Review Article

The Emerging Role of the Gut Microbiome in the Cancer Response to Immune Checkpoint Inhibitors: A Narrative Review

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ABSTRACT

The discovery of immune checkpoint inhibitors (ICIs) has revolutionized the care of cancer patients. However, the response to ICI therapy exhibits substantial interindividual variability. Efforts have been directed to identify biomarkers that predict the clinical response to ICIs. In recent years, the gut microbiome has emerged as a critical player that influences the efficacy of immunotherapy. An increasing number of studies have suggested that the baseline composition of a patient's gut microbiota and its dysbiosis are correlated with the outcome of cancer immunotherapy. This review tackles the rapidly growing body of evidence evaluating the relationship between the gut microbiome and the response to ICI therapy. Additionally, this review highlights the impact of antibiotic-induced dysbiosis on ICI efficacy and discusses the possible therapeutic interventions to optimize the gut microbiota composition to augment immunotherapy efficacy.

Keywords: immune checkpoint inhibitors, microbiome, cancer treatment, immunotherapy response, dysbiosis

INTRODUCTION

Immune checkpoint inhibitors (ICIs) that target programmed cell death-1 (PD-1), programmed cell death-ligand 1 (PDL-1), or cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) have revolutionized the treatment of several malignancies owing to their higher efficacy and lower toxicity as compared with traditional cytotoxic drugs. They are now being used in the standard care of several malignancies.[1,2] These drugs are monoclonal antibodies that target specific proteins on cells, namely PD-1, its ligand (PDL-1), or CTLA-4, that are involved in inhibitory regulation of immune responses.[3] This in turn allows immune cells to better target tumor cells presenting non–self-antigens, thus overcoming tumor immune subversion, which is often seen in malignancies. Despite these advances, the clinical efficacy of ICIs is highly variable among cancer patients. A considerable proportion of patients show primary resistance to ICIs, and others show disease progression or relapse due to secondary resistance after an initial response.[4–6] Several of these resistance mechanisms have been described, and they can be classified as intrinsic (related to characteristics of the cancer cells) or extrinsic (related to interactions among the ICI, T cells, macrophages, and the enteric microbiome, among other elements). Initially, PDL-1 and PD-1 receptor expression were the biomarkers used to predict a response to immunotherapy. More recently, additional predictive biomarkers have included DNA mismatch repair, microsatellite instability, and tumor mutational burden.[7] Most of the evidence has focused on tumor-related characteristics. However, another important determinant of the response to ICIs is the host immune system. Accordingly, factors that modulate the immune system, such as the gut microbiome, could also affect ICI efficacy.[8]

The microbiota that resides in the gastrointestinal tract provides essential health benefits to its host, particularly by regulating immune homeostasis. Moreover, a growing

body of evidence suggests that alteration of this gut microbial community can cause immune dysregulation.[9] Recently, multiple clinical studies have demonstrated that the gut microbiome is an important regulator of systemic immune reactions and is involved in the response to ICI immunotherapy.[10–13] The first study linking the gut microbiome composition to ICI efficacy was a preclinical study using murine models published in 2015 that revealed a role for *Bifidobacterium* in enhancing antitumor immunity and augmenting the effect of ICIs in vivo.[14] Interestingly, such findings paved the way for numerous clinical studies published since 2017, which have established the association between the gut microbiome and response to ICIs in cancer patients. In this review, we summarize currently available data addressing the impact of the baseline gut microbiome, and its alteration with antibiotics, on ICI efficacy, and discuss the therapeutic implications of these findings in the context of cancer immunotherapy using ICIs.

SEARCH STRATEGY

References for this review were identified by using PubMed, Medline, and Embase with the search terms: *immune checkpoint inhibitors*, *immunotherapy*, *cancer*, *responders*, *nonresponders*, *microbiota*, *gut microbiome*, *antibiotics*, *antimicrobials*, *immune-related adverse events*, *fecal microbiota transplant*, and *probiotics*. We included only full text publications written in English. No time restriction was applied. Articles were initially selected by the title and the abstract. The full text was subsequently analyzed. The reference list of the included articles was also screened by FAA and RZ for relevant papers.

GUT MICROBIOTA AND RESPONSE TO IMMUNE CHECKPOINT INHIBITORS (ICIs)

The gut microbiome is a dynamic ecosystem that plays an essential role in maintaining immune homeostasis by tailoring the local and the systemic immune system.[10] Recently, many studies have suggested a role for the microbiome in the response of tumors to ICIs. Although the exact mechanisms have not been fully elucidated, current hypotheses suggest that the gut microbiome produces several molecules that affect the growth of cancer cells and modulate anticancer immunity. These molecules act as messengers that enter the circulation and signal to enhance the systemic immune cell responsiveness.[15] Such products include 1) bacterial toxins that induce immunogenic and necrotic cell death, 2) pathogen-associated molecular patterns (PAMPS) that activate Toll-like receptors and stimulate innate immunity, and 3) bacterially derived metabolites, the best known of which are the short-chain fatty acids (SCFAs) like butyrate, which can trigger cancer cell apoptosis by activating dendritic cells and cytokine production.[16–21]

Several studies have also suggested possible mechanisms by which specific bacterial species of the gut microbiota exert their effect on the immune system and thus affect the response to ICIs (Table 1).

The key to understanding the role of baseline gut microbiome and its disruption in human diseases and response to treatment is the stability of the microbiota over time. Sequencing studies have revealed that each individual harbors a unique collection of gut microbial species. Several studies have also shown remarkable long-term within-individual stability in the gut microbial abundance over years.[22–25] For example, by using 16S rRNA and whole genome sequencing techniques to characterize bacterial strain composition in the fecal microbiota, Faith et al[26] showed that on average 60% of the microbial strains harbored in each adult's intestine were retained over the course of 5 years. Moreover, a gut microbial composition with higher baseline diversity was more stable over time,[23,25] and members of the *Bacteroidetes*, *Bifidobacterium*, and *Actinobacteria* were more stable components of the microbiota than members of other phyla.[25,26] On the other hand, several studies have shown that the gut microbiota varied by various factors such as age, diet, geographic location, smoking, exercise, infections, and antibiotic administration.[27–31] This raises an important question: If the gut microbiome can be influenced by so many factors, how can it be used as a biomarker to predict response to ICIs? The study of metabolites as common outputs of bacterial metabolic function introduces the notion of a functional classification of the gut microbiome rather than a taxonomic one. It is plausible that different taxonomic groups of bacteria have evolved to perform similar metabolic functions, and thus can be classified by the type of metabolites they produce. Despite the variation in microbiota composition among individuals and over time, the gut microbiome metabolic pathways are what remain stable and mediate their interaction with the immune system to modify the response to ICIs. To date, most of the studies have focused on the baseline microbiota composition as a predictive marker of response to ICIs. Few studies have tried to establish the role of specific bacterial metabolites such as SCFAs, glycerophospholipids, and inosine. For example, Nomura et al[32] examined fecal and plasma levels of SCFAs in patients with solid tumors treated with anti–PD-1 in a prospective cohort study. They found that high concentrations of SCFAs were associated with longer progression-free survival (PFS). On the contrary, Coutzac et al[33] demonstrated that anti-CTL4 therapy efficacy was negatively influenced by systemic SCFAs. Xu et al[34] showed that better efficacy of anti–PD-1 treatment was associated with the glycerophospholipid pathway. Mager et al[35] found that mice administered with inosine showed improved antitumor effects from anti-CTL4 therapy. However, the exact mechanisms by which these metabolites modify antitumor immunity are still under investigation. A possible mechanism is related to the

Table 1. Responder bacteria associated with a positive response to immunotherapy and the proposed mechanisms by which they modulate the anticancer efficacy of ICI therapy

Main Responder Bacteria	References	Checkpoints Targeted	Proposed Mechanism
Akkermansia			
A. muciniphila	Routy et al, 2018 [55]	CTLA-4, PD-1	Stimulate secretion of cytokines by MHC Class II restricted CD4+ T cells and DCs in the peripheral blood
A. muciniphila	Wind et al, 2020[47]		
A. muciniphila	Zheng et al, 2019[59]		
A. muciniphila	Salgia et al, 2020[46]		
A. muciniphila	Botticelli et al, 2018[50]		
Bacteroides			
B. thetaiotaomicron, B. fragilis	Vétizou et al, 2015[48]	CTLA-4, PD-1	Induce TH1 immune responses in tumor-draining lymph nodes and maturation DCs
B. thetaiotaomicron, B. caccae	Frankel et al, 2017[41]		
B. plebeius	Botticelli et al, 2018[50]		
B. eggerthii, B. thetaiotaomicron, B. massiliensis	Wind et al, 2020[47]		
B. eggerthii	Salgia et al, 2020[46]		
Bifidobacterium			
B. breve, B. longum	Sivan et al, 2015[14]	CTLA-4, PD-1	Increase accumulation of antigen-specific CD8+ TILs and MHC Class II DCs
B. longum	Botticelli et al, 2018[50]		
B. longum	Matson et al, 2018[51]		
B. longum	Jin et al, 2019[52]		
B. pseudolongum	Mager et al, 2020[35]		
B. adolescentis	Salgia et al, 2020[46]		
Ruminococcaceae			
Unspecified	Routy et al, 2018[55]	PD-1	Increase CD8+ TILs and increase levels of CD4+ and CD8+ T cells in the peripheral blood
Unspecified	Gopalakrishnan et al, 2018[43]		
R. obeum, R. bromii	Zheng et al, 2019[59]		
Ruminococcaceae UCG 13	Hakozaki et al, 2020[56]		
Faecalibacterium			
F. prausnitzii	Frankel et al, 2017[41]	CTLA-4, PD-1	
F. prausnitzii L2-6	Chaput et al, 2017[44]		
Unspecified	Gopalakrishnan et al, 2018[43]		
F. prausnitzii	Botticelli et al, 2018[50]		

ICI: immune checkpoint inhibitor; DC: dendritic cell; MHC: major histocompatibility complex; TH1: T-helper 1; TIL: tumor-infiltrating lymphocyte

accumulation of CD8+ T cells in the tumor microenvironment and increased production of IFN-γ in response to SCFA.[36–38] Further mechanistic studies are needed to define the immunomodulatory role of these metabolites. Such level of understanding of the molecular details of the immune system–microbiota interaction would open a new area in this field, resulting in numerous possibilities for the prognostic and therapeutic use of the gut microbiome.[39,40]

Responders vs Nonresponders

To evaluate the effect of the gut microbiome on ICI response, it is important to determine the composition of the individual's gut flora. Sequencing technologies such as metagenomic shotgun sequencing and 16S RNA gene sequencing have been widely used for this purpose. Subsequently, patients treated with ICI therapy are typically classified as "responders" (R) or "nonresponders" (NR) based on the RECIST (Response Evaluation Criteria in Solid Tumors) criteria in most cases.[41,42] As a result, multiple correlations have been drawn that link particular bacterial species to the observed clinical responses. Most studies thus far have been conducted in patients with melanoma, although more recent ones have included patients with other solid tumors such as non–small cell lung cancer (NSCLC) and renal cell carcinoma (RCC).

Preclinical studies have been largely suggestive of the role of certain microbes in the clinical response to ICIs. Some of these correlations were later re-established in human studies. For example, a study of 43 melanoma patients found *Faecalibacterium* to be correlated with a positive response as demonstrated by prolonged PFS. In addition to responding better to anti–PD-1 therapy, the study revealed that transplanting fecal microbiota rich in *Faecalibacterium* from R patients to mice resulted in significant reduction in tumor growth, as well as an increase in the number of CD45+ immune and CD8+ T cells in the gut, as compared with mice with fecal microbiota transplant (FMT) from NR patients.[43] Other studies have also confirmed this positive correlation.[44,45]

Bacteroides species, particularly *B. fragilis* and *B. thetaiotaomicron,* have also been correlated with a positive response.[45–50] This was demonstrated in a murine study, whereby germ-free or antibiotic-treated mice did not respond to CTLA-4 blockade until gavage with *B. fragilis*, immunization with *B. fragilis* polysaccharides, or adoptive transfer of *B. fragilis*–specific T cells. The immunostimulatory role of *Bacteroides* was further

demonstrated when treatment with CTLA-4–blocking antibodies in mice treated with FMT from R patients favored the outgrowth of *B. fragilis*.[48] As in murine studies, a study on melanoma patients also determined a positive correlation between *Bacteroides* species, namely *B. massiliensis*, and *Streptococcus parasanguinis* and outcome. Patients with large concentrations of these two bacteria had prolonged PFS and overall survival (OS), respectively. However, patients with large numbers of *Peptostreptococcaceae* had a shorter duration of both PFS and OS.[47]

Bifidobacterium is another important genus that has been consistently shown to be correlated with a positive response, as defined by the RECIST criteria.[51,52] This was established in early studies, whereby *Bifidobacterium*-treated mice displayed significantly improved tumor control in comparison with their non–*Bifidobacterium*-treated counterparts. In one study, oral administration of *Bifidobacterium* alone improved tumor control to the same degree as PD-L1 therapy blockade.[14] Another study conducted on a murine model of colorectal cancer also confirmed a significant role for *Bifidobacterium pseudolongum*. In this study, monocolonization of mice with either *Lactobacillus johnsonii*, *Olsenella* species, or *B. pseudolongum* significantly enhanced CTLA-4 blockade activity. *B. pseudolongum* had the most robust ICI response, owing to its correlation with an elevated level of inosine, a metabolite that promotes Th1 cell activation through adenosine 2A (A2A) receptor signaling.[35]

Correlation studies conducted on human subjects have also identified numerous bacterial species that impact the response to ICIs (Table 1). *Bifidobacterium* was again noted to favor a positive response, particularly *B. longus* and *B. adolescentis* in NSCLC and melanoma studies, respectively. These two studies also noted other bacteria correlated with R patients, including the previously noted *Lactobacillus* and *Enterococcus faecium*. Other R species included *Prevotella copri*, *Alistipes putredinis*, *Parabacteroides*, *Methanobacteriaceae*, *Clostridium*, *Collinsella aerofaciens*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, and *Syntrophococcus*. Importantly, both studies noted *Ruminococcus* to be associated with the NR group, particularly *R. obeum*. Other NR species included *Sutterella*, *Bilophila*, and *Roseburia intestinalis*.[48,51–54] Intriguingly, some studies have found *Ruminococcaceae* to have a general positive prognostic significance, which raises the question as to the cause of this difference.[43,55,56]

Akkermansia muciniphila was also consistently found to be associated with favorable clinical outcomes and improved PFS in NSCLC and RCC studies conducted by Routy et al[55] and Derosa et al.[57] A positive correlation was also noted in a study on melanoma patients.[51] In addition, a study comparing a control group with NSCLC patients found that *A. muciniphila* was more abundant in the gut of control participants.[49]

Chronology has been an interesting aspect to examine as well. A study on metastatic RCC included temporal profiling of the microbiome in patients treated with ICIs. In addition to identifying *A. muciniphila*, *B. adolescentis*, *Barnesiella intestinihominis*, *Odoribacter splanchnicus*, and *Bacteroides eggerthii* as correlated with clinical benefits, the study revealed a general increase in the relative abundance of *A. muciniphila* in patients deriving those benefits, with a relative decrease in abundance in those not deriving benefit. Other studies differ in reporting temporal changes, whereby some found no significant change in the microbiome composition over the course of ICI therapy, whereas others determined that microbial diversity changes in the first few weeks of treatment could be an early predictor of response.[44,46,58,59] This raises hope for the potential utility of microbiome modulation in improving outcomes of patients receiving ICI treatment.

The difference in results of all these studies is noteworthy. As detailed above, different studies have identified different responder bacteria. Although for the most part congruent, some inconsistencies were noted with certain bacteria such as *B. eggerthii* and *Ruminococcaceae*—among others—whereby different studies identified them as R or NR bacteria.[47] No clear explanation has yet been provided for these variations. The type of ICI used may be a possible explanation, as not all studies encompassed both PD-1/PD-L1 and CTLA-4 blockade. In the case of *Ruminococcaceae*, all studies have included only PD-1/PD-L1 inhibition. Despite most studies identifying these bacteria as R, a study by Jin et al[52] found them to be associated with the NR group, thus disputing the theory that specific R bacteria are associated with the subtype of the ICI at hand. Another possibility is that geographic location or cancer type may play a role as well. However, a few studies that have examined these variables have noted that the diversity of the microbiome per se is the main factor affecting response, rather than geographic locations.[47] In addition, multiple bacteria have been independently shown to be responders in studies conducted in vastly separate countries such as China, The Netherlands, Italy, and France.[47,49,55,59] Similarly, the same bacteria were found to be R bacteria in patients with different types of malignancy, such as the case of *A. muciniphila* in NSCLC, RCC, and melanoma.[47,55] It is true that currently available studies have not sufficiently accounted for confounding variables such as age or body mass index (BMI) which may in part play a role in the minor inconsistencies of some study results.[47] What remains most consistent, however, is the correlation between the diversity of the microbiome and favorable anticancer efficacy of ICIs. Abundance of R species is also favorable, but more studies are needed to identify implicated species and the means of interpreting their presence.[49,52] Although the exact mechanisms have not yet been fully elucidated, it may be related to the intricate cross talk between microbes through signaling molecules such as SCFAs.[49,52]

The role of the gut microbiome in human health extends further to influence the tumor microenvironment. A new trend to study how the intratumor microbial ecosystem—particularly in tumor locations that the gut microbes can reach—affects clinical outcome has emerged in recent years. A study conducted by Riquelme et al[60] examined the role of intratumor microbiome on long-term survival of pancreatic adenocarcinoma patients. They analyzed the tumor microbiome composition of 68 patients who underwent surgical resection of pancreatic adenocarcinoma. They found that greater intratumor microbial diversity (defined by the number of species) and higher abundance of *Pseudoxanthomonas*, *Saccharopolyspora*, *Streptomyces*, and *Bacillus clausii* were associated with better survival outcome. They also found a strong correlation between CD8+ and granzyme B tissue densities with the intratumor microbial diversity, which suggested that the tumor microbiome might influence the degree of CD8+ T-cell infiltration and activation to affect antitumor immune response. Interestingly, they demonstrated that the intratumor microbiome cross-talks with the gut microbiome. By performing FMT in mice previously treated with antibiotics and challenged with orthotopic implantation of syngeneic cancer lines, they were able to detect human donor bacteria in the murine tumor microbiome post FMT, whereas it remained absent from mice who did not receive FMT, suggesting that potentially the gut microbiome has the capacity to translocate to pancreatic tumors and this colonization can modify the tumor microbiome and antitumor immunity. However, studies of this topic are limited and the impact of tumor microbiome on prognosis is still not clear.

Manipulation of the Gut Microbiome Impacts ICI Efficacy

Antibiotic-induced dysbiosis and the therapeutic effect of probiotics

Intestinal dysbiosis is defined as the imbalance in the baseline structure and function of the gut microbiota. This is typically represented by major shifts in microbial composition, resulting in decreased overall diversity, decreased abundance of anti-inflammatory species such as *Faecalibacterium prausnitzii* and increased abundance of different *Enterobacteriaceae* species. As such, changes in the abundance of particular species can be considered as markers of dysbiosis.[61] Dysbiosis can be induced by exposure to various environmental factors such as antibiotics, diet, and infections. It has been associated with the pathogenesis of multiple intestinal diseases such as inflammatory bowel disease and colorectal cancer.[12,62–65] Several studies have demonstrated the important role played by antibiotics in intestinal dysbiosis. It has been shown that antibiotics restrict the microbial diversity in the gastrointestinal tract and displace certain bacterial taxa.[66–68] This alteration of taxonomic composition appears shortly after the administration of antibiotics and can persist for long peri-

ods.[68–71] A study of healthy human volunteers before, during, and after a 5-day course of oral ciprofloxacin demonstrated decreased abundance of about a third of the bacterial taxa in the gut during treatment. Although the microbiota largely returned to its pretreatment composition in about 4 weeks after treatment, several taxa failed to recover within 6 months.[68] Another study of the human fecal microbiota showed that a 7-day course of clindamycin reduced the diversity of *Bacteroides*, which did not return to the pretreatment composition for up to 2 years after treatment.[69] These studies suggest that although antibiotic-induced dysbiosis can be reversible, it can lead to persistent long-term impacts on the gut microbiome. The altered composition of the gut microbiome affects the mutual relationship between the microbiome and the host immune system.[10,72] Antibiotics are also shown to alter gene expression and metabolite production by the gut microbiome.[73,74] The administration of antibiotics can reduce the expression of antimicrobial peptides by intestinal cells, downregulate genes encoding MHC class I and II, and reduce the frequency of CD4+ T cells expressing IFN-γ and interleukin-17. Antibiotics also impact host immunity by reducing the production of bacterial metabolites like SCFAs, which have been implicated in regulating many aspects of intestinal immunity.[10,75] Interestingly, the impact of antibiotics on the gut microbiome exhibits significant interindividual variability.[68] Both drug-related factors (such as antibiotic class, timing of exposure, and route of administration) and host-related factors (such as age and microbiota composition) influence the perturbations of the gut microbiome caused by antibiotics. For example, oral vancomycin is a powerful modulator of the microbiota but not intravenous vancomycin. The microbiota of infants has been shown to be more affected by antibiotics than adults.[76] The initial state of the gut microbiota appears to influence its reshaping by antibiotics in adults. In a study of 18 healthy volunteers, a 7-day regimen of cefprozil led to similar qualitative alterations of microbiota in most subjects, but to drastically different alterations in a subset of individuals with a predominant *Bacteroides* enterotype.[77]

On the other hand, probiotics have demonstrated a beneficial role on human gastrointestinal diseases through influencing the intestinal microbiota.[78] They are suggested to restore microbial balance and recover the intestinal homeostasis, thus serving as a therapeutic option in several gastrointestinal disorders.[79,80] The mechanisms by which probiotics exert their therapeutic effects include: improvement of cell barrier function; antagonist activity against pathogenic bacteria; modulation of intestinal cytokine signaling; and exhibition of anti-inflammatory properties.[81–83] Probiotics' anti-inflammatory effect is mediated by the downregulation of proinflammatory cytokines like TNF-α and attenuation of the NF-kB signaling pathway.[79] Moreover, they can induce regulatory T cells, which inhibit the effector T cells that would cause inflammation.[83] Certain pro-
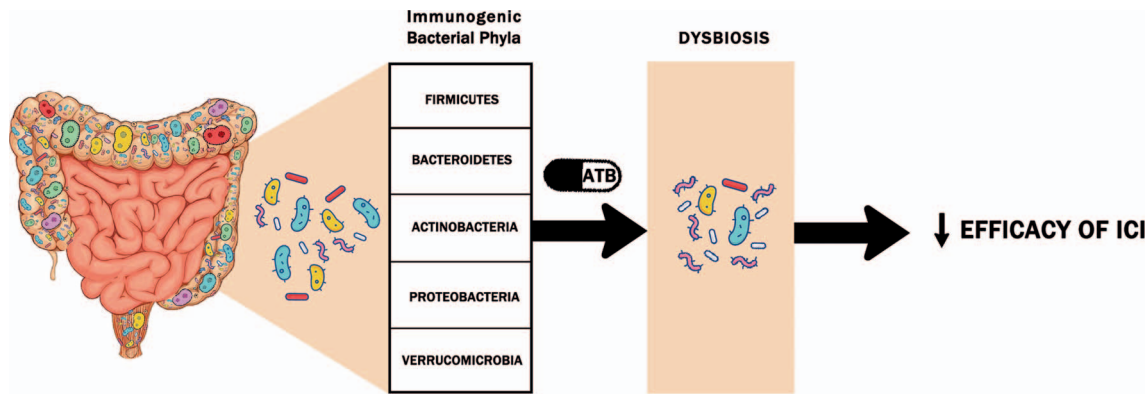
Figure 1. The five immunogenic bacterial phyla of the gut microbiome implicated in the response to immune checkpoint inhibitors (ICIs). It is speculated that antibiotic (ATB) use induces a dysbiosis that alters the concentrations of these immunogenic bacteria required to engage the immune system in the response to ICIs, thus reducing their efficacy.

biotics have been shown to reduce the rate of infection with *Clostridium difficile* and the incidence of antibiotic-associated diarrhea.[84–87] Accordingly, the association between the gut microbiome and the clinical response to ICIs makes it logical to hypothesize that the manipulation of the gut microbiome by antibiotics or probiotics would influence ICI efficacy.

Antibiotic-related dysbiosis impact on ICI efficacy

Antibiotic-related dysbiosis can eliminate the most immunogenic bacteria required to engage the immune system in the response to ICIs (Figure 1).[57] Moreover, by downregulating the expression of MHC class I and II, which are necessary for antigen presentation, they help tumor cells avoid immune recognition and thus decrease the efficacy of ICI therapy. To date, multiple clinical studies have attempted to determine the impact of antibiotics on the clinical efficacy of ICIs (Table 2).[55,57,88–98] In most of these studies, antibiotics were associated with reduced ICI efficacy, and both univariant and multivariant analyses confirmed that antibiotics received during a critical timeframe in patients receiving ICIs are independently associated with worse clinical outcomes, suggesting that an intact gut microbiome is needed to reinforce the immune system during immunotherapy. Interestingly, several meta-analyses have shown through subgroup analysis that this association is independent of cancer type, age, sample size, therapeutic strategy, ICI type, and timing of antibiotics.

To further determine which type of antibiotics most significantly affected ICI efficacy, Ahmed et al[96] divided the patients into narrow-spectrum antibiotics recipients and broad-spectrum antibiotics recipients. Narrow-spectrum antibiotics were defined as antibiotics only covering gram-positive bacteria, whereas broad-spectrum antibiotics included antibiotics that would cover gram-positive and gram-negative with or without anaerobic bacteria. It was found that narrow-spectrum antibiotics did not affect the response rate (RR) to ICIs. In contrast, patients who received broad-spectrum antibiotics had a lower RR (25% vs 61%) and took longer time to respond to immunotherapy. These findings suggested that broad-

spectrum antibiotics might have impoverished the microbes involved in stimulatory immune responses, creating a habitat for the microbes that induce suppressive immune responses, reducing ICI efficacy.

The study by Tinsley et al[95] was the first to shed light on the importance of antibiotic duration and cumulative antibiotic use. Recipients of antibiotics were categorized as either having had a single course of antibiotics, or cumulative courses of antibiotics, defined as concurrent or successive antibiotics for more than 7 days. The latter had significantly lower PFS and OS. Similar results were reported by Iglesias-Santamaría[98] whereby patients with higher "antibiotic exposure," defined as % "days of antibiotic/days of ICI," had significantly reduced clinical outcomes.

Derosa et al[57] examined the impact of antibiotic timing with respect to ICI initiation on the prognostic role of antibiotic administration. After finding that antibiotics administered within 30 days of starting ICIs were associated with decreased OS and PFS, they examined the effect of antibiotics within 60 days of ICI initiation. Although they were still associated with a worse clinical outcome, the effect was not as pronounced as within 30 days before ICI. These findings can be justified by evidence showing subsequent recovery of the gut microbiome within 1–3 months after antibiotic discontinuation.[99] This implies that there is a specific timeframe during which antibiotics will have the greatest impact on ICI efficacy. However, it remains difficult to define this timeframe considering the differences in the timing of antibiotic administration between the different studies. Ruiz-Patiño et al[92] also investigated whether antibiotic timing plays a role, to find if the effect of antibiotics would differ between patients who received them before ICI initiation and those who received them concomitantly with ICIs. In both groups, antibiotic use was associated with reduced OS with no significant difference observed between them. In contrast, Schett et al[90] showed that antibiotics had a negative impact on OS only when administered 2 months prior to ICI initiation, but not when administered during treatment or after ICI discontinuation.

Table 2. Summary of available studies addressing the impact of antibiotics on patients with cancer treated with ICI therapy

Study	Cancer Type	Pts, n	ICI	Antibiotic Timing With Respect to ICI Initiation	Outcomes	Statistics, p or HR (95% CI)
Kaderbahi et al, 2017[89]	NSCLC	74	Anti-PD-1	Within 3 mo before ICI initiation	No change in PFS No change in RR	$p = 0.72$ $p = 0.75$
Hakozaki et al, 2019[88]	NSCLC	90	Anti-PD-1	Within 1 mo after ICI initiation	No change in OS	2.0 (0.7–5.8)
Schett et al, 2020[90]	NSCLC	218	Anti-PDL-1	Within 2 mo before ICI initiation	PFS (1.4 vs 5.5 mo) OS (1.8 vs 15.4 mo) PD (72.7 vs 43.9%)	2.2 (1.5–3.3) 2.6 (1.7–4.0) $p = 0.02$
Chalabi et al, 2020[91]	NSCLC	757	Anti-PDL-1	Within 1 mo before and after ICI initiation	OS (8.5 vs 14.1 mo)	1.3 (1.1–1.6)
Ruiz-Patino et al, 2020[92]	NSCLC	140	Anti-PDL-1 with chemotherapy, anti-PDL-1 alone	Within 1 mo before ICI initiation During treatment Anytime within treatment period	OS (20.3 vs 40.6 mo) OS (24.7 vs 40.6 mo) No change in PFS No change in RR	$p < 0.05$ $p < 0.05$ $p > 0.05$ $p > 0.05$
Lalani et al, 2020[93]	RCC	146	Anti-PD-1, anti-PDL-1 alone or with chemotherapy	Within 1 mo before or after ICI initiation	RR (12.9 vs 34.8%) PFS (2.6 vs 8.1 mo)	$p = 0.026$ 2.0 (1.2–3.2)
Elkrief et al, 2019[94]	Melanoma	74	CTLA-4 with chemotherapy, CTLA-4 alone, anti-PD-1	Within 1 mo before ICI initiation	RR (0 vs 34%) PFS (2.4 vs 7.3 mo)	$p = 0.01$ $p = 0.01$
Derosa et al, 2018[57]	NSCLC RCC	239 121	Anti-PD-1, anti–PD-1 + CTLA-4	Within 1 mo before ICI initiation	NSCLC: PFS (1.9 vs 3.8 mo) OS (7.9 vs 24.6 mo) RCC: PD (75 vs 22%) PFS (1.9 vs 7.4 mo) OS (17.3 vs 30.6 mo)	1.5 (1.0–2.2) 4.4 (2.6–7.7) $p < 0.01$ 3.1 (1.4–6.9) 3.5 (1.1–10.8)
Routy et al, 2018[55]	NSCLC RCC UC	140 67 42	Anti-PD-1, anti-PDL-1	Within 2 mo before or 1 mo after ICI initiation	PFS (3.5 vs 4.1 mo) OS (11.5 vs 20.6 mo)	$p = 0.017$ $p < 0.001$
Tinsley et al, 2020[95]	NSCLC RCC Melanoma	291	Anti-PDL-1, CTLA-4	Within 2 wk before or 6 wk after ICI initiation	PFS (3.1 vs 6.3 mo) OS (10.4 vs 21.7 mo)	$p = 0.003$, HR 1.6 $p = 0.002$, HR 1.7
Ahmed et al, 2018[96]	Multiple	60	ICI with chemotherapy, anti–PD-1 or anti–PDL-1 alone	Within 2 wk before and/or after ICI initiation	RR (29.4 vs 62.8%) PFS (0 vs 22.5 wk) OS (24 vs 89 mo)	1.9 (1.2–3.0) 1.6 (0.8–3.0) 2.9 (1.1–8.1)
Pinato et al, 2019[97]	Multiple	196	Anti-PD-1 Anti-PDL-1	Within 1 mo before ICI initiation Anytime within treatment period	OS (2 vs 26 mo) PD (81 vs 44%) No change in OS	7.4 (4.2–12.9) $p < 0.001$ $p = 0.76$
Iglesias-Santamaría, 2020[98]	Multiple	102	Anti-PDL-1, CTLA-4	Within 4 wk before or after ICI initiation anytime within treatment period	No change in PFS No change in OS PFS with ↑ AE* (3.1 vs 8.2 mo) OS with ↑ AE* (9.4 vs 17.8 mo)	1.4 (0.9–2.3) 1.5 (0.9–2.7) 2.3 (1.2–4.6) 2.3 (1.2–4.9)

ICI: immune checkpoint inhibitor; Pts: patients; HR: hazard ratio; NSCLC: non-small cell lung cancer; PFS: progression-free survival; RR: response rate; OS: overall survival; PD: primary progressive disease; RCC: renal cell carcinoma; UC: urothelial carcinoma; AE, antibiotic exposure; ↑ indicates increase.
*AE defined as number of days of antibiotic use divided by the number of days of ICI use.

Similar results were also reported in the prospective study of Pinato et al.[97]

While multiple studies have concluded that, in general, antibiotic-induced dysbiosis can potentially dampen the antitumor effects of ICIs, some studies have produced evidence that is at odds with such findings. In a retrospective study by Kaderbhai et al,[89] investigators assessed the treatment outcomes of patients with NSCLC who were receiving anti–PD-1 immunotherapy with respect to antibiotic usage within the 3 months prior to the first dose; the study demonstrated a lack of association between response to ICI and antibiotic use. However, these results might have been affected by the long cutoff of 3 months, considering that the gut microbiome repopulation can occur within 4 weeks after antibiotic discontinuation. Another study by Hakozaki et al[88] on NSCLC patients also showed no effect of antibiotics administered within 1 month after initiation of ICI. Taking it a step further, a study by Metges et al[100] examined the outcomes of antibiotic use prior to and during treatment with ICIs in a cohort of 325 patients with advanced NSCLC and deemed that a survival advantage was appreciated in patients who had received antibiotics up to 60 days before or during immunotherapy (median survival, 16.2 months vs 11.5 months). Equivalently, the use of antibiotics during immunotherapy correlated with increased survival in a study by Masini et al.[101] In a similar vein, one study by Vétizou et al[48] that analyzed the factors that affect CTLA-4 blockade in patients with melanoma concluded that vancomycin could potentially augment the antitumor effect observed with anti–CTLA-4 treatment; this was attributed to a potentially induced favorable representation of certain components of the microbiota such as *Bacteroidales* as opposed to *Clostridiales*.

In summary, it has been well established that altering the gut microbiome can potentially impair the response to ICIs. However, if gut microbiome is to be used as a biomarker to predict the response to ICIs, we need to better understand in depth how it is affected by antibiotics. The above-mentioned studies need to be interpreted in the context of their study design. First, they are all retrospective observational studies, and thus their results reflect a general prognostic association and not causation, and they can be affected by selection bias and unmeasured confounders. The conflicting findings among the different studies are difficult to explain and may result from the inherent limitations of retrospective analysis such as small population size, variation in patient characteristics between groups, and different definitions of studied variables such as the antibiotic use window and antibacterial spectrum. Additional studies are needed to answer controversial questions regarding the effect of several variables that were not taken into account in most of the aforementioned studies, such as the duration of antibiotic treatment, the class of the antibiotic and its spectrum, the route of administration (intravenous versus oral), the indication

for administration (infection treatment vs prophylaxis), and the severity of the infection. It is challenging to infer a causal link between the use of antibiotics and decreased response to ICIs. A confounding factor could be that frail patients who are less likely to benefit from cancer treatment are more likely to acquire infections that need antibiotic treatment. Moreover, it may be possible that the infections themselves have an immunosuppressive effect that alters the response to ICIs. Therefore, future studies are warranted to focus on the experimental designs to control for confounding factors and further delineate the mechanistic basis of the effects of antibiotics on ICI therapy.

Use of probiotics to modify the microbiome to a pattern favorable for ICI therapy

Due to the beneficial effect of probiotics on dysbiosis-related intestinal disorders, several studies investigated the role of probiotics in improving efficacy of ICI. Studies using mice models demonstrated that the efficacy of PDL-1 inhibitors was ameliorated with supplementation of probiotics containing *Bifidobacterium* species by augmenting the activity of tumor-specific CD8 T cells.[14] Similarly, in addition to a noteworthy association between the presence of *A. muciniphila* and favorable clinical outcomes, preclinical studies have demonstrated that supplementation with *A. muciniphila* improved PD-1 efficacy in mice models.[55] A study by Vétizou et al[48] conducted on mice models deemed that the antitumor efficacy of CTLA-4 antibodies improved after oral administration of *B. fragilis* with *B. thetaiotaomicron* or *Burkholderia cepacia*. The enhanced efficacy is associated with increased dendritic cell maturation and Th-1 predominant response.

A clinical single-center retrospective study was performed by Tomita et al[102] to investigate the role of probiotic treatment on PFS and OS in patients with advanced NSCLC treated with ICI. The study included 118 patients, 39% of whom received probiotics within 6 months of ICI therapy or during treatment. Probiotics were prescribed for alleviation of constipation or diarrhea. To minimize bias arising from participants' baseline characteristics, the authors conducted univariate analysis and survival analysis. They adjusted for several factors including age, sex, smoking history, initial staging, first- or second-line ICI therapy, monotherapy or combination therapies, use of antibiotics, and others. The authors were able to identify the use of probiotics as an independent prognostic factor associated with improved PFS and OS in patients with advanced NSCLC. This study also showed that patients who received antibiotics followed by probiotics had improved survival outcomes when compared with patients who received antibiotics alone. Another study by Takada et al[103] showed that probiotics administration improves PFS in patients with advanced or recurrent NSCLC treated with a PD-1 inhibitor. The study retrospectively analyzed data of 294 patients with NSCLC from multiple centers across Japan and used inverse probability of treatment weight-
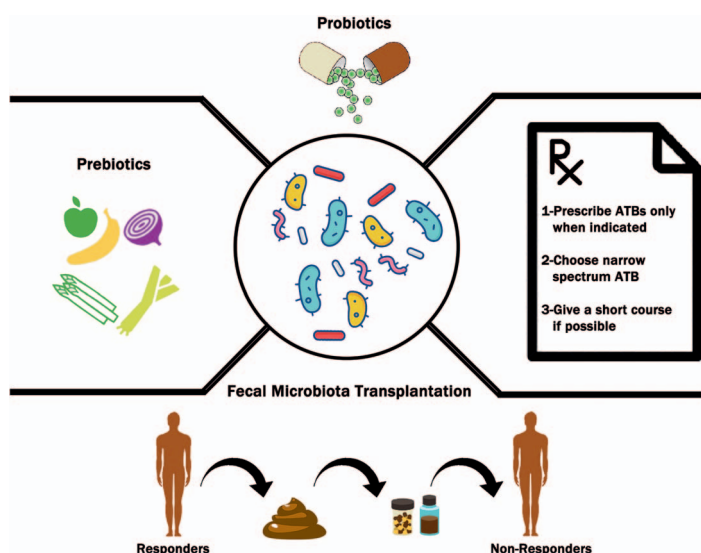
Figure 2. The possible therapeutic interventions to optimize unfavorable gut microbiome into a favorable composition in order to improve the clinical outcomes of immune checkpoint inhibitors (ICIs). These interventions range from complex microbiome transfers in the form of fecal microbiota transplant (FMT), to delivery of microbes by probiotics. Additional interventions include prebiotic use to favor the growth of beneficial gut microbiome and following specific considerations when prescribing antibiotics (ATBs) in patients treated with ICIs.

ing. This study revealed that the use of probiotics is an independent prognostic factor associated with increased PFS but not OS. Overall, the current clinical evidence is still limited. In light of the available promising data, future randomized clinical trials are required to investigate the role of probiotics in modulating response to immunotherapy and assess its therapeutic potential.

Fecal microbiota transplant

In recent years, increased effort uncovered the rising role of FMT as a method of microbiota modulation with therapeutic implications for ICI therapy. A study by Baruch et al[104] highlighted the promising role of FMT in improving therapy outcome. The authors performed an open-label single-armed randomized clinical trial on patients with metastatic melanoma resistant to at least one line of PD-1 inhibitors. Patients initially received oral antibiotics to deplete their baseline intestinal microbiota. Then, they received FMT from metastatic melanoma patients who were previously treated with PD-1 inhibitors and achieved at least 1 year of remission. Three patients had response to treatment, including one patient with complete remission. Davar et al[105] similarly conducted an open-label single-armed study that highlighted the improved clinical benefit in PD-1 refractory melanoma patients after responder-derived FMT, with increase in the abundance of taxa associated with a positive response.

The mechanisms through which a subsequent response to anti–PD-1 therapy was obtained following microbiome manipulation via FMT are interconnected to the remodeling of the gut microbiome–host immunity

interface. Brauch et al[104] readily revealed that participants had an upregulated expression of genes correlated with the innate immune system and the antigen presentation machinery. Moreover, all participants had augmentation of T-cell activation. This pointed out the novel possibility of alternative mechanisms independent of PD-1 signaling that could further contribute to the enhanced antitumor activity. This encompasses a myriad of possible mechanisms that pertain to a noted enhanced signaling through antitumoral immune pathways, that is, increased IFN-γ signaling, escalated CD8+ cellular infiltration into the tumoral microenvironment, production of immunomodulatory metabolites by the microbiome constituents, and exclusion of potentially immunosuppressive microbes via microbiome reconstitution.[106]

Several randomized controlled trials are currently in progress to further investigate the role of FMT as an intervention to enhance the response to immunotherapy. However, to confirm its therapeutic efficacy, future research should focus on conducting double-blinded studies to account for bias or confounding variables that might be affecting the results.

CONCLUSION

In recent years, the intricate interplay between the gut microbiome and overall health has become an area of increasing interest. Growing evidence introduces the notion of relying on microbiome characterization as a prognostic marker capable of forecasting a patient's clinical response or resistance to ICIs. Several studies set out to determine the bacterial composition that influences the clinical response to ICIs, culminating in promising results that linked the presence of certain taxa such as *Akkermansia*, *Ruminococcaceae*, *Faecalibaterium*, *Bacteroides*, and *Bifidobacterium* to positive outcomes including reduction in tumor growth and increase in prolonged PFS and OS in some studies. These results opened doors for further examination as to how the manipulation of the microbiome could potentially affect ICI efficacy. Antibiotic-related dysbiosis has generally been linked to reduced ICI efficacy in most studies. On the other hand, supplementation with probiotics such as *Bifidobacterium*, which restores the gut dysbiosis, has emerged as a potential method through which augmentation of response to ICIs is possible. Additionally, alteration of the microbiome via FMT is being investigated in multiple randomized clinical trials to further elucidate its role as a therapeutic intervention able to shift outcomes in patients receiving ICIs.

Figure 3 provides a brief graphic summary of this review. We examined the most up-to-date evidence establishing the association between the gut microbiome and response to ICIs, the factors that influence the composition of the gut microbiome, possible therapeutic interventions to modulate the microbiome (Figure 2), and we presented some key unanswered questions to be
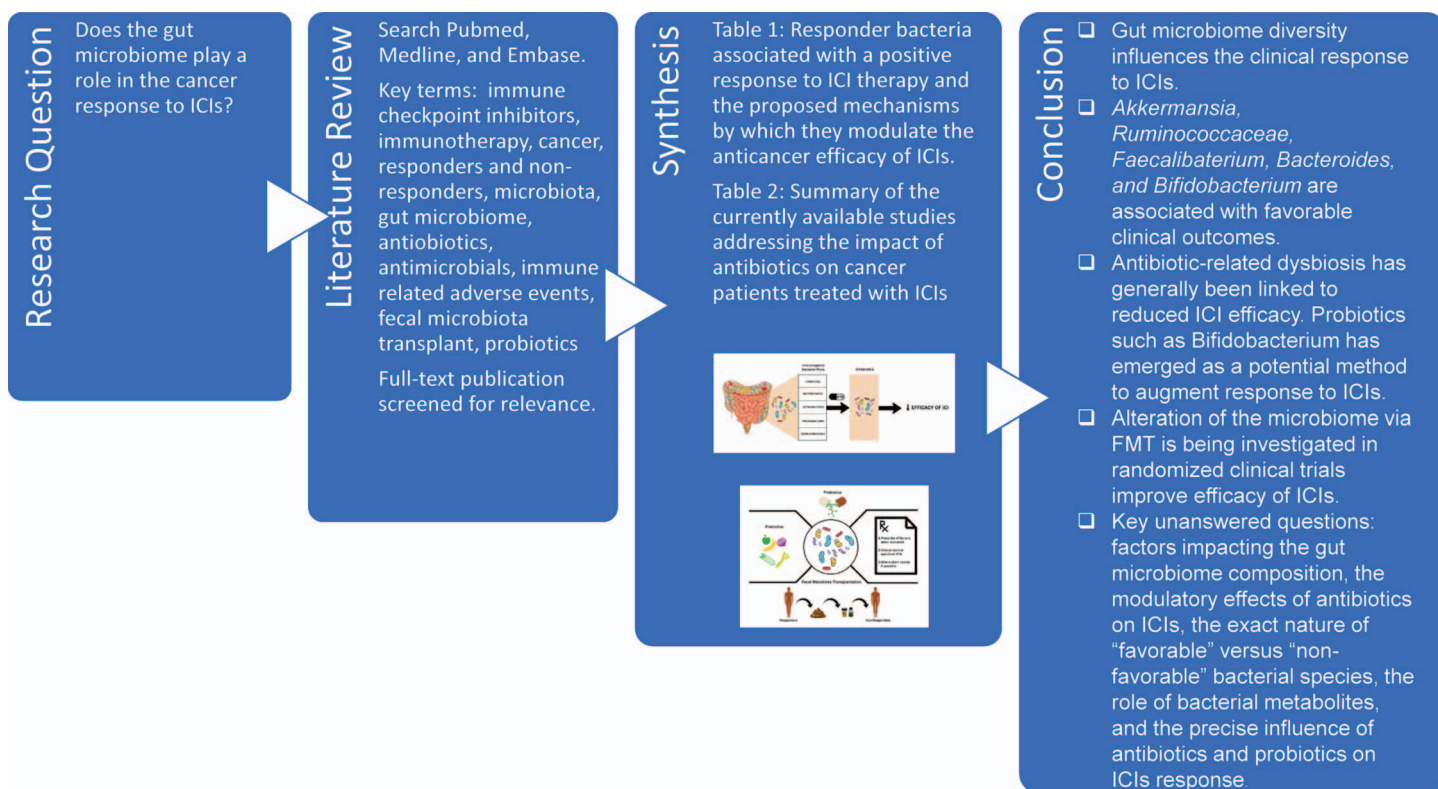
Figure 3. Graphic summary of the emerging role of the gut microbiome in the cancer response to immune checkpoint inhibitors (ICIs). The baseline gut microbiome and its alteration with antibiotics have been shown to affect ICI efficacy by tailoring the immune system. Probiotics and fecal microbiota transplant (FMT) provide promising therapeutic interventions to enhance the response to ICIs.

addressed in future research (Figure 3). Complexity arises from the presence of several uncertainties on the matter, such as the factors impacting the gut microbiome composition, the modulatory effects of antibiotics on ICIs, the exact nature of "favorable" versus "non-favorable" bacterial species, the role of bacterial metabolites, and the precise influence of antibiotics and probiotics on ICIs response. More studies are warranted to better characterize the exact nature of the microbiome-ICI interaction, to propose novel and reliable biomarkers that enable prediction of future response or resistance to ICIs. It is important that future studies focus on standardization of sampling, sequencing techniques, data analysis, and study design in order to minimize the inconsistencies amongst the present data.

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Author Interview

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