REVIEW

# The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies

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**The fine line between human health and disease can be driven by the interplay between host and microbial factors. This "metagenome" regulates cancer initiation, progression, and response to therapies. Besides the capacity of distinct microbial species to modulate the pharmacodynamics of chemotherapeutic drugs, symbiosis between epithelial barriers and their microbial ecosystems has a major impact on the local and distant immune system, markedly influencing clinical outcome in cancer patients. Efficacy of cancer immunotherapy with immune checkpoint antibodies can be diminished with administration of antibiotics, and superior efficacy is observed with the presence of specific gut microbes. Future strategies of precision medicine will likely rely on novel diagnostic and therapeutic tools with which to identify and correct defects in the microbiome that compromise therapeutic efficacy.**

Cancer cells frequently express tumor-associated antigens that are targetable by T lymphocytes; however, concomitant immune regulatory molecules often suppress these functional immune responses. Therapeutic agents uncoupling these immune checkpoints have recently been shown to have a major impact on patient treatment outcomes. In particular, monoclonal antibodies (mAbs) that block the engagement of the inhibitory receptor PD-1 by its main ligand PD-L1 have to date been approved by the U.S. Food and Drug Administration (FDA) for use in the treatment of patients with 10 distinct tumor types (*1*). However, the majority of tumors appear to lack infiltration with T cells, and expression of immune genes indicative of an active immune response. Major efforts are being made to overcome the mechanisms of primary resistance to immunotherapy (*1*). Besides tumor cell–intrinsic oncogenic pathways, additional host and environmental factors can have a major impact on the degree of endogenous immune responses and hence the efficacy of cancer immunotherapeutics (*1*). The composition of the gut microbiome has emerged as one major factor that exerts a profound impact on the peripheral immune system, including in the cancer context (*2*). The mammalian microbiome represents all host-associated microorganisms, a complex and diverse ecosystem residing at portals of entry on all epithelial barriers. It encompasses the bacterial microbiome, the archaeal microbiome, the virome (bacteriophages and eukaryotic viruses), the mycobiome (fungi), and the meiofauna (unicellular protozoa and helminthic worms) (*3*) and is acquired after birth through vertical transmission and then shaped by environmental exposure throughout life. Disrupting the repertoire of the gut microbiome, which is sometimes referred to as "intestinal dysbiosis," has been epidemiologically (and sometimes causally) associated with a variety of chronic inflammatory disorders (*4*). Here, we discuss the impact of the bacterial microbiome on the relationship between cancer and the immune system, and the potential therapeutic utility of directly manipulating commensal microbiota as an approach to enhance the efficacy of cancer immunotherapy.

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## Early steps: A role for the microbiome in cancer

The cardinal role of the intestinal microbiota in regulating health and diseases has only recently been fully appreciated (Fig. 1) (*4*). The human gut microbiome contains ~$3 \times 10^{13}$ bacteria, most of which are commensals (*5*). From birth, the intestinal microbiota plays a crucial role in the life-long programming of innate and acquired immune responses; it fine-tunes the delicate balance between inflammation, infection, and tolerance of food and commensal antigens (*4*, *6*). Beyond effects on intestinal and local immune physiology, the gut microbiome has systemic effects throughout the meta-organism (*6*). Exemplifying this notion, critical host fitness-promoting traits are missing in laboratory mice maintained under specific pathogen–free (SPF) conditions, compared with wild free-living animals. Transfer of the wild gut microbiome to laboratory mice induces long-lasting immune modulatory effects (over several generations), which improves disease outcome against viral infection and mutagen- and inflammation-induced carcinogenesis (*7*).

The microbiome has been discovered to be involved in the initiation and progression of various types of cancer, both at epithelial barriers and within sterile tissues (*8*). Commensal ecosystems inhabiting the intestine or other mucosae play a role in both local and distant carcinogenesis. Microbes can directly act as cancer-transforming agents, by providing a toxic metabolite or an oncogenic product, or indirectly by inducing inflammation or immunosuppression. Moreover, fecal microbial transplantation can transfer the neoplasia-prone phenotype from knockout mice lacking some immune-relevant genes (such as *Tbx21*, *Nod2*, *Nlrp6*, or *Tlr5*) of wild-type mice (*8*). By contrast, accumulating evidence supports a positive role for bacteria in combating cancer located at sites that are distant from the gut, through potentiation of host antitumor immune responses (table S1) (*9*). Epidemiological studies supported by experiments in rodents suggest a dose-dependent association between antibiotic use and risk of cancer (*9*). Taken together, these studies laid the theoretical framework to identify microbes that may bestow anticancer activities.

## The gut microbiome and immuno-oncology

An early hint suggesting an immunotherapeutic effect of the microbiome came from studies of total-body irradiation, which enhanced the efficacy of tumor-specific T cell transfer through translocation of a bacterial product, the toll-like receptor 4L (TLR4L) lipopolysaccharide, from the intestinal lumen to secondary lymphoid organs (*10*). Soon after, it was observed that several anticancer treatment modalities showed reduced therapeutic effects in germ-free mice as well as in mice treated with broad-spectrum antibiotics (*11*–*14*), or in mice lacking specific immune-potentiating bacterial species originating from different vendors (*15*). Such results were obtained with metronomic cyclophosphamide (*12*), chemotherapy with platinum salts (*11*), immunotherapy through a combination of TLR9 antagonist and antibody to interleukin-10R (IL-10R) (*11*), or administration of mAbs to CTLA-4 and/or PD-1/PD-L1 (*13*, *14*). In each case, therapeutic efficacy was curtailed when the gut microbiota was absent or manipulated. The fine mechanisms explaining the contribution of the microbiome to therapy-induced anticancer immune responses may differ for each treatment modality. For instance, cyclophosphamide increases the permeability of the upper gastrointestinal (GI) tract, leading to translocation of the small intestine–residing *Enterococcus*

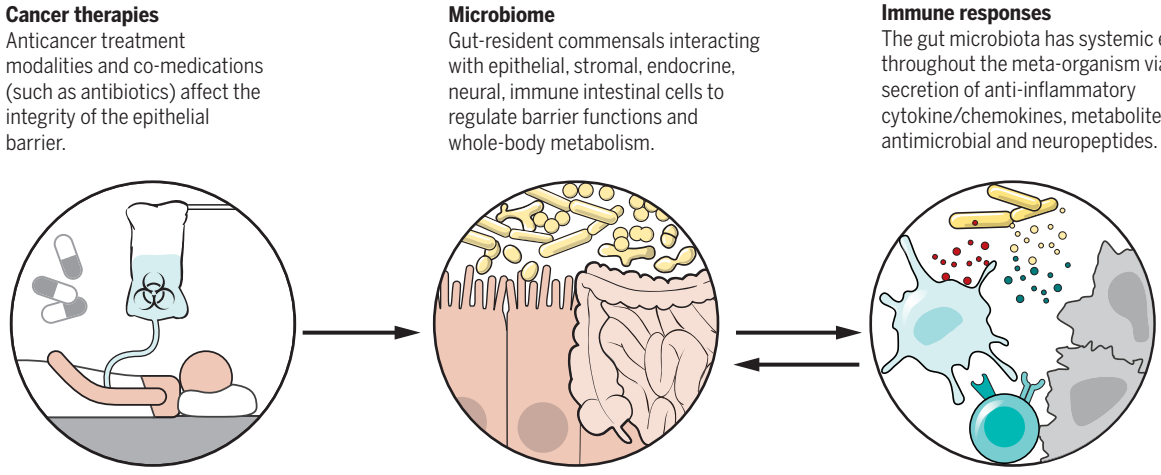


**Fig. 1. The microbiome at the crossroads between physiology and pathology in cancer.** The intestinal microbiota plays a crucial role in the life-long programming of innate and acquired immune responses because it fine-tunes the delicate balance between inflammation, infection, and tolerance of food and commensal antigens. Several therapeutic modalities could be harnessed to restore the homeostasis of the gut and the metaorganism during cancer progression and treatment.

*hirae* to the spleen, but also the accumulation of *Barnesiella intestinihominis* in the colon, which together exert a coordinated immunostimulatory effect on antitumor immune responses (*16*). Upon CTLA-4 blockade, intraepithelial lymphocytes damage ileal epithelial cells, stimulating the accumulation of *Bacteroides fragilis* and *Burkholderiales* spp., activating IL-12–producing dendritic cells (DCs) and T helper 1 ($T_H1$) immune responses (*13*). Therapeutic efficacy of PD-1/PD-L1 blockade was associated with the presence of *Bifidobacterium* spp., which activate antigen-presenting cells (*15*). The immunizing effects of antibody to IL-10R + the TLR9 agonist CpG were linked to the activation of myeloid cells and tumor necrosis factor–α (TNFα) secretion within the tumor microenvironment (*11*). The causal relationship between the dominance of distinct commensals and the efficacy of anticancer therapies in these examples has been proven with mouse cohousing experiments or oral gavage with defined species (table S1).

Corroborating these experimental findings, several independent retrospective analyses in human cohorts of metastatic lung, kidney, and bladder cancer patients indicated the deleterious role of different classes of antibiotics taken around the initiation of mAbs to PD1/PDL-1 (*14*). These retrospective analyses of patients treated with second-line therapies for FDA-approved indications needs to be confirmed in future prospective clinical trials and validated in other treatment contexts (for example, first-line versus second-line immunotherapies, and additional cancer types). In hematological malignancies, intestinal bacteria also modulate the risk of infection and graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (ASCT). Early administration of systemic broad-spectrum antibiotics in ASCT was associated with increased GVHD, and worse transplant-related mortality, presumably by depleting protective *Clostridiales* and *Blautia* in the intestinal microbiota (*17*).

Perhaps the most provocative data suggesting the importance of commensal microbiota in clinical efficacy of cancer immunotherapy have been derived from the sequencing of baseline stool samples from patients being treated with antibody to PD-1–based therapies. Recent advances in sequencing technologies have improved our capacity to stratify patients on the basis of their microbial metagenomic fingerprint (*13*–*15*, *18*–*20*). 16*S* ribosomal RNA (rRNA)–based sequencing of gene amplicons and shotgun DNA sequencing of patient stool samples have identified subsets of bacteria more abundant in responding versus nonresponding patients. In some cases, decreased α diversity or richness of fecal bacterial composition was correlated with worse patient survival. It should be highlighted that variations exist between the different research studies, which included patients with distinct genetic and nutritional patterns, clinical trials that were conducted in different geographic locations within the United States or Europe, and different types of tumors, including lung cancer, renal cell cancer, and melanoma. Given these considerations, it is perhaps even more striking that bacteria generally associated with health (such as *Clostridiales*, *Ruminococcaceae*, *Faecalibacterium* spp., *Akkermansia muciniphila*, *B. fragilis*, and *Bifidobacteria*) (*13*, *14*, *18*–*20*) or immunogenicity (*Enterococci*, *Collinsella*, and *Alistipes*) (*11*, *13*, *14*, *20*) were found to be abundant in responding patients. Further corroborating these findings of solid malignancies, a study performed in 541 patients with hematologic malignancies reported that the gut microbiome at diagnosis influenced the probability of relapse within 2 years after ASCT. In this context, a high abundance of a bacterial group composed mostly of *Eubacterium limosum* had a positive prognostic impact (*21*).

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Data supporting a causal role for improved immunotherapy efficacy have been derived by using transfer of patient fecal samples into germ-free (GF) or antibiotics-treated SPF mice, that subsequently were inoculated with mouse syngeneic tumors and then treated with mAbs to CTLA-4 and/or PD-1/PD-L1 (table S1) (*13*, *14*, *19*, *20*). Notably, fecal microbial transplantation (FMT) of feces from patients (who showed clinical response to immune checkpoint blockade) transferred a "responder" phenotype to recipient mice, whereas "nonresponder" patient feces tended to confer nonresponsiveness to recipients (*13*, *14*, *19*, *20*). These results highlight that the responder/nonresponder status of recipient mice was derived from the composition of the donor microbiome via FMT. Several defined bacteria species were identified that conferred improved immune-mediated tumor control in reconstituted mouse systems in vivo. This effect depended on distinct *Bacteroides* species in melanoma treated with ipilimumab (*13*) and at least partly on *Faecalibacterium* (*19*) in melanoma and on *Verrucomicrobiacae* [more specifically, *A. muciniphila* (*14*)] in lung cancer patients treated with the PD-1 inhibitors pembrolizumab or nivolumab.

Uncoupling efficacy from toxicity has always been a holy grail in clinical oncology. Evidence suggests that a microbiome rich in *Blautia* and *E. limosum* (which results from avoiding antibiotics that kill anaerobic bacteria) favors longer

survival after ASCT because such a microbiome simultaneously reduces GVHD and boosts graft-versus-leukemia effects (*17*, *21*). In patients with metastatic melanoma treated with the checkpoint inhibitor ipilimumab (antibody to CTLA-4), the abundance of *Bacteroidetes* inversely correlated with the severity of colitis (*22*). Accordingly, *B. fragilis* and *Burkholderia cepacia* administered via gavage to mice reduced the severity of colitis induced by mAb to CTLA-4 (*13*, *22*).

In parallel lines of investigation beyond immunotherapy, there is growing awareness that microbial metabolism of anticancer drugs may promote tumor chemoresistance (table S1). For example, the majority of pancreatic adenocarcinomas were reported to be invaded by high densities of *Gammaproteobacteria* expressing the long isoform of cytidine deaminase, an enzyme that deactivates gemcitabine. Colorectal cancers enriched in *Fusobacterium nucleatum* also demonstrated worse prognosis, in part because the bacteria conveyed resistance to oxaliplatin and 5-fluorouracil by inducing autophagy as a cellular defense mechanism in malignant cells (table S1). Altogether, these observations support the impact of intestinal microbiome composition—and that of individual phyla and species with contrasting activities—on the evolution of cancers and their response to treatment with immunotherapy or chemotherapy.

## Immunostimulation by the microbiome: Mechanisms of action

Defining the mode of action of microbes will likely become crucial for monitoring their beneficial effects in the context of cancer treatments. On theoretical grounds, the gut flora may activate anticancer immune responses in numerous ways. The main hypothetical mechanisms are (i) through the stimulation of T cell responses against microbial antigens, which either provide help for tumor-specific immune responses, or may cross-react against tumor-specific antigens; (ii) through engagement of pattern recognition receptors that mediate pro-immune or anti-inflammatory effects; or (iii) via small metabolites that mediate systemic effects on the host.

Peptide or lipid structures from bacteria can activate a range of distinct T cell receptors, thus selecting a surge of T lymphocytes that might be expanded and enter the circulation. Recent data have suggested that bacterial epitope-specific T cells can be found within the tumor microenvironment in mouse models, perhaps because of the high level of chemokines that can be produced by tumor cells, which in turn recruits the normal gut-homing $CCR9^+$ T cells (*14*). In principle, such T cells could produce cytokines or express CD40L and thereby provide help to tumor antigen–specific $CD8^+$ T cells. Alternatively, it is conceivable that they could recognize cross-reactive antigens expressed by normal or cancer cells. Such a molecular mimicry across the meta-organism has been insinuated to support the development of autoimmune diseases (*23*). However, thus far there are very scarce reports indicating that such cross-reactivities may provide an etiological link between the microbiome and responses to tumor-associated antigens expressed by malignant cells (*24*).

***"One of the striking findings that distinguishes cancer patient responders from nonresponders after PD-1 blockade immunotherapy is the ratio of putatively favorable to unfavorable bacteria."***

In terms of effects on pattern recognition receptors, DCs exposed to microbes (such as *B. fragilis* or *A. muciniphila*) associated with anticancer properties induce systemic IL-12–dependent $T_H1$/Tc1 immune responses beneficial against tumors treated with antibody to CTLA-4 (*13*) or PD-1/PD-L1 (*14*). In the presence of *Bifidobacteria*, type I interferon (IFN)–related immune genes are up-regulated in antigen-presenting cells of secondary lymphoid organs (*15*). Ligands of TLRs or Nod-like receptors (NLRs) may mediate the effects of such bacteria, as documented for *E. hirae* (*16*, *25*), *B. fragilis* (*13*), and *A. muciniphila* (table S1). Indeed, *Alistipes shahii* stimulates TNFα production by tumor-associated myeloid cells during immunotherapy combining TLR9 agonists with IL-10R blockade in a TLR4-dependent fashion (*11*). However, the microbial stimulation of anticancer memory $T_H1$/Tc1 cell responses only partially rely on TLR2/TLR4 in the context of treatments with cyclophosphamide (*16*) or CTLA-4 blockade (*13*). What is not yet clear is whether DC precursors attain exposure to bacterial-derived products in the vicinity of the intestinal mucosa and then traffic to the tumor and tumor-draining lymph node, or whether systemically circulating mediators dependent on specific gut bacteria can have distant effects on DCs elsewhere in the host. On a different note, nociceptive neuropeptides produced by bacteria may also affect the host, not only through the activation of sensory neurons but also by eliciting immunoregulatory T cell subsets in the gut (*26*). These findings suggest that the enteric nervous system may constitute yet another target for local (and perhaps systemic) immunomodulation.

The gut microbiome has a major impact on host metabolism, including immunometabolism. Polyamines generated in the gut—such as spermidine, as well as vitamin B6—can stimulate autophagy at distant sites of the body, eliciting anticancer immune responses in the context of chemotherapy (*27*). The capacity of the microbiome to generate polyamines has also been associated with reduced toxicity of antibody to CTLA-4 in melanoma patients (*22*). Short-chain fatty acids produced by gut bacteria are sensed by a variety of cell types, including DCs and regulatory T cells expressing the G protein–coupled receptors GPR41 or GPR43 (*28*). A microbe-associated metabolite, desaminotyrosine (DAT), derived from *Clostridium orbiscindens* has been reported to protect from influenza virus–mediated lung immunopathology through type I IFN signaling (table S1). Bacterium-derived dipeptide aldehydes mediate cathepsin L inhibition, which may enable gut mutualists to stably occupy a niche in the phagolysosome and interfere with antigen presentation of epithelial or immune cells (*28*). It can be anticipated that these and other, yet-to-be-discovered bacterial metabolites may profoundly

**Box 1. Culturomics approaches to discover new biotherapeutics.**

The description of cancer-associated intestinal dysbiosis constitutes an unmet medical need. So far, techniques aimed at identifying microbes were based almost exclusively on metagenomic studies. Metagenomics has several limitations, mostly related to DNA extraction methods, amplification steps, and big-data computerized analyses. Only 15% of bacteria grown from feces are detectable with metagenomics. The absence of clear definitions of uncultivatable species led to a considerable number of operational taxonomic units with a marginal taxonomic value. A large part of the bacterial cells in stools analyzed with metagenomics are nonviable at the time of defecation. The sensitivity of polymerase chain reaction is often incompatible with the detection of bacterial species located in the upper GI tract, where the vast majority of metabolic and immune functions are regulated. Automatic sampling of small intestinal content and mucosal specimens by means of ingested capsules containing miniaturized devices may constitute a technique to circumvent this limitation. Culturomics, the cultivation of all microbes living in mucosae, was developed in 2008 by using a combination of diversified culture media and rapid identification of bacterial colonies by means of matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry alone or combined with 16S rRNA sequencing (*40*). Thus, the development of new culture media for anaerobic bacteria or metanogenic oxygen-sensitive archae or slowly growing populations forming microcolonies has been carried out (*40*), enabling the description of more than 400 new species. To render the microbiome "druggable," one needs to develop good manufacturing processes to grow commensals by using chemically defined media, without natural products originating from animals. Last, the ability to lyophilize the microorganisms while maintaining their viability after freeze-drying remains crucial for future implementations.

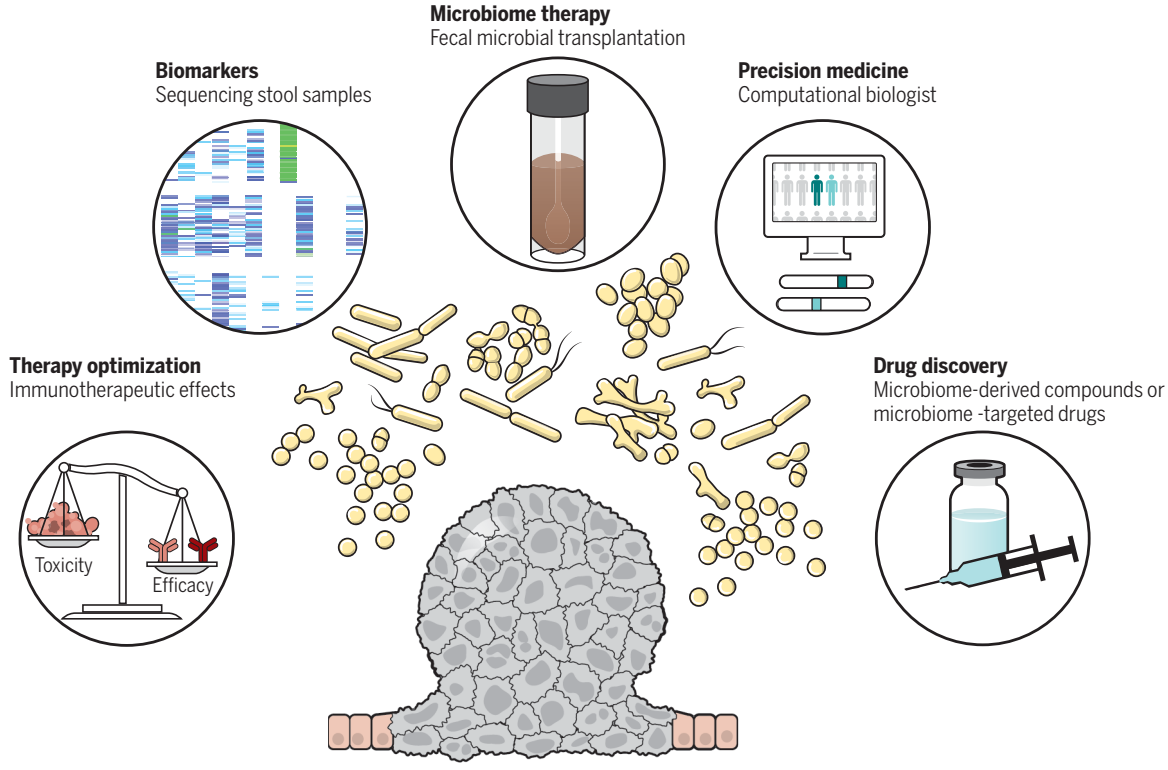


**Fig. 2. Harnessing the microbiome for the discovery of diagnosis and therapeutic tools in cancer.** The complex interplay between cancer, immunity, and microbiota may be partially elucidated by novel "omics" technologies: metagenomics, metatranscriptomics, culturomics, metabolomics, co-occurrence analyses, and three-dimensional crypt stem cell–derived enteroid in contact with distinct species of the microbiome and immune subsets, all integrated through computational biology. Such an apothecary of omics technologies will generate diagnosis tools for dysbiosis, for drug screening and novel therapeutics, such as artificial ecosystems or new microbial species, as well as small bioactives that either act on the microbiota to induce favorable shifts in its composition or mimic desirable microbial effects on the host. Microbial intervention as a cancer therapeutic includes prebiotics, probiotics or live bacteria (and associated phages), and natural products (autologous or allogeneic fecal microbial transplantation). These interventions will have to be adapted according to the patient's life style, comorbidities, comedications, and genetic inheritance for an optimized personalization of his or her therapy. Building on pioneering studies (*14*, *19*, *20*), new biomarkers based on microbial composition of the stool will likely emerge. Monitoring the microbiome will also be essential as a pharmacokinetic and dynamic parameter, longitudinally on new interventional studies aimed at targeting the intestinal ecosystem.

influence the host immune system. The generation of diagnostic tools and novel therapeutics will provide a challenge that will require molecular analysis of the microbiome, metabolism, and immunity cycle via novel "omics" technologies and subsequent integration through systems biology methods (Fig. 2).

## Microbial interventions as a cancer therapeutic

If patients lack colonization with a community of commensal microbes that support endogenous T cell priming against tumor antigens, it is somewhat intuitive to consider FMT from a donor patient who has a favorable microbiome. Impressive results of FMT (up to 81% response rate) are well known to be effective for the treatment of refractory *Clostridium difficile* diarrhea (*29*). However, there are multiple critical parameters to consider for this approach, most notably the selection of the ideal donor. In principle, it should be an individual with a diverse microbial composition that includes bacteria associated with favorable treatment outcomes. One consideration could be to use material from cancer patients that had a major clinical response to mAbs to PD-1. However, there are reasons to approach this strategy with caution. In addition to modulating immune responses, the composition of commensal microbiota has been linked to chronic diseases, with a theoretical risk of transferring obesity from donor to recipient (*30*). Transfer of pathogens is also a potential concern—either bacterial, viral, or parasitic—necessitating careful screening. In addition, some bacteria appear to contribute to inflammation-induced carcinogenesis. FMT from colorectal patients into germ-free mice can elicit dysplasia and polyp formation, which did not occur with transplantation from normal donors (*31*). Additional variables are whether to use fresh or frozen donor fecal material, identification of optimal storage conditions, and whether a single FMT is sufficient, or multiple will be required (*32*).

A desirable alternative to transfer of a mixed population of commensal bacteria from a given donor is to use defined bacteria, either singly or in combination. This strategy will depend on identification of the precise bacterial isolates capable of supporting improved antitumor immunity in the human host, combined with culture conditions that can support their expansion in vitro and encapsulation protocols that preserve biologic activity upon oral administration. The current 16*S* rRNA and shotgun sequencing strategies are likely preferentially detecting the most abundant bacteria correlated with favorable clinical outcome. However, it is conceivable that less abundant bacterial entities that coexist with the more abundant species are functionally important. Therefore, careful culture, isolation, and mechanistic testing of rare species (some of which may reside in the small intestine and be less abundant in stool samples) will need to be considered. Communities of bacteria also may be involved in the immune-potentiating effects of gut microbes, rather than a single major species. For protection against vancomycin-resistant *Enterococci feacium*, a consortium of five bacteria was identified that functioned together in vivo (*33*). Because only a minority of bacterial entities identified through sequencing appear to be culturable with standard methods, improvement of protocols for optimal

in vitro growth will become a critical component for moving this strategy forward (Box 1). It is essential that great care be taken to use actual bacterial isolates that have the desired biological properties, which will necessitate focusing beyond the species and down to the strain level. A panel of cultured *Bifidobacterium* and *E. hirae* species and strains showed major distinctions based on genome sequencing, and clearly not all strains will be efficacious in vivo (*16*, *34*). Once specific bacteria candidates are identified, then a final challenge would be to use preclinical systems for functional screening. For in vivo testing, several mouse models have been used, including SPF mice having defined commensal bacterial compositions or preconditioned with antibiotics, or germ-free mice that lack commensals at baseline. To date, *Bifidobacteria* spp. (*15*, *20*), *Akkermansia muciniphilia* (*14*), *E. hirae* (*16*), and *Bacteroides* spp. (*13*) have been shown to improve antitumor T cell responses and support better tumor control in vivo. Although it is likely that mice cannot support colonization with all commensals that have been adapted to the human GI tract—and thus represent an imperfect system—it may be sufficient to focus on bacteria that do successfully thrive in both hosts during these early days of therapeutic development.

One of the striking findings that distinguishes cancer patient responders from nonresponders after PD-1 blockade immunotherapy is the ratio of putatively favorable to unfavorable bacteria (*20*). Thus, it is conceivable that a subset of commensal organisms have a negative impact on immunotherapy efficacy. Strategies aimed at specifically eliminating unfavorable bacteria while providing immune-potentiating effects should be pursued. While standard antibacterial antibiotics may lack specificity and pose a risk, more precise strategies are warranted. It is noteworthy that bacteriophages can be highly selective for a given bacterial species and are already being used in the food industry to eliminate unfavorable bacteria (*35*). Dietary or chemical entities that support the colonization and expansion of selected bacteria are collectively referred to as prebiotics. In principle, prebiotics should favor the relative expansion of specific bacterial entities that could have a favorable impact on antitumor immunity. Much investigation has been done with dietary fiber, components of which are metabolized to short-chain fatty acids that can have immunomodulatory properties (*36*). However, prebiotics rely on expansion of the types of bacteria that are already present in the host or ingested naturally over time. Hence, controlling for interpatient heterogeneity and experimental variables may make pharmacologic development of prebiotics as a stand-alone therapy challenging. Combinatorial administration of a prebiotic with specific bacteria (called "synbiotics") may be attractive as an integrated approach. Dietary interventions are already being evaluated for GVHD, with the goal of modulating host microbiota (ClinicalTrials.gov, NCT02763033). Besides diet interventions, other factors such as exercise, concomitant medications, and likely sleep cycles can modulate gut microbial composition (*37*). Therefore, at minimum these parameters should be tracked in clinical trial databases and evaluated for correlation with efficacy of checkpoint inhibitors and other immunotherapies.

## Future challenges and regulatory considerations

The regulation of microbial consumption as a category of drug provide a therapeutic challenge. In the United States, current commercial probiotics can be purchased over the counter; however, they are only regulated as food products/dietary supplements, not as drugs (*38*). Yet if the intent of the probiotic is to have therapeutic impact such as for cancer immunotherapy, then the FDA has indicated that development and regulation as a drug is indicated, including filing of an Investigational New Drug application and the usual reporting requirements. General guidelines include the clear identification of the genus and species of the probiotic strain, including genomic sequencing, potency and mechanistic laboratory studies, human clinical trials with efficacy endpoints, human safety and adverse event evaluation, and potential for infectivity. The latter point has been made relevant by studies of the yeast-based probiotic *Saccharomyces boulardii*, which has been marketed as a probiotic and also investigated for the treatment of *C. difficile* infection. Multiple cases of disseminated infection have been identified in apparent association with this probiotic, particularly in immune-suppressed individuals (*39*). Future challenges that merge the microbiome and oncology fields will include the development of rapid and cost-effective methods for the diagnosis of intestinal dysbiosis and the precise mapping of the biological effects and modes of action of pre-, pro-, and synbiotics for each cancer type. Addressing these challenges should forge a path toward a reproducible way of manipulating the intestinal ecosystem for optimizing precision medicine and improved patient survival.

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## SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/359/6382/1366/suppl/DC1
Table S1
References (*41*–*74*)

10.1126/science.aar6918

# The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies

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