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Gut Microbiome and Breast Cancer in the Era of Cancer Immunotherapy

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Abstract

Purpose of Review Immune checkpoint inhibitors (ICIs) are an emerging therapy in breast cancer, but not all patients will have benefit with these medications. It has been proposed that certain gut microbes may play a role in protecting the host against inappropriate inflammation and modulating the immune response. Here, we review the current evidence on the association of the gut microbiome, antitumor immunity, and response to immunotherapy and discuss open questions, ongoing trials, and future directions for modulating the gut microbiome as part of breast cancer treatment.

Recent Findings Several groups have showed that the composition of gut microbiota modulates responses to ICI in preclinical cancer models, and the composition of gut microbiota can predict which patients with solid tumors are more likely to respond to ICI. In addition, it was also showed that fecal microbiota transplant was able to make non-responder animals into responders when they received feces from patients who had benefited to ICI.

Summary Recent studies suggest that ICIs can be active in breast cancer but identifying the patients who are most likely to benefit remains a challenge. In other tumor types, the gut microbiome differs between responders and non-responders, suggesting that it can be used as a predictive biomarker of response. In addition, future investigations will determine whether manipulating the gut microbiota can improve responses to ICIs in breast cancer.

Keywords Breast cancer · Fecal microbiota transplantation · Immune checkpoint inhibitors · Immunotherapy · Microbiome · Gut microbiota

Introduction

Accumulating preclinical and clinical evidence suggests that the immune system is critical for the outcome of patients with breast cancer treated with standard chemotherapy [1]. Both immune-related gene signatures and increased tumor infiltrating lymphocyte (TIL) concentration predicted response to neoadjuvant chemotherapy in all subtypes of breast cancer [2]. Moreover, both in triple-negative breast cancer (TNBC) and in HER2-positive breast cancer, TILs are associated with a survival benefit [2]. Therefore, modulating the immune response is thought to be a good candidate for the development of more effective therapies in breast cancer.

Initial clinical trials assessing the efficacy of PD-1/PD-L1 inhibitors given as monotherapy showed that only a small fraction of patients with breast cancer derive benefit from immunotherapy in the metastatic setting. The objective response rates range from 5 to 23% [3–6] in TNBC and from 3 to 12% [6, 7] in ER+/HER2 tumors, depending on the number of prior lines of CT in the metastatic setting and on the expression of PD-L1 in the tumor microenvironment. Such results suggest that additional immunosuppressive mechanisms contribute to primary resistance to immunotherapy and that different drugs must be combined with PD-1/PD-L1 inhibitors to increase the likelihood of success with immunotherapy in breast cancer. Importantly, approval of the anti-PD-L1 agent atezolizumab in combination with nab-paclitaxel for patients with metastatic TNBC PD-L1-positive tumors opens an era of immunotherapy in the treatment of breast cancer [8•]. However, only a minority of patients will derive benefit from this combination, and additional studies must be taken to better understand which immunosuppressive mechanisms must be turned off to increase the efficacy of immunotherapy in breast cancer.

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Humans are colonized by trillions of microbes comprising the human microbiota. The vast majority of the human microbiota is composed of commensal bacteria living in the gastrointestinal tract, although archaea, viruses, and eukaryotes (such as yeast and protozoans) are also represented within the gut and at other body sites, including the mouth and skin [9]. The totality of the genome of the entire microbiota is referred to as the microbiome. Notably, the human microbiome has approximately 150-fold more genes than the human genome [10]. Several factors influence the composition of the human microbiota, including age, race, diet, birth method, maternal colonization, and hygiene, as well as host genetics and environmental exposures to xenobiotics, antibiotics, and other drugs.

In particular, the gut microbiota has been recognized as an important player in the metabolism of essential nutrients and hormones, as well as a master modulator of immune system development [10–12]. Events that cause a perturbation of the normal microbiota, referred to as dysbiosis, will change the interactions between the gut microbiota, intestinal epithelium, and host immune system and are associated with many diseases, including cancer [13].

Recently, preclinical and clinical studies provided strong evidence for the role of gut microbiota in modulating response and resistance to immune checkpoint inhibitors (ICIs) in several types of cancers, raising the possibility that the stool microbiota could be used as a predictive biomarker to benefit immunotherapy [14•, 15•, 16••, 17••, 18••]. In patients with breast cancer, there is a lack of knowledge about the landscape of the gut microbiome and how this can affect response to systemic therapy [19]. In this review, we will summarize the available data on this topic with an emphasis on the implications of the microbiome in the success of cancer immunotherapy and discuss future perspectives in the field of breast cancer immunotherapy.

Gut Microbiota and Breast Cancer Carcinogenesis and Progression

Importance of Gut Microbiota in Estrogen Metabolism

Several microbes that live in the gut have genes whose products metabolize estrogen and its metabolites. These genes have been collectively called the "estrobolome" [19]. In this context, bacterial species with β-glucuronidase activity in the gut have a crucial role in estrogen metabolism. In the liver, estrogens are conjugated and excreted into the gastrointestinal lumen together with the bile; via the action of β-glucuronidase activity by some bacterial species in the gut, estrogen is deconjugated and available for reabsorption through the enterohepatic circulation. Therefore, perturbations of the gut microbiota can result in dysregulation of the so-called estrobolome, leading to increased levels of estrogen in the peripheral circulation and theoretically being associated with an increased risk of hormone-dependent breast cancer [19].

However, only a few clinical studies have evaluated the association between breast cancer and gut microbiota [20]. In the largest study, Goedert et al. found that postmenopausal breast cancer patients presented with a lower alpha-diversity (the richness of microbiota (i.e., the number of organisms and the evenness of distribution of those organisms)) and a higher beta-diversity (defined as the extension of absolute or relative overlap in shared taxa between samples) than patients without cancer [21, 22]. In addition, patients with breast cancer had an abundance of *Clostridiaceae*, *Faecalibacterium*, and *Ruminococcaceae* and lower levels of *Dorea* and *Lachnospiraceae* than did patients without cancer. Importantly, after adjusting, these results were estrogen independent.

Given the importance of the gut "estrobolome," it would be of great interest to evaluate whether the gut microbiota affects the success of both adjuvant and palliative endocrine therapy, as well as its impacts on endocrine therapy-induced toxicity. However, to date, there are no studies addressing these issues.

Gut Dysbiosis-Induced Tumor Microenvironment Inflammation

Recently, Buchta Rosean et al. demonstrated in a mouse model of hormone-receptor-positive breast cancer that dysbiosis enhances fibrosis and collagen deposition both systemically and locally within the tissue and tumor microenvironment and promotes cancer cell dissemination to metastatic sites [23]. The authors used two different regimens of antibiotics by oral gavage, a broad spectrum of antibiotics that were absorbable and another regimen that was non-absorbable. In addition, the authors also showed that gut dysbiosis promoted the early recruitment of inflammatory myeloid cells into the mammary tissue microenvironment during tumor progression. Notably, the same effects were recapitulated by fecal microbiota transplantation (FMT) of dysbiotic cecal contents. The authors did not find significant differences in estrous cycling in animals with dysbiosis. It can be hypothesized that external factors modulating the gut microbiota could have an impact on the outcomes of patients with breast cancer.

Gut Microbiota and Immune Checkpoint Inhibitors

Association with Response

In 2015, two different groups showed that the composition of the gut microbiota affected responses to ICIs in preclinical models [14•, 15•]. In addition, several groups in different

geographic regions have published studies showing a difference in the gut microbiota composition found in responders versus non-responders among patients with advanced melanoma, renal cell carcinoma, and non-small cell lung cancer treated with ICIs (Table 1 [16••, 17••, 18••, 24, 25••];). Altogether, these observations have led to the hypothesis that gut microbiota signatures could be used as predictive biomarkers of response to ICIs. Importantly, although there was not a higher concordance in the type of microbes associated with ICI benefit across these studies, it is known that there is functional redundancy between bacterial species [26]. The most common taxa associated with favorable outcomes are *Clostridiales*, *Ruminococcaceae*, *Faecalibacterium* spp., *Akkermansia muciniphila*, *Bacteroides fragilis*, *Bifidobacteria*, *Enterococci*, *Collinsella*, and *Alistipes.*

Perhaps more important is the fact that some of these preclinical studies also showed that FMT was able to make non-responders into responders when they received feces from patients who had benefited from ICIs [16•, 17••, 18••]. In addition, these studies also showed that treatment of mice with specific bacterial taxa could modulate their response to ICIs.

The mechanisms by which the gut microbiota modulates the response to ICIs are not fully known. Different mechanisms have been proposed, including the interaction of microbial-derived peptides with antigen-presenting cells, which can trigger an antitumor immune response; the role of local or distant effects of microbial metabolites is also under investigation [27•].

Association with Colitis

Two different groups have found that a baseline gut microbiota enriched in bacteria belonging to the *Bacteroidetes* phylum is inversely correlated with colitis in patients with metastatic melanoma who started therapy with ipilimumab (Table 1). Data regarding the association of the gut microbiota and PD-1/PD-L1-induced colitis, as well as its association with other immune-related adverse events, are lacking. Interestingly, just recently, FMT was used to successfully treat a patient with severe immunotherapy-induced colitis refractory to immunosuppressive therapies, including high-dose corticosteroids, anti-tumor necrosis factor alpha, and anti-integrin therapies [28].

Modulation of the Gut Microbiota and Its Importance to Cancer Therapy

Both antibiotics and probiotics are commonly used by patients for different reasons and contexts. There are data suggesting

Table 1 Clinical studies showing the association of gut microbiota and response to, or adverse events (colitis) following, immune checkpoint inhibitors (ICI) in solid tumors

Reference	Design of the study and genomic tools used	Study population	Major clinical results
• Dubin et al. Nature Communications 2016 [24].	• Prospective cohort	• Patients with metastatic melanoma who received ipilimumab	• An increased representation of bacteria belonging to the *Bacteroidetes* phylum was correlated with less ipilimumab-induced colitis. • A paucity of genetic pathways involved in polyamine transport and B vitamin biosynthesis was associated with an increased risk of colitis
• Chaput et al. Annals of Oncology 2017 [25••].	• Prospective cohort	• Patients with metastatic melanoma who received ipilimumab and/or nivolumab	• Patients whose baseline microbiota was enriched with *Faecalibacterium* genus and other Firmicutes (had longer PFS and OS) • Most of the baseline colitis-associated phylotypes were related to *Firmicutes*, whereas no colitis-related phylotypes were assigned to *Bacteroidetes*
• Gopalakrishnan et al. Science 2018 [18••].	• Retrospective cohort	• Patients with metastatic melanoma who received PD-1 inhibitors	• It was showed significantly higher alpha diversity and relative abundance of bacteria of the *Ruminococcaceae* family in responding patients
• Matson et al. Science 2018 [16••].	• Retrospective cohort	• Patients with metastatic melanoma who received PD-1/PD-L1 inhibitors	• There was a significant association between commensal microbial composition and clinical response • Bacterial species more abundant in responders included *Bifidobacterium longum*, *Collinsella aerofaciens*, *and Enterococcus faecium*
• Routy et al. Science 2018 [17••].	• Retrospective cohort	• Patients with metastatic urothelial carcinoma, NSCLC, and RCC who received PD-1/PD-L1 inhibitors	• It was found a correlation between clinical responses to ICIs and the relative abundance of *Akkermansia muciniphila* • Use of antibiotic was associated with diminished response to ICI

NSCLC non-small cell lung carcinoma; *PFS* progression-free survival; *OS* overall survival; *RCC* renal cell carcinoma

that patients with advanced non-small cell lung cancer, bladder cancer, or renal cell carcinoma who started on antibiotics shortly before or after initiating anti-PD-1/PD-L1 therapy have worse outcomes than those who did not take antibiotics [17••]. While these differences in outcome may be related to antibiotic-induced dysbiosis, more studies are needed to definitely establish this association. Moreover, a prospective evaluation of the benefit of using probiotics to modulate ICI responses is also needed. Spencer and coauthors [29] showed that over-the-counter use of probiotic supplements in patients with metastatic melanoma was associated with a significant 70% lower likelihood of response to ICIs than that seen in patients who did not use probiotics. Furthermore, prospective clinical studies have already started testing whether FMT or the use of prebiotics or probiotics could increase the success rate of ICIs in clinical practice [27•]. However, caution is needed to optimize the manufacturing of the feces-derived products used in FMT, as well as to select optimal FMT donors. Recently, the Food and Drug Administration released a safety communication about bacterial infections caused by multi-drug resistant organisms (MDROs) that have occurred in two immunocompromised patients due to transmission of an MDRO from the use of investigational FMT (https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse). Unfortunately, one of these patients died.

Future Perspective in Breast Cancer and Conclusions

The recognition that the gut microbiome affects antitumor immunity requires the evaluation of its composition among patients with breast cancer. It will be important to establish whether the gut microbiome differs according to molecular subtype, staging, and prior lines of therapy. Of great interest is also to determine whether the gut microbiome can be used as a predictive biomarker for chemotherapy and/or immunotherapy. Finally, the results of FMT and other gut microbiome-modulating approaches used to potentiate the action of ICIs in other cancer types are expected and could open a new pathway for treating patients with so far incurable metastatic breast cancer.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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