

**THE VAGINAL AND GASTROINTESTINAL MICROBIOMES IN GYNECOLOGIC CANCERS:
A REVIEW OF APPLICATIONS IN ETIOLOGY, SYMPTOMS AND TREATMENT**

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Abstract

The human microbiome is the collection of microorganisms in the body that exist in a mutualistic relationship with the host. Recent studies indicate that perturbations in the microbiome may be implicated in a number of diseases, including cancer. More specifically, changes in the gut and vaginal microbiomes may be associated with a variety of gynecologic cancers, including cervical cancer, uterine cancer, and ovarian cancer. Current research and gaps in knowledge regarding the association between the gut and vaginal microbiomes and the development, progression, and treatment of gynecologic cancers are reviewed here. In addition, the potential use of probiotics to manage symptoms of these gynecologic cancers is discussed. A better understanding of how the microbiome composition is altered at these sites and its interaction with the host may aid in prevention, optimization of current therapies, development of new therapeutic agents and/or dosing regimens, and possibly limit the side effects associated with cancer treatment.

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Fig. 1. The core human microbiome is variable, depending on a myriad of factors. These factors (blue) include host environment, lifestyle, physiology, genotype, and immune system. Other conditions (red) that can impact the microbiome composition include cancer, chemotherapy, and radiation treatment, all of which can contribute to GI and vaginal symptoms in cancer patients.

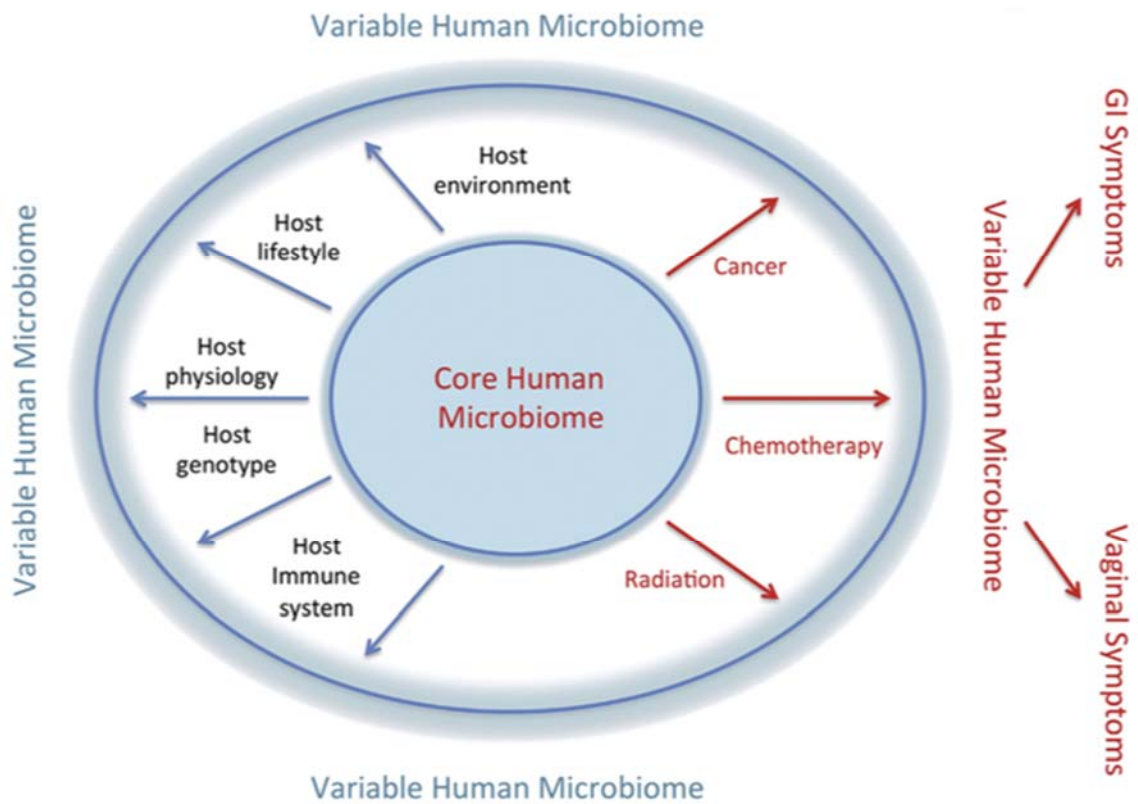


Fig. 2. Factors that impact the gut and vaginal microbiomes that can result in toxicity during cancer and cancer-related treatment. The gut of a healthy female is dominated by Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Fusobacteria. Cancer treatment, environmental factors, and pre-existing medical conditions can alter the GI microbiota and cause GI symptoms like nausea, vomiting, diarrhea, constipation, bloating, and abdominal pain. The vaginal microbiome of a healthy female is dominated by Firmicutes, specifically Lactobacilli spp. Perturbations of the vaginal microbiota can contribute to vaginal symptoms including pain, sexual dysfunction, and urinary symptoms.

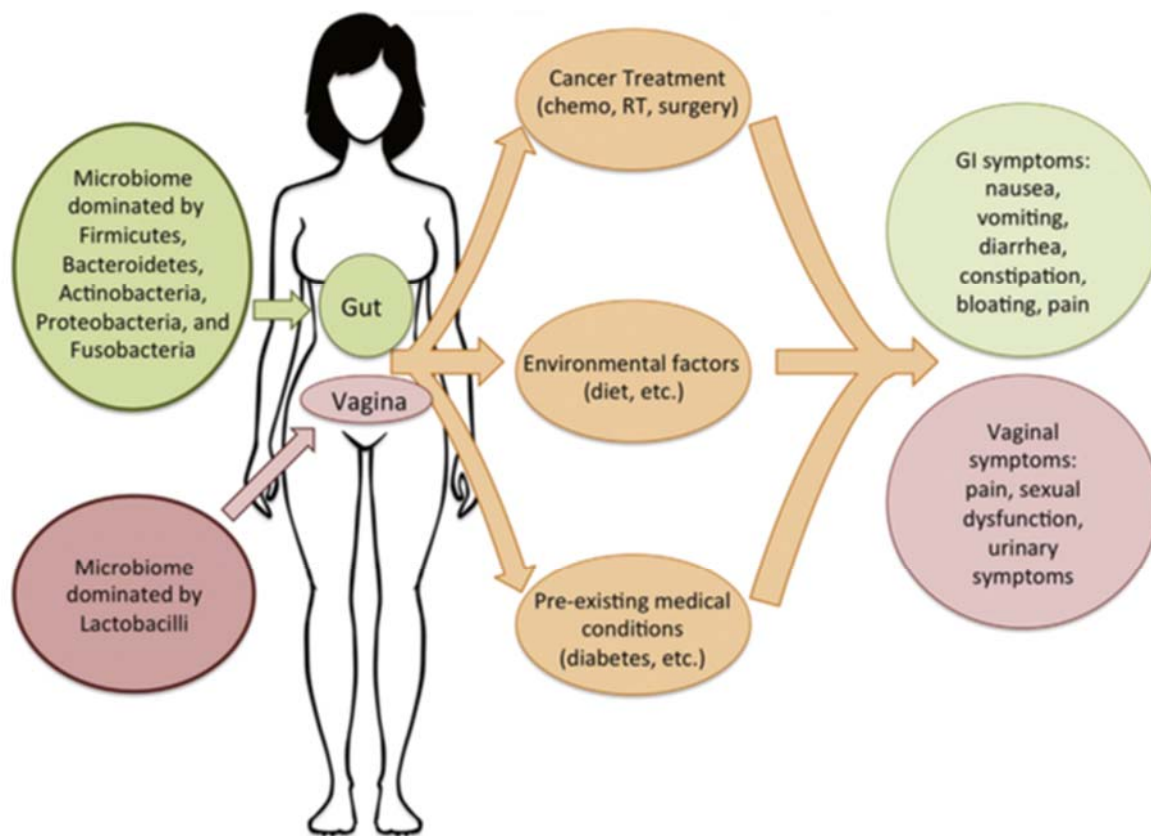


Fig. 3. There is a complex relationship between probiotic, pathogenic, and commensal microbiota in the gut and vagina. At both mucosal sites, probiotic bacteria (yellow) have beneficial functions including lowering the pH locally, bolstering mucosal immunity by interacting with IgA and altering antimicrobial peptide (AMP) secretion, blocking pathogen (red) growth in the mucus layer, and blocking pathogen adhesion to epithelial cells. In addition, probiotic bacteria may serve a beneficial role by enhancing commensal (green) colonization. In both the gut and vagina, these beneficial functions help to enhance epithelial barrier function. When the microbiota are disrupted, however, pathogenic bacteria are able to proliferate in the mucus and adhere to the gut and vaginal epithelial cells. In this state of dysbiosis, epithelial barrier function is disrupted and pathogenic bacteria are able to translocate through the epithelium resulting in inflammation and infection. Microbial translocation can also result in chronic, low-grade inflammation in the gut and vagina, which may cause GI and vaginal symptoms and may even drive carcinogenesis.

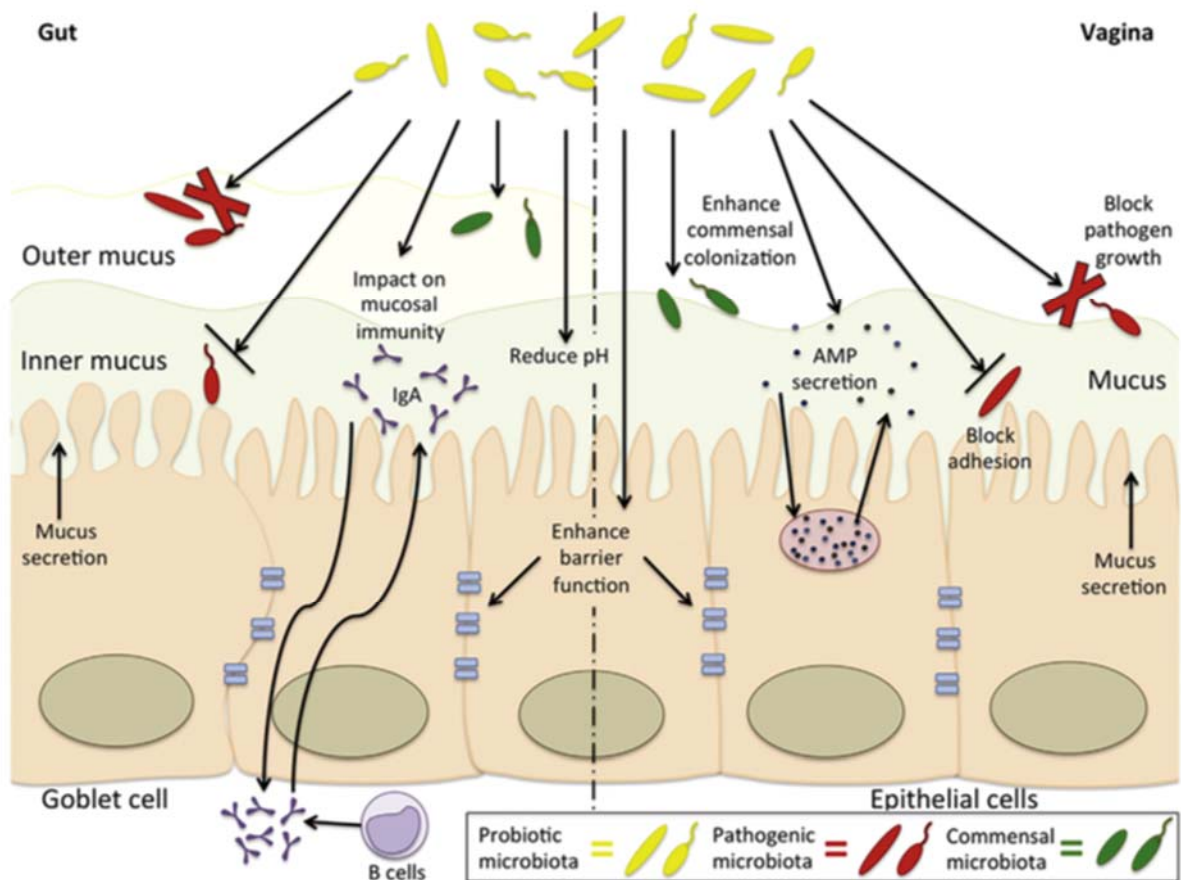


Table 1. Clinical trials investigating the impact of probiotics on preventing or alleviating symptoms following gynecologic cancer treatment. PubMed was used to search for the following terms: “cervical cancer and radiation and probiotics,” “uterine cancer and radiation and probiotics,” “endometrial cancer and radiation and probiotics,” “ovarian cancer and radiation and probiotics,” “pelvic cancer and radiation and probiotics,” “cervical cancer and chemotherapy and probiotics,” “uterine cancer and chemotherapy and probiotics,” “endometrial cancer and chemotherapy and probiotics,” “ovarian cancer and chemotherapy and probiotics,” and “pelvic cancer and chemotherapy and probiotics.” The results from all of these searches were combined, and we identified a total of 4 relevant primary research articles published in English within the last 10 years. The design and results of these studies are presented below. R = randomized, DB = double blind, PC = placebo controlled.

References	No. of patients (probiotics/control)	Type of study	Bacterial strain investigated/formulation/frequency of treatment	Clinical setting	% of patients with treatment-induced diarrhea	Severity of treatment-induced diarrhea	Anti-diarrheal medication use following probiotic treatment	Stool consistency
Chitapanarux et al. [64]	63 (32/31)	R, DB, PC	<i>L. acidophilus</i> plus <i>Bifidobacterium bifidum</i> /capsule/twice a day before meals (morning and evening)	Women undergoing radiation with concurrent cisplatin for cervical cancer	-	-	Use decreased in placebo group (p = 0.03)	Improved in probiotic group (p < 0.001)
Delia et al. [63]	482 (243/239)	DB, PC	VSL#3 (combination of <i>L. casei</i> , <i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>B. longum</i> , <i>B. breve</i> , <i>B. infantis</i> , and <i>L. salivarius</i> subsp. <i>thermophilus</i>)/sachet/three times a day	Patients undergoing adjuvant radiation after surgery for sigmoid, rectal, or cervical cancer	51.8% of placebo group vs. 31.6% of probiotic group (p < 0.001)	Less severe in probiotic group (p < 0.001)	Mean time to medication use was longer in probiotic group (p < 0.001)	-
Giralt et al. [66]	85 (44/41)	R, DB, PC	<i>L. casei</i> DN-114 001/probiotic drink/three times a day	Women undergoing radiation for cervical carcinoma or endometrial adenocarcinoma	-	-	-	Improved in probiotic group (p = 0.04)
Demers et al. [65]	229 (140/89) Standard dose probiotic: n = 81 High dose probiotic: n = 59	R, DB, PC	<i>L. acidophilus</i> LAC-361 plus <i>Bifidobacterium longum</i> BB-536/capsule/standard dose of 1.3 billion CFU twice a day OR high dose of 10 billion CFU three times a day	Patients undergoing radiation, with or without chemotherapy, for gynecologic, rectal, or prostate cancer	-	Less severe in group receiving standard dose of probiotic (p = 0.04)	-	-

Introduction

The human microbiome is the aggregate of microorganisms that reside at the body's mucosal surfaces [1]. These microbes help perform essential functions that include nutrient absorption, establishing/regulating the immune system, and protecting against pathogenic insults. These microbiota also occupy critical niches to prevent pathogen invasion. While these microbial communities populate all mucosal surfaces, the composition of each community varies from site to site within the body depending upon a myriad of host-derived factors (**Figure 1**). These factors include available nutrients (e.g. diet), hormone levels, host genetics, race and age [1-3]. This core human microbiome can also be altered by cancer and cancer treatment, which may result in the variable human microbiome as depicted in **Figure 1**.

Our knowledge of the human microbiome and its implications in health and disease has grown exponentially in recent years, largely due to advancements in sequencing technology and the establishment of the several international consortia to characterize and understand the role of the human microbiome in health and multiple types of diseases. The most common sequencing methods to characterize the microbiome are pyrosequencing and 16S ribosomal RNA (rRNA) sequencing [4]. The latter method relies on the identification of hypervariable regions in the 16S rRNA gene, which are unique from species to species. This method is advantageous because ribosomes and rRNA are present in all cells, and rRNA sequences are highly conserved. This method can also facilitate the identification of new or lesser-known bacterial species [5].

These sequencing methods have been used to characterize diverse microbial ecosystems, including the human microbiome. A healthy gastrointestinal (GI) microbiome is typically populated by five major bacterial phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Fusobacteria*. These bacteria make up approximately 90% of the total microbiota in the gut (**Figure 2**), although the relative abundance of GI microbiota may vary throughout the host's lifespan [6]. In contrast, a healthy vaginal microbiome is typically populated by members of the *Firmicutes* phylum (**Figure 2**), dominated by *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, and *Lactobacillus jensenii* [1]. Similar to GI microbiota, the relative abundance of each of these bacteria may change over time [1].

The sequencing and characterization of GI microbiota has greatly expanded our understanding of how the human microbiome impacts the overall health of the host. For example, the GI microbiome supports functions that include immune system development, digestion, fat metabolism, epithelial homeostasis, enteric nerve regulation, and angiogenesis. As such, disruption of the microbiome, termed dysbiosis, can impact these important processes and result in disease, including cancer [7]. In a healthy female, microbiota in the gut and vagina are separated from the host by a multi-level barrier system that includes, among other factors, a mucus layer, secretion of soluble immune mediators, and intact epithelium with tight junctions (**Figure 3**). This barrier system is also supported by immune cells. For example, B cells produce IgA that helps to neutralize pathogenic bacteria. When this multifaceted barrier system fails, pathogenic bacteria can translocate across the gut and vaginal epithelia, causing low-grade chronic inflammation that leads to disease, including cancer [8]. Conversely, cancers of the GI and reproductive tracts cause inflammation (**Figure 3**) that results in dysbiosis, resulting in a positive feedback loop that may result in promoting disease [8].

A better understanding of how dysbiosis contributes to disease will allow for the development of novel bacterial therapies that can manipulate the microbiome to reinstate homeostasis. One such example is the use of fecal microbiota transplantation (FMT) in the treatment of *C. difficile* infection (CDI). CDI often follows antibiotic use and is characterized by a decrease in the amount of resident, beneficial bacteria in the gut, which allows *C. difficile* to proliferate and cause dysbiosis of the GI microbiota [9]. FMT reintroduces the beneficial bacteria from a healthy donor into the gut of the infected patient and this aids in recolonization [10]. This technique has been successful for treating these infections and supports the concept that microbiota can be manipulated to restore homeostasis in states of dysbiosis.

This review highlights the existing knowledge from the literature and gaps in our current understanding of the gut and vaginal microbiomes related to specific gynecologic cancers and therapy-related side effects. This review focuses on the three most common gynecologic malignancies and is organized accordingly: cervical, uterine, and ovarian. Within each of these primary sections, there are subsections dedicated to carcinogenesis and therapy-induced symptoms as they relate to microbiota perturbations. We close this review with a section

dedicated to symptom management and the potential use of probiotics. The purpose of this review is to highlight the importance of future research focused on studying the gut and vaginal microbiota (VMB) composition and impact on the host during carcinogenesis, progression and treatment of gynecologic cancers. Targeting the microbiota could possibly influence patient-reported quality of life (QOL) by reducing cancer- and treatment-related complications in these women.

Methods

A comprehensive literature search was conducted using PubMed to search for articles reported in English and published within the last 10 years. The search terms used are detailed below in their respective sections. Relevant abstracts and full-text articles were considered for inclusion in this review. Review articles were also examined to identify additional relevant studies. Studies using human subjects were prioritized, but studies using animal models were included when human data was unavailable.

Results

CERVICAL CANCER

Carcinogenesis

The microbiome and HPV infection

Human papillomavirus (HPV) is a well-established causative agent of cervical cancer. More specifically, persistent infection with high-risk HPV (especially HPV type 16 or 18) is directly involved in the pathogenesis of approximately 70% of cervical cancer cases [11]. A PubMed search for the term “vaginal microbiome and HPV” returned 11 results published within the last 10 years, 7 of which were in English and involved human subjects. Four of those 7 papers were primary research articles and are discussed here. The first paper followed 32 women who collected vaginal swabs twice weekly for 16 weeks [12]. These samples were analyzed using pyrosequencing to determine the VMB composition and HPV status of each patient. Results of this study showed that women with *Lactobacillus gasseri*-dominated microbiota had the fastest HPV remission rate. Additionally, women whose VMB were low in *Lactobacillus gasseri* and high in *Atopobium* had the slowest rate of HPV remission. Overall, the composition of the VMB was significantly associated with changes in HPV status ($p < 0.001$) [12]. In the second paper, the vaginal microbiota of 70 women—32 HPV-negative and 38 HPV-positive—were sequenced using PCR [13]. Results of the sequencing showed that there was significantly greater diversity in the microbiota of HPV-positive women ($p < 0.001$) and that *L. gasseri* and *Gardnerella vaginalis* were found in significantly higher frequencies in HPV-positive women ($p = 0.005$ and $p = 0.031$, respectively) [13]. The third paper examined the association between vaginal pH, which is determined by the microbiota composition, and HPV infection. In this study, the vaginal pH and HPV status was determined for 9165 women. Results showed that an elevated vaginal pH, associated with decreased abundance of *Lactobacilli*, was associated with a 30% greater risk of infection with LSIL and with multiple HPV types [14]. The fourth paper was a twin study that followed 68 HPV-infected or uninfected females [15]. The researchers used 454-pyrosequencing to determine the microbiota composition of each woman and found that HPV-positive women had significantly higher microbial diversity and a lower

proportion of *Lactobacilli* species, specifically *L. iners* ($p = 0.03$) [15]. Given that the VMB composition has been shown to play a role in HPV infection and the rate of HPV clearance, the vaginal microbiome structure may be associated with the development of cervical cancer secondary to a persistent HPV infection.

Therapy-induced symptoms

The microbiome and radiation-induced enteritis

Radiation therapy, used either alone or in conjunction with surgery and/or chemotherapy, is the standard of care for most locally advanced cervical cancers. Unfortunately, radiation therapy can secondarily affect the bowel. Up to 80% of patients experience bowel symptoms –including abdominal pain, urgency, diarrhea, fecal incontinence, and bloating throughout their radiation treatment [16]. While these symptoms often resolve spontaneously within 8 weeks following cessation of treatment, as many as 50% of patients may have chronic radiation enteritis (CRE), defined as symptoms persisting for at least 3 months post-treatment [16]. Using the search term “radiation enteritis and cervical cancer,” we identified a key study that followed 117 women who underwent radiotherapy for endometrial or cervical cancer to determine how treatment impacted their QOL [17]. Of the 117 women in this study, 47% indicated that they experienced CRE and that it negatively impacted their QOL. Younger women and women with cervical cancer were most likely to experience CRE. There was no association found between patient-reported severity of CRE and dose of radiation or stage of cancer, therefore suggesting that alteration of the local GI microbiota could be playing a role in this radiation-mediated toxicity [17].

The search term “microbiome and radiation therapy” yielded a key study that demonstrated that the GI microbiome is altered by radiotherapy specifically in gynecologic cancer patients [18]. Fecal samples from patients undergoing pelvic radiotherapy were compared using 454-pyrosequencing to samples from healthy women, and it was found that the GI microbiota between the two groups differed significantly. The relative abundance of *Actinobacteria* was 30 times higher in cancer patients prior to treatment compared to healthy individuals ($p = 0.001$), whereas the relative abundance of *Fusobacteria* was 7.4 times lower (p

= 0.001). The relative abundance of each of these bacteria is important because *Actinobacteria* are dominant in the gut and help prevent symptoms like bloating and abdominal pain [19], whereas *Fusobacteria* include invasive, proinflammatory bacteria associated with diseases like inflammatory bowel disease [20]. The study also monitored changes in microbiota at several time points: before treatment (T0), after first radiotherapy fraction (T1), after fifth radiotherapy fraction (T2), and at the end of radiotherapy treatment (T3). The relative abundance of *Fusobacteria* was 6 times higher at T2 compared to T0 ($p = 0.05$), and the abundance of unclassified bacteria increased by 10% between T0 and T3 ($p = 0.04$). While the sample size in this study was small these data suggest that there is a link between the composition of the GI microbiota, health status, and pelvic radiotherapy [18]. It is important to note that health status changes through gynecologic cancer can impact both local and distal mucosal site (e.g. gut) microbiota composition as shown in this study.

Collectively, there has been very little research that has directly examined GI microbiome composition during radiotherapy and how changes in composition correlate to patient-reported QOL. Given the compelling research demonstrating that CRE has a negative impact on patient QOL and that the GI microbiome is significantly altered by radiotherapy, it is important that future studies investigate how changes in the microbiome correlate with patient-reported QOL.

The microbiome and radiation-induced cystitis

Cystitis, inflammation of the bladder wall, is another common side effect of radiation therapy for cervical cancer due to unintentional irradiation of the bladder during treatment. There has been little research on perturbations of the GI microbiome specifically related to radiation-induced cystitis; our literature search for the term “microbiome and radiation-induced cystitis” did not yield any results. However, literature does suggest that there is significant cross-talk between the GI tract and the bladder [21, 22]. A clinical study of irritable bowel syndrome (IBS) patients found that rectal lidocaine provided relief not only from bowel symptoms, but also from abdominal pain [21]. While the exact mechanism for this phenomenon is unknown, animal studies have shown that the spinal nerves that respond to

colon stimulation also respond to bladder stimulation and vice versa [22]. While these studies must be replicated in humans, this neuronal cross-talk suggests that the bowel may be able to modulate bladder function and pain. With regard to the GI microbiome, perturbations of the microbiota have been implicated in a number of bowel inflammatory and/or pain conditions, including Crohn's patients, whom exhibit diminished bacterial diversity among *Firmicutes* in their GI tracts [23]. There is also growing evidence of the link between human-microbial interface along the human GI tract and the inclusion of the enteric nervous system, which through dense projections carried within the vagus nerve to the brain, can potentially modify behaviors or neurological signals through changes in neuro-peptide release [24]. Given that alterations in the GI microbiome are associated with inflammation and pain of the bowel, and given that the bowel and bladder have significant cross-talk through neuronal networks, it is possible that perturbations in the GI microbiome may also contribute to the bladder pain characteristic of interstitial cystitis [25]. Therefore, GI microbiome perturbations during radiotherapy may be partially responsible for the bladder-specific pain of radiation-induced cystitis. However, additional research in this area is necessary to support this hypothesis.

The microbiome and other symptoms associated with cervical cancer treatment

There are additional symptoms commonly associated with cervical cancer treatment, including pelvic pain and sexual dysfunction. However, there were no relevant results found using the search terms "microbiome and pelvic pain" and "microbiome and sexual dysfunction." Notably, pelvic pain is a common symptom of cervical cancer and may persist throughout treatment, possibly due to unintentional irradiation of healthy pelvic structures [26]. Sexual dysfunction can also be a side effect of treatment for cervical cancer and may be due to pain following treatment, among other factors [27]. Since conventional photon radiotherapy for cervical cancer irradiates parts of the healthy tissue of the reproductive tract, including the vagina, it is possible that this treatment perturbs the VMB. This perturbation may disrupt epithelial barrier function, permitting translocation of pathogenic bacteria and causing an inflammatory response [8]. This dysbiosis and local inflammation may contribute to pelvic pain and sexual dysfunction in cervical cancer patients. Given the relative lack of current

literature related to these topics, it is important that additional research be conducted to investigate these areas.

UTERINE CANCER

Carcinogenesis

The microbiome and pelvic inflammatory disease

Pelvic inflammatory disease (PID) results when pathogenic bacteria ascend through the cervix into the upper genital tract and cause inflammation of the uterus, fallopian tubes, and/or ovaries [28]. Recent studies have indicated that dysbiosis of the VMB, specifically as seen with bacterial vaginosis (BV), is associated with an increased risk of PID [28, 29]. BV is a disease state characterized by a decrease in the abundance of *Lactobacilli* and an increase in the abundance of anaerobic organisms including *Gardnerella*, *Prevotella*, *Atopobium*, *Mobiluncus*, *Ureaplasma*, and *Mycoplasma* [29]. A PubMed search for “bacterial vaginosis and pelvic inflammatory disease” yielded 80 results published within the past 10 years. Of these 80 papers, 61 were in English and involved human subjects; 28 were review articles and were thus excluded from this review. One of the most comprehensive and relevant studies was a multi-center study of 1140 women. In this study, vaginal swabs were used to investigate the association between the dysbiosis seen in BV and the development of PID [29]. Results of the study showed that women with a decrease in *Lactobacilli* species and a relative abundance of *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and anaerobic Gram-negative rods were significantly more likely to develop PID [29]. Therefore, perturbations in the VMB are risk factors for PID. The chronic inflammatory state in the upper genital tract caused by PID may result in uterine endothelial dysfunction. As discussed previously, endothelial dysfunction and chronic inflammation drive carcinogenesis [8]. Therefore, one hypothesis may be that vaginal dysbiosis leads to PID and thus could be associated with the development of endometrial cancer, but additional research is necessary to support this theory.

The microbiome and obesity

Our literature search for “microbiome and obesity and uterine cancer” did not yield any relevant results. However, obesity is a well-known risk factor for uterine cancer [30] and has recently been associated with alterations in the GI microbiota [31]. One hypothesis for the association between GI microbiota and obesity is that the composition of the microbiota influences nutrient absorption, which influences adiposity and body composition [32]. A twin study consisting of 154 individuals was conducted recently to characterize the GI microbiome in lean and obese humans [31]. Results showed a lower relative abundance of *Bacteroidetes* ($p = 0.003$) and a higher abundance of *Actinobacteria* ($p = 0.002$) in obese individuals compared to lean individuals. There was no significant difference in the relative abundance of *Firmicutes* ($p = 0.09$). Obese individuals also had decreased diversity of the GI microbiota in general [31]. These results indicate that alterations in the GI microbiota are associated with obesity. Given that obesity is an established risk factor for uterine cancer, it is possible that perturbations in the GI or vaginal microbiota, or even upper reproductive tract microbiota (as discussed above) are involved in development of this cancer.

The microbiome and sex hormones

The composition of the VMB varies significantly as women undergo hormonal changes throughout their lifetime. A recent study of 87 women characterized the composition of the VMB in premenopausal, perimenopausal, and postmenopausal women [33]. The researchers found that the VMB of premenopausal women were dominated by *L. crispatus* and *L. iners*. In contrast, perimenopausal women had higher levels of *L. gasseri*, and postmenopausal women had higher levels of *Streptococcus* and *Prevotella* and an overall decrease in *Lactobacilli spp.* [33]. These results suggest that the VMB composition fluctuates as women undergo hormonal changes throughout their lifetime.

The vaginal microbiome is not the only microbial community associated with hormonal changes in women. Human GI microbiota are closely associated with sex hormones via the estrobolome, defined as the collection of bacterial genes whose products metabolize estrogens [34]. More specifically, enzymes produced by gut bacteria can conjugate and deconjugate estrogen, thus impacting circulating and excreted estrogen levels [34]. This association is

important in gynecologic oncology because excess estrogen is a well-established risk factor for the development of uterine cancer [35-37]. Perturbations of GI microbiota have been shown to impact metabolism and excretion of estrogen. Because the composition of the GI microbiome is crucial for the proper metabolism and excretion of estrogen, it is possible that perturbations of the microbiota may result in an increased risk of estrogen-driven cancers, including uterine cancer.

Therapy-induced symptoms

The microbiome and vaginal atrophy

Vaginal atrophy is a significant concern for women undergoing treatment for uterine cancer, because atrophy can result from cellular damage due to radiation therapy [38]. A PubMed search for “microbiome and vaginal atrophy” returned 6 results, one of which was a review and thus was not included in this paper. Of the 5 remaining studies, one of the papers specifically examined the association between the VMB and vulvovaginal atrophy. The authors found that women with vulvovaginal atrophy had significantly less *Lactobacillus* than women without the condition [33]. Given that radiation therapy damages the vaginal epithelium [38], pathogenic bacteria may be able to cross the epithelium and cause chronic inflammation that contributes to vaginal atrophy. Vaginal atrophy contributes to sexual dysfunction, which is known to affect QOL post-treatment [39].

Another study identified in this literature search further supported the finding that women with vulvovaginal atrophy have significantly less *Lactobacillus* in their vaginal microbiome than women without the condition [40]. In this 2011 study, the VMB of 32 post-menopausal women were analyzed. From that group of 32 women, 6 healthy women and 4 women with vaginal dryness were selected to identify whether there were any differences in VMB associated with atrophy [40]. Results showed an inverse relationship between the amount of *Lactobacillus* and degree of dryness, and an increase in bacterial diversity among women experiencing moderate to severe dryness [40]. While the sample size of this study was small, the results suggest that vaginal atrophy and dryness are associated with changes in the VMB.

These data support the continued investigation of host-VMB interactions in the context of the vaginal epithelium and vulvovaginal atrophy.

OVARIAN CANCER

The GI microbiome is of particular interest in ovarian cancer patients since ovarian cancer often presents initially with GI symptoms, including abdominal pain, bloating, indigestion, constipation, and early satiety [41-43]. Patient-reported abdominal discomfort scores are highest at presentation, and they improve slightly throughout treatment. However, compared to other gynecologic cancers, GI symptoms remain heightened throughout ovarian cancer treatment in general [44, 45].

Carcinogenesis

The microbiome of the upper reproductive tract

The etiology of ovarian cancer is still largely unclear, although a chronic inflammatory state has been implicated in the carcinogenesis [46]. Some hypothesize that BV promotes the ascension of dysbiotic VMB to the upper reproductive tract or that BV could indicate colonization of the upper tract [47-49]. In addition, BV-associated bacteria is a risk factor for pelvic infection and studies have shown that BV-associated bacteria are found in the upper reproductive tract (uterus and fallopian tubes) suggesting a direct link [50-52]. Inflammation in the upper tract could establish a chronic inflammatory state that could influence the development of ovarian cancer, especially in light of recent studies indicating that this process may arise in the fallopian tube [53]. Additional studies are required to determine if there is a link between the microbiome and inflammation at these previously considered “sterile” sites of the upper female reproductive tract and carcinogenesis.

Therapy-induced symptoms

The microbiome and changes with chemotherapy

It is important to understand how the GI microbiota change during chemotherapy, because the composition of the GI microbiome may impact the effectiveness of chemotherapy

used to treat ovarian cancer. Our literature search for “microbiome and chemotherapy and ovarian cancer” did not yield any relevant results with human subjects. However, this search identified several key animal studies. Alkylating agents like cyclophosphamide are chemotherapy agents used to treat advanced, recurrent and/or persistent ovarian cancer [54]. A recent mouse study examined how cyclophosphamide alters the composition of the microbiota in the small intestine and how these microbiota facilitate an anti-cancer immune response [55]. In this study, cyclophosphamide treatment resulted in disruption of the epithelial barrier of the small intestine and a decrease in the relative abundance of *Lactobacilli* and *Enterococci*, suggesting that this drug facilitates the translocation of certain bacterial species across the gut epithelium [55]. These bacteria were detected in mesenteric lymph nodes and the spleen within 48 hours of treatment [55]. By homing to lymphoid organs, bacteria from the gut helped stimulate an immune response characterized by a significant increase in helper and memory T cells, which may have facilitated the antitumor effects of cyclophosphamide. Additionally, germ-free mice lacking commensal GI microbiota showed a reduction in T cell activation, and their tumors were significantly less responsive to cyclophosphamide [55]. This study demonstrates the importance of GI microbiota in the effectiveness of chemotherapy and suggests that perturbations in GI microbiota may have an impact on chemotherapy effectiveness.

Another recent study used a mouse model to determine whether disruption of the GI microbiota affects the efficacy of CpG-oligonucleotide immunotherapy and platinum chemotherapy, two common anticancer treatments [56]. Compared to wild type mice, mice treated with antibiotics to disrupt the GI microbiota had a diminished response to immunotherapy [56]. This therapy inhibits tumor growth by stimulating cytokine production, particularly tumor necrosis factor (TNF) from tumor-associated myeloid cells; the cytokines induce a hemorrhagic necrosis of the tumor [56]. In antibiotic-treated mice, cytokine production was significantly diminished and there was minimal tumor necrosis compared to untreated mice [56]. In addition, TNF production was correlated with microbiota composition. It was determined that the presence of *Lactobacillus* species, which have anti-inflammatory effects and reduce TNF production, attenuated tumor response to immunotherapy, while the

presence of *Ruminococcus* and *Alistipes* species reconstituted TNF production [56]. A similar response was seen in mice treated with platinum chemotherapy instead of immunotherapy; antibiotic-treated mice had significantly reduced tumor regression and survival [56]. The anti-tumor effects of platinum chemotherapy are mediated by reactive oxygen species (ROS) and inflammatory responses independent of TNF [56]. In antibiotic-treated mice, expression of pro-inflammatory genes was downregulated and ROS production decreased, accounting for the diminished response to chemotherapy [56]. The results of this study suggest that commensal bacteria in the gut affect the inflammatory responses required for immunotherapy and platinum chemotherapy to be effective, thus indicating that manipulation of particular communities of GI microbiota may enhance cancer treatment and could be a potential target to optimize therapeutic outcomes (**Figure 2**).

The microbiome and chemotherapy-induced symptoms

Chemotherapy-induced nausea and vomiting (CINV) and chemotherapy-induced fatigue are among the most common side effects of treatment for ovarian cancer. There does not appear to be any current literature directly investigating the association between these symptoms and perturbations in the GI microbiota. However, given how much the microbiome is altered during chemotherapy, it is possible that these changes are associated with nausea/vomiting and fatigue. Therefore, further research is needed in these areas.

The microbiome and cachexia

Cachexia is another concern in ovarian cancer patients; malnutrition and cachexia markedly reduce the effectiveness of treatment and increase morbidity and mortality [57]. A PubMed search for “microbiome and cachexia” yielded 5 results, two of which were relevant for this review. One of these articles described a possible mechanism by which cancer-related cachexia and the GI microbiome are related [58]. This report suggested that cachexia in cancer patients is due to increased intestinal permeability secondary to microbial pathogens and/or intestinal inflammation [58]. As discussed above, chemotherapy can result in increased intestinal permeability, resulting in microbial translocation of gut microbes out of the intestine,

which then triggers a systemic immune response [58]. This immune response may lead to chronic inflammation that may contribute to metabolic dysfunction, malnutrition, and cachexia. Maintenance of gut barrier function may help counteract symptoms of cancer cachexia, thus illustrating the importance of a healthy GI microbiome in cancer patients [58].

The second relevant article from the literature search for “microbiome and cachexia” discussed how the GI microbiome and cachexia may also be associated via the “GI microbiota-muscle axis,” which has both positive and negative effects on muscle mass [59]. A positive effect is that GI microbiota contribute to amino acid bioavailability, which is critical for the production and maintenance of adequate muscle mass to combat cachexia [59]. However, a negative effect of the GI microbiota-muscle axis is that a variety of bacteria in the gut can activate toll-like receptors (TLRs) that activate NF- κ B, which in turn can cause muscle wasting [59]. Specific TLRs are triggered by certain microbial products in the gut [60]. NF- κ B, a downstream target of TLRs, promotes muscle wasting and atrophy by stimulating the production of proinflammatory cytokines that mediate the cachectic process [61]. Therefore, GI microbiota are closely involved in the TLR/NF- κ B pathway that results in muscle atrophy and cachexia [59].

An additional study aimed to characterize the GI microbiota in a mouse model of leukemia with signs of fat loss, muscle atrophy, anorexia, and inflammation. This report showed that the relative abundance of *Lactobacillus* in the gut, which has immunomodulatory properties, was decreased in the mice with cachexia compared with healthy mice [62]. The number of total bacteria and relative abundance of *Bacteroidetes* species did not change significantly [62]. Most telling, however, was the fact that following supplementation with *L. reuteri* and *L. gasseri*, levels of inflammatory cytokines that contribute to muscle atrophy and cachexia were reduced, and the weight of the tibialis muscle increased by 8% ($p = 0.05$) [62]. These results suggest that there are specific changes in the composition of the GI microbiota that accompany cachexia in cancer patients, and treatment of this dysbiosis may be beneficial for cachectic patients. However, while these data are promising, it is still necessary to validate the results using human patients by comparing the relative abundance of *Lactobacillus* in the guts of healthy patients to those of cachectic ovarian cancer patients.

SYMPTOM MANAGEMENT

Probiotics

As discussed throughout this review, perturbation of the microbiome may be implicated in many GI and vaginal symptoms associated with gynecologic cancer and treatment. Probiotics, defined as “a product or preparation containing viable and defined microorganisms in a number thought to be sufficient to alter by implantation or colonization the host’s microbiota and thereby exert beneficial effects,” [63] are currently under investigation as a method of reinstating microbiome homeostasis (**Figure 3**). We searched PubMed for the following terms: “cervical cancer and radiation and probiotics,” “uterine cancer and radiation and probiotics,” “endometrial cancer and radiation and probiotics,” “ovarian cancer and radiation and probiotics,” “pelvic cancer and radiation and probiotics,” “cervical cancer and chemotherapy and probiotics,” “uterine cancer and chemotherapy and probiotics,” “endometrial cancer and chemotherapy and probiotics,” “ovarian cancer and chemotherapy and probiotics,” and “pelvic cancer and chemotherapy and probiotics.” The results of these searches were combined, and we identified a total of four relevant primary research articles published in English within the last 10 years (**Table 1**). All four of these papers examined whether probiotics are effective at reducing radiation-induced diarrhea in gynecologic cancer patients [63-66]. The first paper was a randomized, double blind, placebo controlled trial of 63 advanced cervical cancer patients that investigated whether a probiotic containing live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* could reduce the incidence of diarrhea as the patients underwent radiotherapy with concurrent cisplatin. Grade 2-3 diarrhea was initially reported in 45% of the placebo group and 9% of the study group. Upon follow-up, anti-diarrheal medication use was significantly decreased in the placebo group ($p = 0.03$), while the patients in the study group had a significantly improved stool consistency ($p < 0.001$) [64]. The second study was a double blind, placebo controlled trial that followed 482 patients with sigmoid, rectal, or cervical cancer and gave them either a probiotic or a placebo beginning the first day of radiation therapy [63]. The patients given the probiotic had significantly less diarrhea than those given the placebo ($p < 0.001$), and the severity of the diarrhea of patients given the probiotic was significantly lower ($p < 0.001$) [63]. Furthermore, daily bowel movements among

the placebo group were 14.7 +/- 6, and only 5.1 +/- 3 in the probiotic group ($p < 0.05$) [63]. The third paper was a randomized, double blind, placebo controlled trial that demonstrated the beneficial effects of probiotics in reducing GI symptoms in patients with either cervical carcinoma or endometrial adenocarcinoma. Forty-one patients were assigned to the placebo group and 44 were assigned to the probiotic group, which involved drinking a probiotic drink daily [66]. The probiotic drink did not significantly reduce the incidence of radiation-induced diarrhea or use of anti-diarrheal medication ($p = 0.568$), but patients in the probiotic group had significantly better stool consistency ($p = 0.04$) [66]. The fourth and final paper was a randomized, double blind, placebo controlled trial that followed 229 patients with pelvic cancer (either gynecologic, rectal, or prostate) undergoing radiation with or without chemotherapy [65]. Of the 140 patients assigned to the probiotic group, 81 patients took a standard dose (1.3 billion CFU twice a day) and 59 took a high dose (10 billion CFU three times a day). After 60 days, 35% of patients in the standard dose group had no symptoms of moderate to severe diarrhea, compared to only 17% of patients in the placebo group ($p = 0.04$) [65]. Collectively, the results of these studies indicate that probiotics may mitigate radiation-induced diarrhea in gynecologic cancer patients and warrants further study (**Table 1**).

Another major area of probiotic research is the potential use and beneficial effects on the VMB. As illustrated in **Figure 3**, probiotics including *Lactobacilli* may improve vaginal health through a variety of mechanisms [67]. A variety of *Lactobacilli* strains have been studied for reinstating vaginal health in humans. Each strain and combination functions differently. *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains have been shown to modulate host defense, interfere with pathogen growth, induce anti-adhesion and antivirulence factors and disrupt biofilm formation [68-71]. Oral administration of the GR-1/RC-14 combination in humans was shown to decrease urinary tract infection (UTI) recurrence and to increase the probability of a *Lactobacilli* dominant VMB [68-71]. Intravaginal delivery of *L. crispatus* CTV05 has shown to decrease levels of bacterial vaginosis-associated bacteria when this strain persists more than 28 days and significantly reduced UTI recurrence [72]. A recent, well-designed meta-analysis also focused on the role of probiotics in the treatment of BV [73]. This meta-analysis identified 12 high-quality studies in the field and specifically singled out 4 papers as being the highest quality

of these 12 [74-77], due to appropriate randomization, double-blind structure, and description of withdrawals and dropouts. Two of these 4 papers reinforced the efficacy of oral GR-1/RC-14 probiotics in treating BV [74, 75]. The other 2 papers described the efficacy of different bacterial strains as probiotics, including *L. brevis* CD2, *L. salivarius* FV2, and *L. plantarum* [76, 77]. Taken together, these limited studies support the continued investigation of probiotics and bacterial therapeutics in the treatment of BV and vaginal dysbiosis. However, more research is needed to determine the optimal strain(s), dosage, formulation, timing and route of administration to reinstate vaginal health.

Probiotics have also been investigated for their beneficial role in the clearance of HPV, a virus associated with the development of cervical cancer. Our literature search on PubMed for “probiotics and HPV” yielded 6 results published within the last 10 years. Of these 6 articles, one was a review article and thus is not discussed in this paper. Two of the papers examined whether recombinant and non-recombinant lactic acid bacteria could be used for vaccination against HPV; since these studies investigated the role of probiotics as vectors, not as a therapeutic treatment, they were not included in this section. The remaining 3 papers regarding probiotic treatment and HPV clearance are discussed here. The first in vitro study examined the impact of probiotic treatment on the expression of HPV16 E6 and E7 in SiHa cells. Results indicated that after 48 hours, both E6 and E7 mRNA transcript levels decreased following treatment with *Bifidobacterium adolescentis* in cervical cancer cells ($p < 0.01$ and $p < 0.05$, respectively); however, E6 and E7 protein expression was not significantly changed [78]. The significant decline in mRNA transcript levels indicates that *B. adolescentis* may exhibit some level of antiviral activity against HPV E6 and E7 [78]. The second study investigated the cytotoxic effects of *Lactobacillus* on normal cervical cells compared to HeLa (cervical cancer) cells [79]. Normal cervical and HeLa cells were exposed to culture supernatants, cytoplasmic extracts, cell wall extracts, and live cells of *Lactobacillus gasseri* and *Lactobacillus crispatus*. Cell growth inhibition was measured using an MTT assay. Upon exposure to the supernatant of *Lactobacilli*, HeLa cell growth was significantly inhibited compared to normal cells ($p < 0.05$), indicating that *Lactobacilli* exert cytotoxic effects on cervical cancer cells, but not on healthy cells [79]. However, it is important to note that the effects of bacteria on cultured cells may not

accurately approximate the effects of probiotics on cells in vivo, therefore more research is needed in this area. In the third study, 54 women with HPV+ low-grade squamous intraepithelial lesions were monitored for 6 months; the intervention group consumed a daily probiotic drink containing *Lactobacillus casei*, while the control group received no treatment [80]. After 6 months, the probiotic users had double the chance of clearance of cytological abnormalities (60% vs. 31% in women who received no treatment, $p = 0.05$). Additionally, HPV was cleared in 29% of probiotic users and only 19% of control patients, although not significant ($p = 0.41$) [80]. These results suggest that probiotics may play a role in promoting the clearance of HPV, although the mechanism is unknown. Collectively, these studies highlight the need to further study the pleiotropic mechanisms of beneficial bacteria and their application to promoting vaginal health. Additional research is needed to investigate the mechanisms of specific bacterial strains delivered orally or locally and how dosage and formulation may impact VMB composition, recolonization and restoration of vaginal health and how it could be applied to the prevention and/or treatment of gynecologic cancer.

Necessity of a personalized approach to symptom management

Despite the strong evidence indicating the efficacy of probiotics in reducing GI symptoms in gynecologic cancer patients, it is necessary to take a personalized approach when considering this treatment. One important consideration is whether a patient is immunocompromised, as the *Lactobacillus* found in probiotics may be an opportunistic pathogen and a rare cause of sepsis in immunocompromised patients [81, 82]. The primary concern is that immunocompromised cancer patients may have GI endothelial barrier dysfunction that allows the *Lactobacillus* from the probiotic to translocate across the epithelium and cause sepsis [83]. However, it is important to note that cases of *Lactobacillus* sepsis are very rare and probiotics are often used in cancer patients with no adverse effects [83, 84].

Other considerations for the use of probiotics in GI symptom management should include factors like the age, race, diet, and hormonal status of the patient, because all of these factors have been shown to affect the composition of the GI microbiome [1-3]. Therefore, these

factors may also impact the efficacy of probiotics. There does not appear to be any research directly investigating the impact of these factors on the efficacy of probiotics, but these variables may be another aspect to consider when recommending probiotic treatment for gynecologic cancer patients.

Future directions

As demonstrated in this review, there are large gaps in knowledge regarding the microbiome and gynecologic cancers. Continued investigation of the core and variable microbiome in the context of gynecologic cancer is warranted (**Figures 1 and 2**). In addition, further investigations into the host-microbiota and metabolome is needed to improve treatment and develop new interventions for women's health.

Animal models will be critical in studying the role of the microbiome in cancer development and progression. As discussed previously in this review, mouse models have been used to assess different aspects of the relationship between dysbiosis, cancer, and GI/vaginal symptoms. These models may also be useful for evaluating the possible association between probiotics and sepsis in immunocompromised patients.

Human cell culture models and human clinical trials will also be of critical importance for future research. Human cell culture models are being utilized to investigate host-microbiome interactions [85] and gain a better understanding of the relationship between epithelial barrier dysfunction, bacterial translocation, and carcinogenesis [8]. Furthermore, it is of critical importance that we identify how these bacteria are interacting with host cells through evaluation of host cell-microbe interactions. Host factors that may impact the microbiome composition, including ethnic and/or socioeconomic diversity, will also be important for addressing health disparities related to cancer. Most clinical trials to date have focused on the efficacy of probiotics for reducing GI symptoms in gynecologic cancer patients [63, 64, 66, 78, 80, 86, 87], but there is little research on vaginal symptoms or the microbiota. Additional clinical trials will be useful for determining the efficacy of these agents for reducing GI and vaginal symptoms and whether they should be recommended for symptom management in gynecologic cancer patients.

Currently, our group is in the final stages of a project whose aim is to determine cervicovaginal immune signatures and microbiome composition in patients at various stages of cervical cancer. We hypothesized that perturbations in the vaginal microbiome may contribute to mucosal inflammation in the female reproductive tract, which in turn could affect the progression of precancerous cervical lesions to invasive disease. We collected cervicovaginal

lavages (CVL) and swabs from premenopausal, non-pregnant women with histologically confirmed low (LGD) and high grade cervical dysplasia (HGD), ICC, or healthy controls. Age, ethnicity and BMI were not significantly different among analyzed groups. Using ELISA and cytometric bead arrays, over 80 CVLs were quantified for 34 secreted immune mediators with a variety of immune functions, including proinflammatory, hematopoietic, adaptive immunity, immunoregulation, antimicrobial peptides (AMP), protease inhibitors, iron-limiting proteins and cancer biomarkers. DNA extracted from vaginal swabs was analyzed by 16S sequencing using the Illumina MiSeq platform. Microbiome analysis is ongoing, but analysis of preliminary data demonstrates significantly increased levels of proinflammatory mediators and cancer biomarkers, as well as decreased levels of AMPs, across groups as dysplasia progressed to ICC. Furthermore, we observed a significant correlation between levels of particular proinflammatory immune mediators and several demographic variables (vaginal pH, ethnicity, and BMI). Initial microbiome analysis also revealed that vaginal pH, ethnicity and age were significantly correlated with beta-diversity differences in VMB. Additional microbiome analysis may allow us to further correlate VMB dysbiosis with progressive mucosal inflammation and cervical dysplasia.

Conclusions

Foundational studies on the human microbiome are increasing, largely due to technological advances with human in vitro models, sequencing technology and large scale international research consortia, such as the Human Microbiome Project. New knowledge in this field has the potential to revolutionize our approach to health and disease. Much of the current research has focused on the GI microbiome and how it impacts the health of the host in general. For example, the relationship between the GI microbiome and colorectal cancer is well documented. However, there is an important gap in the studies investigating the association between the gut and vaginal microbiomes and gynecologic cancers. Studies to date are encouraging, but there must be more research conducted on the topic of the human microbiome and gynecologic cancer. Dysbiosis or community structure may directly impact development, progression, and persistence of cancer. Additional research in this field is also necessary to help determine whether the relationship between microbiota perturbations and gynecologic cancers is correlational or causal, as research in this area is still in its infancy. Employing clinical, translational, and basic science strategies and partnerships are required to better understand the role of the human microbiome in gynecologic cancer and develop new preventatives or treatment strategies. Enhanced understanding of the microbiota composition and host-microbiota interactions will ultimately aid in improving therapeutics, development of new agents and/or dosing regimens and possibly limit toxicities associated with treatment.

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