Trends in Cancer

CellPress

Review

Gut Microbiota in Cancer Immune Response and Immunotherapy

Cheng-Bei Zhou,[1] Yi-Lu Zhou,[1] and Jing-Yuan Fang[1,*]

The gastrointestinal tract (GIT) is the largest immune organ and maintains systemic immune homeostasis in the presence of bacterial challenge. Immune elimination and immune escape are hallmarks of cancer, both of which can be partly bacteria dependent in shaping immunity by mediating host immunomodulation. In addition, host immunity regulates the microbiome by altering bacteria-associated signaling to influence tumor surveillance. Cancer immunotherapy, including immune checkpoint blockade (ICB), appears to have heterogeneous therapeutic effects in different individuals, partially attributed to the microbiota. Thus, the microbiome signature can predict clinical outcomes, prognosis, and immunotherapy responses. In this review, we summarize the intricate crosstalk among the gut microbiome, cancer immune response, and immunotherapy. Interactive modulation of the host microbiota provides new therapeutic strategies to promote anticancer therapy efficacy and/or reduce toxicity.

Highlights

Immunotherapy is a promising treatment for cancer, the responses to which might be affected by the gut microbiota. Demonstrating its underlying mechanism could lead to new strategies to treat cancer.

Previous studies observed a mutual relationship between the gut microbiota and gastrointestinal immunity. Immunity shapes gut microbes, and vice versa, mainly through adaptive immunity, either in a beneficial or harmful manner.

The gut microbiota might also act as a modulator of gut immunity. Fecal microbiota transplantation and antibiotics administration might influence the efficacy and toxicity of immunotherapy via the microbiota.

Increasing research is being performed in the field of microbiota precision medicine. Using precise microbial modulation could make ICB therapy safer and more effective.

Introduction

The GIT is not only an important site of digestion and absorption, but also the largest immune organ in the body, harboring 60–80% of the general immune cells of the host, as well as structures that maintain gut immune homeostasis in the presence of bacterial challenge [1]. The GIT immune system confronts an array of challenges compared with other organs because of its constant microbial load. Carcinogenesis is interrelated with human immune status and environmental factors, among which the gut microbiota and its metabolites have been discussed widely over the past decade. Commensal and pathogenic bacteria have an intricate immunoregulatory impact on systemic cancer immunity [2]. In turn, cancer can also affect the gut bacterial composition, which regulates the tumor microenvironment (TME), contributing to immune inhibition [3]. Therefore, cancer and self-specific immune cells might cross-react with bacteria.

Microbiota can influence both tumor development and treatment response [4]. Cancer immunotherapy has been described as a major scientific breakthrough in cancer treatment. ICB is at the forefront of immunotherapy development, mostly owing because of its extensive bioactivity among distinct histopathological cancer types and efficacy against metastatic tumors [5]. The most prominent immune checkpoints include programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which suppress T cell activation and, thus, diminish immune responses against cancer. Inhibitors of these immune checkpoints could reverse this unfavorable situation. Regrettably, <30% of patients benefit from ICB [6]. The heterogeneous clinical response to ICB is multifactorial and can be divided into two aspects: (i) tumor-intrinsic factors (mutational status and oncogenic signaling); and (ii) tumor-extrinsic factors. The TME, metabolic factors, host (age and genetics), and environmental factors (microbiota and diet) are major tumor-extrinsic factors [7].

[1]Division of Gastroenterology and Hepatology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University; Shanghai Institute of Digestive Disease; State Key Laboratory for Oncogenes and Related Genes, Key Laboratory of Gastroenterology & Hepatology, Ministry of Health, 145 Middle Shandong Road, Shanghai, China

*Correspondence: jingyuanfang@sjtu.edu.cn (J.-Y. Fang).

In this review, we emphasize the crosstalk among the gut microbiome, cancer immune response, and cancer immunotherapy, by providing evidence from both preclinical studies and clinical trials. We discuss the intrinsic mechanisms involving the gut microbiota and highlight promising technologies that might lead to new therapeutic avenues for cancer immunotherapy.

Immunomodulatory Effects between the Host and the Gut Microbiota in the Cancer Immune Response

Host immunity regulates the gut microbiome to maintain homeostasis and, in turn, the microbiota also shapes host immunity (Table 1) [8–29]. The gut microbiome can affect not only gut immunity, but also immune responses in distal mucosal sites via the circulation, systemic metabolism, and immune modulation (Figure 1) [1].

The GIT is heavily exposed to microorganisms and foreign antigens. Therefore, the mucosal immune system (MIS) emerges as the main barrier against pathogen invasion [1]. Intestinal epithelial cells (IECs), as one of the barriers in the MIS, widely express classical pattern recognition receptors (PRRs), such as NOD domain-like receptors (NLRs) and Toll-like receptors (TLRs), which communicate with microorganisms via initial recognition of lipopolysaccharide (LPS), flagellins, bacterial peptidoglycans, and cell wall lipoproteins [30] (Figure 1).

The relationship between immunity and tumor cells can be divided into three phases: (i) tumor immune elimination; (ii) immune equilibrium; and (iii) immune escape. Immune elimination refers to the recognition and removal of tumor cells by immune cells, whereas immune equilibrium is a dynamic balance whereby cancer cells cannot be either completely removed or grow rapidly because they are still controlled by immunity. Immune escape is the ultimate phase, in which escaped tumor cells grow independently of the host immune system (Figure 2). Thus, immunity has a dual role in tumorigenesis. Here, we focus on the gut microbiota in adaptive and innate immune responses in cancer.

Adaptive Immune Response between the Host and the Gut Microbiota in Cancer

The adaptive immune response is more specific to antigens, and is separate from the innate immune response, which can be affected by the gut microbiota in a beneficial or harmful way. For example, fecal bacteria, especially *Bacteroides fragilis*, from $Apc^{Min/+}$ mice, were associated with mucosal dysplasia, increased numbers of polyps, and increased proportions of T helper (Th) 17 ($CD4^+$ IL-17^+) and Th1 ($CD4^+$ IFN-γ^+) cells, thus triggering signal transducer and activator of transcription 3 (STAT3) stimulation in colorectal cancer (CRC) [31]. Enterotoxigenic *B. fragilis* (ETBF) also promoted oncogenesis via its toxin BFT and interleukin (IL)-17 on colon epithelial cells. This resulted in the recruitment and differentiation of myeloid cells into myeloid-derived suppressor cells (MDSCs), which can upregulate nitric oxide synthase 2 (NOS2) and arginase 1 (ARG1), generate NO, and inhibit T cell proliferation in the TME [32] (Figure 1).

Fusobacterium nucleatum is also associated with CRC, and has been found to inhibit anticancer T cell-mediated adaptive immunity [33]. The interaction between *F. nucleatum* fibroblast activation protein 2 (FAP2) adhesin and human T cell immunoglobulin and ITIM domain (TIGIT) expressed on natural killer (NK) cells, blocked NK cell activities to enable *F. nucleatum* to evade antitumor immunity [34]. *F. nucleatum* also selectively recruits tumor-infiltrating myeloid cells, thus promoting inflammation in the TME, which favors colon neoplasia. In this regard, MDSCs were enriched in *F. nucleatum*-fed $Apc^{Min/+}$ mice compared with controls, and could significantly inhibit T cells [35] (Figure 1). T cells recognized certain bacteria, indicating the possibility of bacteria sharing common antigens with tumor cells [36].

Table 1. Interplay between the Gut Microbiota and Host Immune System to Maintain Homeostasis[a]

Bacteria/bacterial metabolites	Immune system components	Major findings	Refs
Gut microbiota shape immunity			
Innate immunity			
Commensal microbiota	Innate immunity	Indole-derivatives decomposed by commensals promote barrier functions mediated by AhR	[8]
	ILCs	Commensal bacteria induce NKp46$^+$ RORγt$^+$ ILC cell differentiation	[9]
SFB	ILCs	SFB enhances IL-22 production from ILC3	[10]
Lactobacillus casei	Innate immune system	*L. casei* stimulates anti-inflammatory effects by suppressing NF-κB pathway to mitigate *Shigella flexneri* infection	[11]
SCFAs	IECs	SCFAs intensify epithelial barrier function through PXR	[12]
		SCFAs prompt myelopoiesis	[13]
	Myeloid cells	SCFAs change gene expression profile of local macrophages	[14]
	Plasma cells	SCFAs promote immune function by IgA generated by plasma cells	[15]
Butyrate	IECs	Downregulation of proinflammatory cytokine expression in IECs requires signal from butyrate	[16]
Flagellin	ILCs, DCs	IL-23-mediated IL-22 generation by ILCs requires flagellin sensing by CD103+CD11b+ DCs	[17]
	DCs	DCs interact with flagellin via expression of chemokines, cytokines, and antimicrobial peptides	[18]
LPS	Monocytes	LPS induces migration of monocytes from bone marrow dependent on CCR2	[19]
Adaptive immunity			
Clostridia	Tregs	CD4$^+$ Tregs can be induced by Clostridia spp. *Clostridium* induces Treg differentiation and their expression of IL-10	[20]
Bacteroides fragilis	Tregs	*B. fragilis* induces Treg production and differentiation via TLR2 receptor activation	[21]
	Th17 cells	*B. fragilis* PSA stimulates IL-10 response in T cells to prevent Th17 cell expansion and gut barrier damage	[21]
	CD4$^+$ T cells, Th1 cells	*B. fragilis*-produced PSA induces accumulation of CD4$^+$ T and Th1 cells in circulation	[22]
Bifidobacterium adolescentis	Th17 cells	*B. adolescentis* induces accumulation of Th17 cells	[23]
Alcaligenes	IgA of B lymphocytes	*Alcaligenes* induces IgA generation by interacting with CD11$^+$ DCs	[24]
Commensal microbiota	Tregs	Commensal microbiota produce a specific set of Tregs, which present TCRs against commensal antigens, in murine intestines	[25]
SFB	Th17 cells	SFB assists in induction and differentiation of intestinal Th17 cells and its active sampling by DCs	[26]
Immunity control gut microbiota			
Commensal microbiota	Innate immune system	Fucosylated proteins shedding into gut lumen become an energy source for commensal microbiota when intestinal ecosystem is temporarily disturbed	[27]
Alcaligenes	ILCs	The containment of *Alcaligenes* is induced via IL-22 produced by ILCs	[28]
Bacteroidetes	IECs	Mice deficiency in IEC expression of NLRP6 develop immune-driven dysbiosis with higher abundance of *Bacteroidetes*	[29]

[a]Abbreviations: AhR, aryl hydrocarbon receptor; DC, dendritic cell; IECs, intestinal epithelial cells; ILCs, innate lymphoid cells; PSA, polysaccharide-A; PXR, pregnane X receptor; SFB, segmented filamentous bacteria; TCR, T cell antigen receptor; Treg, regulatory T cell.

Under certain circumstances, the commensal microbiome can promote immune clearance by facilitating tumor immunosurveillance. Bacteria-reactive responses of circulating CD8$^+$ T cells, depending on antigen-presenting monocytes, were identified in patients with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) compared with healthy controls. In addition, the disease-free survival duration after tumor resection associated positively with the frequencies of

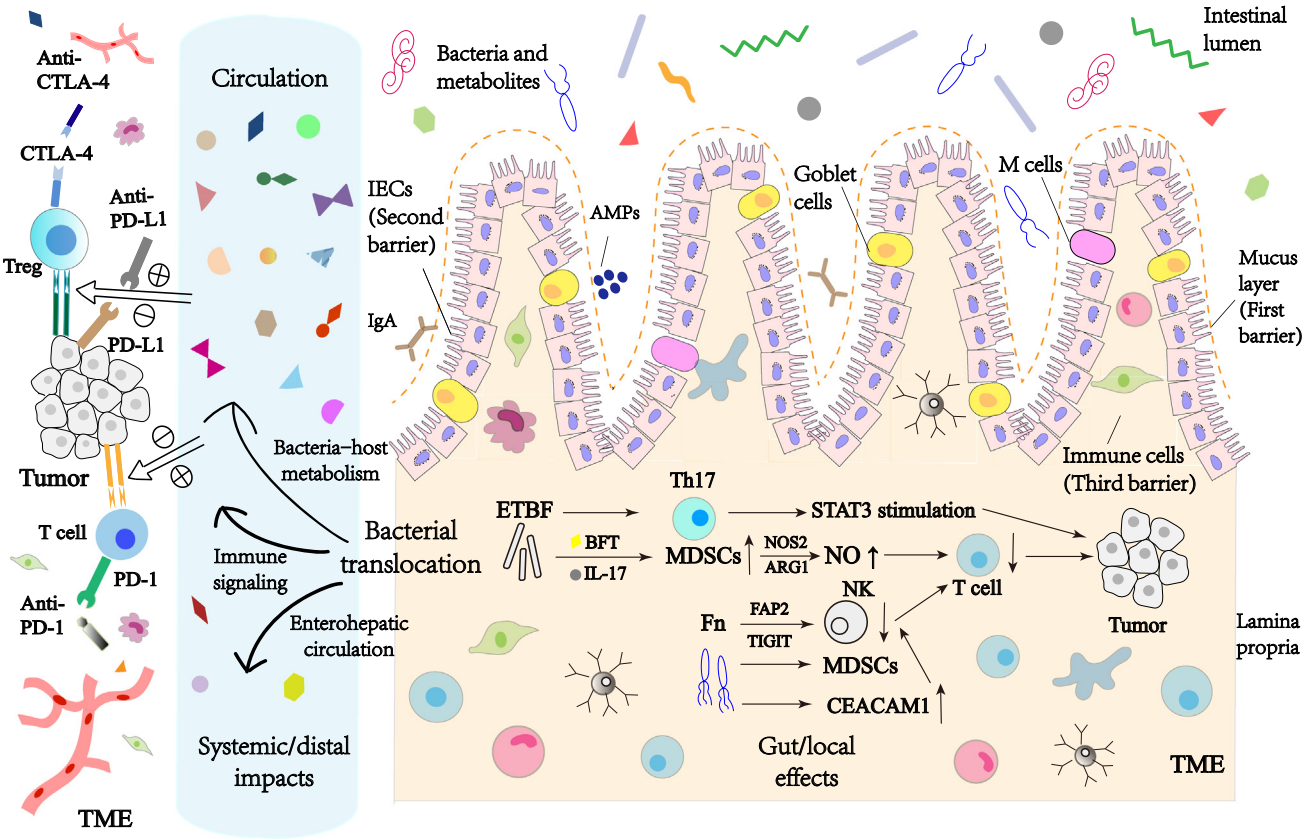

Figure 1. The Interaction between the Gut Microbiota and Cancer Immune Response. In the colon, the mucosal immune system (MIS) emerges as the main barrier defense against pathogen invasion [1]. The mucus layer acts as the first barrier and helps keep microbes away from intestinal epithelial cells (IECs; second layer, mainly comprising columnar epithelial cells, goblet cells and M cells), which activate intraepithelial lymphocytes to generate effector cytokines that collaboratively function on immune cells (third layer) in Peyer's patches and mesenteric lymph nodes residing on lamina propria [1,69]. MIS is critical for bacteria phagocytosis and provides secretory immunoglobulin (Ig)-A and IgM [15,16]. The antitumor/procarcinogenesis local effects in the gut may be expanded to distal sites through bacterial translocation into host circulation mainly by means of immune signaling, metabolism of microbial metabolites, and enterohepatic circulation. The systemic effects are displayed when associated signaling pathways are up/downregulated. Thus, the immune response to programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) of tumors outside the gut can be enhanced/reduced. Also shown are the major mechanisms currently known to be related to colon tumorigenesis. Enterotoxigenic *Bacteroides fragilis* (ETBF) triggers signal transducer and activator of transcription 3 (STAT3) stimulation in a T helper (Th)-17-dependent pathway in colitis-associated colorectal cancer (CRC) [32]. ETBF also promotes oncogenesis driven by *Bacteroides fragilis* toxin (BFT) and interleukin (IL)-17 on colon epithelial cells through the recruitment of myeloid cells to the tumor microenvironment (TME), and induces them to differentiate into inducible nitric oxide synthase $(iNOS)^{hi}$ monocytic myeloid-derived suppressor cells (Mo-MDSCs), which can upregulate nitric oxide synthase 2 (NOS2) and arginase 1 (ARG1), generate nitric oxide (NO), and inhibit T cell proliferation [63]. In addition, the interaction between *Fusobacterium nucleatum* fibroblast activation protein 2 (FAP2) adhesin and human T cell immunoglobulin and ITIM domain (TIGIT) blocks natural killer (NK) cell activities to enable *F. nucleatum* to evade antitumor immunity [45]. *F. nucleatum* binds to, and induces, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) expression to inhibit activities of NK and T cells [65]. It also electively recruits tumor-infiltrating myeloid cells, thus regulating the TME to promote inflammation conducive for colon neoplasia. In this regard, MDSCs are enriched and significantly inhibit T cells [46]. Abbreviations: AMPs, antimicrobial proteins; M cells, membranous cells; Fn, *Fusobacterium nucleatum*; Treg, regulatory T cells.

Enterococcus hirae-reactive and *Bifidobacterium longum*-reactive $CD8^+$ T cells [37]. Patients with tumor-resected pancreatic ductal adenocarcinoma (PDAC) and long-term survival displayed a more diverse microbiome landscape, including *Streptomyces*, *Pseudoxanthomonas*, and *Saccharopolyspora*, which induced $CD8^+$ T cell-dependent antitumor immune responses [38], suggesting that the composition of the tumor microbiome influences host immune responses and patient outcome.

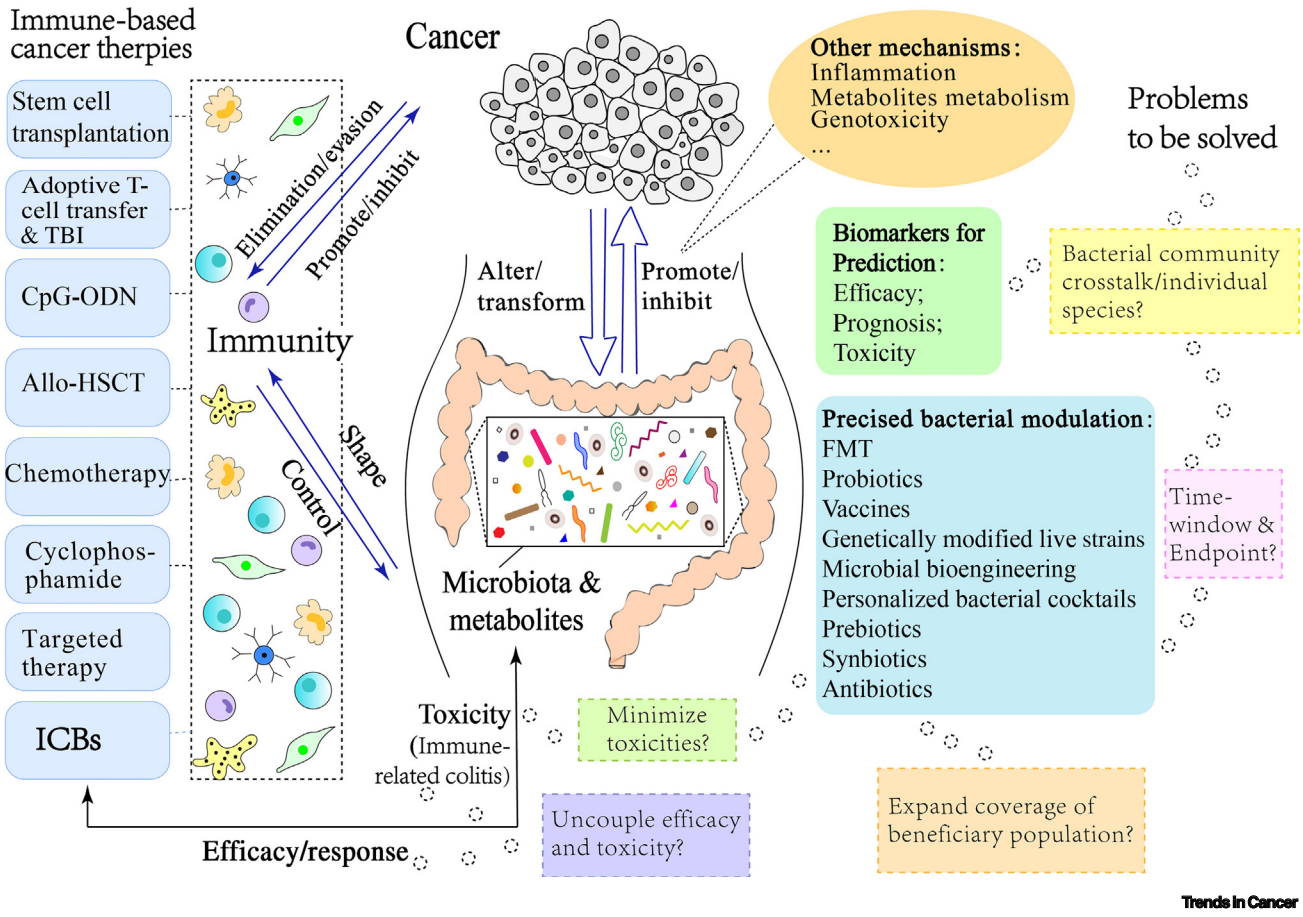

Figure 2. The Interplay among Gut Microbiota, Cancer Immune Response, and Cancer Immunotherapy and Perspectives for Precise Microbiota Manipulation. There exists a complex association among the enormous microbiota community colonizing the gut and their metabolites, host immune system, and cancer. From the inside out, microbiota and their metabolites shape host immunity by mediating host immune disorders and consequently affecting tumor onset and progression. From the outside in, the host immunity controls the microbiome by altering microbe-related signaling or metabolic functions to influence tumor surveillance. Carcinogenesis is promoted/inhibited through immune evasion/elimination. Moreover, gut microbiota can also induce carcinogenesis via other mechanisms, such as genotoxicity, inflammation and metabolism. Conversely, cancer also transforms the gut microbiome to intensify/reduce responses to immune-based therapies, including immune-checkpoint blockade (ICB). Occasionally, ICB results in immune-related adverse effects (irAEs)/toxicity in the host of, which the most severe is immune-related colitis. This figure shows the role of microbiota in future applications involving biomarkers for immunotherapy efficacy, prognosis, and toxicity, as well as précised manipulation, such as fecal microbiota transplantation (FMT), probiotics, prebiotics, vaccines, antibiotics, and bacterial bioengineering. However, multiple gaps and problems remain to be solved in future research. Abbreviations: allo-HSCT, allo-hematopoietic stem cell transplantation; CpG-ODN, CpG-oligodeoxynucleotides; TBI, total body irradiation.

Innate Immune Response between the Host and the Gut Microbiota in Cancer

Innate lymphocytes enriched in the gut mucosa or other digestive organs assist the coordination of immune equilibrium and express cytokines to exert immunoregulatory activities [39]. In a cohort of patients with CRC, bacteria-dependent activation of transcription factor 6 (ATF6) induced early gut dysbiosis, epithelial barrier damage, and innate immune signaling that promoted tumorigenesis. In nATF6IEC MyD88/TRIF-knockout mice, bacterial penetration into the mucus induced MYD88 innate immune signal transduction adaptor (MYD88)/TLR adaptor molecule 1 (TRIF)-dependent Stat3 activation to promote tumor growth [40]. Compared with controls, a dominant distribution of *B. fragilis* and *Escherichia coli* existed in the colonic mucosa of patients with familial adenomatous polyposis. Co-colonizing azoxymethane (AOM) and $Apc^{Min\Delta716/+}$ GF mice with enterotoxigenic *B. fragilis* and *E. coli* jointly led to tumor susceptibility, with an increase in IL-17

that was released by both γδT17 and Th17 cells [41]. Likewise, colon polyposis in *Apc*$^{Min/+}$ mice was stimulated by bacterial accumulation in polyps, inducing local inflammatory responses. IL-10 derived from T cells and regulatory T cells (Tregs) inhibited colonic inflammation, bacterial accumulation in polyps, and polyp growth [42]. Commensal bacteria accelerated tumorigenesis by inducing differentiation and maturation, which led to immunocytokine production [43].

In patients with PDAC, bacterial ablation by oral antibiotics was found to reprogram the TME via TLR signaling [44]. In this context, MDSC infiltration was reduced, macrophage 1 differentiation was enhanced, and Th1 differentiation of CD4+ T cells and CD8+ T cell activation was stimulated. Taken together, these findings imply an intricate interplay between the innate immunomodulating role of specific bacteria and multiple tumors.

Gut microbes can also benefit the innate antitumor immunity. Mice lacking ubiquitin ligase ring finger protein 5 (RNF5), a major molecule in T cell affusion, displayed a transformed gut microbiota with anticancer immunity capability against melanoma. Eleven bacterial strains, mainly members of *Bacteroides* and *Parabacteroides*, were enriched in *Rnf5*$^{-/-}$ mice, which suppressed tumor growth. Altered IEC immunogenicity and antimicrobial peptides both influenced the gut microbiome constitution and activation of dendritic cells (DCs) and T cells to inhibit melanoma growth [45]. Broad-spectrum antibiotics could eliminate symbiotic microflora, thereby inducing engrafted melanoma and lung cancer progression in mice. In antibiotic-treated mice, the γδT17 cell response was impaired and the subsequent release of IL-17, IL-23, and IL-6 was reduced [46].

There is also a correlation between microbial immunomodulatory metabolites and immune responses, suggesting future tumor therapeutic manipulation [47]. Certain metabolites produced by microbes might affect adaptive and innate immunity. As one of the byproducts of microbial fermentation of fiber, short-chain fatty acids (SCFAs) facilitate colonic Treg expansion by suppressing histone deacetylase (HDAC) activity and targeting Forkhead box P3 (FOXP3), the major nuclear transcription factor of Tregs [48,49]. Tregs either exhibit anti-inflammatory effects against carcinogenesis or mitigate anticancer responses when they infiltrate the TME [50]. SCFAs can also increase the number of macrophage precursors and intensify CD8$^+$ T cell function by influencing cellular metabolism, maintaining an equilibrium between innate and adaptive immunity [51]. In particular, butyrate can boost activated T cell elimination via Fas cell surface death receptor (FAS) upregulation [14,49,52].

Other microbial metabolites, such as polyamines, restrain anticancer immunity by inhibiting lymphocyte proliferation and inducing the generation of tumor-derived proteases that heighten tumor cell invasiveness. In patients with obesity-associated HCC, lipoteichoic acid (LTA), derived from Gram-positive bacteria, aggravated tumor burden through its translocation to the liver. Mechanistically, LTA induces a senescence-associated secretory phenotype together with the bacterial metabolite deoxycholic acid, thereby upregulating cytochrome C oxidase subunit 2 (COX2) expression via TLR2-mediated signaling. Subsequent prostaglandin E2 (PGE2) production mediated by COX2 inhibits anticancer immunity via the prostaglandin E receptor 4 (PTGER4) [53].

Collectively, the impact of gut microbiota on the cancer immune response mainly manifests as follows: activation of regulatory T cell proliferation and differentiation; induction of IgA expression; and the influence of antibacterial peptide expression, microbial metabolism, the regulation of systemic inflammation, and bacterial translocation. Notably, key questions remain (see Outstanding Questions), which will be vital for mechanistic research and will affect subsequent research directions.

The Impact of Gut Microbiota on the Responses to Cancer Immunotherapy

Cancer immunotherapy can effectively control tumors by restarting the tumor–immune loop and restoring host antitumor immune responses. Currently, classical tumor immunotherapies include ICB, therapeutical monoclonal antibodies (mAbs), cancer vaccines, adoptive T cell transfer, small-molecule inhibitors, and immune system modulators. ICB appears to exert heterogeneous therapeutic efficacy on different individuals [5]. In recent years, a growing number of studies have jointly underlined that fecal and/or gut microbiome signatures are predictive of clinical outcomes, prognosis, and responses to immunotherapy. Indeed, modulation of the host–microbe interaction provides new therapeutic strategies to target certain specific bacteria to promote anticancer therapy efficacy or reduce toxicity.

The Effects of Gut Microbiota on ICB Responses

Recently, efforts have centered on exploiting the effective clinical efficacy or prognosis indicators for ICB for immunotherapy responses. Antibodies targeting PD-1 and PD-L1, namely atezolizumab, nivolumab, pembrolizumab, durvalumab, avelumab, toripalimab, sintilimab, and camrelizumab, and CTLA-4 blockers, such as ipilimumab, are approved by the US Food and Drug Administration (FDA) to treat lymphoma, melanoma, non-small cell lung cancer (NSCLC), prostate cancer, bladder cancer, and kidney cancer [54]. There is mounting evidence that members of the gut microbiome can serve as prognostic biomarkers to predict patient response to ICB therapies [55–57]. A marked difference in microbiomes was observed between patients with different ICB prognoses. In the first global prospective study in 2017, human gut microbiota, and metabolomic and metagenomic outlines were analyzed in 39 patients with melanoma who received anti-CTLA-4 (ipilimumab) combined with anti-PD-1 (nivolumab) immunotherapy (IN) or just anti-PD-1 therapy (P) [55]. *Holdemania filiformis*, *Bacteroides thetaiotaomicron*, and *Faecalibacterium prausnitzii* were enriched among IN responders, whereas *Dorea formicogenerans* was enriched among P responders [55]. 16S ribosomal RNA (rRNA) sequencing in stool samples previously revealed a relevant increase in *Burkholderiales* and *Bacteroidales*, as well as a decrease in *Clostridiales* in anti-CTLA-4 responders [56], and suggested that mucosal damage mediated by CTLA-4 blockade was the main cause of gut microflora alteration. In addition to melanoma, another larger-scale cohort included patients with advanced epithelial tumors [e.g. renal cell carcinoma (RCC, n = 67), NSCLC (n = 140), and urothelial carcinoma (n = 42], which revealed that patients with higher fecal abundance of *Akkermansia muciniphila* achieved a better response to anti-PD-1 therapy [57]. Moreover, fecal microbiota transplant (FMT) from responders (R-FMT) and nonresponders (NR-FMT) into germ-free (GF) mice confirmed the microbial modulation of antitumor immune responses [56,57]. Compared with R-FMT mice, NR-FMT mice had more rapid tumor growth and poorer responses to anti-PD-1 therapy, which indicated that the gut microbiota could be a modulator of the responses to ICB therapies.

The gut microbiota might have a close relationship with ICB treatment responses; however, the underlying mechanisms remain unknown. Most of studies focus on the adaptive immunity induced by the gut microbiota during ICB treatment. One suggested mechanism is that gut microbes promote antitumor $CD8^+$T cell responses during ICB treatment. Early in 2015, 16S rRNA gene sequencing in mice found that *Bifidobacterium OTU_681370* was enriched most in JAX-fed transverse aortic constriction (TAC) mouse feces, displaying the strongest positive correlation with peripheral and intratumoral $CD8^+$T cell responses. Oral supplementation of a probiotic cocktail containing live *Bifidobacterium* spp. (*Bifidobacterium longum* and *Bifidobacterium breve*) inhibited melanoma growth and facilitated tumor-specific antitumor $CD8^+$T cell responses, which almost paralleled the efficacy of anti-PD-L1 therapy [58]. In addition, *E. hirae* strain 13144, which was increased in responders (R) compared with nonresponders (NR) among 32 patients

with NSCLC, elevated peripheral $CD8^+$ or $CD4^+$ T cell responses, enhanced IFNγ production, and extended progression-free survival (PFS) in the R group [57]. Moreover, 11 bacterial strains, containing seven *Bacteroidales* spp. from healthy donor stools [59], was shown to facilitate the accumulation and recruitment of intestinal $IFN\gamma^+$ $CD8^+$ T cells, without causing innate immunomodulation, which depended on lamina propria $CD103^+$ DCs and MHC class Ia.

Another mechanism could be that gut microbes influence the Th1 immune response. Immunogenic *B. fragilis* or *Bacteroides thetaiotaomicron* influence IL-12-dependent Th1 immune responses and are associated with anti-CTLA-4 efficacy. In a RET melanoma mouse model, the anti-CTLA-4 effect was diminished in GF mice and specific-pathogen-free (SPF) mice that were treated with a 2-week course of broad-spectrum antibiotics. Oral administration of *Bacteroides romine* combined with *B. thetaiotaomicron* or *Burkholderia cepacia* to GF mice stimulated Th1 immune responses in draining lymph nodes and maturation of intratumoral DCs, resulting in restoration of anti-CTLA-4 efficacy [56].

Gut microbes might also modulate TH17 cells, which in turn affect the TME. Researchers investigated whether PDAC metastasis contained an abundant microbiome that matched the intestinal microbiota. The unique TME in PDAC was infiltrated by $IL\text{-}17A^+$ $CD4^+$ $Th17^+$ cells at the cost of anticancer $IFN\text{-}\gamma^+CD4^+$ Th1 cells to block differentiation. Oral antibiotics significantly mitigated the tumor burden and reprogrammed TME immunogenically, especially via the adaptive immune responses [44].

Moreover, *Akkermansia muciniphila* was found in various studies to influence ICB responses by modulating innate immunity, although the mechanism remains unclear. However, *A. muciniphila* could be used to predict or enhance the ICB response, given that it was reported to have a mucosal healing capacity. When *A. muciniphila* was fed orally to NR-FMT mice, anti-PD-1 efficacy was preserved, which depended on IL-12 by promoting $CCR9^+CXCR3^+CD4^+$ T lymphocyte recruitment into the tumor beds [60]. Inoculation of *A. muciniphila* and *E. hirae* into GF mice intensified anti-PD-1 efficacy in metastatic melanoma, NSCLC, and RCC [57]. Another study addressed the positive role of *A. muciniphila* in inducing T follicular helper cell-dependent IgG1 responses in mice [61]. The specific mechanism of by which *A. muciniphila* exerts its effects were not clearly demonstrated in either study; however, it appears to correlate with type 1 immunity.

Future research for clinical translation is expected to exploit the tremendous potential of appropriate bacteria for the prevention, target acquisition, and treatment of cancers (Figure 2).

Antibiotics and ICB Efficacy

Antibiotics are commonly used prophylactically or therapeutically in patients with cancer because of their susceptibility to infections. Nonetheless, studies have shown that antibiotic administration at inappropriate times could decrease the efficacy of immunotherapies [46,57,62–70]. Therefore, clinicians should cautiously weigh the pros and cons of antibiotic use.

It was suggested that broad-spectrum antibiotic administration, even its chronic excessive exposure under definite or latent infections, would lead to intestinal dysbiosis and impaired immune cell responses [63–65]. The median survival of patients treated with antibiotics before or immediately after anti-PD-1 treatment was almost half that of those who did not receive antibiotics [57]. Poorer response rate and shorter overall survival (OS) or progression-free survival (PFS) were also observed when antibiotics were used before or after ICB therapy in patients with advanced cancer [71,72]. The positive correlation between long-term exposure to antibiotics and cancer risk has

been reported in breast, gastric, esophageal, renal, and lung cancers [69,70,73–75]. Similarly, combined broad-spectrum antibiotic (vancomycin, ampicillin, metronidazole, and neomycin) injection into mouse models of either NSCLC or melanoma inhibited the protective IL-17-producing γδT17 cell response and ultimately enhanced the development of metastasis [46]. These results provide support for the adverse effects of antibiotics in tumor progression.

Nevertheless, these studies were unable to confirm the direct negative impact of antibiotics on anti-PD-1 efficacy because most were either animal experiments or retrospective studies, the results of which must be confirmed by prospective studies. In this regard, a larger sample size, multicenter, prospective cohort study, which included 196 patients with NSCLC, melanoma, RCC, and head and neck cancer, indicated a worse response and OS in recipients of PD-1/PD-L1 antibodies after antibiotic use [62]. Surprisingly, patients achieved worse clinical outcomes when they were administered broad-spectrum antibiotics once a month before, rather than concurrent with, ICB therapies [62]. ICB therapy following antibiotics appeared to be even worse than no primary treatment. Another clinical trial examined the clinical outcome of antibiotics (quinolones or β-lactam) administration within 30 days of therapy outset in patients with advanced RCC or NSCLC who received anti-PD-(L)1 mAbs alone or together with anti-CTLA-4 mAbs [68]. The results showed that antibiotics reduced the OS in NSCLC and PFS in RCC. Additionally, the clinical benefit to those patients with antibiotics usage within 30 days before ICB therapy was poorer than in those who received antibiotics 60 days before ICB [68]. Collectively, these findings indicate the significance of the timing of antibiotic use during immunotherapy.

Some studies have reached different conclusions. One retrospective study among 74 patients with NSCLC undergoing nivolumab and prior antibiotic medication found no obvious difference in efficacy or PFS [76], whereas other studies indicated that oral antibiotics could block the development of melanoma, pancreatic, and liver cancers [77,78].

Despite such conflicting outcomes, most researchers consider that antibiotics diminish the ICB response. Thus, the dosage and duration of antibiotics treatment should be considered thoroughly by clinicians before starting such treatment. It is also confusing whether patients should take probiotics or prebiotics to reverse the diminishing ICB response caused by antibiotics treatment. Such problems remain unsolved.

Gut Microbiota and ICB Therapy Toxicity

Similar to other cancer therapies, ICB can lead to immune-related adverse effects (irAEs), which include gut mucosal injury, intestinal barrier damage, and bacterial translocation resulting from increased gut permeability [79]. One of the high-risk toxicities is ICB-related colitis. Currently, there are few approaches that can be explored or applied to treat patients with ICB-related colitis.

Two prospective studies analyzed the association between ICB-related colitis and gut bacteria. In the studies, 34 patients with metastatic melanoma treated with ipilimumab with a lower fecal abundance of *Bacteroidetes* developed an increased risk of colitis because of the stimulation of Treg differentiation [80]. Shotgun metagenomic sequencing also found that a lower prevalence of two modules [i.e., microbial polyamine transport and biosynthesis of vitamin B (B1, B2, and B5)] could lead to higher susceptibility to colitis following anti-CTLA-4 therapy, with a sensitivity of 70% [80]. Among 26 patients with metastatic melanoma undergoing ipilimumab treatment, *Faecalibacterium* and *Firmicutes* enrichment in feces appeared to partially account for ipilimumab-mediated colitis, despite a better clinical response. This is the so-called efficacy–toxicity coupling effect [81]. This response might be associated with decreased baseline circulating $CD4^+$ Tregs and inflammatory cytokines, such as IL-6, IL-8, and sCD25 [81]. The occurrence of adverse

Table 2. Clinical Trials on Gut Microbiota Modulation in Cancer Immunotherapy[a]

Clinicaltrials.gov. identifier	Cancer	Type of trial	Population (*N*, age)	Intervention	Objective	Primary outcome/endpoint	Status	Location
FMT								
NCT04056026	Metastatic mesothelioma	Phase I	1; age: child/adult	FMT from healthy family donor (transferred by colonoscopy): concurrent with anti-PD-1 therapy	Treatment: to enhance efficacy of pembrolizumab with single-dose FMT	PFS	Completed (September 2018–December 2018)	USA
NCT03353402	Advanced melanoma	Phase I	40; age ≥18	FMT from responders (transferred by colonoscopy and capsule): after anti-PD-1 therapy	Treatment: to evaluate efficacy of FMT in stage III/IV patients who failed anti-PD-1	Proper implant engraftment	Recruiting (November 2017–December 2021)	Israel
NCT03341143	Advanced melanoma	Phase II	20; age ≥18	FMT from responders (transferred by colonoscopy): concurrent with anti-PD-1	Treatment: to evaluate efficacy of FMT in patients resistant to pembrolizumab	ORR	Recruiting (January 2018–July 2021)	USA
NCT03772899	Advanced melanoma	Phase I	20; age ≥19	FMT capsule from healthy donors: prior to anti-PD-1 therapy	Treatment: to examine effect and safety of combining FMT and pembrolizumab/nivolumab	ORR	Recruiting (March 2019–December 2023)	UK, Canada
NCT04163289	RCC	Phase I	20; age ≥18	FMT capsule from healthy donors: prior to and concurrent with anti-CTLA-4 and anti-PD-1 therapy	Treatment: to prevent immune-related colitis of ipilimumab/nivolumab	ORR	Recruiting (February 2020–March 2024)	UK
NCT04130763	GIT cancers	Phase I	5; age 18–70	FMT capsule from healthy donors: prior to and concurrent with anti-PD-1	Treatment: to improve efficacy in patients with PD-1-resistant GIT tumors	ORR	Recruiting (October 2019–December 2021)	China
Probiotics								
NCT03829111	Advanced RCC	Phase I	30; age ≥18	*Clostridium butyricum* CBM 588: concurrent with anti-PD-1 and anti-CTLA-4	Treatment: to increase effect of nivolumab/ipilimumab	Constitutional change in *Bifidobacterium*	Recruiting (April 2019–June 2022)	USA
NCT03637803	Advanced solid tumors	Phase I, Phase II	132; age ≥18	MRx0518 (a Live bio-lyophilized *Enterococcus gallinarum*): concurrent with anti-PD-1	Treatment: to improve immune responses in patients resistant to pembrolizumab	Safety and clinical benefit	Recruiting (January 2019–March 2023)	USA
NCT03817125	Metastatic melanoma	Phase I	30; age ≥18	SER-401: prior to and concurrent with anti-PD-1 after antibiotic (vancomycin) treatment	Treatment: to evaluate safety of treatment with SER-401 combined with nivolumab	Adverse events	Recruiting (January 2019–February 2022)	USA
NCT03686202	Solid tumors	Phase I	65; age ≥18	Microbial ecosystem therapeutics (MET-4, defined mixture of pure live cultures of gut bacteria isolated from stools of healthy donor): concurrent with ICBs	Treatment: to assess safety, tolerability and engraftment of MET-4 in combination with ICBs	Change of response related to species abundance and bacterial diversity; and adverse events	Recruiting (November 2018–December 2020)	Canada
NCT03595683	Advanced melanoma	Phase II	70; age ≥18	EDP1503 (monoclonal microbial product with *Bifidobacterium* spp.): concurrent with anti-PD-1	Treatment: to enhance response to pembrolizumab	Response rate and adverse events	Recruiting (July 2018–November 2021)	USA

Prebiotics								
NCT04009122	Metastatic NSCLC	Not applicable	280; age ≥18	IGEN0206 (dietary nutritional product): concurrent with therapies	Treatment: to improve life quality of patients with NSCLC with immunotherapy, chemotherapy, and biological therapy	Assessment of life quality	Recruiting (June 2019–December 2021)	Spain
Antibiotics								
NCT03891979	Pancreatic adenocarcinoma	Phase IV	25; age: 18 months–100 years	Antibiotics (ciprofloxacin and metronidazole): combined antibiotics preoperative days 1–29 with pembrolizumab on days 8 and 29	Treatment: to determine changes in immune activation in tumor tissues	Changes in immune activation in tumor tissue measured by activation of HLA-DR	Withdrawn (suspended due to primary investigator's decision) (May 2019–June 2020)	USA
NCT02366884	Advanced cancer	Phase II	250; age: 1–75 y	Antibiotics (antibacterial, antifungal, antiprotozoal agents)	Treatment: to determine benefit of these agents or their combination	Clinical efficacy	Recruiting (July 2011–December 2022)	Mexico
Vaccines								
NCT03421236	Nonmuscle-invasive bladder cancer	Phase I	25; age ≥18	Ty21a (typhoid)	Treatment: to test safety of intravesical Ty21a	Adverse events	Recruiting (February 2018–March 2021)	Switzerland
NCT02625857	Metastatic castration-resistant prostate cancer	Phase I	26; age ≥18; male	JNJ-64041809 (live attenuated double-deleted *Listeria monocytogenes*); once a cycle	Treatment: to identify dose of JNJ-64041809 when administered intravenously	Incidence of dose-limiting-toxicity and antigen-specific T cell response	Completed (December 2015–July 2018)	USA
NCT02718430	CRC with liver metastasis	Phase I	6; age ≥18	VXM01 (live attenuated *Salmonella typhimurium* carrying *VEGFR2*)	Treatment: to assess effect of VXM01	Safety and tolerability of VXM01	Completed (February 2016–March 2018)	Germany
Others								
NCT02002182	HPV(+) oropharyngeal cancer	Phase II	30; age ≥18	ADXS11-001 (using a genetically-modified live strain of *L. monocytogenes*): before surgery	Treatment: to assess effect of ADXS11-001 before surgery	HPV-specific T cell response rate and toxicity	Active, not recruiting (December 2013–August 2022)	USA
NCT01924689	Solid tumor	Phase I	24; age ≥18	*Clostridium* Novyi-NT spores (intratumoral injection)	Treatment: to examine efficacy and safety in patients with treatment-refractory solid tumors	Adverse events	Completed (October 2013–October 2017)	USA

[a]Abbreviations: DCR, disease control rate; DOR, duration of response; ORR, objective response rate; RR, response rate.

effects was related to host immune status, tumoral genetic factors, the TME, and microbial regulation.

The combination of *B. cepacia* and *B. fragilis* mitigated the histopathological intestinal mucosal inflammation related to the anticancer response in mouse models [56]. In a successful case series of FMT amelioration of ICB-related refractory colitis in two patients [82], clinical symptoms and endoscopic manifestations were relieved at 53 days and 78 days after each dose of FMT, respectively. After FMT, a significant decrease in $CD8^+$ cytotoxic T cell levels with an elevation of $CD4^+$ $Foxp3^+$ T cells was observed in one patient, and a reduction of all T cell subtypes was observed in the other patient. The gut microbial signatures were altered following FMT, with a notable increase in *Akkermansia, Bifidobacterium*, and *Bacteroides*, and a reduction in *Escherichia*. This successful attempt provided insights into gut microbiome modulation via FMT to improve ICB-associated toxicities. How can we forecast and minimize severe ICB-related adverse effects? Can we intervene to uncouple the toxicity and efficacy in ICB therapy [34]? To answer these questions and evaluate the profit of this approach, further large-scale cohorts and studies of potential mechanisms are required [82] (Figure 2).

Concluding Remarks

The gut microbiota has a significant role in the cancer immune response and immunotherapy. Therefore, microbiota precision medicine, including FMT, prebiotics, probiotics, antibiotics, and vaccines, has been proposed as the ideal microbial therapeutic application in cancer treatment, which represents a more selective and safer microbial therapy facilitating immune elimination of tumor cells (Figure 2). Many strategies are being tested in clinical trials [83,84] (Table 2), such as more live immunogenic commensal bacteria, antibiotics that specifically target unfavorable bacteria, genetically modified vaccines comprising cancer epitopes with microbial adjuvants, monoclonal microbial products with specific strains, FMT, probiotics, prebiotics or dietary immunostimulating products, and adjuvants that can enhance the antitumor effect of bacteria [5,56,58,83–85] (Table 2).

Attempts at microbiome-based therapeutics could concentrate on the identification of individual bacterial strains or synthetic engineering of target bacteria to deliver products or drugs to target organs [86]. Indeed, engineered bacterial immunotherapy has been proposed, whereby bioengineered antitumor bacteria strains enhance adaptive and innate immunity [87,88]. Moreover, systemic administration of bacterial outer membrane vesicles (OMVs) might represent a new strategy for cancer immunotherapy by means of generating anticancer cytokines, without obvious adverse effects [89].

In summary, the gut microbiota and its metabolites act as adjuvants that determine the 'tone' of the TME. Host immunity also modulates and controls the microbiome by altering microbe-related signaling or metabolic functions to influence tumor surveillance. Moreover, cancer transforms the gut microbiome and either intensifies or reduces its response to immunotherapies, particularly ICB (Figure 2). In recent years, microbiota precision medicine has become a highly anticipated therapy. Nonetheless, there remain multiple knowledge gaps or problems for future exploration (see Outstanding Questions). High-quality, large-scale studies may supply strong evidence that the gut microbiota could serve as a promising prognostic marker or therapeutic candidate as a cancer immunotherapy adjuvant.

Author Contributions

C-B.Z. was responsible for collecting and arranging references, writing, and revising the manuscript. Y-L.Z. assisted in revising the manuscript. J-Y.F. provided ideas and helped organize the structure and main content of this manuscript.

Outstanding Questions

How does immunity specifically discriminate commensal from pathogenic bacteria?

How can the cancer immune response be specifically regulated between the gut microbiome and tumor-associated antigens?

How can the microbiota transmit signals of immune responses in different intestinal positions from the intestines to distant extraintestinal organs?

Can we extend in-depth mechanistic studies to cancers other than GIT tumors?

What types of patient are at a higher risk for poor efficacy of immunotherapies?

How can we expand the coverage of ICB efficacy to most patients with cancer?

How can we predict, and more importantly, reduce, therapy resistance to the maximum extent regarding the response heterogeneity to ICB therapies?

What is the proper time window and endpoint of antibiotic medication during immunotherapy?

What is the course of action if patients have severe infections and must use antibiotics during their cancer treatment?

Is it necessary to take probiotics concurrently with antibiotics during cancer treatment?

Do antibiotics reduce ICB efficacy via direct mutual interference or by destroying the microflora?

Is it the collective microbial community or individual species, through continuous crosstalk, critical for patient outcomes?

How can bacteria-specific immune cells gather at a remote TME and assist the mounting of a tumor-specific immune response to ICB therapies?

How can we better manipulate the gut microbiome? Individualized microbial biomarkers should be explored to

assist clinicians to identify the host ecological imbalance associated with adverse outcomes of immunotherapy.

How can we prevent tolerance to cancer immunotherapy by regulating the gut microbiome?

How can bacteria be used as prognostic biomarkers or even therapeutic targets?

Declaration of Interests

None declared by authors.

References

1. McDermott, A.J. and Huffnagle, G.B. (2014) The microbiome and regulation of mucosal immunity. *Immunology* 142, 24–31
2. Wong, S.H. and Yu, J. (2019) Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat. Rev. Gastroenterol. Hepatol.* 16, 690–704
3. Jin, C. *et al.* (2019) Commensal microbiota promote lung cancer development via gammadelta T cells. *Cell* 176, 998–1013
4. Elinav, E. *et al.* (2019) The cancer microbiome. *Nat. Rev. Cancer* 19, 371–376
5. Pitt, J.M. *et al.* (2016) Resistance mechanisms to immune-checkpoint blockade in cancer: tumor-intrinsic and -extrinsic factors. *Immunity* 44, 1255–1269
6. Hamid, O. *et al.* (2013) Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N. Engl. J. Med.* 369, 134–144
7. Park, Y.J. *et al.* (2018) Future prospects of immune checkpoint blockade in cancer: from response prediction to overcoming resistance. *Exp. Mol. Med.* 50, 109
8. Stockinger, B. *et al.* (2014) The aryl hydrocarbon receptor: multitasking in the immune system. *Annu. Rev. Immunol.* 32, 403–432
9. Vonarbourg, C. *et al.* (2010) Regulated expression of nuclear receptor RORgammat confers distinct functional fates to NK cell receptor-expressing RORgammat(+) innate lymphocytes. *Immunity* 33, 736–751
10. Atarashi, K. *et al.* (2015) Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* 163, 367–380
11. Tien, M.T. *et al.* (2006) Anti-inflammatory effect of *Lactobacillus casei* on *Shigella*-infected human intestinal epithelial cells. *J. Immunol.* 176, 1228–1237
12. Venkatesh, M. *et al.* (2014) Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* 41, 296–310
13. Trompette, A. *et al.* (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20, 159–166
14. Singh, N. *et al.* (2014) Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40, 128–139
15. Pabst, O. (2012) New concepts in the generation and functions of IgA. *Nat. Rev. Immunol.* 12, 821–832
16. Segain, J.P. *et al.* (2000) Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* 47, 397–403
17. Sawa, S. *et al.* (2011) RORgammat+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat. Immunol.* 12, 320–326
18. Kinnebrew, M.A. *et al.* (2012) Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity* 36, 276–287
19. Shi, C. and Pamer, E.G. (2011) Monocyte recruitment during infection and inflammation. *Nat. Rev. Immunol.* 11, 762–774
20. Atarashi, K. *et al.* (2013) Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 500, 232–236
21. Round, J.L. *et al.* (2011) The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science (New York, N.Y.)* 332, 974–977
22. Mazmanian, S.K. *et al.* (2005) An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122, 107–118
23. Tan, T.G. *et al.* (2016) Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. *Proc. Natl. Acad. Sci. U. S. A.* 113, E8141–E8150
24. Obata, T. *et al.* (2010) Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 7419–7424
25. Lathrop, S.K. *et al.* (2011) Peripheral education of the immune system by colonic commensal microbiota. *Nature* 478, 250–254
26. Ivanov, I.I. *et al.* (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498
27. Pickard, J.M. *et al.* (2014) Rapid fucosylation of intestinal epithelium sustains host-commensal symbiosis in sickness. *Nature* 514, 638–641
28. Sonnenberg, G.F. *et al.* (2012) Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science (New York, N.Y.)* 336, 1321–1325
29. Elinav, E. *et al.* (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145, 745–757
30. de Kivit, S. *et al.* (2014) Regulation of intestinal immune responses through TLR activation: implications for pro- and prebiotics. *Front. Immunol.* 5, 60
31. Wong, S.H. *et al.* (2017) Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. *Gastroenterology* 153, 1621–1633
32. Thiele Orberg, E. *et al.* (2017) The myeloid immune signature of enterotoxigenic *Bacteroides fragilis*-induced murine colon tumorigenesis. *Mucosal Immunol.* 10, 421–433
33. Nosho, K. *et al.* (2016) Association of *Fusobacterium nucleatum* with immunity and molecular alterations in colorectal cancer. *World J. Gastroenterol.* 22, 557–566
34. Gur, C. *et al.* (2015) Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 42, 344–355
35. Kostic, A.D. *et al.* (2013) *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 14, 207–215
36. Buchta Rosean, C.M. and Rutkowski, M.R. (2017) The influence of the commensal microbiota on distal tumor-promoting inflammation. *Semin. Immunol.* 32, 62–73
37. Rong, Y. *et al.* (2017) Reactivity toward *Bifidobacterium longum* and *Enterococcus hirae* demonstrate robust CD8(+) T cell response and better prognosis in HBV-related hepatocellular carcinoma. *Exp. Cell Res.* 358, 352–359
38. Riquelme, E. *et al.* (2019) Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell* 178, 795–806
39. Tait Wojno, E.D. and Artis, D. (2016) Emerging concepts and future challenges in innate lymphoid cell biology. *J. Exp. Med.* 213, 2229–2248
40. Coleman, O.I. *et al.* (2018) Activated ATF6 induces intestinal dysbiosis and innate immune response to promote colorectal tumorigenesis. *Gastroenterology* 155, 1539–1552
41. Dejea, C.M. *et al.* (2018) Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science (New York, N.Y.)* 359, 592–597
42. Dennis, K.L. *et al.* (2013) Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10-producing T cells. *Cancer Res.* 73, 5905–5913
43. Abed, J. *et al.* (2016) Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* 20, 215–225
44. Pushalkar, S. *et al.* (2018) The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov.* 8, 403–416
45. Li, Y. *et al.* (2019) Gut microbiota dependent anti-tumor immunity restricts melanoma growth in Rnf5(-/-) mice. *Nat. Commun.* 10, 1492
46. Cheng, M. *et al.* (2014) Microbiota modulate tumoral immune surveillance in lung through a gammadeltaT17 immune cell-dependent mechanism. *Cancer Res.* 74, 4030–4041
47. Zhou, C.B. and Fang, J.Y. (2018) The regulation of host cellular and gut microbial metabolism in the development and prevention of colorectal cancer. *Crit. Rev. Microbiol.* 44, 436–454
48. Arpaia, N. *et al.* (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504, 451–455

49. Furusawa, Y. *et al.* (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450
50. Wolf, D. *et al.* (2015) Treg(s) in cancer: friends or foe? *J. Cell. Physiol.* 230, 2598–2605
51. Trompette, A. *et al.* (2018) Dietary fiber confers protection against flu by shaping Ly6c(-) patrolling monocyte hematopoiesis and CD8(+) T cell metabolism. *Immunity* 48, 992–1005
52. Smith, P.M. *et al.* (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science (New York, N.Y.)* 341, 569–573
53. Loo, T.M. *et al.* (2017) Gut microbiota promotes obesity-associated liver cancer through PGE2-mediated suppression of antitumor immunity. *Cancer Discov.* 7, 522–538
54. Bhatt, A.P. *et al.* (2017) The role of the microbiome in cancer development and therapy. *CA Cancer J. Clin.* 67, 326–344
55. Frankel, A.E. *et al.* (2017) Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. *Neoplasia* 19, 848–855
56. Vetizou, M. *et al.* (2015) Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science (New York, N.Y.)* 350, 1079–1084
57. Routy, B. *et al.* (2018) Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science (New York, N.Y.)* 359, 91–97
58. Sivan, A. *et al.* (2015) Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science (New York, N.Y.)* 350, 1084–1089
59. Tanoue, T. *et al.* (2019) A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* 565, 600–605
60. Alam, A. *et al.* (2016) The microenvironment of injured murine gut elicits a local pro-restitutive microbiota. *Nat. Microbiol.* 1, 15021
61. Ansaldo, E. *et al.* (2019) *Akkermansia muciniphila* induces intestinal adaptive immune responses during homeostasis. *Science (New York, N.Y.)* 364, 1179–1184
62. Pinato, D.J. *et al.* (2019) Association of prior antibiotic treatment with survival and response to immune checkpoint inhibitor therapy in patients with cancer. *JAMA Oncol.* 5, 1774–1778
63. Yoon, M.Y. and Yoon, S.S. (2018) Disruption of the gut ecosystem by antibiotics. *Yonsei Med. J.* 59, 4–12
64. Gui, Q.F. *et al.* (2015) Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. *Genet. Mol. Res.* 14, 5642–5651
65. Blaser, M.J. (2016) Antibiotic use and its consequences for the normal microbiome. *Science (New York, N.Y.)* 352, 544–545
66. Keeney, K.M. *et al.* (2014) Effects of antibiotics on human microbiota and subsequent disease. *Annu. Rev. Microbiol.* 68, 217–235
67. Peterson, C.T. *et al.* (2015) Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. *Clin. Exp. Immunol.* 179, 363–377
68. Derosa, L. *et al.* (2018) Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann. Oncol.* 29, 1437–1444
69. Lalani, A.A. *et al.* (2019) Effect of antibiotic use on outcomes with systemic therapies in metastatic renal cell carcinoma. *Eur. Urol. Oncol.* 3, 372–381
70. Ueda, K. *et al.* (2019) The impact of antibiotics on prognosis of metastatic renal cell carcinoma in Japanese patients treated with immune checkpoint inhibitors. *Anticancer Res.* 39, 6265–6271
71. Zhao, S. *et al.* (2019) Antibiotics are associated with attenuated efficacy of anti-PD-1/PD-L1 therapies in Chinese patients with advanced non-small cell lung cancer. *Lung Cancer* 130, 10–17
72. Ahmed, J. *et al.* (2018) Use of broad-spectrum antibiotics impacts outcome in patients treated with immune checkpoint inhibitors. *Oncoimmunology* 7, e1507670
73. Velicer, C.M. *et al.* (2004) Antibiotic use in relation to the risk of breast cancer. *JAMA* 291, 827–835
74. Friedman, G.D. *et al.* (2006) Antibiotics and risk of breast cancer: up to 9 years of follow-up of 2.1 million women. *Cancer Epidemiol. Biomarkers Prev.* 15, 2102–2106
75. Boursi, B. *et al.* (2015) Recurrent antibiotic exposure may promote cancer formation–another step in understanding the role of the human microbiota? *Eur. J. Cancer* 51, 2655–2664
76. Kaderbhai, C. *et al.* (2017) Antibiotic use does not appear to influence response to nivolumab. *Anticancer Res.* 37, 3195–3200
77. Sethi, V. *et al.* (2018) Gut microbiota promotes tumor growth in mice by modulating immune response. *Gastroenterology* 155, 33–37
78. Yoshimoto, S. *et al.* (2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499, 97–101
79. Michot, J.M. *et al.* (2016) Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur. J. Cancer* 54, 139–148
80. Dubin, K. *et al.* (2016) Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat. Commun.* 7, 10391
81. Chaput, N. *et al.* (2017) Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann. Oncol.* 28, 1368–1379
82. Wang, Y. *et al.* (2018) Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat. Med.* 24, 1804–1808
83. Baiden-Amissah, R.E.M. and Tuyaerts, S. (2019) Contribution of aging, obesity, and microbiota on tumor immunotherapy efficacy and toxicity. *Int. J. Mol. Sci.* 20, 3586
84. McQuade, J.L. *et al.* (2019) Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol.* 20, e77–e91
85. Viaud, S. *et al.* (2013) The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science (New York, N.Y.)* 342, 971–976
86. Schwabe, R.F. and Jobin, C. (2013) The microbiome and cancer. *Nat. Rev. Cancer* 13, 800–812
87. Zheng, J.H. *et al.* (2017) Two-step enhanced cancer immunotherapy with engineered *Salmonella typhimurium* secreting heterologous flagellin. *Sci. Transl. Med.* 9, eaak9537
88. Chowdhury, S. *et al.* (2019) Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nat. Med.* 25, 1057–1063
89. Kim, O.Y. *et al.* (2017) Bacterial outer membrane vesicles suppress tumor by interferon-gamma-mediated antitumor response. *Nat. Commun.* 8, 626