Microbiome-based interventions: therapeutic strategies in cancer immunotherapy

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The composition of the commensal microbiota has recently emerged as a key element influencing the efficacy of cancer treatments. It has become apparent that the interplay between the microbiome and immune system within the host influences the response to immunotherapy, particularly immune checkpoint inhibitor therapy. Identifying the key components of the gut microbiota that influence this response is paramount for designing therapeutic interventions to enhance the response to cancer therapy. This review will discuss strategies being considered to modulate the gut microbiota, including fecal microbiota transplantation, administration of defined bacterial isolates as well as bacterial consortia, supplementation with probiotics, and lifestyle modifications such as dietary changes. Understanding the influence of the complex variables of the human microbiota on the effectiveness of cancer therapy will help drive the clinical design of microbial-based interventions in the field of oncology.

Key words: immunotherapy, biomarkers, resistance, microbiome

INTRODUCTION

Host immunity typically involves a coordinated response of the innate and adaptive immune systems to ward off threats from both external (e.g. pathogens) and internal (e.g. cancer) sources. Factors frequently considered to impact on quantitative and qualitative aspects of immune responses include germline polymorphisms in immunoregulatory genes, history of prior antigen or pathogen exposures, and whether acquired immunosuppression (either pharmacologic or pathogenic) is present. However, recent work has indicated an additional host-associated factor that has a major regulatory influence on immune responses — the commensal microbiota. Originally acquired after birth, the intestinal microbiota exceeds $3 \times 10^{13}$ bacterial cells and plays a crucial role in modulating innate and adaptive immunity.1–3 The composition of the gut microbiota has been demonstrated to influence immune responses in a variety of disease model systems, including autoimmune processes,1–8 viral infection,9–11 solid organ transplantation,12,13 allogeneic bone marrow transplantation14,15 and cancer.16–20 Modulation of antitumor immunity by the intestinal microbiota has prompted investigation into the potential for specific gut bacteria to potentiate the efficacy of immunotherapy with anti-CTLA-4 antibody or PD-1/PD-L1 blockade in mouse models.18,19,21–23 These preclinical studies have shown that manipulation of the microbiome can improve therapeutic efficacy over immune checkpoint blockade therapy alone. These exciting early results have motivated clinical exploration of microbiota-based interventions as a mechanism to optimize the clinical response to cancer immunotherapies in patients. This review will discuss various strategies being explored to enhance the therapeutic efficacy of immune checkpoint inhibitors: fecal microbiota transplantation (FMT), probiotics (isolated bacteria as well as bacterial consortia) and dietary modifications. These various approaches are illustrated in Figure 1.

THERAPEUTIC STRATEGIES

Fecal microbiota transplantation

One strategy for altering the composition of the intestinal microbiota is through FMT. FMT is the process of transferring fecal material from a donor into a recipient via a nasogastric tube, nasojejunal tube, upper tract endoscopy, colonoscopy, enema or as a prepared capsule. Transfer of fecal material from healthy donors has been shown to be effective in treating recurrent Clostridium difficile infection, and has been incorporated into the C. difficile infection management guidelines from the Infectious Diseases Society of America and the Society for Healthcare Epidemiology...
FMT has also been investigated in clinical trials for the treatment of ulcerative colitis, irritable bowel syndrome and other gastrointestinal conditions. The therapeutic potential of FMT in cancer immunotherapy has been supported by preclinical models in which patient-derived microbiota was transferred by gavage into...
germ-free mice (GFM). GFM colonized with FMT from responder patients exhibited slower tumor growth compared with those colonized with FMT from non-responder patients. In addition, reconstitution of GFM with responder-derived FMT resulted in greater efficacy of anti-PD-L1 therapy, while it was ineffective in mice with non-responder-derived FMT. In another cohort of patients with epithelial tumors, the therapeutic response to anti-PD-1 therapy was abrogated in mice that received FMT from non-responders or were treated with broad-spectrum antibiotics. Response to anti-PD-1 therapy could then be restored with FMT transfer from either responder-derived FMT or non-responder-derived FMT enriched with Akkermansia muciniphila. Similar correlation of improved anti-tumor response was conferred to mice that were administered FMT with Faecalibacterium spp. These findings suggest a functional association between the gut microbiome and the efficacy of anti-PD-1 immunotherapy, and the potential for therapeutic modulation.

Several independent research groups are currently investigating the effectiveness of FMT in enhancing the clinical response to anti-PD-1 therapy (Table 1). At the University of Pittsburgh Hillman Cancer Center, a clinical trial is exploring the delivery of FMT in combination with pembrolizumab in patients with melanoma refractory to anti-PD1 treatment (NCT03341143). This phase II feasibility study is exploring whether FMT from donor patients who responded to PD-1 blockade is able to improve the therapeutic outcome in patients with anti-PD-1 refractory melanoma. Functional and phenotypic changes in the innate and adaptive immune systems will be studied in parallel. Early data were presented by Giorgio Trinchieri at the American Association for Cancer Research 2019 annual meeting, where he reported stable disease or tumor regression in two of three patients with melanoma after receiving FMT, resulting in one death. One of these individuals was enrolled on a clinical trial of FMT for the treatment of hepatic encephalopathy, and the other was receiving an allogeneic stem cell transplant for myelodysplastic syndrome. Clearly these were complex cases with multiple concomitant medical conditions, but nevertheless, these occurrences introduce an element of caution for FMT in patients with advanced cancer. FMT involves the transfer of not only commensal bacteria, but also viruses, protozoa, archaea and fungi, which are often not accounted for due to inadequate methods to characterize these organisms fully. There is growing evidence that, collectively, these microbial communities impact the development of cardiovascular disease, neurological disorders, metabolic disorders, psychiatric conditions and lung disorders. Theoretically, the transfer of fecal microbiota may lead to unrecognized transmission and predisposition to these chronic health disorders, and thus may warrant more judicious selection of FMT donors as well as additional studies to better understand the long-term health impact of FMT. A recent detailed analysis of stool donors as candidates for FMT protocols, who underwent intensive screening for medical illnesses, subclinical presence of infectious pathogens in the specimen, or detection of antibiotic-resistant bacteria, revealed that only 3% of donors would qualify based on rigid criteria. Thus, FMT remains investigational, and it is anticipated that strict donor screening will be expected in future studies.

**Defined bacterial isolates**

Another microbiome-based intervention being explored for its potential to improve the efficacy of cancer immunotherapy is the administration of defined bacteria with immunomodulatory properties. In 2015, two independent research groups reported the identification of single bacterial species that could potentiate the response to checkpoint blockade (e.g. anti-CTLA-4 and anti-PD-1/PD-L1 antibodies) in preclinical models. In a study by Zitvogel and her team, mice with established sarcomas that were treated with antibiotics or housed in germ-free conditions respond to subsequent anti-PD-1 therapy even without any microbiome-based intervention. Nonetheless, thus far, these early results support the safety and feasibility of FMT in combination with anti-PD-1 therapy.

Although FMT is one potential strategy to modulate the gut microbiome with the goal of enhancing clinical responses to immunotherapy, there are some risks and uncertainties with the use of this modality. The first major issue is safety; asymptomatic donors can harbor unrecognized pathogens, including parasites and pathogenic bacteria. In addition, they may be colonized with antibiotic-resistant bacteria (multi-drug-resistant organisms). The US Food and Drug Administration issued a safety communication in June 2019 warning healthcare professionals of the potential risk of life-threatening infections following investigational FMT. This safety alert emerged as a result of two patients who developed serious bacterial infections after receiving FMT, one of whom died. One of these patients was a 65-year-old individual who was receiving an allogeneic stem cell transplant for myelodysplastic syndrome and her team, mice with established sarcomas that were treated with antibiotics or housed in germ-free conditions...
showed tumor progression following treatment with CTLA-4 blockade.22 The anticancer efficacy of CTLA-4 blockade was restored when the mice were recolonized with specific *Bacteroides* spp. (i.e. *B. fragilis* and *B. thetaiotaomicron*), but not with other bacterial isolates, suggesting that *Bacteroides* spp. may play a key role in driving immune responses to anti-CTLA-4 therapy. It should be noted, however, that while reconstitution with live bacterial species was shown to restore the efficacy of anti-CTLA-4 therapy, intestinal epithelial cells, when exposed to microbial products (in the form of Toll-like receptor agonists), induced a response from intraepithelial lymphocytes in mice treated with anti-CTLA-4, suggesting the need for bacterial components alone to induce an inflammatory response from intestinal lymphocytes. This form of molecular mimicry may result in a beneficial or detrimental patient outcome (e.g. autoimmune reaction).32 Similarly, modulation of the intestinal microbial flora with oral administration of *Bifidobacterium* spp. was shown to augment the response to PD-1 blockade in a murine melanoma model.18 The therapeutic effect of *Bifidobacterium* spp. was associated with increased intratumoral and circulating tumor-antigen-specific CD8⁺ T cells, which could be attributed to increased ‘pooing’ of dendritic cells throughout the animals.18

A similar increased abundance of *Bifidobacterium* spp. was observed in a cohort of patients with metastatic melanoma with improved responsiveness to anti-PD-1 therapy.22 Among multiple species of bacteria showing differential abundance in the stool of responders and non-responders was *Bifidobacterium longum*, which correlated with enhanced T-cell infiltration in the tumor microenvironment. The lack of detectable bifidobacterial sequences in the majority of patients with metastatic melanoma is in keeping with previous studies indicating loss of bifidobacterium colonization with aging,33,34 and also preferential loss in a cohort of patients with colorectal cancer compared with control individuals with non-cancerous intestinal illnesses.35 In a second study, analysis of the composition of the gut microbiota of patients with non-small cell lung carcinoma (NSCLC) and renal cell carcinoma similarly exhibited over-representation of distinct bacterial genera that were associated with improved clinical response.21 In that particular patient population, *A. muciniphila* was abundant in checkpoint inhibitor responders, and mice treated with antibiotics recolonized with *A. muciniphila* (alone or in combination with *Enterococcus hirae*) were shown to have a restored response to PD-1 blockade, emphasizing the influence that specific bacterial taxa can have on modulating the response to immunotherapy. Collectively, these studies provide further insight into the impact and immunostimulatory effect of isolated bacteria on regulating the response to immune checkpoint inhibitors. However, the most critical species and strains of bacteria for potentiation of antitumor T-cell responses are still unclear, and predicting the clinical outcome of patients from mice reconstituted in controlled environments with non-physiological levels of specific bacterial species has yet to be determined.

Despite these limitations, clinical trials aiming to improve the effectiveness of immunotherapy with defined bacterial strains have been initiated (Table 2). Evelo Biosciences is enrolling patients with various cancers in clinical trials investigating the oral administration of a bifidobacterial strain. Supplementation with *Bifidobacterium animalis lactis* (EDP1503) in the form of a capsule is administered for a 2-week period, followed by continued administration in combination with pembrolizumab (NCT03595683, NCT03775850). Preliminary data reported at the 2020 European Society for Medical Oncology World Congress on Gastrointestinal Cancer Virtual Meeting demonstrated safety and tolerability across various solid tumors, including colorectal cancer, triple-negative breast cancer and NSCLC.56 Notably, the overall response rate in the cohort with triple-negative breast cancer was 25%, which is encouraging given that the response with single-agent anti-PD1 therapy in this patient group ranges between 5% and 10%. Similarly, in a phase 1 study, the National Cancer Institute in collaboration with the City of Hope Medical Center are investigating the addition of *Clostridium butyricum* (CBM 588) to patients with advanced renal cell carcinoma undergoing combination therapy with nivolumab and ipilimumab (NCT03829111). Overall, single bacterial interventions as a tool to enhance the immunotherapeutic response are in the early stages of development, and questions remain regarding which precise bacterial isolate would best improve antitumor immunity, which assay is ideal to identify the relative abundance and functional

| Trial no. | Phase | Cancer type | Intervention | Sponsor/investigator |
|----------|-------|-------------|--------------|----------------------|
| NCT03341143 | II | Melanoma | FMT (via colonoscopy) + immune checkpoint inhibitors | UPMC Hillman Cancer Center |
| NCT03353402 | I | Melanoma | FMT (via colonoscopy and stool capsules) + immune checkpoint inhibitors | Sheba Medical Center |
| NCT03772899 | I | Melanoma | FMT + immune checkpoint inhibitors | Lawson Health Research Institute |
| NCT04577729 | — | Melanoma | Autologous or allogeneic FMT + immune checkpoint inhibitors | Medical University of Graz |
| NCT04521075 | I/II | Melanoma | FMT (stool capsules) + immune checkpoint inhibitors | Sheba Medical Center |
| NCT04056026 | I | Mesothelioma | FMT (via colonoscopy) + immune checkpoint inhibitors | ProgeniBiome |
| NCT04116775 | II | Prostate | FMT (via endoscopy) + immune checkpoint inhibitors + enzalutamide | VA Portland Health Care System |
| NCT04130763 | I | Gastrointestinal | FMT (stool capsules) + immune checkpoint inhibitors | Peking University |

NSCLC, non-small cell lung cancer.
status of specific bacteria (e.g. 16S sequencing, metagenomics, metatranscriptomics, culturomics, metabolomics), and which animal model is optimized to represent the interaction among human commensals and to detect single bacterial species with immune-potentiating effects.

**Bacterial consortia and other probiotics**

Administration of a consortium of commensal bacteria strains has emerged as an attractive method for manipulating the host microbiome to affect the therapeutic response to cancer immunotherapy (Table 3). Rather than transplanting the complete gut microbiome from human donors (e.g. FMT), which could produce unwanted side-effects from pathogenic strains, transfer of specific strains identified empirically as critical to the enhancement of immunotherapeutic intervention could allow for an ‘off-the-shelf’ approach for transferring multiple bacterial strains concurrently. To this end, work by Honda and colleagues identified 11 bacterial strains (seven Bacteroidales spp. and four non-Bacteroidales spp.) from healthy human donors that promoted the generation of interferon-γ-producing CD8⁺ T cells in mouse intestine.⁵⁷ When this bacterial consortium was reconstituted in a mouse syngeneic tumor model, enhancement of the anti-CD103 therapeutic response was observed in conjunction with an increase in tumor-infiltrating CD8⁺ T cells. Interestingly, when the 11-strain consortium was analysed further, it was shown that the seven Bacteroidales spp. did not induce a CD8⁺ T-cell response whereas the four non-Bacteroidales spp. did, albeit at a much lower rate than the complete set of 11 species, pointing to a true consortium effect as well as highlighting the incomplete understanding of the interplay between multiple members of a bacterial community. Based on these data, a clinical trial is planned using this bacterial consortium in combination with anti-CD103 therapy.⁵⁸

While the application of a bacterial consortium to enhance cancer immunotherapy is relatively new, augmenting the antitumor response using scientifically designed clinical-grade probiotics or over-the-counter probiotics is currently under evaluation. A number of studies in colorectal cancer have shown changes in bacterial composition within the tumor, as well as differing cytokine profiles, after probiotic administration, but these results need to be investigated further regarding their impact on antitumor immunity (NCT01895530, NCT03072641, NCT03782428).⁵⁹,⁶⁰ A phase I trial is currently underway investigating the safety and engraftment of a defined mixture of bacterial species (MET-4) administered orally in combination with immune checkpoint inhibitors (NCT03686202). In another phase I study, the tolerability and preliminary efficacy of SER-401, a microbial cocktail correlated previously with response to immunotherapy in patients with melanoma, is being assessed (NCT03817125). In contrast, early retrospective data have indicated that over-the-counter probiotics are associated with decreased response to immune checkpoint inhibitors based on data collected from lifestyle surveys in patients with melanoma.⁶¹ Clinical trials assessing microbial manipulation with over-the-counter probiotics have been initiated (NCT03358511); however, particular attention is required when promoting their off-protocol use for patients with cancer until safety profiles of probiotic formulations are better understood. Together, these studies should help to identify microbial consortia that favor the responsiveness of immunotherapy, and will guide the design of next-generation microbiota-based therapeutics.

There is some question regarding the level of engraftment afforded by probiotics in healthy individuals and the resulting effect on the host.⁶² A recent study exploring the effect of an 11-strain probiotic regimen revealed differential colonization patterns (i.e. permissive versus non-permissive) within volunteers influenced by strain and

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**Table 2. Clinical trials investigating bacterial isolates**

| Trial no.  | Phase | Cancer type | Intervention | Sponsor/investigator |
|------------|-------|-------------|--------------|----------------------|
| NCT03775850 | I/II  | Solid tumors | EDP1503 (bifidobacterium capsule) + pembrolizumab | Evelo Biosciences |
| NCT03595683 | II    | Melanoma    | EDP1503 (bifidobacterium capsule) + pembrolizumab | Evelo Biosciences |
| NCT03829111 | I     | Renal cell carcinoma | CBM588 (Clostridium butyricum probiotic) + nivolumab and ipilimumab | City of Hope Medical Center |
| NCT03637803 | I/II  | Solid tumors | MRx0518 (Enterococcus gallinarum capsule) + immune checkpoint inhibitors | 4D Pharma |

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**Table 3. Clinical trials investigating bacterial consortia and probiotics**

| Trial no.  | Phase | Cancer type | Intervention | Sponsor/investigator |
|------------|-------|-------------|--------------|----------------------|
| NCT03686202 | I     | Solid tumors | MET-4 (defined bacterial consortia) + immune checkpoint inhibitors | University Health Network, Toronto |
| NCT03817125 | Ib    | Melanoma    | SER-401 (defined bacterial consortia) + immune checkpoint inhibitors | Parker Institute for Cancer Immunotherapy |
| NCT03919530 | I     | Colorectal cancer | Supplement with probiotic Saccharomyces boulardii | Federal University of Minas Gerais |
| NCT030772641 | —     | Colorectal cancer | Supplement with Probion Clinica (Bifidobacterium lactis, Lactobacillus acidophilus, inulin) | Vastra Gotaland Region |
| NCT03782428 | —     | Colorectal cancer | Probiotic with six viable micro-organisms of Lactobacillus spp. and Bifidobacterium spp. | National University of Malaysia |
| NCT03358511 | —     | Breast cancer  | Over-the-counter probiotic with 13 bacterial species | Mayo Clinic |
Clinical trials investigating immunotherapy in combination with dietary modifications

| Trial no. | Phase  | Cancer type | Intervention | Sponsor/investigator |
|----------|--------|-------------|--------------|---------------------|
| NCT03192059 | II | Cervical/uterine | Pembrolizumab + RT + immunomodulatory cocktail + dietary supplement (curcumin) | University Hospital, Ghent |
| NCT03700437 | — | NSCLC | Carboplatin/pemetrexed + pembrolizumab, fasting-mimicking diet | Indiana University |

RT, radiation therapy; NSCLC, non-small cell lung cancer.

Cancer Center suggest that diet may influence the gut microbiome and, in turn, affect anticancer immune responsiveness. Analysis of dietary surveys completed by a subset of 46 patients with melanoma who received anti-PD-1 therapy revealed that patients who consumed a high-fiber diet were more likely to have a favorable response to anti-PD-1 therapy than patients who consumed a low-fiber diet. Among patients who consumed diets rich in processed meats and sugars, their gut microbiome was not enriched with bacteria that have been correlated with an improved immunotherapeutic response. Additional studies on the role of dietary patterns (e.g. Mediterranean, Japanese, vegetarian diet) on alterations to the composition of the microbiota and their effects on the function of the immune system are being evaluated. Preliminary evidence further supports a link between diets low in fiber, high in animal protein and high in saturated fat with an unfavorable bacterial signature and lower amounts of beneficial bacteria, such as *Bifidobacterium* spp.

Further studies are needed to support a causal role linking specific dietary interventions to immunotherapy-related clinical outcomes. Several groups are actively assessing the dietary impact on patients with cancer undergoing combination treatment with immunotherapy and dietary interventions. A phase II study in Belgium is examining the effects of diet supplementation with curcumin in addition to an immunomodulatory cocktail (vitamin D, aspirin, cyclophosphamide and lansoprazole) followed by pembrolizumab in patients with cervical, endometrial and uterine sarcoma (Table 4). Similarly, patients with metastatic NSCLC receiving chemo-immunotherapy enrolled in a clinical trial at Indiana University have been randomized to a dietary modification arm (consuming a fasting-mimicking diet) or a placebo arm (consuming a regular diet) to determine clinical efficacy via response rate and progression-free survival (Table 4). Importantly, accurate collection of the dietary data of patients needs to be standardized to enable accurate comparison of treatments and outcomes. Additional limitations of dietary interventions include difficulty regulating patient compliance and interpretation of subjective information obtained through patient-reported surveys. Furthermore, clinical trials with sequential and multistage interventions will help simplify confounding variables, and allow better understanding of the relationship between food and commensal bacteria. Finally, nutrition-based approaches are not selective for modulating specific bacteria, and have the potential to result in divergent effects on varying patient populations (e.g. geographical or genetic differences altering the

Table 4. Clinical trials investigating immunotherapy in combination with dietary modifications

| Trial no. | Phase | Cancer type | Intervention | Sponsor/investigator |
|----------|-------|-------------|--------------|---------------------|
| NCT03192059 | II | Cervical/uterine | Pembrolizumab + RT + immunomodulatory cocktail + dietary supplement (curcumin) | University Hospital, Ghent |
| NCT03700437 | — | NSCLC | Carboplatin/pemetrexed + pembrolizumab, fasting-mimicking diet | Indiana University |

RT, radiation therapy; NSCLC, non-small cell lung cancer.

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baseline gut microbiome and thus the response to dietary modifications).

CONCLUSIONS AND FUTURE DIRECTIONS

With this new treatment paradigm, many questions have yet to be answered, one of the foremost being how to match microbial-based interventions effectively with the appropriate patient population. For those who fail a cancer immunotherapy regimen (such as anti-PD-1 therapy), the reason for lack of efficacy may be due solely to suboptimal microbiota in a subset of patients. For other patients, poor tumor antigenicity, tumor-cell-intrinsic oncogenic events that are immune evasive, or germline genetic variants that bestow a high threshold for immune system activation may be causal, and consequently may not require any modification of the microbiome. Therefore, selection of patients who might benefit from microbial manipulation has yet to be defined. In addition, even if suboptimal microbiota is indeed causal, there are likely to be multiple mechanisms by which gut microbes impact systemic antitumor immunity. Some individuals may possess an abundance of immunoregulatory bacteria, whereas others may lack immune-potentiating bacteria. As such, selection of the most suitable microbiome donor is equally important, and clear demarcation of how these donors are identified should be provided in clinical trials as they may introduce additional variables that could impact clinical outcome. For example, FMT donors identified as ‘responders’ can be classified as achieving a response ranging from stable disease to partial or complete response within a defined time period. As one might imagine, such interpatient variability and differences across studies on how these patients are classified could influence the perceived conclusions. Until the deep biological mechanisms are elucidated, it will be challenging to identify the proper intervention that will optimally complement the existing microbial community in a given patient to improve immunotherapeutic efficacy.

While still in its infancy, manipulation of the microbiome has already shown great promise in modulating the therapeutic effect of cancer immunotherapy pre-clinically. Beyond the interventions discussed above, additional strategies under evaluation include depletion of pathogenic bacteria through the use of antibiotics, and selective elimination of targeted bacterial species with strain-specific bacteriophages. Critical to the advancement of these treatment options will be understanding the underlying mechanisms of how bacteria regulate antitumor immunity, in the presence and absence of specific immunotherapeutic interventions. As these mechanisms become more defined, and metabolites and/or specific bacteria-derived molecules are identified, new drug-based treatments could form the next generation of microbiome-informed therapies. This would allow for personalization of existing immune checkpoint inhibitor regimens; that is, patients presently on therapy whose response is unknown would either start therapy with a high probability of responding (e.g. based on proxy models) or receive microbiome modulation specific to their microbial composition in order to enhance their response. As with any new drug class, emphasis must also be placed on determining the safety parameters associated with delivering therapy. Overcoming these challenges will pave the way towards precise mapping of ‘ideal’ immunotherapy-potentiating microbiome interventions.

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