these organisms can be accurately measured by RNA sequencing. Though microbial and human cells are clearly different, less commonly acknowledged is the large difference in expression of different types of human cells. The swab may include epithelial cells and wide variety of immune cells, each of which is expressing a distinct set of genes. Efforts to quantify these different types often rely on microscopy and staining, but the resolution is limited to a few cell types. Other cell-counting methods such as flow-sorting and single cell sequencing are challenging due to the small sample sizes available from simple, non-invasive procedures. An alternative strategy for identifying and quantifying different cell types present in a sample uses cell-type specific expression patterns to deconvolve RNAseq reads from mixtures of cells into fractions of different cell types (Kirchheiner et al., 2014; Van Le & McCormack, 2016). This deconvolution also effectively de-noises heterogeneous datasets by greatly reducing the number of dimensions (Nam, Kim, Seo, Kang, & Bae, 2013). With careful computational processing that we and others have described, RNAseq from a swab can be used to simultaneously measure (1) the quantities of microbes, (2) the overall expression of the human

host, and (3) the quantities of immune cells (Daniel Spakowicz et al., 2020; Zhou et al., 2019), making it a cost-effective method of testing hypotheses related to microbe abundances and generating-correlations with other parts of the data.

The goal of this study is to use a simple clinical tool to evaluate vaginal changes prior to, during and after a course of radiation and to collect patient reported outcomes of sexual function, partner communication and intimacy in order to have a better understanding of physical changes and symptoms over time.

III. Procedures

A. Research Design

This study is a longitudinal, observational study. Participants will have standard of care serial pelvic exams and be asked to complete a panel of patient reported outcomes measures as repeated measures over two years.

B. Sample and Setting

Inclusion criteria:

- Any patient with anal, rectal, cervical, endometrial, vaginal, or vulvar cancer receiving external beam radiation alone, or both external beam radiation and brachytherapy
- Concurrent or prior chemotherapy is allowed, including those participating in OSU-16166
- Any prior gynecologic surgery is permitted
- Rectal surgery, including lower anterior resection and abdominoperitoneal resection, is permitted

Exclusion criteria:

- Patients with scleroderma, mixed connective tissue disorder, and lupus will be excluded
- Patients who have received prior pelvic radiation

Potential participants will be recruited from The Ohio State University James Cancer Hospital Radiation Oncology clinic. This is an observational, longitudinal study of 89 women with gynecologic and colorectal cancers who are receiving either external beam radiation or brachytherapy. Participants will be identified in Radiation Oncology or Gynecologic Oncology prior to their initial consultation. All potential participants (new gynecologic radiation consultations) will be approached about participation in-person in clinic or via phone/video call.

The **sample size** was estimated based on the primary outcome of decreased sexual function (FSFI) from baseline to one-month post-radiation. Based on the literature (Moroney et al., 2018) and the observational nature of the study (e.g., large variability in the sample), a moderate effect size of 0.3 was used to calculate a desired sample of 71, which would detect effects with a power of 0.80 and a one-sided significance level of 0.05. Effect size here is the mean difference (post-pre) divided by standard deviation of the difference. . With an expected 20% attrition from baseline to one-month post-radiation, a minimal sample of 89 participants will be needed to reach the desired power for this study. The proposed sample size is also a reasonable number to accrue in 1-2 years.

C. Measurement/Instrumentation

Vulvovaginal symptoms. The Vaginal Assessment Scale (VAS) and Vulvar Assessment Scale (VuAS) are recently validated clinical measurement tools to identify vulvovaginal symptoms. Patients are asked about specific symptoms over the past 4 weeks including vaginal dryness, soreness, irritation, and dyspareunia and are asked to rate them as mild, moderate, or severe. Each item is scored from 0 (none) to 3 (severe) and a composite score is calculated by taking the mean of the items. A lower score indicates better function. In cancer patients and survivors, the two scales were found to have high internal consistency (Eaton et al., 2017). The scale was initially designed so that the physicians asks the patient the questions but a patient reported version was developed by Jeanne Carter, who has given permission for it to be used in this study. Both the patient reported VAS and VuAS together will be referred to as the PRO-VAS.

Sexual function. Sexual function will be measured using the Female Sexual Functioning Index (FSFI), The FSFI is a 19 item self -reported instrument that has been previously validated to measure sexual functioning in women in clinical trials. It measures six domains of sexual functioning including arousal, orgasm, satisfaction and pain. Overall test-retest reliability coefficients are high for each domain and the internal consistency is high with a Cronbach's alpha of 0.82 and higher. The total maximum score is 36 with a higher score indicating better functioning. Total scores and individual domain scores will be obtained. A total score of ≤ 26.0 indicates sexual dysfunction. It also has good construct validity. (Rosen et al., 2000) It has been validated in gynecologic cancer survivors. (Baser, Li, & Carter, 2012)

Sexual Distress. Sexual distress will be measured using the Female Sexual Distress Scale Revised (FSDS-R), which has been shown to effectively measure sexually related personal distress in women (Derogatis, Rosen, Leiblum, Burnett, & Heiman, 2002). Higher scores indicate more distress. The revised version with 13 items was used in this study. Studies show the scale is valid, with discriminant validity, test-retest reliability, and Cronbach's coefficient alpha of >0.86 (Derogatis, Clayton, Lewis-D'Agostino, Wunderlich, & Fu, 2008). The FSDS has been used repeatedly and successfully in studies of women with cancer (Brotto et al., 2008, 2012; Classen et al., 2013).

Self-efficacy to communicate about sex and intimacy. The Self-Efficacy to Communicate about Sex and Intimacy (SECSI) scale is a list of 10 statements for which respondents rate their degree of confidence to communicate with a romantic partner about changes in sex and intimacy after cancer treatment, scored on a Likert-type response scale (Arthur, Wills, Browning, Overcash, & Menon, 2019). Scores range 0 to 30, with higher scores indicating greater self-efficacy. In the original study of 250 partnered women treated for cancer, the SECSI scale was highly reliable with a Cronbach alpha coefficient of 0.94 and test-retest reliability of r = 0.82. Construct validity of the SECSI scale, including discriminant, convergent, and divergent validity, was also supported.

Intimacy. The Miller Social Intimacy Scale is a 17-item measure of relationship intimacy (Miller & Lefcourt, 1982). The Cronbach alpha coefficient in initial assessment was 0.86 and 0.91, and the test-retest reliability r = 0.84 over a 1-month interval. The scale authors also provide evidence for convergent, discriminant and construct validity. The scale has been used previously in the context of sexual wellbeing after cancer (Barsky Reese et al., 2014).

Sexual behaviors. We measured sexual behaviors by asking women to report whether they have had interest in, have engaged in, have declined, or have their partner declined sexual activity in the last four weeks. We asked participants to report frequency of sexual activities

over the last four weeks, and whether their partner has physical limitations limiting his/her ability to engage in sexual activity.

Satisfaction with provider communication. Communication with a provider will be assessed with six items developed by Flynn and colleagues in response to the qualitative data to address communication about sexual issues with oncology providers (Flynn et al., 2012). Psychometrics have not been published for these items.

Sociodemographic factors. We will ask participants to report sociodemographic characteristics. This included age, race/ethnicity, employment status, income, education level, years with partner and number of children.

Clinical cancer characteristics. Data will be recorded from the medical record regarding diagnosis, stage at diagnosis, cervical cancer histology, initial surgery & surgical details, initial treatments (chemotherapy, radiation, immunotherapy, observation). Details regarding the radiation treatment, including dose, fractionation, and type of treatment will be recorded. We will record response to primary treatment, completion date of primary treatment, recurrence, site of recurrence, date of recurrence, treatment of recurrence, and current status. We will record CBC with differential lab results if the lab was collected, as part of standard of care, over the participant's diagnosis, treatment, and surveillance. The Common Terminology Criteria for Adverse Events (CTCAE*), version 5 (2017)* will be used for evaluation of acute and delayed toxicities related to radiation therapy. Menopausal status prior to radiation and smoking status will be documented.

Vaginal Assessment. The Vaginal Health Assessment, the examination tool used in the clinical validation of the VAS, will be utilized to for the gynecologic physical examination for all patients (Eaton et al., 2017). The assessment evaluates the physical aspect of the vagina including agglutination, adhesions, pH, dryness, rugosity, elasticity, length, thickness, epithelial integrity, vascularity, and irritation. In the current study, we will focus on vaginal diameter and length. Vaginal diameter will be assessed during the manual portion of the exam as would be done to determine the approximate diameter of a vaginal cylinder to use for brachytherapy (Small et al., 2012). Estimates will be <2.5, 2.5, 3.0, 3.5, 4.0 or >4 cm. Vaginal length will be estimated based on manual exam (i.e. finger length) and a ruler external to the patient for confirmation. Estimates will be <4cm, 4-6cm, and >6cm.

Vaginal Swab. Swabs will be collected using DNAgenotek OMR-130 DNA/RNA collection kit by clinicians during standard of care exam or self-collected following manufacturer's instructions. Briefly, each kit contains a collection tube containing RNA-stabilizing solution and a sterile swab. Clinicians or participants will insert the swab 3-5 cm into the vaginal opening and move the swab in full circles along the vaginal walls for 20 seconds. The swab will then be placed in the RNA-stabilizing solution and the swab shaft snapped off at the tube lip. Tubes will be set aside at room temperature for pickup by Spakowicz lab staff. In the Spakowicz lab, samples will be aliquoted into two cryovials, barcoded and put in -80 storage to be processed in batches for sequencing.

D. Detailed Study Procedures

Study enrollment and data collection:
After confirming eligibility for the trial, a study team member will approach potential participants in-person in clinic or via phone/video call. Enrolled participants will receive information about the study activities, participant rights, and any potential risks to participation. Participants will be

asked to sign an informed consent to participate in the research study. The data collected for study purposes as outlined below will be stored in an electronic password protected study database or a locked room when completed in person, that will only be accessible by study investigators and study staff. After collection of data, it will be coded so as not to contain any patient identifiers.

Demographics, pertinent clinical data, and inclusion/exclusion criteria will be obtained and recorded for all patients. Details regarding the radiation treatment, including dose, fractionation, and type of treatment will be recorded. The Common Terminology Criteria for Adverse Events (CTCAE*), version 5 (2017)* will be used for evaluation of acute and delayed toxicities related to radiation therapy. Menopausal status prior to radiation and smoking status will be documented.

At the time of the initial visit (T0) after signing informed consent, participants will be asked to complete a panel of patient reported outcome measures (Table 1). In order to minimize bias, a different study team member will collect the patient reported outcomes (either in REDCap or place in sealed envelope). Participants will receive reminder calls and emails 5 and 10 days after non-completion of the surveys. Participants will receive text messages sent through Outlook thanking them for their participation, asking if they have any questions, and asking them if everything went okay with completing the questionnaires and if applicable, collecting the at home sample and shipping it back. These text messages will be sent a week after each timepoint. Participants will receive a small incentive for participating in the study and completing the surveys. Participants will also have a pelvic exam as standard of care for an initial consultation for radiation therapy for gynecologic cancers. As part of the study, the provider will complete the Vaginal Health Assessment and collect a vaginal swab. If the investigator is unable to collect the vaginal swab in clinic, a home swab kit can be given to the patient or mailed to the patient. For patients who receive an at home kit, a member of the research team will call them to collect the VAS/VuAS. In order to improve internal validity, the same physican will do all patient exams for consistency in documenting physical findings.

Patients will receive their radiation treatment as prescribed by the radiation oncologist. Patients will return for one month (T1), three month (T2), six month (T3), 12 month (T4), and 24 month (T6) follow-ups and the physical exam, vaginal swab and patient-reported outcome measures will again be completed in a similar manner as T0. Though no physical exam will be performed, participants will be asked to complete the patient-reported outcomes at 18 months and will be given or mailed a home swab kit (T5).

Vaginal Swab Analysis:
Cells in nucleic acid stabilizer solution are disrupted with Powerlyzer 24 and RNA purified using QIAGEN RNeasy kit on an automated liquid handler. Total RNA will be depleted of human ribosomes using QIAseq FastSelect -rRNA HMR kit, converted to cDNA and sequencing libraries created using the QIAseq Stranded RNA library kit. Sample integrity and quality is verified using BioAnalyzer chips (Agillent) through the OSUCCC Genomics Shared Resource. Data will be generated using short-read next-generation sequencing (e.g. NovaSeq 6000 (2x150bp) or similar)to a depth of >= 50e6 reads / sample at the OSUCCC-James/Nationwide Genomics Core or other approved vendor using as few kits as possible to minimize batch effects. Data are be transferred to the Ohio Supercomputer Center for demultiplexing, cleaning and further processing through the ExoTIC pipeline. Cleaned and filtered reads are serially aligned to contaminant databases followed by the human reference genome and then unaligned reads to complete genomes of bacteria, fungi, viruses, archae, and a small number of eukaryotyes. Microbes and metabolic pathways are calculated using standard tools (Kraken2/Bracken (Lu, Breitwieser, Thielen, & Salzberg, 2017; Wood, Lu, & Langmead, 2019;

Wood & Salzberg, 2014), MetaPhlAn2/HUMAnN v3.0) (Franzosa et al., 2018; Truong et al., 2015) and normalized to sequencer output or total microbial signal (relative abundance). Inverse Simpson's Index (alpha diversity) is calculated using the vegan package in R (Oksanen et al., 2018).

The table of gene abundances will be deconvolved using the cell-type specific transcriptomes from flow cytometry-sorted and sequenced immune cell types. Briefly, we will identify gene expression signatures for each cell type and then apply the signature set to the human-aligned gene counts from the vaginal swab RNAseq, solving for the equation: $G_{p,g} = F_{p,f} * S_{f,g}$, where $G_{p,g}$ is the matrix of human protein-coding gene expression, for each p patients and g genes, $F_{p,f}$ is the matrix cell-type fractions, f, and $S_{f,g}$ is the custom cell signature matrix. This method uses support vector regression both to perform variable selection on the gene set in $S_{f,g}$ to reduce the chance of overfitting, and to fit the mixture data to generate $F_{p,f}$ .Reads will aligned to the human reference genome (hg38) using standard tools (STAR (Dobin et al., 2013)), the transcripts per kilobase million (TPKM) calculated for each gene isoform using RSEM (Li & Dewey, 2011), and then variance-normalized across all samples using VOOM (Law, Chen, Shi, & Smyth, 2014).

Data Sharing Upon Publication:
The de-identified data (demographic, clinical, survey, pelvic exam and RNAseq) will be made available upon publication to support the reproducibility of the published analyses.

Table 1 Study Timeline

Assessments	Baseline (T0)	One Month (T1)	Three Months (T2)	Six Months (T3)	12 Months (T4)	18 Months (T5)	24 Months (T6)
Demographics and Clinical History	X				X		X
Pelvic Physical Examination	X	X	X	X	X		X
Patient-ReXported Outcome Measures	X	X	X	X	X	X	X
Vaginal Swab	X	X	X	X	X	X	X

T0 = Prior to RT

T1 = 1 month after RT +/- 2 weeks

T2= 4 months after RT +/- 6 weeks

T3= 7 months after RT +/- 6 weeks

T4 = 12 months after RT +/- 8 weeks

T5 = 18 months after RT +/- 8 weeks

T6 = 24 months after RT +/- 8 weeks

E. Safeguarding of Data and Protection of Participants

Adverse Events. No adverse events are anticipated as a result of the study as there is no intervention being conducted. Any adverse events would be related to their radiation treatments which are not experimental in any way and would be delivered according to standard of care. All pelvic examinations are also standard for care for routine cancer surveillance. Risk for harm associated with study participation is minimal, however if participants become distressed the exam or study measure completion they will be referred to their healthcare provider or mental healthcare provider. If a participant expressed suicidal ideation at any time during the study they will be immediately directed to the closest emergency department.

Removal of patient from protocol. A patient can be removed from the protocol at any time and may choose to voluntarily withdraw. The PI should be notified of a participant withdrawal or removal and the reason for the withdrawal or removal should be documented in the patient chart/REDCap.

Reasonably expected risks, harms, and/or discomforts that apply to this research are minimal. These risks, harms, and/or discomforts are related to answering questions regarding to their sexual life, which may bring about feelings of depression, anxiety, anger, or isolation. Participants may experience mild to moderate emotional discomfort answering questions related to their sexual health and behavior. If a participant becomes emotionally distressed from the study questionnaires, the PI (an oncology advanced practice provider) will follow up with them and they will be directed to seek care from a mental health professional or their primary care provider, if necessary. This information would be documented in REDCap under the "withdraw" section.

Institutional Review Board. The PI will obtain approval from the Clinical Scientific Review committee and the cancer IRB prior to enrolling patients on the study. The study will be reviewed on an annual basis.

Informed consent. All patients will be provided with a copy of the consent for the study, explaining the purpose and details of the study, risk to the participants, incentives for participating, PI contact information, the right to withdrawal from the study at any time, and that their personal information will be kept strictly confidential. Consent will be obtained in a private room in clinic OR over phone/video call on a tablet electronically through REDcap. In order to verify participant identity, we will ask them to verify their email address, and also provide back a passcode we will send to their individual email account. After reviewing the information with a study team member in-person or via phone/video call and asking questions about participation, they will be asked to sign informed consent in REDCap if they would like to participate.

For subjects who decline questionnaires, a pelvic exam/swab collection only option will be offered.

Patient confidentiality. All documents and patient related information related to the study will be kept strictly confidential. The patient data collection forms will be kept in a password protected electronic file using REDCap. All surveys and questionnaires will be stored electronically in REDCap. Data will be coded with participant ID numbers, and personal identifiers with participant ID's (key) will be kept in a separate secure electronic location. HIPAA-protected information will also be coded and kept in password-protected files within the medical center firewall.

Internal Validity
Efforts have been made to minimize threats to internal validity. In order to minimize bias, a different study team member (not the radiation oncologist) will collect the patient reported outcomes (either in redcap or place in sealed envelope). In order to improve internal validity, the same physician will do all patient exams for consistency in documenting physical findings. The survey will be administered in the same way for all participants. Participants are encouraged to be honest about their symptoms and opinions. Results of this study will be primarily generalized to other women receiving radiation for gynecologic cancer.

F. Data Analysis

Primary outcomes (powered)
The study team will determine the pattern and severity of missing data and apply appropriate techniques for handling missing data (e.g. multiple imputation). To answer the research questions proposed, the study team will first employ descriptive statistics on all study variables (demographics and clinical history, physical exam outcomes and patient-reported outcomes) at all time points to examine data distribution and check for outliers. Continuous variables will be reported as mean, standard deviation (SD), median and interquartile range (IQR). Categorical variables will be reported as frequencies and percentages. **For physical exam outcomes** (vaginal length and diameter as categorical variables), the study team will first test bivariate associations between physical exam outcomes and categorical sociodemographic factors (race/ethnicity, employment status, income, education level, number of children) using Fisher's exact test at each time point. The study team will test bivariate associations between physical exam outcomes and continuous sociodemographic factors (age, years with partner) using analysis of variance (ANOVA) at each time point. Next, the study team will employ one-tailed Wilcoxon signed-rank test to assess the effects of radiation by comparing one-month, three-month, six-month, 12-month and 24-month physical exam outcomes to baseline. A non-parametric test is used because of the quality of physical exam outcomes, where measure scales are ordinal. **For patient-reported outcomes** (vulvovaginal symptoms, sexual function, sexual distress, self-efficacy to communicate about sex and intimacy, and intimacy as continuous variables), the study team will first test bivariate associations between patient-reported outcomes and categorical sociodemographic factors (race/ethnicity, employment status, income, education level, number of children) using ANOVA. The study team will test bivariate associations between patient-reported outcomes and continuous sociodemographic factors (age, years with partner) using Pearson's correlation. Next, the study team will employ one-tailed paired sample t-test to assess the effects of radiation by comparing one-month, three-month, six-month, 12-month, 18-month, and 24-month patient-reported outcomes to baseline. In addition to statistical tests, the study team will also report 95% confidence intervals and effect sizes. Such information will guide the sample size calculation for future larger scale studies. Finally, the study team will create line plots to show the longitudinal course of physical

exam outcomes and patient-reported outcomes. The study team will utilize mixed-effects modeling to demonstrate changes in each outcome variable over time, adjusting for demographic/clinical characteristics and clustering of repeated observations within individuals. All analyses will be conducted using SAS version 9.4 (SAS Institute Inc.).

Exploratory outcomes (not powered)

Hypotheses We hypothesize that low LPR (pre-treatment, baseline) will correlate with post-treatment vaginal dryness.

Data analysis The LPR will be calculated by aggregating the *Lactobacillus* genus counts and dividing by the aggregated *Prevotella* genus counts. The vaginal dryness (binary, VAS score <=2 vs >2) will be related to the LPR (continuous) by logistic regression. This formalism supports the inclusion of other demographic variables such as age and cancer. A significant association of LPR with VD will be evaluated by Wald test at alpha = 0.05.

Hypotheses We hypothesize that LPR will negatively correlate with the abundance of macrophages.

Data analysis The quantity of macrophages will be estimated by deconvolution as described in section D. The LPR will be correlated with the abundance of macrophages by nonparametric rank-based (Spearman) correlation. We will first identify: (1) the genes of that expression levels, (2) the immune cells' compositions change after radiation using linear mixed effect model. Spearman correlation will be performed to determine the association between LPR relative abundance and expression of a gene or composition of the immune cells identified above. We will use linear discriminant analysis (LDA) to explore a linear combination of gene expression, microbe relative abundance and immune cell composition to predict vaginal dryness. All data analysis will be conducted in R (open resource).

Hypotheses We hypothesize that LPR will negatively correlate aggregated expression of standard inflammasome markers.

Data analysis The level of inflammation in the tissue will be estimated by aggregating the expression of standard "inflammasome" genes, including ASC, CASP 1, GSDMA, GSDMB, GSDMC, GSDMD, GSDME, GSDMF, HMGB1, NLRC4, NLRP 3. Normalized and scaled gene expression values (as described in section D) will be summed across all listed genes for each swab sample. LPR will be correlated with the aggregated inflammation value by Spearman correlation as described in hypothesis 2. (D Spakowicz et al., 2019)

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The Ohio State University Combined Consent to Participate in Research and HIPAA Research Authorization

Study Title: Longitudinal changes in women's pelvic health and sexual function after pelvic radiation

Principal Investigator: Elizabeth Arthur PhD, APRN-CNP, AOCNP®

Sponsor: Pelotonia and The Ohio State University Wexner Medical Center

- **This is a consent form for research participation.** It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.
- **Your participation is voluntary.** You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.
- **You may or may not benefit as a result of participating in this study.** Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.
- **You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate.** If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

Key Information About This Study

The following is a short summary to help you decide whether or not to be a part of this study. More detailed information is listed later in this form.

The purpose of this study is to evaluate changes in physical measures of pelvic health and patient-reported outcomes of sexual function, intimate relationship and quality of life over time in women receiving radiation for pelvic cancer. Participants will have standard of care pelvic exams, vaginal swab sample collections and be asked to complete a panel of patient reported outcomes measures as repeated measures over two years.

1. Why is this study being done?

The goal of this study is to evaluate vaginal changes prior to and after a course of radiation and to collect patient reported outcomes of sexual function, partner communication and intimacy in order to have a better understanding of physical changes and symptoms over time.

2. How many people will take part in this study?

Approximately 89 people will take part in this study at The Ohio State University.

3. What will happen if I take part in this study?

The study will consist of 6 visits, plus 1 additional survey. In addition to a baseline exam and survey, participants will return for routine one month, three-month, six month, 12 month and 24 month follow-ups and the physical exam, vaginal swab sample collection and symptom surveys will be completed. Though no physical exams will be performed, participants will be asked to complete a symptom survey at 18 months. There will be no appointments necessary other than standard of care follow-up appointments at which time a pelvic exam would routinely be done for surveillance. Therefore, the participant will not undergo any unnecessary appointments or exams.

4. How long will I be in the study?

Your participation in this study will last two years.

5. Can I stop being in the study?

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

6. What risks, side effects or discomforts can I expect from being in the study?

Pelvic exams are done as routine care and swabbing of the vaginal wall is noninvasive, with minimal risk to participants.

Research using your specimens may include measuring your RNA (transcriptome sequencing), which could potentially identify you. A federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you

based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions about your eligibility or premiums.

- Employers with 15 or more employees may not use your genetic information from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this federal law. This law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance. Under Ohio law, health insurance companies cannot ask about the results of a genetic test or use any information obtained from genetic testing to make decisions about providing coverage or benefits for health care services.

7. What benefits can I expect from being in the study?

There are no direct benefits from participating in this study.

8. What other choices do I have if I do not take part in the study?

You may choose not to participate without penalty or loss of benefits to which you are otherwise entitled.

9. What are the costs of taking part in this study?

There is no cost to you for participating in the study.

10. Will I be paid for taking part in this study?

You will receive a ten dollar gift card after completing each survey. There are seven planned surveys, for a maximum of $70.00 total if all surveys are completed.

By law, payments to participants are considered taxable income.

Additionally, you will receive a tote bag, a travel mug after one month of participation, and a portfolio after 12 months of participation.

11. What happens if I am injured because I took part in this study?

If you suffer an injury from participating in this study, you should notify the researcher or study doctor immediately, who will determine if you should obtain medical treatment at The Ohio State University Wexner Medical Center.

The cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

12. What are my rights if I take part in this study?

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of research participants.

13. Will my de-identified information (and bio-specimens) be used or shared for future research?

Yes, de-identified data will be made publicly available upon publication to support the reproducibility of the published analyses.

14. Will my study-related information be kept confidential?

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law.

Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- U.S. Food and Drug Administration;

- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor supporting the study, their agents or study monitors; and
- Your insurance company (if charges are billed to insurance).

If we find information that significantly impacts your health, we will share it with you. This includes physical exam findings, such as from the pelvic exam. Results from sexual wellbeing questionnaires represent your individual patient-reported outcomes, and will not be reported back to you.

15. HIPAA AUTHORIZATION TO USE AND DISCLOSE INFORMATION FOR RESEARCH PURPOSES

I. What information may be used and given to others?

- Past and present medical records;
- Research records;
- Records about phone calls made as part of this research;
- Records about your study visits;
- Information that includes personal identifiers, such as your name, or a number associated with you as an individual;
- Information gathered for this research about:
  Physical exams
  Laboratory, x-ray, and other test results
  Diaries and questionnaires

II. Who may use and give out information about you?

Researchers and study staff.

III. Who might get this information?

- The sponsor of this research. "Sponsor" means any persons or companies that are:
  - working for or with the sponsor; or
  - owned by the sponsor.
- Authorized Ohio State University staff not involved in the study may be aware that you are participating in a research study and have access to your information;
- If this study is related to your medical care, your study-related information may be placed in your permanent hospital, clinic, or physician's office record;

IV. Your information <u>may</u> be given to:

- The U.S. Food and Drug Administration (FDA), Department of Health and Human Services (DHHS) agencies, and other federal and state entities;
- Governmental agencies in other countries;
- Governmental agencies to whom certain diseases (reportable diseases) must be reported; and
- The Ohio State University units involved in managing and approving the research study including the Office of Research and the Office of Responsible Research Practices.

V. Why will this information be used and/or given to others?

- To do the research;
- To study the results; and
- To make sure that the research was done right.

VI. When will my permission end?

There is no date at which your permission ends. Your information will be used indefinitely. This is because the information used and created during the study may be analyzed for many years, and it is not possible to know when this will be complete.

VII. May I withdraw or revoke (cancel) my permission?

Yes. Your authorization will be good for the time period indicated above unless you change your mind and revoke it in writing. You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the researchers. If you withdraw your permission, you will not be able to stay in this study. When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others.

VIII. What if I decide not to give permission to use and give out my health information?

Then you will not be able to be in this research study and receive research-related treatment. However, if you are being treated as a patient here, you will still be able to receive care.

IX. Is my health information protected after it has been given to others?

There is a risk that your information will be given to others without your permission. Any information that is shared may no longer be protected by federal privacy rules.

X. May I review or copy my information?

Signing this authorization also means that you may not be able to see or copy your study-related information until the study is completed.

16. Who can answer my questions about the study?

For questions, concerns, or complaints about the study, or if you feel you have been harmed as a result of study participation, you may contact the Principal Investigator, Dr. Elizabeth Arthur, at 614-293-0811.

For questions related to your privacy rights under HIPAA or related to this research authorization, please contact HIPAA Privacy Officer, 600 Ackerman Road, Suite E2140, Columbus, OH 43201.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Principal Investigator, Dr. Elizabeth Arthur, at 614-293-0811.

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this combined consent and HIPAA research authorization form**.**

Printed name of participant	**Signature of participant**
	AM/PM
	Date and time
Printed name of person authorized to consent for participant (when applicable)	**Signature of person authorized to consent for participant (when applicable)**
	AM/PM
Relationship to the participant	**Date and time**

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent	**Signature of person obtaining consent**
	AM/PM
	Date and time

Witness(es) - *May be left blank if not required by the IRB*

Printed name of witness	**Signature of witness**
	AM/PM
	Date and time
Printed name of witness	**Signature of witness**
	AM/PM
	Date and time