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Mining the Gut Microbiota for Microbial-Based Therapeutic Strategies in Cancer Immunotherapy

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The past two decades witnessed a revolution in our understanding of host–microbiota interactions that led to the concept of the super-organism consisting of a eukaryotic part and a prokaryotic part. Owing to the critical role of gut microbiota in modulating the host immune system, it is not beyond all expectations that more and more evidence indicated that the shift of gut microbiota influenced responses to numerous forms of cancer immunotherapy. Therapy targeting gut microbiota is becoming a promising strategy to improve cancer immunotherapy. In this review, we discuss the role of the gut microbiota in response to cancer immunotherapy, the mechanisms that the gut microbiota influences cancer immunotherapy, and therapeutic strategies targeting gut microbiota to improve cancer immunotherapy.

Keywords: gut microbiota, cancer immunotherapy, MAMPs, microbial metabolites, fecal microbiota transplant

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1 INTRODUCTION

Over the past decades, immunotherapy has emerged as a mainstay in cancer treatment, with the advances in our understanding of cancer immunosuppressive microenvironments. Cancer immunotherapy was applied to a broad range of cancers, but 70% to 80% of patients failed to experience a life-altering durable response (1). To benefit more patients from cancer immunotherapy, efforts are made to evoke the immune response.

The gut microbiota is drawing tremendous attention given its effects on human health. Mounting evidence revealed that the gut microbiota and the immune system constantly interact (2, 3). Since immunotherapy was approved by US Food and Drug Administration (FDA), increasing clinical studies revealed the association between the gut microbiota and response to immunotherapy. Basing on the solid clinical association, the causal/mechanistic link of gut microbiota and immunotherapy was uncovered with preclinical models. Microbe-associated molecular patterns (MAMPs), molecular mimicry of microbial antigens with tumor neoantigen, and microbial metabolites were key factors that gut microbiota depends on to influence the response of cancer immunotherapy. Currently, more and more preclinical and clinical evidence indicated that the shift of gut microbiota influenced responses to numerous forms of cancer immunotherapy (4). As a result, therapeutic strategies targeting gut microbiota, including fecal microbiota transplant (FMT), diet, probiotics, and antibiotics, are regarded as promising candidates in improving cancer immunotherapies. Numerous clinical trials were performed to explore effective strategies to benefit cancer immunotherapy *via* improving gut

microbiota. Thus, this review will mine the gut microbiota for cancer immunotherapy *via* summarizing and discussing the clinical-associated and causal/mechanistic links and clinical trials of gut microbiota and cancer immunotherapy, comparing the advantages and disadvantages of therapeutic strategies targeting the gut microbiota.

1.1 Cancer Immunotherapy

The immune system plays a dominant role in cancer control, attributed to the detection and elimination of cancer cells. On the other hand, some tumor cells escape immune surveillance by i) defecting the expression of antigen-presenting proteins, or antigen processing, or presentation, rendering them invisible to immune cell; ii) expressing proteins in inhibiting inflammation and inducing an immunosuppressive state within the tumor microenvironment; and iii) becoming insensitive to immune effector mechanisms (5). Immunotherapy helps the immune system to better act against cancer, *via* encouraging immune elimination and hindering immune evasion of cancer cells.

Therapeutic advances in immunotherapy have rapidly emerged in the past few years, especially the immune checkpoint inhibitors (ICIs). Currently, ICIs are FDA-approved for the treatment of many cancer types, including advanced-stage melanoma, squamous and non-squamous non-small cell lung carcinoma (NSCLC), Merkel cell carcinoma, head and neck squamous cell carcinoma, urothelial carcinoma, kidney carcinoma, microsatellite instability-high or DNA mismatch repair-deficient cancers, refractory Hodgkin lymphoma, hepatocellular carcinoma, and gastric cancer (6, 7). Now, ICIs are coming to neoadjuvant (presurgical) era. Clinical studies (8–10) have unleashed the promise of neoadjuvant immunotherapy. More than 90% of NSCLC patients were able to undergo surgery within the planned timeframe after neoadjuvant immunotherapy (11). In addition, RNA vaccine could be another effective immunotherapy, which drives immunity by the induction of strong $CD4^+$ and $CD8^+$ T-cell immunity against the vaccine antigens to kill cancer cells (12).

Despite the successful application of cancer immunotherapy across a broad range of human cancers, only 20% to 30% of patients experience life-altering durable response from these therapies, which varies depending on the tumor type (1). Indeed, immunotherapy responses are heterogeneous; most patients manifest primary or secondary resistance to ICIs or even acceleration of the disease, which is called "hyperprogression" (13). Efforts are being made to identify the parameters that govern the threshold of the immunity to evoke the effective anticancer immune response, defined as the "cancer immune set-point" (14).

Numerous factors have been identified to contribute to the "cancer immune set-point" *via* regulating overall immune status, including tumor mutational load, cell metabolism, genomic drivers, and host-specific genetic variation (15, 16). Also, recent investigations highlight the effect of microbiota on the parameters that govern the effectiveness of immunotherapy (17, 18).

1.2 Gut Microbiota and Immunity

The human gastrointestinal tract harbors extremely high densities of microorganisms called the microbiota. A human being is more and more perceived as a super-organism consisting of a eukaryotic part and a prokaryotic part (19, 20). The gut microbiota is populated with as many as 100 trillion cells (21), whose collective gene set is approximately 100 times larger than the human gene complement (22, 23). Since birth, gut microbiota interacts with the host constantly throughout development. In consequence, it is not beyond all expectations that gut microbiota plays an important role in numerous host functions including immunity (2, 17).

In addition to influencing localized immune responses, what is more, gut microbiota contributes to systemic innate and adaptive immunity. On the one hand, the gut microbiota is a main source of MAMPs and ligands of pattern recognition receptors (PRRs). PRRs include the Toll-like receptors (TLRs), the nucleotide-binding oligomerization (NOD)-like receptors (NLRs), the RIG-I-like receptors, the C-type lectin receptors, the absent in melanoma 2 (AIM2)-like receptors, and the OAS-like receptors (24), which are widely expressed innate immune cells. In addition, gut microbiota stimulates the expression of PRRs. For example, gut microbiota orchestrates TLR expression on intestinal epithelial cells (25). MAMPs systemically prime the innate immune system, enhancing killing by bone marrow-derived neutrophils (26, 27) and increasing constitutive production of type I interferons of plasmacytoid dendritic cells (DCs) and cross-priming of DCs (28, 29).

On the other hand, gut microbiota-derived metabolites educate both innate and adaptive immunity. The gut microbiota metabolized the fiber, subsequently increasing the concentration of circulating short-chain fatty acids (SCFAs). SCFAs enhance the generation of macrophage and DC precursors and their phagocytic capacity (30), induce anti-inflammatory regulatory T cells (Tregs) (31), and facilitate antibody production of B cells (32). Polysaccharide A (PSA), a zwitterionic capsular carbohydrate, induces $FOXP3^+$ Treg differentiation and the production of IL-10 (33). Purine metabolite inosine advances Th1 differentiation *via* adenosine 2A receptors ($A_{2A}R$) (34). Therefore, it is not surprising that more and more studies are revealing the associations and mechanisms between gut microbiota and cancer immunotherapy and are exploring the strategies to improve immunotherapy by taking advantage of gut microbiota.

1.3 The Mechanisms of Gut Microbiota Modulating Immunotherapy

Diverse studies revealed that gut microbiota plays a crucial part in cancer immunotherapy. Both of the bacteria colonized in the gut and that translocated in the tumor or lymphoid organs regulate cancer immunotherapy. The mechanism for the immune modulation of gut microbiota is being disclosed. Based on existing researches, there are three ways by which gut microbiota influence systemic cancer immunotherapy: a) evoking the innate immunity and downstream adaptive immunity by MAMPs; b) yielding an endogenous tumor vaccine by molecular mimicry of microbial antigens with tumor neoantigen; and c) stimulating tumor-infiltrating immune cell by microbial metabolites (**Figure 1**).

1.3.1 Microbe-Associated Molecular Patterns

MAMPs, ligands of PRRs mostly expressed on innate immune cells, can act directly on local intestinal tissue cells but also penetrate beyond the mucosa, into circulation to tune immune cells in peripheral tissues (35). MAMPS can trigger at least partial activation of innate immune cells such as DCs. Furthermore, conditional antigen-presenting cells (APCs) enhanced the ability to evoke adaptive immune response and modulates cancer immunotherapy (**Figure 1A**).

Commensal bacteria have been identified in extragastrointestinal tissues typically considered to be sterile. Bacteria were detected in the blood (36), lymphoid organs (37, 38), and various tumor tissues (39, 40). Live bacteria gaining access to tumors or lymphoid organs may initiate a strong immune response by MAMPs. For example, the stimulator of interferon genes (STING) is a direct sensor of bacterial cyclic dinucleotides. Shi et al. revealed that *Bifidobacterium* facilitates translocation in tumor sites, where it facilitated anti-CD47 immunotherapy *via* STING signaling, increasing cross-priming of DCs (28) (**Figure 1A**). Sivan et al. showed that splenic DCs isolated from mice colonized with *Bifidobacterium* showed superior priming of naïve $CD8^+$ T cells *in vitro* (41).

MAMPs can traverse the mucosal barrier and enter the circulation. Stimuli capable of activating a range of TLR and NOD receptors were detected in serum from healthy individuals (42). In cancer immunotherapy, gut microbiota enhanced cancer response to the combination of CpG and anti-IL-10R through increasing tumor necrosis factor (TNF) production, which depends on the activation of TLR4 on tumor amyloid cells. And gavage with bacterial lipopolysaccharide (LPS), a ligand of TLR4, largely restored TNF production in tumors of antibiotic-treated mice (43). In addition, the activation of macrophages by MAMPs enhanced the phagocytic capability (44) and then primed $CD8^+$ T cells to exhibit cytotoxic function (45) (**Figure 1A**).

1.3.2 Molecular Mimicry of Microbial Antigens With Tumor Neoantigen

The theory of "molecular mimicry" posits that T cells elicited by bacteria or viruses accidentally recognize autoantigens as they "escape" from self-tolerance-inducing mechanisms. There were some reports that had demonstrated that microbe-specific $CD4^+$ or $CD8^+$ T lymphocytes attack normal tissues (46–48). Some data revealed a mechanistic role for T-cell epitopes shared between bacteria and tumor cells (37, 49–53). Fluckiger et al. (53) found the MHC-I-binding epitopes in the tail length tape measure protein (TMP) of a prophage. *Enterococcus hirae* 13144 harbored the bacteriophage that improves the response to anti-PD1 *via* activating TMP-specific H-2Kb-restricted $CD8^+$ T cell. In mouse models, administration of enterococci containing the bacteriophage boosted T-cell responses. In humans, the presence of the bacteriophage was associated with improved survival after PD-1

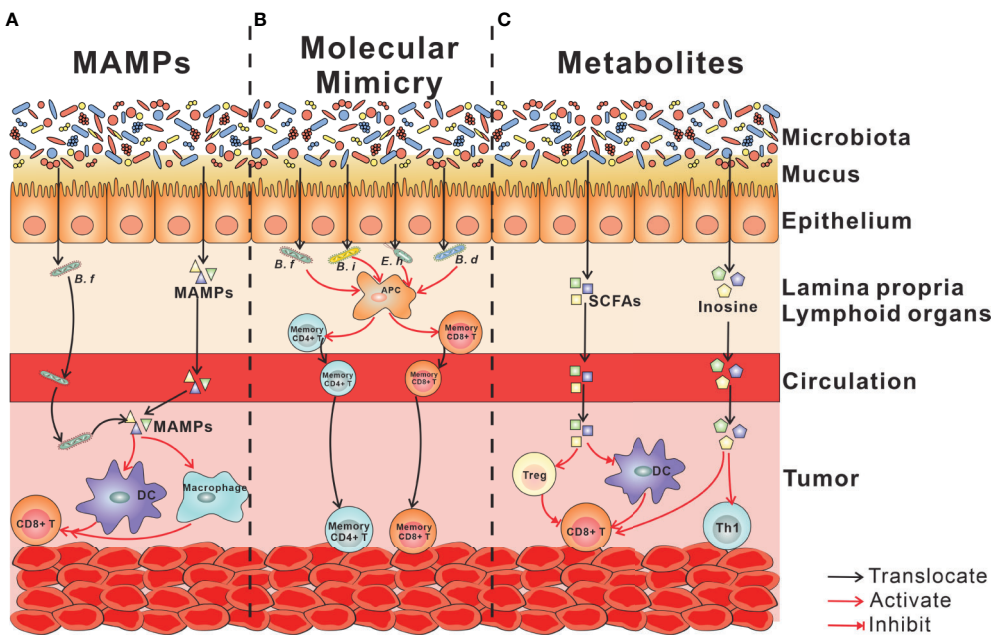

FIGURE 1 | Mechanisms linking gut microbiota with cancer immunotherapy. **(A)** MAMPs. Live bacteria (*Bifidobacterium facilitates*) and MAMPs traverse the mucosal barrier, enter the circulation, and finally locate at the tumor tissue, where MAMPs activate myeloid cells, including DCs and macrophages. The activation of myeloid cells enhances the phagocytosis of macrophages and cytotoxicity of $CD8^+$ T cells downstream. **(B)** Molecular mimicry of microbial antigens with tumor neoantigen. Antigens of commensal bacteria, including *Bifidobacterium facilitate*, *Bifidobacterium intestinihominis*, *Enterococcus hirae* 13144, and *Bifidobacterium breve*, are presented by APCs to $CD4^+$ T cells and $CD8^+$ T cells. By circulations, antigen-specific T cells arrive at tumor tissue and cross-react with tumor neoantigen. **(C)** Microbial metabolites. Microbiota-derived SCFAs play an immune-suppressive role in the tumor microenvironment *via* increasing the portion of Tregs, inhibiting DC maturation and $CD8^+$ T-cell activation. Microbiota-derived inosine acts to advance Th1 differentiation and $CD8^+$ T cytotoxicity. MAMPs, microbe-associated molecular patterns; DC, dendritic cell; APCs, antigen-presenting cells; SCFAs, short-chain fatty acids.

immunotherapy. In addition, *E. hirae* and *Bifidobacterium intestinihominis* specific memory $CD4^+$ T cells were associated with longer progression-free survival (PFS) in cancer patients (37). Memory T-cell responses against *Bifidobacterium fragilis* and anticancer efficacy of anti-CTLA4. Adoptive transfer of *Bi. fragilis*-reactive $CD4^+$ T cells restored anti-CTLA4 efficacy in germ-free (GF) mice (51). Bessell et al. (52) found that T cells targeting an epitope called SVYRYYGL, expressed in *Bifidobacterium breve*, cross-react with a model neoantigen SIYRYYGL. Compared with mice with *Bifidobacterium* colonization, tumors expressing the model SIYRYYGL neoantigen grew faster in mice lacking *Bifidobacterium* (**Figure 1B**).

1.3.3 Microbial Metabolites

Microbiota can metabolize dietary components that cannot be metabolized by the host, thus contributing to the production of primary metabolites and the modulation of secondary metabolites (54). The diverse array of metabolites in the mammalian intestine have the potential to modulate immunity. Several such microbial metabolites include SCFAs, lactic acid, spermidine, niacin, indole, retinoic acid, PSA, bile acid, and taurine (55).

SCFAs, namely, acetate, propionate, and butyrate, are the result of non-digestible carbohydrate fermentation by anaerobic commensal bacteria. In terms of immune regulation, SCFAs modulate cytokine releasing (56–58) and function of innate immune cells (30, 59, 60), B cell (61), and Tregs (31, 62) by acting as a histone deacetylase inhibitor or ligands for G-protein-coupled receptors. In cancer immunotherapy, SCFAs play an immune-suppressive role with an increase in the abundance of Tregs (63). In the mouse model, administration of butyrate diminished the efficacy of anti-CTLA4, *via* inhibiting DC maturation and T-cell activation (**Figure 1C**). In the clinical study, cancer patients with low concentrations of SCFAs showed prolonged PFS, and an association between gut bacteria and systemic concentrations of SCFAs was found (63). However, these results are in contrast those of with two clinical studies showing that high concentrations of fecal and plasma SCFAs were associated with a response to PD-1 treatment (64, 65).

Although there is no evidence showing that lactic acid or spermidine from gut microbiota influence immunotherapy directly, lactic acid derived from cancer cells suppressed the function of T cells, NK cells, and macrophages, resulting in the attenuated efficiency of anti-PD-L1 and anti-CD47 (66, 67). Gut microbiota-derived spermidine preferentially induces naïve T cells to Tregs in the gut tissue (68).

The purine nucleoside inosine is generated by deamination of adenosine or the action of 5′-nucleotidase on inosine monophosphate. He et al. (69) revealed that gut microbiota regulated levels of the purine metabolite inosine that suppressed the differentiation and inflammation of Th1/Th2 cells *via* $A_{2A}R$ on T cells. Intriguingly, Mager et al. (34) discovered that the inhibition of Th1/Th2 cells is dependent on the absence of IFNγ; when this cytokine is present, inosine acted to advance Th1 differentiation *via* $A_{2A}R$ and boost anti-CTLA4 therapy. In addition, the translocation of inosine-producing bacteria in tumors was not required for the enhancement of immune therapy. Thus, microbiota-derived soluble inosine augments cancer immunotherapy through blood circulation. Besides signaling molecules, inosine is an essential cellular energy. Within tumors, cancer cells rapidly deplete glucose such that infiltrating T cells, which require abundant energy substrates for full function, would have been outcompeted if alternative substrates were not present. Wang et al. (70) demonstrated that inosine is an alternative source of energy to glucose within the tumor microenvironment; the combination of inosine supplementation and administration of anti-PD-L1 led to delayed tumor growth and increased survival time in a mouse model of melanoma. Unfortunately, some cancer cells compete with T cells for inosine as an energy source, which diminished the beneficial effect of inosine supplementation together with anti-PD-L1 (**Figure 1C**).

2 GUT MICROBIOTA IN RESPONSE AND TOXICITY TO IMMUNOTHERAPY

2.1 Gut Microbiota and Immunotherapy

2.1.1 Clinical Evidence Linking Gut Microbiota and Immunotherapy

Several clinical studies, involving Americans, Chinese, Japanese, French, and Netherlands, have demonstrated the association between gut microbiota and immunotherapy (**Table 1**). 16S rDNA sequencing or metagenomic shotgun sequencing (MSS) were used to analyze the composition of gut microbiota.

ICIs were first approved by the FDA to cure melanoma. The response of melanoma to ICIs was associated with a range of factors; integrative molecular and clinical modeling was used to predict the response (86). Since 2017, there have been six clinical studies, including 213 patients, that took insight into the association between the gut microbiota and the immunotherapy on metastasis melanoma (63, 71–75). All of them took the baseline (prior ICI treatment) microbiota into the first consideration. Totally, 26 bacteria were found by those studies to be related to a positive response in metastatic melanoma patients, including longer PFS and overall survival (OS). Among those bacteria, *Faecalibacterium prausnitzii* was found enriched in responders by three studies, from the United States and France, respectively (71–73). In addition, Coutzac et al. revealed that responders had increased *Faecalibacterium* (63). Three species of *Bacteroides* (72, 75), two species of *Bifidobacterium* (74), a species of *Clostridium* (71, 73) correlated with immunotherapy response positively. *Gemmiger formicilis* (71) and *Gemminger* (63), *Ruminococcus bromii* (73), and *Ruminococcus* (71) were reported to be enriched in responders. However, only Gopalakrishnan et al. (73) showed increased alpha diversity in responders.

The anti-PD-1/PD-L1 treatment has become the first-line strategy for NSCLC. There were four clinical studies (76–79) about gut microbiota and ICI focused on NSCLC patients, and another two (80, 81) included NSCLC patients. All of those studies recruited East Asian patients, except for Routy et al. (81). In addition, Katayama et al. (78) and Jin et al. (76) took the progression (during ICI treatment) microbiota into

TABLE 1 | Clinical evidence linking gut microbiota and cancer immunotherapy.

Cancer type	Therapy	Sample size	Alpha diversity	Bacteria related to response	Ref.
Metastatic melanoma	Anti-CTLA-4	26	Not mentioned	*Faecalibacterium prausnitzii*, *Gemmiger formicilis*, butyrate-producing bacteria SS2-1, *Ruminococcus*, Lachnospiraceae, *Clostridium* XIVa, *Blautia*	(71)
Metastatic melanoma	Anti-CTLA-4	38	Not mentioned	*Faecalibacterium*, *Gemminger*	(63)
Metastatic melanoma	ICI	39	No significant difference	*F. prausnitzii*, *Bacteroides thetaiotaomicron*, *Holdemania filiformis*, *Bacteroides caccae*	(72)
Metastatic melanoma	Anti-PD1	43	Higher in responders	*F. prausnitzii*, *Ruminococcus bromii*, *Porphyromonas pasteri*, *Clostridium hungati*, *Phascolarctobacterium faecium*	(73)
Metastatic melanoma	Anti-PD1 or anti-CTLA4	42	Not mentioned	*Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp., *Bifidobacterium longum*	(74)
Metastatic melanoma	ICI	25	No significant difference	*Streptococcus parasanguinis*, *Bacteroides massiliensis*	(75)
NSCLC	Anti-PD1	25	Higher in responders	*Alistipes putredinis*, *B. longum*, *Prevotella copri*	(76)
NSCLC	Anti-PD1/PD-L1	70	Higher in responders	Clostridiales, Ruminococcaceae *UCG 13*	(77)
NSCLC	ICI	17	No significant difference	*Lactobacillus*, *Clostridium*, *Syntrophococcus*	(78)
NSCLC	Anti-PD1	63	No significant difference	*Parabacteroides*, *Methanobrevibacter*	(79)
NSCLC and gastric cancer	Anti-PD1	38	Higher in responders	Ruminococcaceae	(80)
NSCLC and RCC	Anti-PD1	100	Not mentioned	*Akkermansia muciniphila*, Lachnospiraceae, *Erysipelotrichaceae bacterium*, *E. faecium*, *Alistipes indistinctus*, *B. caccae*, *Bacteroides xylanisolvens*, *Bacteroides nordii*	(81)
RCC	Anti-PD1	22	No significant difference	*Akkermansia*	(82)
Solid tumors	Chemotherapy/ immunotherapy	26	Higher in responders	*B. xylanisolvens*, *Bacteroides ovatus*, *P. copri*, *Alistipes* spp.	(83)
Thoracic neoplasms	Anti-PD1	42	No significant difference	Akkermansiaceae, Enterococcaceae, Enterobacteriaceae, Carnobacteriaceae, Clostridiales Family XI	(84)
Gastric cancer	Anti-PD1	501	Higher in responders	*Odoribacter*, *Veillonella*	
Gastrointestinal cancer	Anti-PD1/PD-L1	74	No significant difference	*Prevotella*, Ruminococcaceae, Lachnospiraceae	(65)
Hepatocellular carcinoma	Anti-PD1	8	Higher in responders	*A. muciniphila*, Ruminococcaceae spp., *Bifidobacterium dentium*, *Lactobacillus*	(85)

NSCLC, non-small cell lung carcinoma; ICI, immune checkpoint inhibitor; RCC, renal cell carcinoma.

consideration. In general, a positive correlation between alpha diversity and ICI response was found in three of the six clinical studies (76, 77, 80). Nineteen bacteria were related with a positive response, including *Bacteroides* (81), *Bifidobacterium* (76), *Clostridium* (78), and *Ruminococcus* (78, 80), which correlated with immunotherapy response on metastasis melanoma positively, but *Faecalibacterium* was not in the NSCLC list.

In addition, there are some studies that revealed the association between gut microbiota and immunotherapy in the other solid tumor models (65, 80–85). Three of those clinical studies found the responders with higher alpha diversity (80, 83, 85). Worth mentioning is a clinical study involving 501 patients that also revealed the positive correlation between alpha diversity and ICI response (https://meetinglibrary.asco.org/record/193964/abstract). Similar to results of metastasis melanoma and NSCLC, *Bacteroides* (81, 83), *Bifidobacterium* (85), and *Ruminococcus* (65, 85) were enriched in the responders on other solid tumors. Furthermore, *Akkermansia muciniphila* was found enriched in responders by two individual studies (81, 85); Agarwal et al. (82) and Yin et al. (84) showed that *Akkermansia* correlated with beneficial response.

In summary, many clinical studies identified the association between gut microbiota and immunotherapy. Although various sample volumes from different regions, different collection techniques, cancer types, and distinctive sequencing methods limit the accuracy of gut microbial comparisons, we can find some clues from those studies. First, although only a part of the studies showed a positive correlation between alpha diversity and immunotherapy response, none of them showed a negative correlation, indicating the importance of alpha diversity. Second, *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, and *Akkermansia* were frequently found to be associated with beneficial responses, indicating that they may play a role in regulating immunotherapy. Regrettably, there are no available data showing the association between gut microbiota and the efficiency of neoadjuvant immunotherapy. However, Batten et al. revealed that the diversity and composition of gut microbiota were associated with immune-related adverse events in neoadjuvant immunotherapy (87). Rajji et al. reported that antibiotics were associated with less benefit from neoadjuvant immunotherapy on bladder cancer (88).

2.1.3 Mouse Models Showing the Effect of Gut Microbiota on Immunotherapy

There is no available clinical trial that shows the effect of gut microbiota on immunotherapy; therefore, the effect was illustrated by mouse models only (**Table 2**).

First of all, mice with different gut microbiota show distinct responses to immunotherapy. Wild-type (WT) mice from Jackson Laboratory (JAX) and Taconic Biosciences (TAC) were reported to have a distinct gut microbiome that contributes to their distinct immune signatures (91). The JAX

mice carrying B16 melanoma and MC38 showed enhanced response to anti-PD-L1 and anti-CD47, respectively, compared with TAC mice (28, 41). Besides, loss of gut microbiota by using GF mice or treating specific pathogen-free (SPF) mice with antibiotics ablated the response to immunotherapies, including anti-IL-10 receptor plus CpG-oligonucleotide on MC38 tumor-bearing mice (43), anti-CTLA4 on MCA205 sarcoma-bearing mice (51), anti-CTLA4 or anti-PD1 on MC38 tumor-bearing mice (34, 89), anti-CD47 on MC38 tumor-bearing mice (28), and anti-PD1 on CT26 tumor-bearing mice (92). This phenomenon was a window that revealed the association of gut microbiota with immunotherapy.

Second, when given different gut microbiota, gnotobiotic mice appeared to have a distinct response to immunotherapy, which demonstrated the effect of gut microbiota on immunotherapy, as well. As mentioned above, Matson et al. (74) investigated the gut microbiota of 38 metastatic melanoma patients treated with anti-PD1 and found the difference. Reconstitution of GF mice with fecal material from responding patients could lead to improved tumor control, augmented T-cell responses, and greater efficacy of anti-PD-L1 therapy on the B16 melanoma mouse model (74). In addition, a study by Routy et al. exhibited the same benefit from responders' gut microbiota on MCA205 sarcoma (81).

Last but not least, the beneficial effects of defined bacteria on immunotherapy have also been demonstrated by mouse models. Oral supplementation with *Alistipes shahii* or *Ruminococcus* reversed immunotherapy inhibition by the antibiotic treatment, but not *Lactobacillus fermentum* (43). Gavage of TAC mice with *Bifidobacterium* species enhanced the effect of anti-PD-L1 on MC38 colon cancer, with DC activation and increased IFNγ producing $CD8^+$ T cells (41). As well, *Bifidobacterium* species enhanced the effect of anti-CD47 (28). CD47, known as the "don't eat me" signal, is the phagocytosis checkpoint as a new target for cancer immunotherapy (93). *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, and *Burkholderia* effectively aided immunotherapy of anti-CTLA4 on MCA205 sarcomas depending on intratumoral $CD11b^+$ DCs secreting IL-12 and splenic $ICOS^+$ $Ki67^+$ $IFN\gamma^+$ $TNF\alpha^+$ T cells, and tumor-infiltrating T cells, but not *Parabacteroides distasonis* nor *Escherichia coli* nor *Bacteroides uniformis* (51). Oral gavage with *A. muciniphila* after FMT with non-responder feces restored the efficacy of anti-PD-1 on orthotopic Lewis lung carcinoma (LLC) non-small cell lung cancers and MCA205 mouse models, which depended on IL-12, with increasing recruitment of $CCR9^+CXCR3^+CD4^+$ T cell into mouse tumor, in the mechanism (81). A similar phenotype was also revealed on renal cell carcinoma (RCC) tumor-bearing mice (94). Tanoue et al. (89) isolated 11 human gut bacteria that increased colonic $IFN\gamma^+$ T cells, including *Ruthenibacterium lactatiformans*, *Eubacterium limosum*, *Fusobacterium ulcerans*, *Phascolarctobacterium succinatutens*, *B. uniformis*, *Bacteroides dorei*, *Paraprevotella xylaniphila*, *P. distasonis*, *Parabacteroides johnsonii*, *Parabacteroides gordonii*, and *Alistipes senegalensis*. Administration with the 11-bacterium mix (11-mix) recovered efficacy of anti-PD1 or anti-CTLA4 with infiltration with $IFN\gamma^+$ T cells in MC38 tumor. Mager et al. (34) showed that *Bifidobacterium pseudolongum*, *Lactobacillus johnsonii*, and *Olsenella* species significantly enhanced efficacy of anti-CTLA4 on MC38 model and azoxymethane/dextran sodium sulfate (AOM/DSS) model, with increased $IFN\gamma^+CD8^+$ T cells and $IFN\gamma^+$ $CD4^+$ T cells. Roberti et al. (90) found four immunogenic bacteria (*B. fragilis*, a non-enterotoxigenic species, *Erysipelatoclostridium ramosum*, and *Alistipes onderdonkii*), which were able to boost vaccine (oxaliplatin-exposed organoids) efficacy and anti-PD1 efficacy on MC38, in a $CD103^+CD11b^-$DC [conventional type 1 DCs (cDC1)]-dependent manner.

In summary, *via* mouse models, the causal/mechanistic link between gut microbiota and immunotherapy was illustrated.

TABLE 2 | Gut microbiota enhancing cancer immunotherapy in mice.

Tumor model	Therapy	Beneficial bacteria species	Specific mechanisms	Ref.
B16 melanoma	Anti-PD-L1	*Bifidobacterium*	DC, $IFN\gamma^+CD8^+$ T cells	(41)
B16 SIY melanoma	Anti-PD-L1	Responder patient FMT	$CD8^+$ T cells	(74)
RET melanoma	Anti-PD-1	*Akkermansia muciniphila*, *Alistipes*, *Enterococcus hirae*	$CCR9^+CXCR3^+CD4^+$ T cells *via* IL-12	(81)
MC38 colon	Anti-CD47	*Bifidobacterium*	DC *via* STING	(28)
MC38 colon	Anti-IL-10 + CpG	*Alistipes shahii*, *Ruminococcus*	TNF^+ myeloid cell	(43)
MC38 colon	Anti-CTLA4	*Bifidobacterium pseudolongum*, *Lactobacillus johnsonii*, *Olsenella* species	Th1 cell, $IFN\gamma^+CD8^+$ T cells	(34)
MC38 colon	Anti-PD-1 or Anti-CTLA4	*Ruthenibacterium lactatiformans*, *Eubacterium limosum*, *Fusobacterium ulcerans*, *Phascolarctobacterium succinatutens*, *Bacteroides uniformis*, *Bacteroides dorei*, *Paraprevotella xylaniphila*, *Parabacteroides distasonis*, *Parabacteroides johnsonii*, *Parabacteroides gordonii*, and *Alistipes senegalensis*	$CD103^+$ DC, $IFN\gamma^+CD8^+$ T cell	(89)
MC38 colon	Anti-PD-1	*Bacteroides fragilis*, a non-enterotoxigenic species, *Erysipelatoclostridium ramosum*, and *Alistipes onderdonkii*	$CD103^+CD11b^-$ DC	(90)
MCA205 sarcoma	Anti-CTLA4	*B. fragilis*, *Bacteroides thetaiotaomicron*, *Burkholderia*	Memory T cell	(51)
MCA205 sarcoma	Anti-PD-1	Responder patient FMT, *A. muciniphila*, *E. hirae*, *Alistipes*	$CCR9^+CXCR3^+CD4^+$ T cells *via* IL-12	(81)
RENCA RCC	Anti-PD-1 + anti-CTLA4	Responder patient FMT	$CCR9^+CXCR3^+CD4^+$ T cells *via* IL-12	(81)
LLC lung carcinoma	Anti-PD-1	*A. muciniphila*, *Alistipes*, *E. hirae*	$CCR9^+CXCR3^+CD4^+$ T cells *via* IL-12	(81)

RCC, renal cell carcinoma; DC, dendritic cell; FMT, fecal microbiota transplant; STING, stimulator of interferon genes; LLC, Lewis lung carcinoma.

Bacteroides, *Bifidobacterium*, *Ruminococcus*, *Lactobacillus*, *Enterococcus*, and *Akkermansia*, associated with the response to immunotherapy in clinical studies, were revealed to activate immunity and boost the efficiency of immunotherapy in mouse models.

2.2 Gut Microbiota and Immune Response in Chemotherapy

Although not traditionally considered as immunotherapy, effective chemotherapy is also dependent on intact immune responses; therefore, the effect of gut microbiota on conventional chemotherapy depends on modulating the immune response.

Cyclophosphamide, a prominent alkylating anticancer agent, inhibits tumor outgrowth by inducing immunogenic cancer cell death (95, 96), reverting immunosuppressive T cells (97), and promoting Th1 and Th17 cells (98). GF or antibiotic-treated mice carrying MCA205 sarcoma lost cyclophosphamide tumor inhibition, suggesting that the gut microbiota plays a critical role in controlling cancer during cyclophosphamide treatment. While oral gavage with *E. hirae* clone 13144 and *Barnesiella intestinihominis* reinstated cyclophosphamide efficacy, but not *P. distasonis*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *L. johnsonii*, which segmented filamentous bacteria, even other *E. hirae* isolates (37, 38, 53). In mechanism, *E. hirae* clone 13144 translocated into secondary lymphoid organs, where they stimulated the generation of a specific subset of "pathogenic" Th17 cells and memory Th1 immune responses, which cross-react with tumor-associated antigens. Finally, *E. hirae* clone 13144 increased the intratumoral $CD8^+$/Treg ratio and enhanced chemotherapy (37, 38, 53). *Ba. intestinihominis* raised chemotherapy of cyclophosphamide through yielding tumor IFNγ T-cell infiltration (37).

3 STRATEGIES TO IMPROVE GUT MICROBIOTA IN CANCER IMMUNOTHERAPY

3.1 Fecal Microbiota Transplant

FMT is when stool from a healthy donor is made into a liquid mixture and transferred into the gut of a different person to try to reintroduce or boost helpful organisms, which represents the most direct means to manipulate the gut microbiota. Based on results from preclinical studies discussed above, FMT is considered as an intervention to treat patients undergoing immunotherapy, especially those administered with ICIs, aiming for the safety and response of the combo of FMT and immunotherapy. Currently, melanoma, prostate cancer, gastrointestinal system cancer, NSCLC, and mesothelioma are enrolled by several FMT-related clinical trials (**Table 3**).

The key factor of those clinical trials is the criteria of the donor. Six of nine clinical trials treated patients who respond to immunotherapy as donors (NCT04264975, NCT04116775, NCT04521075, NCT03353402, NCT04577729, and NCT03341143). Recently, the result of the phase 1 clinical trial (NCT03353402) was published (99). To assess the safety and feasibility of fecal FMT and re-induction of anti-PD-1 immunotherapy, the trial recruited 10 patients with anti-PD-1-refractory metastatic melanoma. Two FMT donors were included in the trials who had previously been treated with anti-PD-1 monotherapy and achieved a complete response. First of all, the gut microbiota of all recipients significantly differed from their baseline and closed to the donors. In detail, patients who received donor #1 sample had a greater relative abundance of *Ruminococcus* and *Bifidobacterium adolescentis*, whereas those who received donor #2 sample had an overrepresentation of Clostridiaceae (99). In addition, treatment increased multiple immune-related gene sets in the tumor tissue of donor #1 group, including IFNγ-mediated signaling pathway, T-cell activation, MHC-II protein complex, DC differentiation, and Th1-type immune response (99). Most importantly, three of 10 recipients achieved objective responses, all of them from donor #1 group, and only one recipient had a mild temporary bloating considered as an FMT-related adverse event (99). Another phase 2 clinical trial (NCT03341143) showed that six of 15 PD-1-refractory patients with melanoma benefited from the FMT (100). In this study, seven donors were included, including four with a complete response and three with a partial response. Responders' recipient microbiota exhibited a significant shift toward the donor composition compared with the non-responders'. Successful FMT was enriched in Ruminococcaceae, Bifidobacteriaceae, and Lachnospiraceae. A coinciding immune activity after the FMT was found in blood and tumor microenvironment (100). Three of nine clinical trials treated healthy people as donors (NCT04056026, NCT03772899, and NCT04130763). Interestingly, the activation of immune response was also found in advanced or metastatic melanoma patients with FMT from healthy donors (NCT03772899) (101). Most importantly, these three published trials showed a favorable safety profile and represented the first clinical evidence that the gut microbiota may have an impact on antitumor immunity and potentially even responses to immunotherapies.

Besides the criteria of donors, those clinical trials differed on the FMT preparations (**Table 3**). Generally, FMT preparations can be performed *via* oral administration of lyophilized or frozen pills and capsules, or direct delivery by endoscopy. The lower routes of administration (colonoscopy or enema) appeared to be more successful than the upper routes (gastroscopy, or nasogastric and nasointestinal tubes) (102). Maybe this is the reason that most of those clinical trials administer FMT with colonoscopy.

However, to translate FMT into the clinic, there are a number of problems that we need to face. First of all is the safety issue. FDA has reported safety alerts after the death of patients receiving FMT for *Clostridium difficile* infection who developed infections caused by enteropathogenic bacteria contained in the FMT. Besides harmful bacteria, the harmful virus should also be screened before FMT, considering the intestinal epithelium is a tropism of SARS-CoV-2. Second issue is how to define the optimal donors. Several investigators recruit donors from patients who previously responded to immunotherapy, while

TABLE 3 | Clinical trials linking gut microbiota and cancer immunotherapy.

Interventions	Trial number	Conditions	Major microbiota and immune related outcomes	Phases
FMT				
FMT *via* colonoscopy	NCT04264975	Solid carcinoma	Response to immunotherapy plus FMT	Not applicable
FMT *via* colonoscopy	NCT04056026	Mesothelioma	Response to Keytruda plus FMT	Early Phase 1
FMT *via* colonoscopy	NCT03772899	Melanoma	Response to immunotherapy plus FMT	Phase 1
FMT *via* endoscopy	NCT04116775	Metastatic castration-resistant prostate cancer	Response to pembrolizumab plus FMT	Phase 2
FMT *via* oral capsule	NCT04130763	Gastrointestinal system cancer	Response to anti-PD-1 plus FMT	Phase 1
FMT *via* oral capsule	NCT04521075	Metastatic melanoma or NSCLC	Response to nivolumab plus FMT	Phase 1/Phase 2
FMT *via* colonoscopy and oral capsule	NCT03353402	Melanoma Stage IV and unresectable Stage III	Response to immunotherapy plus FMT	Phase 1
FMT	NCT04577729	Melanoma Stage III and IV	Response to checkpoint inhibitor plus FMT	Not applicable
FMT *via* colonoscopy	NCT03341143	PD-1 resistant/refractory melanoma	Response to checkpoint inhibitor plus FMT	Phase 2
Diet				
Fasting mimicking diet	NCT03454282	Breast cancer or melanoma	Tumor-infiltrating lymphocytes, gut microbiota composition	Not applicable
Dietary supplement: IGEN0206	NCT04009122	Non-small cell lung cancer metastatic	Quality of life, changes in the microbiota, interleukin levels, cytokines levels	Not applicable
Probiotics				
Oral Primal Defense Ultra Probiotic Formula	NCT03358511	Breast cancer	Mean number of cytotoxic T cell	Not applicable
Oral MRx0518	NCT04193904	Pancreatic cancer	Tumor infiltrating lymphocytes	Phase 1
5	NCT03817125	Metastatic melanoma	Response to checkpoint inhibitor	Phase 1
Oral MET-4	NCT03838601	Head and neck squamous cell carcinoma	Bacterial composition and diversity, blood immune cell profiling	not applicable
Oral BB536, LA1	NCT00936572	Colorectal cancer	Immune and inflammatory response, bacterial translocation	Phase 2
IV JNJ-64041809	NCT02625857	Metastatic castration-resistant prostate cancer	Immune responses	Phase 1
Oral MRx0518	NCT03637803	Solid tumors	Clinical benefit of MRx0518 in combination with pembrolizumab	Phase 1/Phase 2
Oral RBX7455	NCT04139993	Breast cancer	Intratumoral immunomodulatory	Early Phase 1
Oral GEN-001	NCT04601402	Solid tumors	Response to avelumab	Phase 1
Oral EDP1503	NCT03595683	Melanoma	Response to pembrolizumab	Phase 2
Oral MET-4	NCT03686202	Solid tumors	Relative abundance of immunotherapy-responsiveness associated species of MET-4	Early Phase 1
Oral VE800	NCT04208958	Selected types of advanced or metastatic cancer	Safety and efficacy of VE800 in combination with nivolumab	Phase 1/Phase 2
Oral MRx0518	NCT03934827	Solid tumors	Safety, tolerability, and immune system modulation of MRx0518	Phase 1
Oral EDP1503	NCT03775850	Colorectal cancer, breast cancer, and checkpoint inhibitor relapsed tumors	Safety, tolerability, and efficacy of EDP1503 alone and in combination with pembrolizumab	Phase 1/Phase 2
Antibiotic				
Oral vancomycin	NCT03785210	Refractory primary hepatocellular carcinoma or liver-dominant metastatic cancer from colorectal or pancreatic cancers	Response to nivolumab	Phase 2

FMT, fecal microbiota transplant; NSCLC, non-small cell lung carcinoma.

others prefer healthy volunteers. Now only three positive results have been published. Two of them showed the benefits from responding patients, and one of them showed the benefits from healthy people. Considering that most studies revealed the difference of gut microbiota between responders and non-responders, it seems that responding patients should be better donors. In addition, the kinds of pathologies of the donor should be excluded. In one case, the obese phenotype has been transferred from a donor to a recipient (103). Last, FMT may benefit from host conditioning, including diet, probiotics, and antibiotics. Further studies are needed to make a synergetic combo of the FMT and host conditioning.

3.2 Diet

As a dominant determinant of interindividual microbiota variation (104, 105), diet is the key determinant of the microbiota configuration, through modulation of the abundance of microbial species and their individual or collective functions (106–108). Hippocrates noted "Let food be thy medicine and medicine be thy food." Owing to the advantageous safety, cost, and availability, diet could be a promising clinical intervention to modulate gut microbiota and downstream immune in cancer patient populations.

Prebiotics are a source of diet for your gut's healthy bacteria. They are carbs that our body cannot digest. The well-known prebiotics, microbiota-accessible carbohydrates, have a major impact on gut microbiota composition, diversity, and richness (109). Microbiota-accessible carbohydrates are fermented by gut microbiota to produce SCFAs, which have been discussed above in modulating immunotherapy. It benefited the exclusion of pathogens such as *Citrobacter rodentium* and *C. difficile* (110, 111). Another prebiotic, plant polysaccharide inulin, increased both *Faecalibacterium* and *Bifidobacterium* species in gut microbiota, which are considered potentially favorable for immunotherapy (112). The effect of a dietary supplemental nutritional product (IGEN0206) on the quality of life, nutritional status, and shift in the gut microbiota of patients with NSCLC was investigated by a clinical study (NCT04552418). Unfortunately, we have no idea of the prebiotics in IGEN0206.

Besides prebiotics, the main components of diets shift gut microbiota and immunity, including calorie, protein, and fat. A plant-based, calorie-restricted, low-protein diet, also known as fasting mimicking diet (FMD), modulated gut microbiota composition and immune cell profiles to reduce inflammatory bowel disease pathology (113). It has been proposed as a potential anticancer dietary intervention by enhancing cytotoxic $CD8^+$ tumor-infiltrating lymphocytes (114). At present, NCT03454282 is designed to explore the impact of FMD on the gut microbiota composition, peripheral blood mononuclear cells, tumor-infiltrating lymphocytes, and metabolic parameters of breast cancer or melanoma patients.

The population structure responds to acute dietary change, as evidenced by rapid and substantial increases in populations at the genus and species levels. However, dietary change does not necessarily induce a permanent compositional shift, at least at the phylum level, although evidence for this assertion is limited (115). As a result, diets might not able to reshape the gut microbiota as dramatically as FMT. But the advantage in safety and convenience of diets is obvious. Considering the restricted effect of diets on gut microbiota, the combination of diets and FMT might give their advantages a full play to modulate gut microbiota and immunotherapy.

3.3 Probiotics

Beneficial or immune-modulating bacteria could be administered as a probiotic to manipulate cancer immunotherapy. Probiotics could provide a more feasible method of microbial manipulation in the clinical setting. Many clinical trials using probiotics in cancer patients have been initiated with some completed (**Table 3**).

Most of the probiotics are composed of single strains. MRx0518 is a strain of *Enterococcus gallinarum*, isolated from a healthy human fecal sample (116). EDP1503 is a strain of *Bifidobacterium animalis* subsp. *lactis*, BB536 is a strain of *Bifidobacterium longum*, and LA1 is a strain of *Lactobacillus acidophilus*. JNJ-64041809 is a live attenuated, double-deleted *Listeria* administered intravenously. GEN-001 is a single-strain bacteria isolated from the gut of healthy human volunteers. As mentioned above, *Enterococcus*, *Bifidobacterium*, and *Lactobacillus* are related to immunotherapy (74, 76, 81, 85). Especially, *Bifidobacterium* species have been demonstrated to enhance the response to ICIs in animal models by several studies (28, 34, 41). Currently, initial data from the first six patients of NCT03637803 showed that MRx0518 combined with pembrolizumab is well tolerated in patients with solid tumors who have developed resistance to anti-PD-1/PD-L1. Two patients have shown a partial response with evidence of increased tumor-infiltrating lymphocytes, according to the RECIST v1.1 criteria 1. One additional patient has a stable disease. No drug-related serious adverse events have been noted (https://www.londonstockexchange.com/news-article/DDDD/clinical-observations-from-mrx0518/14295955). Initial data of NCT03775850 show that an overall response rate (ORR) of 25% (2/8) and a disease control rate of 37.5% (3/8) were observed across all triple negative breast cancer (TNBC) subjects receiving high-dose EDP1503. ORR was 33% (2/6) among response-evaluable patients on the high dose, with two patients awaiting first response assessment. Historic studies of anti-PD-1 monotherapy in heavily pretreated TNBC patients have yielded an ORR of 5%–10% (https://ir.evelobio.com/news-releases/news-release-details/evelo-biosciences-present-clinical-data-phase-12-trial-edp1503). NCT02625857 showed that JNJ-64041809 has a manageable safety profile and activation of the immune response. Nevertheless, observed immune activation with monotherapy did not translate into clinical activity (117).

The probiotics could also be a consortium of live bacteria, including Primal Defense Ultra Probiotic Formula, SER-401, and MET-4 and VE800. VE800, which consisted of 11 clonal human commensal bacteria strains, activated the immunotherapy *via* $CD8^+$ T cells in animal models (89). The consortium seems more powerful in shifting gut microbiota than single bacteria; however, it is a pity that there are no available clinical data to show the safety of probiotic consortiums, although five clinical trials are going to reveal the safety and clinical response.

In addition to the strain isolated from humans, synthetically engineered microorganisms can also be implanted as probiotics.

Advances in synthetic biology are enabling the design of microorganisms based on therapeutic needs. Currently, engineered a non-pathogenic *E. coli* strain, expressing encoded nanobody antagonist of CD47, or nanobodies targeting PD-L1 and CTLA4, or STING agonist, were administered to activate systemic antitumor immunity and to regress tumor burden in mouse models (118–120), although, until now, those engineered probiotics were designed to kill tumors, directly. Along with a deep understanding of the role of gut microbiota in cancer immunotherapy, engineered probiotics will be applied to modulating gut microbiota, as an adjuvant of immunotherapy.

We believe that probiotics are the future to improve gut microbiota for immunotherapy. Compared with FMT, probiotics do not need donors nor the criteria for donors. In addition, probiotics contain less harmful and dispensable matter. Last, probiotics are easier for the industry. However, a deeper understanding of the mechanism between gut microbiota and immunotherapy is needed to develop immunotherapeutic probiotics.

3.4 Antibiotics

Antibiotic administration is another straightforward intervention to module gut microbiota and the downstream cancer immunotherapies. By removing harmful bacteria, some antibiotics can provide a positive effect on the gut microbiota and immunotherapy. Vancomycin targets gram-positive bacteria, including butyrate-producing bacteria and decreasing SCFA concentrations. Vancomycin treatment induced an increase of systemic $CD8\alpha^+$ DCs, tumor-associated antigen cross-priming with antitumor $CD8^+$ T cell elicitation, and tumor growth inhibition in mice, *via* decreasing SCFAs (121, 122). Recently, a phase 2 single-arm clinical trial (NCT03785210) was designed to investigate if nivolumab given with tadalafil and vancomycin causes liver tumor to shrink (**Table 3**).

Nevertheless, antibiotic classes should be carefully considered. Due to the lack of specificity, antibiotics decrease bacterial diversity, eliminate beneficial bacteria, and give rise to dysbiosis. As a matter of fact, numerous clinical studies from France, China, Japan, Canada, and the United States unleashed antibiotic treatment prior to immunotherapy was associated with reduced clinical benefit on melanoma (123), NSCLC (81, 124–127), and RCC (81, 94, 124, 128). All of those studies found that patients with antibiotic treatment prior to immunotherapy had decreased diversity of gut microbiota and worse PFS and OS. To some extent, the results are consistent with the investigation of responders and non-responders showing a positive correlation between alpha diversity and immunotherapy response. On the other hand, taking the advantage of broad-spectrum depletion of naïve gut microbiota, antibiotics could be used before FMT to achieve better microbial modulation. For instance, Baruch et al. (99) treated patients with vancomycin and neomycin to deplete their own native microbiota before FMT *via* colonoscopy and *via* oral capsules.

4 DISCUSSION

The dynamic nature of the microbiota makes it an attractive target for therapeutic intervention in a range of conditions, as engraftment or elimination of particular microorganisms. The shift of gut microbiota contributes to altering both innate and adaptive immunity. In addition, many studies incorporating preclinical and clinical studies have gained our insight into the influence of gut microbiota on cancer immunotherapy. Via MAMPs, microbial metabolites, and molecular mimicry, the gut microbiota educates both local and systematic immunity to alter the response to cancer immunotherapy. Therefore, in the age of microbiome, therapeutic strategies targeting gut microbiota, including FMT, diet, probiotics, and antibiotics, are developed to enhance responses to cancer immunotherapy. However, there is still a great deal to investigate the inherent mechanisms, as well as optimal strategies.

To identify causal host–microbiota relationships and mechanisms, there are two approaches generally, the microbiota-based approach and the molecule-based approach (129). The microbiota-based approach is the more often used. First, a complex microbiota is found to promote a given phenotype. Then several methods, including 16S DNA sequence, antibiotic treatment, and *in vitro* culture, are used to narrow down the entire microbiota to a single effector species or consortium. Furthermore, single species intervention and/or bacterial genetic engineering studies are performed to uncover the mechanisms. The molecule-based approach starts from a small molecule, which is proven to promote a given phenotype. Then by searching genomic databases or the literature, the biosynthetic machinery of the molecules and the functional species will be identified. Because many metabolic pathways are conserved in bacteria, the molecule-based approach bacteria may be possible to identify several effector species. If necessary, further investigations are needed to identify the most critical species. Up to now, nearly all of the mechanism studies focusing on the role of gut microbiota in response to immunotherapy belong to microbiota-based approaches (28, 34, 37, 41, 43). No study used a molecule-based approach to explore the host–microbiota relationships in cancer immunotherapy. Given the fact that there are many sensitive *in vitro* models in investigating cancer immunotherapy (130, 131), systematic screening of microbiota-derived molecules with those models is an effective method to identify the molecules associated with the given phenotype. Furthermore, the systematic screen will provide one or more great starts for the molecule-based approach in further revealing the inherent mechanisms. Therefore, the molecule-based approach should be a window to explore causal host–microbiota relationships and mechanisms in cancer immunotherapy.

Furthermore, additional complexities exist as we move forward with optimal microbiota-based strategies to improve therapeutic responses. First, although these clinical studies drew similar conclusions those clinical studies linking gut microbiota and immunotherapy and some beneficial bacteria have been identified by clinical and preclinical studies, there were diverse results; it is not very clear what composition of the gut microbiome is optimal to facilitate antitumor immunity. More researches should be performed to define the ideal gut microbiota for immunotherapy. Second, although there are various range of therapeutic options to

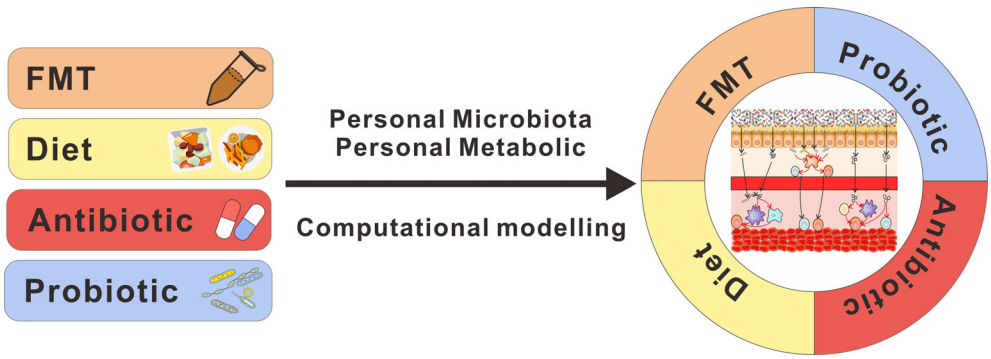

FIGURE 2 | Design of microbial therapeutics to benefit cancer immunotherapy. Stable microbial engraftment is manipulated by intrinsic microbiota, extrinsic nutrients, and colonic metabolic. As a tool, computational modeling can be used to predict the engraftment of microbiota members. Thus, with the deeper insight into this field, the rational design of microbial therapeutics will take all of those factors into consideration and combine those individual therapeutic strategies to improve cancer immunotherapy.

shift gut microbiota, precise modulation with gut microbiota remains difficult owing to the interindividual heterogeneity inherent in humans. Computational models could help in the precise design of microbial therapeutics, which can be used to predict the engraftment of immunomodulatory microbiota members (132). Based on taxonomic analysis of gut microbiota, machine learning can provide new insights to predict disease states and outcomes, which is beneficial for personalized medicine (133). Last, stable microbial engraftment can be manipulated by intrinsic microbiota, extrinsic nutrients (134, 135), colonic metabolic state (136), and immune state (137). Thus, precision medicine interventions in gut microbiota and a rational combo of those individual therapeutic strategies are required to optimize to match the genetic, microbial, and metabolic profiles (**Figure 2**). Unfortunately, we lack the ability to reliably predict how these factors influence bacteria and their immunomodulatory properties, currently. Although the promise of microbial therapy has been revealed in cancer immunotherapy, a number of further studies are still needed to optimize therapeutic strategies.

AUTHOR CONTRIBUTIONS

BL, TG, and YH wrote the paper. XZ and LC revised the paper. All authors contributed to the article and approved the submitted version.

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