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Unlocking the power of the microbiome for successful cancer immunotherapy

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ABSTRACT

In recent years, evidence has shown that the gut microbiome significantly influences responses to immunotherapy. This has sparked interest in targeting it to improve therapy outcomes and predictions of response and toxicity. Research has demonstrated that dysbiosis, often resulting from antibiotic use, can diminish the effectiveness of immune checkpoint inhibitors, and this lack of efficacy could be linked to systemic inflammation. Certain bacterial species have been identified as having beneficial and harmful effects on immunotherapy in the clinic. While a clear consensus has yet to emerge on the optimal species for therapeutic use, introducing a new microbiome into immunotherapy-refractory patients may boost their chances of responding to further treatment attempts. State-of-the-art interventions targeting the microbiome—such as fecal microbiota transplantation—are being assessed clinically for their safety and potential to enhance treatment outcomes, with promising results. Additionally, the microbiome has been leveraged for its power to predict clinical outcomes using machine learning, and surprisingly, its predictive capability is comparable to that of other described multi-biomarker clinical scores. Here, we discuss developing knowledge concerning the microbiome's significance in cancer immunotherapy and outline future strategies for maximizing its potential in immuno-oncology.

INTRODUCTION

The gut microbiome constitutes an ecosystem formed by a complex network of microbial species and their products. The composition of a well-balanced microbiome (eubiosis) is established early in life, and it remains relatively stable throughout adulthood. However, its balance is delicate and environmental and life factors can disturb it, resulting in a temporary or even permanent state of dysbiosis. Depending on the prevalence of certain species, gut microbiomes from different individuals cluster in (quasi-)discrete ecological states defined as microbiotypes.[1] The cross-talk between an eubiotic microbiome and the host is instrumental in maintaining the health of the organism and modulating physiological functions, including inflammation and immunity. Bacteria are associated with certain cancers and may induce genetic instability and progression.[2] We and others have demonstrated that the gut microbiome also modulates the response to different types of cancer therapy in experimental animals and in humans.[3 4] The microbiome enables cancer therapy largely by modulating the anti-tumor immune response by training tumor-infiltrating myeloid and antigen-presenting cells (innate immunity) and by tuning, directly and indirectly, adaptive tumor-specific T and B cells (adaptive immunity).[2–4]

THE GUT MICROBIOME OF CANCER PATIENTS

To tackle the challenges of dimensionality and sparsity and to uncover core microbiome signatures, researchers have used co-abundance networks and machine learning (ML) to analyze guilds—functional groups of bacteria with different taxonomic origins that, by using the same resources, inhabit the same ecological niches.[5] The study of healthy volunteers and patients harboring chronic inflammatory conditions, including cancer—ranging in different geographic regions and ethnicities—identified two major competing guilds: a healthy guild (eubiosis) featuring genes contributing to the utilization of complex carbohydrates and production of short-chain fatty acids (SCFA) such as butyrate that, in turn, protect the integrity of the mucosal barrier, and a pathological guild (dysbiosis) characterized by virulence and antibiotic-resistance genes.[5] Pro-inflammatory states associated with chronic conditions and aging may promote the predominance of the pathological over the healthy guild. Similarly, patients with cancer frequently experience dysbiosis due to the illness itself, medication use, cancer therapy toxicity, nosocomial infections, antibiotic use, and changes in diet and lifestyle.[6]

THE GUT MICROBIOME MODULATES IMMUNOTHERAPY RESPONSE

Dysbiotic perturbations lead to fluctuations in the abundance of microbial species in patients with cancer, which have been shown

to influence immunotherapy outcomes.[1 2 6] Many studies identified in patients with cancer taxa that were associated with either response or non-response, but concern about the conclusions of these studies has been raised by the fact that there was limited species overlap among individual studies.[1 2 6] However, the importance of the role of the microbiome in modulating immunotherapy response was supported by studies in which the transfer of patients' fecal microbiome in germ-free mice mirrored the therapy response observed in the donors and by extensive clinical evidence that antibiotics given prior to immunotherapy adversely affect therapeutic response and reduce survival.[7] Broad-spectrum antibiotics may induce the loss of microbial diversity, alter the balance between favorable and unfavorable bacterial taxa, and favor the expansion of immunosuppressive fungi.[2] Antibiotics may also affect the fitness and permeability of the intestinal mucosa, disrupting local and systemic host-microbiome interaction and its effect on immunity.[2]

Meta-analyses of patients undergoing anti-programmed cell death protein-1 (PD-1) treatment have enabled a better consensus among the data from different cohorts.[1] In melanoma, these studies revealed that beneficial taxa are mostly classified within the phyla Actinobacteria and Firmicutes, whereas detrimental taxa are associated with the gram-negative Bacteroidetes and Proteobacteria phyla.[1] The reason for the identification of different species associated with response has been shown to largely depend on geographically restricted—therapy favorable or unfavorable—microbiotypes differentially represented in the various cohorts.[1] Different bacterial species in different patients may possess shared genes and functions other than taxonomically-restricted genes, which may ultimately influence therapeutic responses.[3] Similarly, in distinct cohorts of non-small cell lung and genitourinary cancers, species-interacting groups (SIGs) based on co-abundance networks correlated with overall survival in patients treated with immune checkpoint inhibitors (ICI).[8] 37 and 45 species, differentially distributed in the patients in the various cohorts, were used as biomarkers to classify the patients into either unfavorable (SIG1) or favorable (SIG2) groups. When combined with a tripartite quantification of Akkermansia species, this procedure was used to define a topological score (TOPOSCORE) that provided predictions for overall survival in patients with renal and lung cancer but was less robust in providing significant predictions for patients with melanoma.[8]

ML models incorporating microbiome data—batch corrected for cohort heterogeneity—have demonstrated accuracy in predicting ICI response, enhanced when using bacterial functions rather than taxonomy (figure 1).[1 9] The robust role of the gut microbiome to regulate the response to ICI therapy is validated by the fact that the prediction accuracy in those models is comparable to the recently published model LORIS, which is based on six clinical markers, including neutrophil-to-lymphocyte ratio (NLR) known to be influenced by the gut microbiome.[1 10]

SYSTEMIC INFLAMMATION PREDICTS POOR IMMUNOTHERAPY RESPONSE

Markers of systemic inflammation such as high NLR and increased serum levels of interleukin (IL)-8, amyloid A, or C-reactive protein are linked to an immunosuppressive cancer microenvironment and reduced response to ICI.[1 2] High NLR and serum IL-8 correlate with a high abundance of gut gram-negative bacteria.[1 11] An analysis of the gene expression of host mucosal cells that exfoliate into the feces revealed an inflammatory signature driven by Lipopolysaccharides (LPS)-induced Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and characterized by pro-inflammatory cytokines in melanoma patients who failed anti-PD-1 therapy.[1] In mouse models of cholangiocarcinoma, vancomycin-induced dysbiosis with expansion of gram-negative Proteobacteria induces in the liver an LPS/Toll-like receptor 4 (TLR4)-mediated induction of IL-8 that attracts immunosuppressive neutrophils that favor tumor growth.[12] Thus, by producing LPS and activating TLR4 signaling in myeloid cells, residing gram-negative bacteria may trigger local immunosuppressive inflammation in the gut while inducing systemic inflammation, ultimately compromising the effectiveness of ICI. Noteworthy, taxa of the gram-negative Bacteroides, Prevotella and Alistipes genera, unlike those associated with a favorable ICI response, contribute to the ML model's prediction of clinical response across most cohorts.[1] These findings suggest that associations between outcomes and microbial signatures are more robust and universal for taxa contributing to inflammation-mediated immunosuppression than taxa enabling cancer immunity, which tend to be more cohort-specific.

TARGETING THE MICROBIOME TO IMPROVE IMMUNOTHERAPY RESPONSE

Despite the lack of consensus on identifying specific bacterial taxa enhancing cancer therapy, a few clinical trials targeting the gut microbiome to improve immunotherapy efficacy have been conducted. The use of single bacterial strains or small consortia, which have been shown to enable immunotherapy response in experimental animals, has not been reported yet to be successful in clinical trials. More encouraging results have been reported in early-stage trials using the anti-inflammatory probiotic strain *Clostridium butyricum*[13] and in fecal microbiome transplant (FMT) trials, including single-arm studies transplanting fecal microbiome from patients with cancer who successfully responded to therapy into patients with anti-PD-1 unresponsive melanoma.[11 14] In these two studies, FMT proved safe and effective in addressing dysbiosis and overcoming resistance to ICI, with about 40% response to anti-PD-1 following FMT and at least one patient achieving a complete response in each study.[9 14] After FMT, the microbiome of the responders aligned more closely to their donors than non-responders, suggesting that the difference between responders and

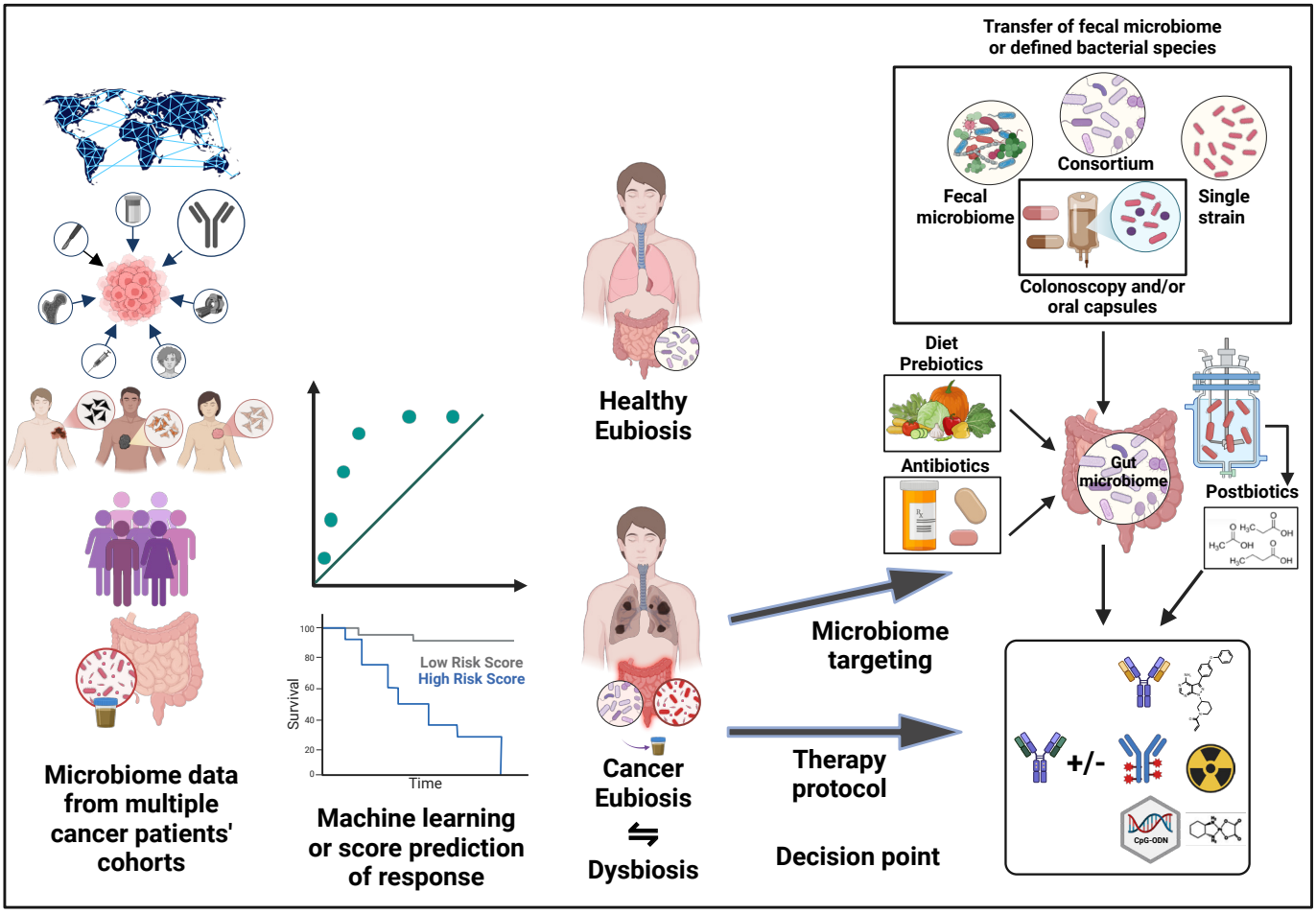

Figure 1 The gut microbiome is a tool for predicting therapy response and patient stratification, as well as a target for therapeutic intervention. Microbiome data and other clinical biomarkers from large cohorts of cancer patients representative of diverse cancer types, therapy protocols, and geographical origins may be used to train machine learning models or to determine clinical scores for predicting responses to different immunotherapy protocols. This prediction is expected to become useful in stratifying patients for the probability of response to immunotherapy as single agents or as combination therapy with other agents. The prediction may also assist in deciding whether and which type of microbiome targeting co-therapy may be advisable. *Created in Biorender.com.*

non-responders may be attributed to the successful engraftment of the transplanted taxa.[9 11] The gut microbiomes of responders showed a significant increase in taxa from the phyla Firmicutes (*Ruminococcaceae* spp and *Lachnospiraceae* spp) and Actinobacteria (*Bifidobacteriaceae* spp and *Coriobacteriaceae* spp), mirroring the composition of the donors, while some Bacteroidetes and Proteobacteria decreased.[9 11] Responders also exhibited reduced levels of serum IL-8 and decreased frequencies of IL-8-producing myeloid cells in tumors, paralleled by a lower number of intratumoral immunosuppressive myeloid cells.[9 11] Additional studies in anti-PD-1-refractory patients with advanced gastrointestinal cancers and as first-line treatment in patients with melanoma provided support for the effectiveness of FMT in combination with anti-PD-1 therapy.[15 16] Recently, partial results from a randomized, placebo-controlled trial of patients with renal cell carcinoma, using fecal microbiome from healthy volunteers as a first-line treatment in combination with anti-PD-1, showed a significantly improved effect, evidenced by an objective response rate of 54% compared with 27% in patients treated with anti-PD-1 monotherapy.[17]

CONCLUSIONS AND FORESIGHT

ML models trained by gut microbiome data and microbiome-based immunotherapy response scores may soon become able to robustly predict patients' responses to different types of immunotherapy and perturbation of the gut microbiome.[1 5 8 10] However, the bacterial taxa affecting immunotherapy responses are context-dependent and distinctive mechanisms may be involved in different types of immunotherapies or even tumor types.[1 8 9] Also, because of the heterogeneity of the human gut microbiome due to geography or other microbiome-affecting factors, different bacterial taxa and mechanisms may be involved in modulating the response to therapy in different patients/cohorts, and further data collection is essential for more accurate prediction.[1] We anticipate that these ML models could ultimately be used to

select the most effective immunotherapy combinations for each patient and to assist in deciding whether to integrate immunotherapy with microbiome-targeted therapy. Because the induction of immunosuppressive chronic inflammation as modulated by the gut microbiome appears to be a pathway of therapy resistance shared by many patients,[1 5 6] understanding the mechanisms may help to plan rational procedures to overcome it. Prebiotics, diets, and food supplements that would favor the conversion of the microbiome to a non-inflammatory and/or anti-PD-1 therapy-favoring state may be identified. Increased dietary fiber intake by patients with type 2 diabetes was able to perturb the two-guild dynamics promoting conversion to the health-associated guild.[5] Fiber-rich diets or supplementation with inulin or pectin favor tumor immunity and ICI therapy in patients and experimental animals.[18 19] FMT trials have provided proof of concept that with replacement therapy, it is possible to change in a persistent way the gut microbiome, thus reducing systemic inflammation and reversing anti-PD-1 unresponsiveness.[11 14] FMT, however, poses risks of unintended transfer of pathogens or antibiotic-resistant opportunistic bacteria. Additionally, the need to identify the ideal donor for each patient and type of immunotherapy might be a drawback. The clinical treatment with better-defined and standardized pharmaceutical preparations of single taxa or small bacterial consortia would be preferable, but so far has provided disappointing results in clinical trials. The reason for this lack of success might reside in human *versus* mouse immunity differences but also in the inability of the selected bacterial composition to colonize the patients successfully or to establish a new gut microbiome ecology that would overcome the immunosuppressive effects of the recipient microbiome. The only clinical treatment that has shown some promise has been the administration of the anti-inflammatory and butyrate-producing probiotic *C. butyricum*, which is known to reduce intestinal and systemic inflammation.[13] Although they have received limited attention, postbiotics could also be considered for ameliorating the microbiome's effect on intestinal and systemic inflammation. For example, short-chain fatty acids (SCFA) such as butyrate may improve mucosa fitness and decrease local and systemic inflammation. Moreover, high NLR associated with increased abundance in the gut microbiome predominantly of gram-negative bacteria[1] predicts poor response to immunotherapy not only with anti-PD-1 alone but also in combination with anti-Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4) and anti-Lymphocyte-activation gene 3 (LAG-3). However, in patients with high-risk resectable melanoma, the taxa necessary for the efficacy of the anti-PD-1 and intratumoral Toll-like receptor 9 (TLR9) agonist combination are opposite to those required for anti-PD-1 monotherapy.[9] In a neoadjuvant trial, the gut microbiome of patients responding to anti-PD-1 and TLR9 agonists with major pathological responses had an increased abundance of gram-negative bacteria, particularly species from the Bacteroidaceae and Enterobacteriaceae families, and, intriguingly, higher NLR than non-responders.[9] The antitumor mechanisms in these patients involve intratumoral myeloid cells, conversion of the microenvironment from immunosuppressive to immunostimulatory, and hemorrhagic necrosis, closely resembling those observed in tumor-bearing mice treated with an intratumoral TLR9 agonist.[3 9] These findings suggest that, in addition to targeting the gut microbiota using prebiotics, probiotics, or postbiotics, it may be possible to identify combination therapies that are more effective in patients with microbiome-induced systemic inflammation who are refractory to other types of immunotherapies.

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