The microbiome and ovarian cancer: insights, implications, and therapeutic opportunities

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Abstract

Ovarian cancer is the leading cause of gynecologic cancer death in the United States. Most ovarian cancer patients are diagnosed with advanced-stage disease, which poses a challenge for early detection and effective treatment. At present, cytoreductive surgery and platinum-based chemotherapy are foundational for patients with newly diagnosed ovarian cancer, but unfortunately, most patients will recur and die of their disease. Therefore, there is a significant need to seek innovative, novel approaches for early detection and to overcome chemoresistance for ovarian cancer patients. The microbiome, comprising diverse microbial communities inhabiting various body sites, is vital in maintaining human health. Changes to the diversity and composition of the microbial communities impact the microbiota-host relationship and are linked to diseases, including cancer. The microbiome contributes to carcinogenesis through various mechanisms, including altered host immune response, modulation of DNA repair, upregulation of pro-inflammatory pathways, altered gene expression, and dysregulated estrogen metabolism. Translational and clinical studies have demonstrated that specific microbes contribute to ovarian cancer development and impact chemotherapy’s efficacy. The microbiome is malleable and can be altered through different approaches, including diet, exercise, medications, and fecal microbiota transplantation. This review...
provides an overview of the current literature regarding ovarian cancer and the microbiome of female reproductive and gastrointestinal tracts, focusing on mechanisms of carcinogenesis and options for modulating the microbiota for cancer prevention and treatment. Advancing our understanding of the complex relationship between the microbiome and ovarian cancer may provide a novel approach for prevention and therapeutic modulation in the future.

**Keywords:** Ovarian cancer, gynecological cancer, microbiota, microbiome, platinum-based chemotherapy, dysbiosis

**INTRODUCTION**

Ovarian cancer (OC) is the most lethal gynecologic cancer in the United States\(^1\). In 2022, approximately 20,000 new cases were diagnosed, with 13,000 estimated deaths\(^2\). The five-year survival in patients with advanced OC is estimated at 30% and has been relatively unchanged over recent years\(^3,4\). The poor prognosis associated with OC is because 70% of patients are diagnosed with advanced-stage disease because of limited effective screening options and often indolent or non-specific symptoms at diagnosis\(^1,5\). Moreover, chemotherapy resistance is one of the most significant factors contributing to the poor prognosis and increased mortality rate of patients diagnosed with ovarian cancer\(^6\).

Notably, in recent years, the remarkable heterogeneity of OC has been increasingly realized, with significant variations in histology, genomic drivers, and molecular classification, which impact carcinogenesis, treatment selection, and disease outcomes. The vast majority of OC are of epithelial origin, and can be subdivided into several different histological subtypes, accounting for either low (30%) or high-grade (70%) tumors\(^7,8\). Low-grade serous OC tends to be relatively chemoresistant and harbor mutations in Kirsten rat sarcoma virus (KRAS), Phosphatase and TENsin homolog deleted on chromosome 10 (PTEN), and PIK3CA oncogenes\(^7\). In contrast, 90% of high-grade OC are serous carcinomas with mutations in tumor protein 53 (TP53), Breast Cancer gene 1 (BRCA1), and Breast Cancer gene 2 (BRCA2) oncogenes\(^7\). Notably, approximately 20% of OCs are hereditary with germline mutations, including BRCA1 and BRCA2\(^7\).

The microbiome has been increasingly studied for its role in maintaining human health and disease. The microbiomes of the gut and the female reproductive tract have been linked to many diseases, including OC\(^8\). In recent years, pre-clinical and translational studies have demonstrated that specific microbes may contribute to carcinogenesis, toxicity, and efficacy of cancer therapies in patients with OC\(^8\). The microbiota is dynamic and may be altered through various mechanisms, including diet, exercise, medications, and fecal microbiota transplantation. This review provides an overview of the current literature detailing the relationship between OC and the microbiome, focusing on mechanisms of carcinogenesis and strategies for modulating the microbiota to improve treatment efficacy and toxicity.

**REVIEW OF CURRENT TREATMENT PARADIGM FOR ADVANCED AND RECURRENT OVARIAN CANCER**

While historically, most patients with OC were treated similarly in the front-line setting, the landscape of therapeutics has changed dramatically in recent years\(^10\). The front-line treatment paradigm for advanced OC currently includes a multimodal approach of cytoreductive surgery and chemotherapy with carboplatin and paclitaxel. Further, in patients with advanced OC, bevacizumab concurrently with platinum-doublet chemotherapy, followed by bevacizumab maintenance, is approved based on the results of GOG-0218 and ICON-7\(^11\). Similarly, maintenance with poly (ADP-ribose) polymerase (PARP) inhibitors is approved as
monotherapy or in combination with bevacizumab, following results of the SOLO-1, PRIMA, VELIA, and PAOLA-1 trials\(^1\,\text{[}11\text{]}\,\text{[}15\text{]}\). Notably, OC tumors with homologous recombination deficiency (HRD) exhibit greater sensitivity to PARP inhibitors and have been shown to have the most significant benefit\(^3\,\text{[}10\text{]}\).

Despite treatment for advanced ovarian cancer with cytoreductive surgery and platinum-based chemotherapy, most patients will recur and die of their disease\(^1\,\text{[}16\text{]}\,\text{[}17\text{]}\). The main cause of chemoresistance in ovarian cancer is the increased antioxidant capacity of cancer cells against platinum-derived compounds\(^6\). Both chemotherapy drugs and cell metabolism produce significant levels of reactive oxygen species (ROS) in tumor cells, resulting in DNA damage and various cellular responses. Consequently, the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is activated to combat oxidative stress, which is associated with ovarian cancer development and resistance to platinum-based treatments\(^19\). Patients with recurrent, platinum-resistant ovarian cancer have limited therapeutic options, decreased chemotherapy response rates, and poor prognosis, with overall survival (OS) of 9–12 months\(^19\). There is a significant unmet need for innovative therapeutic strategies to improve the survival rate of patients with advanced OC.

MECHANISTIC INSIGHTS INTO THE ROLE OF THE MICROBIOME IN THE PATHOGENESIS OF OVARIAN CANCER

The microbiome as a mediator of human health and disease has been increasingly studied. The human microbiome is a collection of microorganisms, including bacteria, viruses, fungi, archaea, and protozoa, that live within and upon the body’s mucosal surfaces\(^20\,\text{[}21\text{]}\). Further, microbial-derived metabolites can impact immune function, cell proliferation and signaling, gene expression, and hormonal and nutrient metabolism. Each microbiota has unique properties, including the composition and diversity of microbes. The gut and the female reproductive tract microbiomes are especially relevant to gynecologic cancers, including OC [Figure 1]. The female reproductive tract has two distinct microbial environments. The lower female reproductive tract microbiota includes the vagina and cervix and is generally colonized by *Lactobacillus* species\(^9\). In contrast, the microbiota of the upper female reproductive tract, including the uterus, fallopian tubes, and ovaries, was historically considered sterile but is now understood to harbor a diverse array of anaerobes\(^9\).

The microbiome can significantly contribute to cancer development through several mechanisms, such as chronic inflammation infections, integration into the human genome, and the production of genotoxic metabolites. Some examples, like *Helicobacter pylori*-mediated inflammation, are linked with mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer\(^22\). Similarly, hepatocellular carcinoma was found to be more frequent in patients infected with Hepatitis B and C viruses\(^23\) and *Fusobacterium nucleatum* with colorectal carcinoma\(^24\,\text{[}25\text{]}\). OC is a complex and multifactorial disease with genetic, epigenetic, immunologic, and environmental risk factors. Given that significant knowledge gaps exist in OC pathogenesis, the microbiome has been increasingly studied as an environmental risk factor for several gynecologic cancers, including OC. While the pathogenesis of OC is not yet fully understood, growing evidence suggests that the microbiota may play a role in the development and progression of OC\(^25\).

Changes in the composition of the gut microbiome and the intestinal mucosal barrier can lead to cancer development through various mechanisms, including chronic inflammation, altered immune response and barrier function, dysregulated nutrient and hormone metabolism, and upregulation of oncogenic signaling. Additionally, certain microbes can induce DNA damage and apoptosis by releasing genotoxic metabolites or indirectly by producing reactive oxygen species [Figure 2].
Observational studies have demonstrated a link between history of prior infections and OC, especially pelvic and sexually transmitted diseases. In a study led by Lin et al., pelvic inflammatory disease was associated with an increased risk of OC\textsuperscript{[26]}. Specifically, studies have identified that a history of infection with specific pathogens, including \textit{Chlamydia trachomatis} and \textit{Mycoplasma genitalium}, is associated with OC development\textsuperscript{[27-30]}. Prior infections may induce the activation of oncogenic pathways and increased production of inflammatory cytokines, such as disruption of the cell cycle via heightened mitogen-activated protein kinase (MAPK) signaling, degradation of p53, and the modulation of DNA damage repair proteins\textsuperscript{[31]}. Specifically, studies have demonstrated that \textit{Chlamydia trachomatis} contributes to carcinogenesis through multiple mechanisms, including blocking mitochondrial caspase-3 mediated cytochrome C release, inhibiting apoptosis and immune response, inducing DNA damage due to reactive oxygen species (ROS), and altering cellular tight junctions\textsuperscript{[31]}. The persistence of human papillomavirus (HPV) infection is a well-established determinant for developing the majority of invasive cervical carcinomas via E6 and E7 viral oncogenes, which inactivate tumor suppressor genes p53 and Rb, respectively\textsuperscript{[31]}. However, recent evidence suggests that HPV infection might also influence the pathogenesis of OC, potentially via a similar mechanism\textsuperscript{[35]}.

Previous studies have suggested complex interactions between the microbiota, cancer cells, and the tumor microenvironment, including upregulation of Toll-like (TLR) and nucleotide oligomerization domain (Nod)-like receptors (NLRs) signaling pathways\textsuperscript{[32-36]}. Several carcinogenic signaling pathways may be activated in OC, including p53, Human epidermal growth factor receptor 2 (HER2), Epidermal growth factor receptor (EGFR), and Vascular endothelial growth factor receptor (VEGFR)\textsuperscript{[37]}. Upregulation of pro-inflammatory pathways leads to increased IL-6, activation of the Janus kinase/signal transducers, and
activators of the transcription 3 (JAK/STAT3) pathway, resulting in tumor proliferation. IL-6 induces proliferation through increased production of matrix metalloproteinase (MMPs) and loss of E-cadherin expression, which promotes cell proliferation, increased autocrine and paracrine cytokine production, promotion of epithelial-mesenchymal transition and metastasis, and potentially development of chemoresistance. Lactobacillus species may serve as tumor suppressors through the downregulation of Wnt/β-catenin signaling and may represent a therapeutic strategy in years to come.

Changes to the intestinal mucosal barrier expose the systemic circulation to bacteria and their associated by-products, leading to the production of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), triggering a cascade of inflammatory processes contributing to cancer tumorigenesis. Specific gut microbes, such as Lactobacillus, Bifidobacterium, and Akkermansia, have been shown to stimulate healthy gut mucus production. Factors promoting a healthy mucosal barrier layer, including short-chain fatty acids and a high-fiber diet, may act as tumor suppressors.

TLRs are important mediators of inflammatory pathways in the gut, which have a significant role in mediating immune responses and linking adaptive and innate immune responses. TLR-4 and TLR-5/7 have been proposed as mediators in OC carcinogenesis via PI3K activation. In a human-derived OC cell line (Human Epithelial Ovarian cancer cell line: CP70) study, bacterial lipopolysaccharide (LPS) binding to TLR-4 triggered multiple carcinogenesis events. Similarly, TLR-4 activation in metastatic ovarian human tissue-derived cells (SKOV3 cells) resulted in NF-κB activation, p65 DNA binding, and the generation of pro-tumoral molecules (IL-6, IL-8, VEGF, and MCP-1), promoting cancer development and chemoresistance. TLR-4 inhibition decreased MMP-2 and MMP-9 gene expression and enzymatic...
activity, preventing the epithelial-mesenchymal transition metastasis and providing antineoplastic effects\(^{[46]}\). TLR-5 recognition of commensal microbiota is linked to metastatic OC and systemic tumor-promoting inflammation in pre-clinical mouse models with TLR-5 deficiency treated with antibiotics to induce microbial dysbiosis\(^{[33]}\). TLR-5 activation leads to the production of immune-suppressive protein galectin-1, upregulation of IL-6, promotion of myeloid-derived suppressor cells (MDSCs) mobilization, and accelerated tumor growth. The importance of IL-6 production via TLR-5 activation in the first phases of ovarian cancer tumorigenesis is highlighted by the fact that TLR-5-deficient mice had much slower tumor growth. Notably, antibiotic-induced dysbiosis revokes TLR-5-dependent variations in tumor development.

The gut microbiome influences estrogen metabolism and systemically available estrogen levels, which may play a role in gynecologic cancer development [Figure 2]. Estradiol undergoes first-pass metabolism in the liver and is excreted through the bile, urine, or feces\(^{[47]}\). The “estrobolome” refers to microorganisms that can deconjugate estrogens excreted within the stool via beta-glucuronidase and beta-glucosidase enzymes, leading to intestinal reabsorption of estrogens and increased systemic estrogen levels\(^{[48]}\). In a study by Peters, shotgun metagenomic sequencing was performed on stool samples of 2,300 patients, including 295 pre-menopausal women, 1,027 post-menopausal women, and 978 men. They identified significant differences in the microbial diversity and composition between the two groups with post-menopausal women with decreased Akkermansia muciniphila, Clostridium lactatifermentans, and Parabacteroides johnsonii species. Specifically, post-menopausal women had a decreased abundance of microbial β-glucuronidase, which correlated with serum progestin metabolite levels\(^{[49]}\). Therefore, the interactions between estrogen, progesterone, gut microbiome, and OC warrant further investigation\(^{[50]}\).

**MICROBIAL SIGNATURES IN OVARIAN CANCER**

In recent years, the gut and female reproductive tract microbiomes have been associated with OC development and treatment responses. While historically, the upper female reproductive tract was considered sterile, preliminary data also supports that microbe within the peritoneal cavity and OC tumors, referred to as the tumor microbiome, may also contribute to carcinogenesis and chemoresistance\(^{[51],[52]}\). In a study by Banerjee et al., it was demonstrated that microbial colonization of OC tumors was frequent, with representative species including Brucella (76%), Chlamydia (60%), and Mycoplasma (74%)\(^{[53]}\). Additionally, they observed a significant increase in the ratio of Proteobacteria to Firmicutes in OC tumors, distinct from the composition in healthy tissues\(^{[54]}\). Similarly, Miao et al. evaluated the peritoneal microbiota in patients with benign ovarian masses (\(n = 20\)) or OC (\(n = 10\))\(^{[55]}\). They identified that patients with OC had a distinct peritoneal microbial signature, including microorganisms responsible for estrogen metabolism (Rikenellaceae), vascular permeability and metastasis (Alphaproteobacteria), and anti-inflammatory responses (Akkermansia)\(^{[56]}\). Most recently, Asangba et al. evaluated the microbiota in OC patients compared to controls\(^{[57]}\). They systematically sampled the microbiome of the female reproductive tract, ascites or peritoneal fluid, urine, and stool in both benign and OC patients. They determined a distinct OC microbiome with enrichment of several microbes at all body sites except the stool and omentum, including Dialister, Corynebacterium, Prevotella, and Peptostreptococcus in the OC cohort. Notably, these microbes were more prominent in low-grade, early-stage OC patients, indicating a potential avenue for early detection strategies\(^{[58]}\). Similarly, they associated specific microbial populations with histologic subtypes. Patients with non-serous showed increased Lactobacillus iners, Fusobacterium nucleatum, Prevotella buccalis, and Dialister propionicificaciens versus patients with serous OC.

The female reproductive tract microbiome has been associated with the development of OC. Studies have identified that the absence of Lactobacillus in the microbiota of the female reproductive tract may impact OC development and oncologic outcomes. In a case-control study of 176 patients with OC compared to 115
healthy controls, 16S rRNA gene sequencing, decreased Lactobacillus within the female reproductive tract microbiota was strongly associated with increased risk for OC. Further analysis demonstrated that in age-matched women with BRCA1 mutations, those with a non-Lactobacillus dominant microbiota were more likely to have OC [Table 1] [54].

Lastly, preliminary data has linked the gut microbiota composition with the development of OC. In a study by Chen et al., two cohorts of mice were treated with tamoxifen to induce ovarian cancer over the course of one year and were supplemented with either antibiotics for five months or a placebo [55]. Antibiotic treatment led to significant changes to the microbiota of the vagina and the gut in mice, and mice that received antibiotics had significantly fewer and less advanced tumors than control animals at the study endpoint [55]. Jacobson et al. identified an inverse relationship between Lactobacillus species and OC, with only 24% of OC patients having Lactobacillus species present compared to healthy controls [Table 1] [56]. In contrast, studies have suggested that certain gut microbes may have anti-tumor activity in OC. In a study by Wang, supplementation of Akkermansia in pre-clinical mouse models of OC was associated with decreased tumor growth, increased T-cell activation, and enhanced interferon (IFNy) secretion from CD8+ T cells [57]. Further investigation is needed to understand how these microbes could be targeted for early diagnosis, therapeutic modulation, and to overcome chemoresistance [56].

**IMPACT OF THE MICROBIOME ON TREATMENT RESPONSE AND DEVELOPMENT OF CHEMORESISTANCE IN OVARIAN CANCER**

Over the last decade, studies across multiple cancer types suggest that the microbiome may impact the efficacy and toxicity of both systemic chemotherapy and immunotherapy [58-68]. Platinum-based chemotherapy remains the most effective chemotherapy agent for OC patients. It is utilized both in the front-line setting and in patients with recurrent, platinum-sensitive disease in combination with other agents, including paclitaxel, gemcitabine, pegylated liposomal doxorubicin with or without bevacizumab [16]. Platinum-based chemotherapy exerts its antineoplastic effects through the formation of platinum-DNA adducts or crosslinks, which block DNA replication and provoke the production of ROS, resulting in cell death [69,70].

Several studies have assessed the impact of the microbiome on platinum response in pre-clinical cancer models, including OC. A landmark study by Iida et al. administered antibiotics to mouse models of melanoma, leukemia, and colon adenocarcinoma before treatment with oxaliplatin and cisplatin [69]. Antibiotics were associated with reduced treatment response and survival compared to control animals [69]. Mice treated with antibiotics had downregulation of genes related to adaptive immune response, antigen presentation, DNA damage repair, and upregulation of genes related to cancer metabolism. Specific gram-positive microbes, such as Lactobacillus fermentum, were associated with improved anticancer activity [69]. One potential mechanism for this effect is, in prior studies, translocation of gram-positive bacteria during mucositis (characterized by epithelial barrier inflammation and cell loss), which leads to increased production of cytotoxic ROS and the infiltration of Th-17 cells into tumors, resulting in improved anticancer activity [70].

Several studies have demonstrated that in pre-clinical models of cancer, antibiotic treatment to alter the gut microbiome leads to global changes in immune response and activation of pro-inflammatory genes, which supports the gut microbiome’s impact on chemotherapy response through direct or indirect actions on the immune system [69-71]. These data suggest that the gut microbiota may have a role in modulating the immune response for platinum chemotherapy efficacy. Additionally, the gut microbiota might trigger the production of ROS in tumor-infiltrating myeloid cells, leading to increased oxidative stress and heightened cytotoxicity of platinum compounds [69,72,74].
In pre-clinical models of OC utilizing syngeneic ID-8 cells, Chambers et al. demonstrated that broad-spectrum antibiotic treatment (with neomycin, ampicillin, vancomycin, and metronidazole) resulted in increased tumor growth, cisplatin resistance, and decreased survival compared to non-antibiotic treated control mice. Additionally, RNAseq analysis of OC tumors of antibiotic-treated mice demonstrated significant changes in DNA damage repair, cell death, angiogenesis, and cancer stem cell gene pathways compared to controls. Notably, the chemoresistant phenotype observed in the antibiotic-treated mice was overcome through cecal microbiota transplantation of control mice stool. In this study, several gut-derived tryptophan metabolites were significantly decreased compared to healthy controls, including indole-3-propionic acid. These studies provide background to support the production of anticancer metabolites and may represent a target for intervention in women with recurrent, platinum-resistant ovarian cancer in years to come. Subsequently, clinical studies have demonstrated that antibiotics during platinum chemotherapy are associated with worsened oncologic outcomes in several cancers, including OC.

These studies associated anti-gram-positive antibiotics with reduced progression-free survival (PFS) and OS. In a study by Pflug et al., 800 patients with chronic lymphocytic leukemia and lymphoma were undergoing treatment with either cyclophosphamide or cisplatin, respectively, on two clinical trial protocols. Investigators assessed the use of antibiotics targeting gram-positive bacteria during treatment and demonstrated that of the patients with relapsed lymphoma, those who were treated with anti-gram-positive antibiotics (n = 21/122) had a significantly lower response rate (70.3% vs. 42.9%, P = 0.016) and decreased PFS (median 2.3 vs. 11.5 mo, P = 0.001). Additionally, on multivariate analysis controlling for multiple confounders, anti-gram-positive antibiotics were independently associated with reduced PFS (HR 2.237, 95% CI 1.28-3.91, P = 0.004).

**Table 1. Clinical studies overview**

<table>
<thead>
<tr>
<th>Author</th>
<th>Study type</th>
<th>Study population</th>
<th>Study findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asangba et al., 2023</td>
<td>Cohort</td>
<td>18 years of age or older and undergoing hysterectomy for ovarian cancer (n = 34) or a benign gynecologic condition (n = 30)</td>
<td>Microbial taxa were identified as differentially abundant between the benign cohort and early- and advanced-stage OC patients. The presence of mostly detrimental microbes was observed in early-stage, low-grade OC patients, with a decrease in advanced-stage, high-grade OC patients.</td>
</tr>
<tr>
<td>Nené et al., 2019</td>
<td>Case-control</td>
<td>OC patients (n = 176) compared to healthy controls (n = 115)</td>
<td>Non-lactobacilli-dominated cervicovaginal microbiomes were more prevalent in patients with ovarian cancer and in women with BRCA1 mutations who had yet to develop cancer, compared with age-matched healthy women and women without BRCA1 or BRCA2 mutations, respectively.</td>
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<tr>
<td>Jacobson et al., 2021</td>
<td>Retrospective</td>
<td>Platinum-free interval (PFI) &lt; 6 months (n = 17), PFI &gt; 24 months (n = 23), benign (n = 5)</td>
<td>Observed the inverse relationship between a Lactobacillus-dominant vaginal microbiome and ovarian cancer, with only 24% of OC patients having Lactobacillus species present compared to healthy controls (47%).</td>
</tr>
<tr>
<td>Chambers et al., 2021</td>
<td>Retrospective</td>
<td>Patients with recurrent EC, CC, and OC treated with ICIs (n = 101)</td>
<td>Antibiotics given 30 days prior to ICI treatment, but not during, are linked to reduced response and worse progression-free survival (PFS) and overall survival (OS) in women with recurrent endometrial, cervical, and ovarian cancer.</td>
</tr>
<tr>
<td>Pflug et al., 2022</td>
<td>Prospective cohort</td>
<td>n = 800; patients with chronic lymphocytic leukemia and lymphoma were undergoing treatment with either cyclophosphamide or cisplatin</td>
<td>A potential negative impact of anti-Gram-positive antibiotics on the anticancer activity of cyclophosphamide and cisplatin in a clinical setting.</td>
</tr>
<tr>
<td>Chambers et al., 2020</td>
<td>Retrospective</td>
<td>Patients diagnosed with epithelial ovarian cancer stage III/IV (n = 424) underwent cytoreductive surgery and Platinum chemotherapy</td>
<td>Antibiotic treatment during platinum chemotherapy leads to a significant reduction in both progression-free survival (PFS) and overall survival (OS). Additionally, the use of antibiotics targeted against gram-positive bacteria is strongly associated with worse oncologic outcomes.</td>
</tr>
<tr>
<td>Wang et al., 2022</td>
<td>Community based observational</td>
<td>n = 1,417; 10% were considered sarcopenic based on physical performance and biometric testing</td>
<td>Significant differences in the microbial populations and microbial-derived metabolites were observed between sarcopenic and non-sarcopenic patients.</td>
</tr>
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</table>

In pre-clinical models of OC utilizing syngeneic ID-8 cells, Chambers et al. demonstrated that broad-spectrum antibiotic treatment (with neomycin, ampicillin, vancomycin, and metronidazole) resulted in increased tumor growth, cisplatin resistance, and decreased survival compared to non-antibiotic treated control mice. Additionally, RNAseq analysis of OC tumors of antibiotic-treated mice demonstrated significant changes in DNA damage repair, cell death, angiogenesis, and cancer stem cell gene pathways compared to controls. Notably, the chemoresistant phenotype observed in the antibiotic-treated mice was overcome through cecal microbiota transplantation of control mice stool. In this study, several gut-derived tryptophan metabolites were significantly decreased compared to healthy controls, including indole-3-propionic acid. These studies provide background to support the production of anticancer metabolites and may represent a target for intervention in women with recurrent, platinum-resistant ovarian cancer in years to come. Subsequently, clinical studies have demonstrated that antibiotics during platinum chemotherapy are associated with worsened oncologic outcomes in several cancers, including OC. These studies associated anti-gram-positive antibiotics with reduced progression-free survival (PFS) and OS. In a study by Pflug et al., 800 patients with chronic lymphocytic leukemia and lymphoma were undergoing treatment with either cyclophosphamide or cisplatin, respectively, on two clinical trial protocols. Investigators assessed the use of antibiotics targeting gram-positive bacteria during treatment and demonstrated that of the patients with relapsed lymphoma, those who were treated with anti-gram-positive antibiotics (n = 21/122) had a significantly lower response rate (70.3% vs. 42.9%, P = 0.016) and decreased PFS (median 2.3 vs. 11.5 mo, P = 0.001). Additionally, on multivariate analysis controlling for multiple confounders, anti-gram-positive antibiotics were independently associated with reduced PFS (HR 2.237, 95% CI 1.28-3.91, P = 0.004).
During 16 FMT with stool from patients whose tumors responded well to treatment. FMT overcame the Landmark phase I clinical trials by two groups gave patients with PD-1 refractory metastatic melanoma an exercise, fecal microbiota transplantation (FMT), and probiotic supplementation to improve response to cancer treatment in OC patients. Potential strategies include dietary modifications, exercise, fecal microbiota transplantation (FMT), and probiotic supplementation. The next frontier in microbiome science is to develop therapeutic approaches to modulate the microbiome for the treatment of OC, antibiotic treatment prior to immunotherapy was associated with a significantly lower response rate, and postoperative complications, including ileus, infection, wound separation, and anastomotic leaks. Still, additional data are necessary to understand the potential morbidity associated with FMT. In a retrospective cohort study of 101 women with recurrent gynecologic cancers, including 26 patients with OC, antibiotic treatment prior to immunotherapy was associated with a significantly lower response rate, PFS, and OS. Notably, in patients with melanomas and various solid malignancies, the gut microbiome has been shown to modulate response to immunotherapy. In a retrospective cohort study of 101 women with recurrent gynecologic cancers, including 26 patients with OC, antibiotic treatment prior to immunotherapy was associated with a significantly lower response rate, PFS, and OS. Further research is needed to understand how the microbiome mediates immunotherapy response in women with gynecologic cancers and whether modulation of the microbiota may be a strategy to improve immunotherapy response in these patients.

**IMPACT OF THE MICROBIOME UPON SURGICAL MANAGEMENT AND POSTOPERATIVE OUTCOMES IN OVARIAN CANCER**

Cytoreductive surgery, which normally includes the removal of the uterus, cervix, fallopian tubes, ovaries, and omentum, is an essential component of the care of OC patients. Optimal cytoreduction, to remove all visible disease to less than one centimeter and ideally no gross residual disease, is consistently associated with improved PFS and OS. Due to the often widespread nature of OC involving the peritoneal surfaces, upper abdominal surgical procedures, including diaphragm resection or splenectomy, as well as small bowel resection and large bowel resection, are often required to achieve optimal cytoreduction. Given the extensive nature of these surgical procedures, it is not uncommon for patients to experience postoperative complications, including ileus, infection, wound separation, and anastomotic leaks. Data on how the gut and female reproductive tract microbiome may influence a woman’s risk for postoperative complications are limited. In a study by Tong et al., significant differences were noted between stool samples taken pre-operatively, postoperatively, and during chemotherapy. Further, they appreciated that OC patients significantly increased in anaerobic species such as Bacteroides, Collinsella, and Blautia during chemotherapy. Studies in patients with colorectal cancer undergoing cytoreductive surgery with bowel resection have demonstrated that probiotic administration before surgery may be protective against surgical site infection and anastomotic leaks. Still, additional data are necessary to understand the potential mechanism and whether this will similarly extend to patients with OC.

**MODULATION OF THE MICROBIOME IN OVARIAN CANCER AND FUTURE DIRECTIONS**

The next frontier in microbiome science is to develop therapeutic approaches to modulate the microbiome to improve response to cancer treatment in OC patients. Potential strategies include dietary modifications, exercise, fecal microbiota transplantation (FMT), and probiotic supplementation. Studies in non-gynecologic cancers have demonstrated promising early responses to FMT in immunotherapy patients. Landmark phase I clinical trials by two groups gave patients with PD-1 refractory metastatic melanoma an FMT with stool from patients whose tumors responded well to treatment. FMT overcame the
immunotherapy resistance in certain patients, with clinical responses restored in 30%, with one complete response. Therefore, it is plausible that these approaches may yield similar benefits in women with OC, and further study is necessary\cite{89,91}.

In OC patients, many of whom are elderly, fatigued, malnourished, or have diminished appetite related to the stigma of advanced disease, probiotic supplementation holds potential as a practical therapeutic intervention. Studies evaluating probiotics in non-gynecologic cancers have demonstrated promising results, including decreased infectious outcomes and diminished radiation and chemotherapy-induced gastrointestinal toxicities\cite{92-96}. Further, in vitro and early-phase clinical trials of probiotics as an oral supplement or intra-tumoral injection have demonstrated anticancer activity\cite{92-97}. Additional studies are needed to investigate the feasibility of probiotic supplementation during chemotherapy in OC patients, evaluate the impact on the gut microbiota, patient quality of life, and symptoms, and assess the association with oncologic outcomes.

Currently, data regarding microbiome-directed dietary interventions to impact outcomes in OC patients are limited\cite{98-100}. Diet is a key mediator of the composition and function of the microbiota. Microbial-derived metabolites, such as short-chain fatty acids (SCFA), are degradation products of fiber and can influence host immunity, either directly via changes to metabolic signaling pathways or indirectly through the regulation of gut mucosal permeability. Western-style diets, which are defined by high levels of saturated fats and low dietary fiber, have been linked to reduced SCFA levels and altered host immune response\cite{101}. In a study of 128 patients with melanoma, those with high dietary fiber intake (defined as > 20 grams/day) had improved PFS compared to the low fiber cohort, which was most notable in those who did not use concurrent probiotics\cite{86}. These studies provide evidence that diet modulation may be a promising strategy to improve cancer therapy outcomes.

Gynecologic cancer patients, especially the older population with advanced-stage disease, are more susceptible to malnutrition, sarcopenia, and cachexia, impacting approximately 70% of patients\cite{102}. Malnutrition and skeletal muscle depletion have many adverse consequences, including lower quality of life, increased risk of prolonged hospitalization, heightened treatment-related toxicity, re-operations, readmission, and postoperative complications\cite{103-105}. Several retrospective studies have indicated a strong link between malnutrition and decreased OS in OC patients compared to non-malnourished patients\cite{103-106}. Importantly, the microbiome may influence the pathogenesis of cancer cachexia through changes in the gut microbial composition and mucosal barrier function. In a study of 1,417 participants, 10% were considered sarcopenic based on physical performance and biometric testing. Significant differences in the microbial populations and microbial-derived metabolites were observed between sarcopenic and non-sarcopenic patients [Table 1]\cite{107}. An analysis of blood samples of patients with cancer cachexia showed increased bacterial translocation, resulting in heightened inflammation and elevated concentrations of inflammatory markers such as IL-6, TNF-α, and IFN-γ\cite{108}. Approaches focused on restoring microbial balance and enhancing gut barrier function through long-term or acute dietary changes or nutrition interventions may hold promise for addressing malnutrition and sarcopenia in cancer patients.

Prospective clinical trials are urgently needed to assess potential strategies to target the microbiome. In July 2023, we performed a systematic search strategy on the ClinicalTrials.gov website utilizing a librarian-designed search strategy [Supplementary File 1]. We compared the number of active clinical trials for microbiome-mediated interventions (prebiotics, probiotics, live biotherapeutics) for the leading cancer diagnoses in the United States according to the Surveillance, Epidemiology, and End Results (SEER)
program\cite{109}. On a review of available clinical trials on ClinicalTrials.gov in July 2023, we identified that OC and uterine cancer are remarkably underrepresented compared to other disease sites such as colorectal cancer and melanoma, and this highlights a major unmet in future research efforts [Figure 3].

CONCLUSIONS

The microbiome of the gut and female reproductive tracts serves essential functions in maintaining health. The microbiome may also impact the toxicity and efficacy of cancer therapies for OC patients. Furthermore, the microbiota is highly dynamic and may be modulated through various approaches, although significant research gaps exist for gynecologic cancer patients. The connection between the microbiome and ovarian cancer is still being investigated, but the key question of whether they are directly linked remains unanswered. Since ovarian cancer tends to occur later in life, it is challenging to conduct long-term studies that establish causality. This raises concerns about whether the vaginal microbiome contributes to its development and whether it can be leveraged for primary prevention. Moreover, establishing a causal link between the microbiome and ovarian cancer is a challenging task due to the complexity of the disease, the difficulty in conducting large longitudinal studies, and the multifaceted nature of the microbiome. Although research is still underway, there is optimism that we are making progress in the right direction. Targeting the microbiome may lead to innovative ways to treat ovarian cancer, improve outcomes, and reduce toxicity. This emerging field holds promise for developing personalized treatment approaches. Further research may lead to innovative therapies that can transform the prognosis for those affected by recurrent and chemoresistant OC. Advancing our understanding of the complex relationship between the microbiota and OC represents a novel strategy to improve future patient outcomes.

DECLARATIONS

Author’s contributions

Participated in conceptualization, methodology, investigation, data curation, writing the original draft, reviewing and editing, and visualization: Mehra Y

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Participated in project reviewing, editing of the final manuscript, and project supervision: O’Malley D
Participated in conceptualization, methodology, formal analysis, data curation and reviewing, editing, and project supervision: Spakowicz D
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**Conflicts of interest**
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REFERENCES


31. Shanmughapriya S, Senthilkumar G, Vinodhini K, Das BC, Vasanthi N, Natarajaseenivasan K. Viral and bacterial aetiologies of


48. Peters BA, Lin J, Qi Q, et al. Menopause is associated with an altered gut microbiome and estrobolome, with implications for adverse cardiacmetabolic risk in the hispanic community health study/study of latinos. *mSystems* 2022;7:e002732. DOI PubMed PMC


