Gut bacteria affect the tumoral immune milieu: distorting the efficacy of immunotherapy or not?
Pu Xiaoyu a, Ge Chao a, Dong Lihua a,b, and Chang Pengyu a,c

Department of Radiation Oncology & Therapy, Jilin Provincial Key Laboratory of Radiation Oncology & Therapy, The First Hospital of Jilin University, Changchun, China; NHC Key Laboratory of Radiobiology, School of Public Health, Jilin University, Changchun, China; Key Laboratory of Organ Regeneration & Transplantation of the Ministry of Education, Department of Radiation Oncology & Therapy, The First Hospital of Jilin University, Changchun, China

ABSTRACT
Immunotherapy using immune-checkpoint inhibitors is revolutionizing oncotherapy. However, the application of immunotherapy may be restricted because of the lack of proper biomarkers in a portion of cancer patients. Recently, emerging evidence has revealed that gut commensal bacteria can impact the therapeutic efficacy of immune-checkpoint inhibitors in several cancer models. In addition, testing the composition of gut bacteria provides context for prediction of the efficacy and toxicity of immunotherapy. In this review, we discuss the impacts of gut commensal bacteria on the tumoral immune milieu, highlighting some typical bacteria and their associations with immunotherapy.

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Introduction
Immune-checkpoint inhibitors (ICIs) have opened a new era of oncotherapy. Currently, numerous clinical trials investigating ICI efficacy are ongoing across a large range of cancer types. Accordingly, first-generation ICI drugs targeting CTLA-4, PD-1, or PD-L1 have been approved for fourteen indications. In general, the response rates to one of these drugs used alone vary from 20% to 40%. More strikingly, a portion of patients can achieve complete remission of tumors after receiving this therapy.

Although ICI therapy tinctures offer cancer patients hope of a cure, the priority task is determining the biomarkers with high specificity and sensitivity to predict the candidates who can benefit from ICI therapy with more precision. Currently, the available biomarkers for selecting patients receiving ICI therapy include PD-L1, tumor mutational burden (TMB), high microsatellite instability (MSI-H), and deficient mismatch repair (dMMR). On this basis of MSI-H or dMMR, the drug approved by the FDA is pembrolizumab, regardless of cancer type. Nevertheless, different types of cancer have express biomarkers useful for selecting candidates for ICI therapy. For example, PD-L1 positivity and a high TMB value in patients with non-small cell lung cancer (NSCLC) and presentation with MSI-H or dMMR by colorectal cancer patients have been well-established markers. In addition to such biomarkers, several other ICI-therapy-associated biomarkers are being explored.

As revealed by the molecular characteristics common across cancers, immune-associated biomarkers are also being discovered. Hence, intestinal commensal bacteria have attracted public interest because it is believed that intestinal commensal bacteria shape human immunity, presenting that disturbed homeostasis of bacterial ecosystem enables host immunity to be abnormal. This imbalance can be translated into ICI therapy. Emerging data have supported the idea that evaluating intestinal bacteria is a new route by which to predict the therapeutic response to and toxicity of ICI drugs. For example, commensal bacteria that can synergize with ICI therapy in tumoricidal processes include Bifidobacterium longum, Collinsella aerofaciens, Enterococcus faecium, Faecalibacterium genus bacteria and Akkermansia muciniphila in humans. In addition, poor efficacy of ICI therapy appears to be associated...
with increased frequency of *Bacteroides* in the gut.\textsuperscript{20,22} Referring to ICI-associated toxicity, it has been revealed that patients with a higher fecal level of *Bacteroidales* commonly exhibit lower incidences of colitis than those with lower levels.\textsuperscript{23} Conversely, cancer patients who have used antibiotics long term have not only a poor response to ICI therapy but also an increased incidence of ICI-related toxicity.\textsuperscript{24,25} It has been shown that antibiotics are able to reduce the total number and diversity of commensal bacteria in the gut.\textsuperscript{25} In this regard, homeostasis in the ecosystem of commensal bacteria is critical for ensuring the effectiveness of ICI therapy. Thus, a prevailing proposal has been presented suggesting that the richer of diversity of the commensal bacteria, the better response to ICI therapy is likely to be.\textsuperscript{19} Currently, several clinical trials concerning oral supplementation of commensal bacteria for improving the therapeutic efficacies of ICI therapy are ongoing (NCT03772899 and NCT03686202). However, the mechanisms by which gut commensal bacteria mediate the efficacy of ICI therapy are indeed complicated because bacteria-primed immune processes that favor ICI therapy have not been established for certain species. Instead, a community of bacteria elicits the tumoricidal response.\textsuperscript{26} In this review, we discuss the role of commensal bacteria in regulating host immunity and their influence on tumoral immune milieu formation, thus providing a rationale for commensal bacteria in guiding ICI therapy.

### Composition and physiological functions of gut commensal bacteria

The gut contains a microbial world. As estimated, $3.8 \times 10^{13}$ bacteria exist in the lumen,\textsuperscript{27} and most are commensal. Among these bacteria, the number of species ranges from 500 to 1000.\textsuperscript{28} Approximately 98% of these bacteria belong to *Bacteroidetes* and *Firmicutes* phyla, whereas *Fusobacteria*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia* and *Cyanobacteria* account for a only a minor portion.\textsuperscript{28–30} A healthy intestine provides an environment that favors the growth of anaerobes.\textsuperscript{31,32} However, significant differences characterize bacterial diversity among individuals.\textsuperscript{33} Therefore, dietary habits and living environment are factors influencing the bacterial diversity in gut.\textsuperscript{34,35}

At steady state, gut commensal bacteria have beneficial effects on the hosts in several respects, including maintaining epithelial homeostasis and a barrier function,\textsuperscript{36,37} facilitating food digestion and nutrient absorption, synthesizing bioactive substances in favor of cell metabolites, and shaping the host immunity.\textsuperscript{38,39} In terms of host immunity, gut commensal bacteria control the polarization of several T cell subsets, such as Th1, Th2, Th17 and Treg cells, thus enabling the host to defend against foreign stimuli while sustaining an immunotolerant milieu.\textsuperscript{40} During human evolution, the diversity of commensal bacteria was reduced in the gut constantly,\textsuperscript{35} causing abnormal colonization of commensal bacteria, sluggishness in the physical functions of the bacteria, and abnormal immunity.\textsuperscript{41} Consequently, hosts are prone to increased susceptibility to autoimmune diseases or even cancer.\textsuperscript{18} This notion has been typically translated into gastric and colorectal cancer, in which collective commensal bacterial dysbiosis is an intrinsic factor that induces carcinogenesis.\textsuperscript{42,43} In fact, reduced diversity represents a paradigm of bacterial dysbiosis.

#### Gut commensal bacteria in the regulation of host immunity

Gut commensal bacteria shape host immunity. In general, gut bacteria use antigens and metabolites to induce immune cell commitment.\textsuperscript{44} In contrast, the composition of the gut bacteria is maintained at a physical level by the substances provided by the gut immune and epithelial cells.\textsuperscript{45} Therefore, IgA secreted by gut B cells or IgM secreted by plasma cells is critical in defending against the outgrowth of pathogenic bacteria in the gut.\textsuperscript{46} In parallel with these secretions, mature epithelial cells, especially Paneth cells, can produce various anti-microbial peptides to sustain the bacterial ecosystem in the gut, enabling a moderate level of fixed bacteria.\textsuperscript{45} On this basis, this bacterial community is helpful in protecting against the occurrence of immune abnormality.

In general, T cell polarization in the gut induced by commensal bacteria can present in either a dendritic cell (DC)-dependent or DC-independent manner.\textsuperscript{47} DCs are professional antigen-presenting cells in humans.\textsuperscript{47} Especially in a bacterial antigen-enriched milieu, the pattern recognition receptors (PRRs) and
damage-associated molecular patterns (DAMPs) are crucial molecules that mediate DCs in recognizing and subsequently responding to antigens.\(^{48,49}\) Thereafter, DCs process such antigens into adaptive immune cells by manipulating their survival and function through DC-produced cytokines under given conditions.\(^{50}\) After this process, commensal bacteria such as \textit{Lactobacillus sakei} and \textit{Bifidobacteria} are reported to have the capacity to upregulate MHC-II expression by DCs while recruiting them into the gut.\(^{51,52}\) Functionally, the antigens presented by MHC-II molecules are able to activate naïve T cells while priming their differentiation into other T cell subsets (Figure 1). Independent of DCs, gut B cells, macrophages, or other innate immune cells, such as NK cells and innate lymphoid cells, can also be activated by lumen bacteria presenting immunoregulatory phenotypes (Figure 1).\(^{53}\) For example, in gut Peyer’s patches (PPs), bacterial antigen presentation by epithelial M cells serves as a route for inducing B cell activation, allowing for IgA, IgM and IgG to clear pathogens.\(^{54}\) In this situation, the complex of commensal IgG can be cleared by residual macrophages.\(^{55}\) In response, the macrophages increase the production of IL-1β along with stimulating neutrophils and Th17 cells to clear the intestinal infection.\(^{55}\) Similar effects can be induced by NK cells as well. For example, \textit{Bacteroides fragilis} and

**Figure 1.** Contributions of gut commensal bacteria and their metabolites to the host immune system. Based on the function of antigen-presenting cells, such as DCs, NK cells, and macrophages, commensal bacteria mediate the differentiation of naive CD4 + T cells into different subgroups, such as T-bet+ Th1 cells, GATA3+ Th2 cells, RORγt+ Th17 cells, and FOXP3+ Tregs, which further contribute to different immune modulation responses, and the production of various cytokines, such as TGF-β, IFN-γ and ILs. Immune regulation can be mediated not only by bacteria but also by their metabolites, especially SCFAs and AHR ligands, exerting functions by binding GPCRs and AHR on the surface of epithelial cells and immune cells, respectively, which subsequently contribute to augmented epithelial barrier function and improved gut immune tolerance. Conversely, some immune cells and epithelial cells can also mediate the balance of bacteria by secreting antibacterial substances, such as B cells secreting IgA, Goblet cells secreting mucins and Paneth cells secreting antimicrobial peptides, etc. Overall, microbiota-immune cross-talk contributes to gut homeostasis by forming a relatively stable feedback loop. SCFAs, short-chain fatty acids; AHR, aromatic hydrocarbon receptor; GPCRs, G protein-coupled receptors; TCR, T cell receptor; PRR, pattern recognition receptor; DAMP, damage-associated molecular pattern; MHC-II, major histocompatibility complex II; B7, B7.1(CD80)/B7.2(CD86); DC, dendritic cell; NK, natural killer cell; NE, neutrophil; Treg, regulatory T cell; CTL, cytotoxic T lymphocyte; ILC, innate lymphoid cell; IFN-γ, interferon-γ; TGF-β, transforming growth factor; and IL, interleukin.
Salmonella can directly activate NK cells by interacting with TLR4 or TLR9, thus increasing NK cell cytotoxic activity.\textsuperscript{53,56}

Similar to bacterial antigens, the metabolites processed by commensal bacteria are critical in immunity homeostasis.\textsuperscript{44} For example, the most critical substances are short-chain fatty acids (SCFAs) (Figure 1). SCFAs can be generated by gut commensal bacteria such as Lactobacillus, Bacteroides, Bifidobacterium, and Akkermansia muciniphila after they conduct glycolysis of food fibers.\textsuperscript{57,58} SCFAs are able to sense immune cells, including DCs, T cells and B cells, with respect to increasing the gut numbers of Treg cells and DCs with a tolerogenic phenotype, minimizing Th2 cell-associated immune responses and improving the secretion of IgA by gut B cells.\textsuperscript{59} Alternatively, Lactobacillus spp. are able to utilize tryptophan to produce indole-3-aldehyde, which interacts with the aromatic hydrocarbon receptor (AHR) on Figure 2. Potential mechanism that explains the anticancer or pro-cancer effects of some candidate ICI-therapy-associated bacteria by shaping the host immune status. Lactobacilli may upregulate the expression of MHC-II on DCs, enhance the activity of NK cells and macrophages, and improve Th1-mediated immune responses as well as increase the production of IFN-γ in tumors. The effects described above facilitate the anticancer potential of Lactobacilli. Bifidobacteria can improve the efficacy of anti-PD-L1 therapy by upregulating the expression of MHC-II on DCs, promoting Th1 polarization and CTL accumulation in the tumor microenvironments, and reducing the toxicity of anti-CTLA-4 therapy through enhanced Treg cell metabolism. Akkermansia muciniphila can enhance the efficacy of anti-PD-1 therapy in a manner dependent on the enhanced IL-12-dependent Th1-related immune response, along with increased levels of IFN-γ and TNF-α and decreased levels of IL-4 and IL-10. The results from a preclinical trial confirmed that Bacteroidetes can restore anti-CTLA-4 treatment efficacy by enhancing the IL-12-dependent Th1-related immune response, whereas these bacteria are associated with poor clinical outcomes of anti-CTLA-4 or anti-PD-1 therapy in human clinical trials. Fusobacterium nucleatum can promote tumor progression in gastrointestinal cancer and pancreatic cancer, and it is verified to be associated with reduced density of CD3 + T cells, exhaustion of NK cells, and augmentation of M2 polarization along with accumulation of MDSCs in tumor microenvironments. These effects may be closely linked with their colonization in tumors.
innate immune cells to induce their secretion of IL-22 (Figure 1). In addition, SCFAs can induce epithelial cells to upregulate the production of anti-microbial peptides. In summary, gut commensal bacteria are essential for gut immunity.

**Commensal bacteria, tumoral immune milieu and effectiveness of ICI therapy**

Immune deficiency serves as a critical factor for cancer pathogenesis. Mechanically, an action called immunoediting represents cancer cell clone evolution over time after the host immune clearance, thus enabling the clones with low immunogenicity to be preserved. In this setting, commensal bacteria can cross-talk with the residual cancer cells directly or indirectly to facilitate their aggressive behaviors. For example, intestinal bacterial dysbiosis is a state of early gut carcinogenesis, while intestinal bacterial dysbiosis persists during cancer progression. For example, *Fusobacterium nucleatum* (*F. nucleatum*) are regarded as ‘Oncobacteria’ of colorectal cancer because they can self-localize into tumors to facilitate their growth, induce chemoresistance and conduct immunosuppression. In another paradigm, an event called ‘bacterial succession’ suggests that one type of bacterium, followed by the others, continues to perform its oncogenic functions during different periods. As an example of this notion, *Helicobacter pylori* plays a pioneering role in inducing early lesions of gastric cancer, and other bacteria, such as *F. nucleatum*, continue to perform their function in promoting tumor progression. Similar to it effect in colorectal cancer, intestinal bacterial dysbiosis also influences pancreatic cancer pathogenesis. For example, antibiotics can be used to support the management of pancreatic cancer when the tumoral neoantigens share similarity with the antigens on bacteria. In this situation, tumoricidal T cells potentially recognize bacterial antigens, thus misleadingly clearing bacteria instead of tumor cells. In this regard, immunosuppression in tumors naturally requires bacterial participation. In fact, recent data support this notion, suggesting that intestinal bacteria influence the tumoral immune milieu mainly by altering the tumoral density of immune cells and changing their cytokine production. In general, commensal bacteria have discriminating roles in this event. By using the syngeneic melanoma or sarcoma transplantable tumor models, it was revealed that supplying mice with feces of human ICI responders can significantly enhance tumor remission after anti-PD-1 or -PD-L1 therapy. For example, such feces were found to upregulate PD-L1 expression in the tumor microenvironment of melanoma mice. By contrast, feces from non-ICI-responders can even promote tumor progression. Therefore, commensal bacteria are able to distort the effectiveness of ICI drugs. In the following section, we introduce the contributions of some commensal bacteria in altering the tumoral immune milieu and analyze their roles in improving or distorting the efficacy of ICI drugs (Figure 2).

**Lactobacilli**

*Lactobacilli* are the most common probiotics distributed in the gut. They belong to the *Firmicutes* phylum. At the genus level, *Lactobacillus* includes more than 150 species, and most of them are facultative anaerobic bacteria. Among them, *Lactobacillus reuteri* (*L. reuteri*), *Lactobacillus acidophilus* (*L. acidophilus*) and *Lactobacillus casei* (*L. casei*) serve as common bacteria corresponding to intestinal health and disease. At steady state, several strains of *L. reuteri* can exert their functions, including promotion of T cell development and reduction in pro-inflammatory cytokine production, thus protecting against the exacerbated inflammation in gut. In fact, independent of Treg cells, *L. reuteri* also exhibits potency in inhibiting Th1- or Th2-related immune responses because *L. reuteri* are capable of restoring the levels of plasma inosine, which are dedicated to reducing IFN-γ and IL-4 along by inhibiting Th1/Th2 polarization via inosine–adenosine A2A receptor interactions (Table 1). Similarly, the supernatant from in vitro cultured *L. reuteri* is able to reduce TNF-α production by human myeloid cells, suggesting that the metabolites of *L. reuteri* also exert an anti-inflammatory effect. In addition to the aforementioned intrinsic roles, tryptophan catabolites generated by *L. reuteri* can eventually promote the proliferation of CD8^+^CD4^+^ double-positive intraepithelial
lymphocytes (Table 1).

After this proliferation is initiated, tryptophan catabolites act as ligands of AHR. Upon the AHR-ligand interactions, the production of IL-22 by innate lymphoid cells is improved. Functionally, IL-22 can protect the intestine against infection synergistically with IL-17 through the induction of enterocyte expansion and antibacterial peptide production. However, in colorectal cancer, IL-22 is able to promote tumor progression. In the latter situation, administration of the *L. casei* BL23 strain to mice bearing colorectal cancer reportedly reduced the tumoral level of IL-22; thus *L. casei* BL23 serves as a candidate treatment against colorectal cancer progression (Table 1).

Concerning *L. acidophilus*, previously reported data suggested that oral administration of *L. acidophilus* to breast cancer-bearing mice could activate NK cells...
while enhancing Th1-mediated immune responses and increasing the production of IFN-γ in tumors, thus eliciting antitumoral immunity (Table 1). In parallel with \textit{L. acidophilus}, \textit{L. plantarum} and \textit{L. brevis} were found to generate effective immune responses against tumors in breast cancer-bearing mice because administration of these bacteria cause increases of the production of IFN-γ, IL-2, and TNF-α by activating T cells and macrophages and causes increased cytotoxic activity of NK cells (Table 1). In this respect, tumoral upregulation of IFN-γ is believed to be a biomarker that can be used to predict the response of a tumor to ICI therapy. In this regard, despite the lack of valid evidence suggesting the synergistic effect of \textit{Lactobacillus} in ICI therapy, it is reasonable to speculate that maintaining the gut frequency of \textit{Lactobacillus} at a stable level assists in gut immune homeostasis, thereby minimizing the distortion of ICI drugs.

**Bifidobacteria**

\textit{Bifidobacteria} are also commonly believed to be probiotics in the human gut. They are anaerobic bacteria that belong to the \textit{Actinobacteria} phyla. In general, \textit{Bifidobacteria} exert beneficial effects on immunomodulatory effects by using their own bacterial components and various immune-related metabolites, and they maintain immune homeostasis through a cross-feeding mechanism. Typically, different species of \textit{Bifidobacteria} facilitate Th1 and Th2 polarization and CTL accumulation. In addition, bacterial metabolites also play an important role in stimulating immune function. Another study confirmed that \textit{Bifidobacterium longum} BB536 has positive effects on the early establishment of healthy intestinal bacteria and plays a significant role in enhancing the Th1 immune response (Table 1). Additionally, \textit{Bifidobacterium dentium}, as desirable mucus-layer builders, have been shown to enhance the intestinal mucus layer through autophagy and calcium signaling pathways.

In an experimental model, oral administration of \textit{Bifidobacterium} to melanoma-bearing mice was found to synergize with anti-PD-L1 therapy to induce tumor shrinkage. The underlying mechanism is indicated by \textit{Bifidobacterium} administration enhancements of the antigen-presentation function of DCs, thus facilitating CD8+ T cell activation and infiltration into tumors (Table 1). In addition to exerting a synergistic effect, \textit{Bifidobacteria} were found to reduce the toxicity of anti-CTLA-4 therapy by modulating the metabolism of Treg cells rather than altering Treg cell density in the tumors, thus not distorting the efficacy of anti-CTLA-4 therapy (Table 1). Upon integrating the above data, \textit{Bifidobacteria} can be regarded as beneficial for ICI therapy.

**Akkermansia muciniphila**

\textit{Akkermansia muciniphila} (\textit{A. muciniphila}) are bacteria that are initialized in the early life of humans. They are gram-negative and colonize in the outer layer of the mucin covering the intestinal epithelium. In the gut, cecum has the largest number of \textit{A. muciniphila}. However, they only account for 1 ~ 4% of all commensal bacteria. Despite being a small portion overall, they are regarded as promising probiotics. At steady state, \textit{A. muciniphila} are capable of renewing the mucus layer by degrading mucins, providing a route to strengthened intestinal barrier function. Alternatively, the bacterial component \textit{Amuc-1100} can interact with TLR2-positive cells, thereby enhancing intestinal barrier function by upregulating tight-junction-associated proteins. Upon epithelial damage, \textit{A. muciniphila} preferentially localize in the wound to elicit proliferation and migration of enterocytes to this site. Once at the wound, \textit{A. muciniphila} can also increase the levels of IFN-γ and TNF-α while decreasing IL-4 and IL-10 production (Table 1). In this regard, \textit{A. muciniphila} exhibits the capability to improve the effect of ICI therapy (Table 1). In fact, basic research has confirmed that \textit{A. muciniphila} can promote Th1 polarization. To show this effect, \textit{A. muciniphila} was administered to germ-free mice bearing sarcoma to increase the Th1-related immune response significantly, thus enhancing the efficacy of anti-PD-1 therapy in causing tumor shrinkage. Moreover, cancer patients with a high number of \textit{A. muciniphila} in the gut commonly show a better response to ICI drugs than those with a low number of \textit{A. muciniphila}. Therefore, \textit{A. muciniphila} can be regarded as an ICI therapy-favored bacterium.
**Bacteroidetes**

In the healthy human gut, gram-negative *Bacteroidetes* are universally distributed in the colon.\(^{98}\) Herein, *Bacteroidetes fragilis* (*B. fragilis*) are the most prominent bacteria and have long been considered pathogens in humans.\(^{99}\) In fact, they are capable of inducing immune tolerance of the gut.\(^{100}\)

In parallel with tolerance induction, it has also been revealed that nontoxigenic *B. fragilis* can counteract enterotoxigenic *B. fragilis* to protect against colitis and tumorigenesis in the gut.\(^{101}\)

In summary, *Bacteroidetes* are able to induce cell polarization, including Th1, Th17 and Treg cells, in the gut.\(^{102,103}\) In the context of the administration of *Bacteroidetes*, the efficacy of anti-CTLA-4 therapy was restored in germ-free mice bearing melanoma or colon cancer (Table 1).\(^{103}\)

Mechanically, in germ-free mice, pure colonization of *Bacteroidetes* promoted the maturation of IL-12-producing DCs in tumors and induced a Th1-related immune response.\(^{89,103}\) However, melanoma patients with a high fecal *Bacteroidetes* count have poor clinical outcomes after receiving anti-CTLA-4 therapy (Table 1).\(^{22}\) This outcome is translated into anti-PD-1 therapy.\(^{20}\) The underlying mechanism involves a reduced level of MHC-II molecules and a higher number of Treg cells and Th17 cells in nonresponders to anti-PD-1 therapy than are presented by responders (Table 1).\(^{20}\) In this context, different species of *Bacteroidetes* can have different impacts on the efficacy of ICI therapy.

**Fusobacterium nucleatum**

*Fusobacterium nucleatum* (*F. nucleatum*) has been well established as pathogens in the induction of colorectal cancer.\(^{43,66}\) They can infect CRC cells to induce robust proliferation of CRC cells. Imunosuppression is another hallmark of CRC tumors after *F. nucleatum* infection. In general, in CRC specimens, it was revealed that the tumoral density of CD3\(^+\) T cells inversely correlated with *F. nucleatum* number (Table 1).\(^{104}\) This result can be attributed to T cell loss, which is driven by the interaction between bacterial Fap2 and the TIGIT inhibitory receptor on T cells.\(^{105}\) This outcome also translates to NK cells. Thus, it was found that exhaustion of NK cells occurs in early gut carcinogenesis.\(^{105}\) After the initiation of these processes, *F. nucleatum* facilitates M2-like TAM polarization via IL-6/c-MYC/STAT3 axis activation.\(^{106}\) In addition, *F. nucleatum* has been shown to enhance the accumulation of myeloid-derived suppressive cells in CRC tumors (Table 1).\(^{107}\) Thus, the immunosuppressive milieu for CRC tumors is thus formed. Generally, CRC patients with a high number of *F. nucleatum* in their tumors commonly have poor clinical outcomes than those without *F. nucleatum* infection. Referring to its impact on ICI therapy, it was found that the presence of *F. nucleatum* is inversely related to tumoricidal infiltrates, even in MSI-H tumors,\(^{108}\) thus implying that *F. nucleatum* represents an ICI therapy-unfavorable bacteria.

**Concerns related to the prediction of ICI efficacy by testing fecal bacteria**

Above information has exemplified some typical bacteria that can impact the immune milieu in tumors. Yet, there are some concerns still existing in predicting the response of cancer patients to ICI therapy by testing the composition of their fecal bacteria. For example, albeit collecting data from melanoma patients, two separate research groups reported all the bacteria including *Bifidobacterium longum*,\(^{19}\) *Collinsella aerofaciens*,\(^{19}\) *Enterococcus faecium*,\(^{19}\) *Faecalibacterium* genus bacteria\(^{20}\) were highly related to clinical response of patients to ICI therapy. Moreover, high fecal frequency of *A. muciniphila* was associated with well response of NSCLC to ICI therapy.\(^{21}\) Although the specific roles of aforementioned bacteria in improving tumoral immune milieu have been well characterized in corresponding animal models,\(^{19–21}\) an open question is emerged as which will be the most reliable bacteria in predicting or comparing the therapeutic efficacies of ICI drugs within a certain type of cancer, or among different cancers? Remarkably, a basic study has confirmed that a consortium of eleven strains of bacteria function jointly in eliciting CD8\(^+\) cell accumulation in gut, whereas such an effect will be abated if in absence of one or more certain strains of bacteria.\(^{26}\) Otherwise, it should be asked whether the mice can support the colonization of all bacterial species from human feces.\(^{11}\) In...
these situations, it is conceivable that the response to ICI therapy should not be merely attributed to one single major species of bacteria that increase their frequency in cancer patient feces. Probably, other bacteria will assist in this process albeit they do not significantly alter their frequencies in gut. Thus, we believe that defining a group of bacteria may be more precise in predicting ICI response than a certain type of bacteria does.

Another concern will be presented in the methodology. As we know, 16S rRNA or metagenomic whole-genome shotgun sequencing can identify most of the abundant bacteria that impact the efficacy of ICI therapy. On this basis, we should note the rare bacteria in feces, such as those residing in the small intestine but with less frequencies in feces. As estimated, these bacteria may distort the efficacy of ICI therapy as well, but methods concerning culture, isolation, identification and functional testing for these bacteria are technically difficult. Thus, more advanced methods should be developed to fulfill this aim. As we have exemplified the bacteria that can differ their roles in priming immune cells even if they are from the same taxonomy; so in our opinion, more deep sequencing or identification work should be done in the future. For example, a basic study has revealed that the non-toxigenic strain and the enterotoxigenic strain of *B. fragilis* exhibit opposite effects on gut tumor progression.

Collectively, all these concerns will provide new sparks for future research in this field.

**Conclusion**

Gut commensal bacteria can certainly influence the therapeutic effects of ICI drugs. Due to the preponderance of gut bacteria in controlling host immune integrity, maintaining homeostasis of gut commensal bacteria appears to be the basis of ensuring the effectiveness of ICI therapy. Moreover, due to the certainty that some commensal bacteria impact the effectiveness of ICI therapy and are predictive of the response to ICI therapy in cancer patients, the composition of these bacteria provides context in the field of oncotherapy.

**Disclosure of Potential Conflicts of Interest**

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**Contributions**

PX and CP wrote this paper; PX and GC prepared the figures and tables of this review; CP and DL conceived the topic of this review, and CP designed the logic flow.

**ORCID**

Pu Xiaoyu [http://orcid.org/0000-0001-9138-1598]
Ge Chao [http://orcid.org/0000-0002-5976-3462]
Dong Lihua [http://orcid.org/0000-0002-5148-2949]
Chang Pengyu [http://orcid.org/0000-0001-9047-7942]

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