WHITE PAPER

Leveraging the microbiome to strengthen immune therapeutic response.

The therapeutic landscape for the treatment of cancers continues to evolve, with increased attention being paid to differences in treatment responses between patients within the same disease population. Immunotherapies, especially immune checkpoint inhibitors, have gained notoriety for their ability to activate the patient's immune system and break down resistance barriers. Although this therapeutic class has delivered positive results, many patients still remain unresponsive to treatment. Investigating the tumor microenvironment provides researchers and clinicians with insights into how it can be modulated to improve the therapeutic effectiveness of immune checkpoint inhibitors and produce more treatment responders. One approach that researchers are exploring is to leverage the gut microbiome, which acts as an immune response modulator.

The gut microbiome is the primary residential site of bacteria in the body and dysbiosis of the microbiome is associated with various pathologies, including cancer. Researchers are striving to determine where and when relevant bacteria play a role in cancer progression and whether those interactions are localized to the gastrointestinal tract or occur in other tissues before malignancy. Understanding the role bacteria play, whether it be oncogenesis, tumor promotion, or treatment effectiveness, may provide an opportunity for intervention at the microbiome level in favor of cancer regression.

Bacteria: Effective immune response modulators

In a healthy gut microbiome, a state known as eubiosis, bacteria play a role in preventing infection, breaking down food, synthesizing vitamins, regulating gut and brain function, and modulating the immune system. The immune system plays a significant role in homeostatic maintenance and disrupting this endogenous control system can lead to imbalance, disease, and an increased risk for cancer. The microbiome influences local and systemic immunomodulation functions in a non-cancerous state, including reduction of inflammation, anti-tumorigenesis, and tumor suppression. Bacteria act as key modulators by promoting or disrupting immunological functioning through interactions with specialized immune receptors. In scenarios of dysbiosis and carcinogenesis, protective immunomodulation functions are often turned off, resulting in the promotion of inflammation and unchecked tumor growth.

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Table 1: Types, function, and action of immune response influenced by bacteria

	Type 1	Type 2	Type 3
Function	Maintains and protects the integrity of the gut barrier	Provides robust immune activation	Immunosuppressive modulation
Action	▪ Immediate, localized, and inflammatory in nature ▪ Increases phagocytosis, activates macrophage activity, recruits neutrophils, and heightens oxidative burst	▪ Delayed response, requiring activation of secondary immune cells in lymphoid tissues ▪ Anti-inflammatory cytokines: ▪ Promote B-cell proliferation ▪ Increase antibody production and class switching ▪ Increase granulopoiesis and granulocyte infiltration ▪ Induce macrophage activity	▪ Cytokine modulation: ▪ T-cell activation ▪ Inhibits antitumor immunity

There are three different types of immune responses influenced by bacteria, each supporting healthy gut function and suppressing tumor activity, as described in Table 1.[1] In the presence of cancer, there is no consensus regarding a particular genus of bacteria inducing a specific and correlated immune response. The molecular interactions between bacteria in the microbiome and immune cells are complicated and individualistic, and although trends may be isolated, certain factors influence individual differences in treatment response. Researchers are learning that these factors can predict which patients will respond to immunotherapy and, thus, fuel further evaluation.

Bacteria can be induced to colonize different parts of the body at tumor sites as cancer therapeutics, to create a supportive environment for co-administered therapeutic intervention, or as a tool for diagnosis. Upon establishing the role of specific bacteria in immune modulation, researchers can manipulate the system with strains known to enhance immune function. Understanding key players, response type in a healthy or tumor microenvironment, and potential bolstering or inhibitory interactions due to therapeutic intervention is critical to foster positive patient outcomes.

Utilizing the microbiome to enhance therapeutic efficacy

There have been numerous studies to determine patterns of bacteria in different tumor types and whether they act as causative agents or opportunistic tumor inhabitants. Delineating prevalence versus causation helps identify potential influencers of oncogenesis and where therapeutic efforts should be focused to create an environment supportive of remission and long-term survival. Tumor-specific factors supporting bacterial growth include disorganized or leaky vasculature, immune suppression, low oxygen regions, and excessive nutrients.

Independently, Mark Tangney, University College Cork, and Mat Robinson, Microbiotica, are visualizing the site of bacterial growth *in vivo*. Using this technique, they can observe whether the injected strain localizes and flourishes within the tumor site and whether bacterial growth occurs systemically. Work such as this offers supportive evidence of bacterial growth specific to the tumor microenvironment and highlights the potential for microbial manipulation in favor of immune modulation.

The tumor microbiome has also been investigated for its potential influence on therapies, including chemotherapy drugs. For example, specific prodrugs have been shown to exhibit increases or decreases in cytotoxicity in the presence of certain types of bacteria in the body. Tangney and Robinson have used technologies such as high-performance liquid chromatography (HPLC) and mass spectrometry to investigate the effect of bacteria on chemotherapies in mouse models. Their work has confirmed that bacterial types could influence the treatment effectiveness and suggest that bacteria could be administered to manipulate the tumor microenvironment toward positive outcomes for targeted cell and gene therapies. Studies have also shown that certain bacteria favor the tumor microenvironment,

where they can evade the immune system and feed off surrounding cancer cells. Researchers are now exploring the influence of bacteria on the production of tumor necrosis factor-alpha (TNFα), the use of bacterial treatment before immunotherapy as a means of tumor conditioning, and vaccine delivery methods to enable further studies in a clinical setting.

The gut microbiome can also influence patient responses to immune checkpoint inhibitors such as anti-PD-1 therapies. In normal conditions, immune checkpoint receptors are expressed on the surface of T and B cells and suppress inflammatory responses to prevent autoimmunity. In cancer, the immune system's ability to detect and tag cancer cells for degradation is blocked, thus allowing for rampant growth and expansion. Immune checkpoint inhibitors allow the immune system to recognize cancer cells as foreign and trigger a firstand second-order immune response to eliminate the cells. By recruiting cytotoxic T lymphocytes or natural killer cells, the body can degrade cancer cells with high levels of specificity while rendering healthy cells unharmed. Bacterium types that promote anti-tumor activity cause activation of secondary immune response signals, resulting in an increase in CD8+T cells and a subsequent decrease in regulatory T cells and myeloid-derived suppressor cells. Manipulating the bacterial composition of the microbiome can therefore prime patients for immune checkpoint inhibitor treatment with an increased probability of success.[2]

Identifying patient responders through microbiome profiling

Microbiome profiling allows for the stratification of cancer patients into rational treatment groupings based on bacterial profiles. This approach offers insights into potential responses to cancer immunotherapies. Profiling can be performed in large patient groups with unparalleled comprehensiveness and precision. Researchers can identify microbial signatures linked to phenotypes using bioinformatic tools, which identify relationships between patient outcomes and signatures that other investigative tactics may miss. Considering the microbial profile of patients allows for rational targeted treatments to be implemented, with biomarker presence and microbiome specificity driving the type of therapeutic intervention used. Microbiome signatures can be used to stratify patients for personalized drug treatment and identify live bacterial therapeutics and drug targets for testing in defined patient groups.

Optimal responses to cancer therapy require an intact commensal microbiota that mediates its effects by modulating myeloidderived cell functions in the tumor microenvironment. Depending on the quality of the microbiome and its characteristics, patients can be classified as responders or non-responders in clinical trials. Using knowledge of the correlation between the characteristics of patients' gut microbiome and their individualized response allows researchers and clinicians to triage patients and determine the type of treatments they should receive. Biomarker testing provides a window for treatment decision-making by not only helping to select the best treatment option for their scenario but identifying potential ways to exploit underlying microbial profiles to facilitate the best outcome.

Future perspectives of microbiome modulation

The progression of cancer is intricately related to the functioning of the immune system and understanding the role bacteria play in immune modulation offers an avenue for further correlative evaluation. Multiple studies have illustrated that it is possible to manipulate the microbiome in favor of immune-potentiating bacteria while limiting the effectiveness of bacteria associated with increased immune suppression. Determining which organisms promote anti-tumor immunity and which facilitate tumor growth is key to augmenting the microenvironment in favor of immunotherapy efficacy.

Future perspectives and areas for further research include the possibility of bacteria as a drug delivery vehicle, as well as additional ways that bacteria can enhance therapeutic functioning. Microbiome modulation using diet, probiotics, and fecal transplantation offer promise for improving responses to immunotherapies. Further testing to include *in vivo* imaging studies to detect bacterial patterns, the role of microbial metabolites, and complete microbial profiling are also on the revvity.

This whitepaper is based on presentations by Mark Tangney, Professor at University College Cork, and Mat Robinson, Vice President Translational Biology at Microbiotica.

Reference

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