How the Gut Microbiome affects Cancer Immunotherapy

The human gut microbiome modulates many host processes, including metabolism, inflammation, as well as immune and cellular responses. It is now becoming increasingly apparent that the microbiome can also influence the development of cancer and impact the outcome of cancer treatment. Therefore, integrating gut microbiome effects with other tumour and host factors, regulating immunotherapy responsiveness versus resistance, could facilitate optimisation of therapeutic outcomes.

GUT MICROBIOME AND ITS FUNCTION

The human body is a **complex ecosystem** inhabited and influenced by microorganisms including bacteria, yeast, fungi, protozoa, archaea, and viruses, all of which collectively constitute the commensal microbiota. Particularly, the intestinal tract is considered a microbial hotspot consisting of trillion microbial cells with an aggregate 9.9 million microbial genes across the gut microbiome[1]. The exact composition of the microbial community may vary greatly from person to person, however, it generally consists of about a dozen phyla, primarily *Firmicutes* and *Bacteroidetes*, followed by *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and others.

It has been widely established that the gut microbiota influences numerous and diverse physiological functions, hence has a **prevalent role in human health**. Not only by providing nutrients and vitamins but also by preservation of epithelial mucosa homeostasis and by supporting the innate and adaptive immunity.

Recently, it has become more and more elucidated that the **interplay of the gut microbiota with the immune system** has a significant impact on the patient's health. This complex interaction between the gut microbiota and our immune system has evolved as a means to maintain a symbiotic relationship. When operating optimally, this symbiosis ensures a protective response against pathogens and the maintenance of regulatory pathways involved in the immune tolerance to innocuous antigens.

GUT DYSBIOSIS AND CANCER DEVELOPMENT

Although the gut microbiota is essential for human health, alterations of the microbial community have been linked to the development of multiple diseases, both gastrointestinal as well as non-gastrointestinal diseases[2]. In the context of cancer, some specific bacteria have been demonstrated to be **involved in the process of carcinogenesis** through the following effects:

- A direct oncogenic effect of the microorganisms themselves and their products as-is
- Alteration of circulating metabolites which, in turn, become pro-carcinogenic
- Stimulation of the synthesis of trophic factors (e.g. growth factors) by the host, and finally
- Disruption of the host cancer immunosurveillance through the induction of pro-inflammatory and immunosuppressive pathways[3,4].

Unravelling the biological mechanisms of microorganisms in driving carcinogenesis requires great effort and has only been established for certain bacterial species. For example, the association between *Fusobacterium nucleatum* and colorectal cancer (CRC) progression has already been established[4]. Nonetheless, as the gut microbiome is a highly dynamic and complex ecosystem, a lot more research is required to fully unravel its role in disease progression.

GUT MICROBIOTA AND CANCER THERAPY

In addition to pro-carcinogenic effects, the gut microbiota has also been shown to be **involved in the efficacy of cancer therapy**. Indeed, recent studies have associated the gut microbiota with response rate

as well as toxicity across a range of treatments, including chemotherapy, immunotherapy and stem cell transplantation.

Evidence linking the gut microbiome to cancer immunotherapy

The beneficial role of the gut microbiota was first reported by Paulos *et al.*[5], his research showed that the efficacy of anti-tumour CD8+ T-cells adoptive transfer in a melanoma murine model was strongly increased after total body irradiation due to the translocation of gut bacteria into mesenteric lymph nodes. Six years later, the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host were demonstrated both in chemo- and immunotherapy[6,7]. Even more recently, multiple publications have proven a **crucial role for gut microbiota in the efficacy of CPI** (CheckPoint Inhibitor**) therapy across several cancer types**[8–11].

Initial evidence for the contribution of specific bacterial species to CPI therapy was first demonstrated in mouse models[8,9]. Sivan *et al.* reported that oral administration of *Bifidobacterium* promotes anti-tumour immunity and facilitates anti-PD-L1 efficacy by activation of dendritic cells, which in turn supports improved effector function of tumour-specific CD8+ T-cells[9]. Furthermore, Vétizou *et al.* revealed that antibiotic-treated or germ-free mice did not respond to CTLA-4 blockade. However, this defect was overcome by gavage with *B. fragilis*, by immunisation with *B. fragilis* polysaccharides, and by adoptive transfer of *B. fragilis*–specific T-cells, revealing a key role for *Bacteroidetes* in the immunostimulatory effects of CTLA-4 blockade[8]. Both these preclinical models and similar observations across numerous clinical cohorts contributed to the growing consensus that the gut microbiome is linked to CPI immunotherapy efficacy in cancer patients (Table 1)[8–14].

Moreover, these studies broadly demonstrate that **differential gut microbiota 'signatures' exist in patients who respond to CPI therapy** and that these favourable signatures are associated with enhanced systemic immunity and intratumoural immune infiltrates. A significant association was observed between commensal microbial composition and clinical response, notably for *Akkermansia muciniphila*[16], *Bifidobacterium longum*[11], *Collinsella aerofaciens*[11], *Enterococcus faecium*[11], bacteria of the Ruminococcaceae family[12] and many others. Additionally, several of these studies demonstrate that faecal microbiota transplantation (FMT) from cancer patients who responded to CPI immunotherapy into germ-free or antibiotic-treated mice ameliorated the anti-tumour effects, whereas FMT from nonresponding patients failed to do so. Moreover, targeted manipulation of the gut microbiota with specific bacterial taxa could enhance therapeutic response[11,12,16] indicating that specific bacterial species improve CPI therapy efficacy.

Deciphering the biological mechanisms of microbiome-mediated immune modulation

The aforementioned studies indicate that commensal bacteria not only have a direct effect on immune cells in colon cancer, moreover, they also have the capability to affect the development and treatment efficacy of more distal cancer types, such as melanoma.

The exact mechanism of these **long-distance microbiota-mediated effects** are only beginning to be understood, however, some hypotheses are summarised below.

Table 1. Evidence linking gut microbiome composition to cancer therapy efficacy. Adapted from *Fessler et al.*[15].

Major finding	Data origin	Cancer/Therapy	Ref.
Total body irradiation disrupted intestinal barrier and improved outcome of T-cell based therapy by a mechanism dependent on LPS/microbe translocation and TLR4 signaling	Mouse	Melanoma/Adoptive T-cell transfer	5
Commensal microbiota was required for optimal response to therapy	Mouse	CpG-oligonucleotide + anti-IL-10R antibody and platinum chemotherapy	6
Akkermansia muciniphila abundance in baseline stool samples was associated with response to CPI	Mouse	Cyclophosphamide immunostimulatory chemotherapy	7
Bacteroides abundance was associated with response to CPI	Mouse; Human	Metastatic melanoma/Anti-CTLA-4	8
Bifidobacterium abundance was associated with improved spontaneous antitumour immunity and response to CPI	Mouse	Melanoma/Anti-PD-L1	9
A. muciniphila abundance in baseline stool samples was associated with response to CPI	Human; Mouse	Non-small cell lung cancer; Renal cell carcinoma/Anti-PD-1	10
Several dozen bacterial species in baseline stool samples were differentially enriched between patients with strong vs. poor responsiveness to CPI	Human; Mouse	Metastatic melanoma/Anti-PD-1	11
Higher microbiome richness, *Clostridiales*, *Ruminococcaceae*, and *Faecalibacterium* abundance, and enrichment in genes involved in anabolic pathways in baseline stool samples were associated with responsiveness to CPI	Human; Mouse	Metastatic melanoma/Anti-PD-1	12
Faecalibacterium and other *Firmicutes* abundance in baseline stool samples was associated with response to CPI; *Bacteroides* abundance was associated with poor responsiveness to CPI	Human	Metastatic melanoma/Anti-CTLA-4	13
Bacteroides caccae, Faecalibacterium prausnitzii, Bacteroides thetaiotaomicron, Holdemania filiformis, and *Dorea formicogenerans* were associated with response to CPI	Human	Metastatic melanoma/Anti-PD-1; Anti-CTLA-4	14

The effect gut microbiota exert on cancer immunosurveillance results from a combination of antigenicity and adjuvanticity. **Adjuvanticity** is related to the fact that microbiota can act as adjuvants by activating pathogen recognition receptors (PRRs), such as toll-like receptors (TLRs), on innate immune effectors resulting in stimulation of the production of cytokines and interferons. This can evoke an immunostimulatory effect with increased numbers of tumour-infiltrating lymphocytes (TILs) and decreased numbers of myeloid-derived suppressor cells (MDSCs). In contrast, certain bacterial species may suppress anti-tumour immunity by inducing immunosuppressive immune cells such as regulatory T-cells (Tregs) and MDSCs.

Besides adjuvanticity, the gut microbiota may also exert a direct effect: **antigenicity** due to T-cell epitopes that are shared between bacteria and tumour cells[8,10,17–20]. Moreover, it is hypothesised that molecular mimicry between distinct commensal bacteria and tumour neoantigens could account for the toxicity and/or efficacy of CPI immunotherapy[8,17,21,22]. In this case, bacterial antigens with a high similarity to tumour antigens would be recognised by cross-reactive T-cells, resulting in a broadened and stronger immune response against the cancer cells presenting the tumour antigen. Indeed, Bessell *et al.*[22], identified a neoepitope (SIY) that cross-reacts with the SVY-epitope expressed in the commensal bacterium *Bifidobacterium breve*. Moreover, cross-reactive T-cells were able to recognise SIY-expressing melanomas in mice which led to a decreased tumour growth and extended survival.

POTENTIAL CLINICAL APPLICATIONS

The collective evidence linking the gut microbiome to carcinogenesis and immunotherapy efficacy creates exciting opportunities to improve clinical treatment strategies. Both preclinical and clinical data suggest that the modulation of the microbiota could become a novel strategy for improving the efficacy of immune-

based therapies for cancer, in particular checkpoint blockade approaches targeting the CTLA-4 and PD-1 pathways[23].

Biomarker

As stated above, the gut microbiome composition has already been associated with treatment efficacy. Moreover, some bacterial species have been identified as "beneficial" while others seem to have a more "detrimental" effect. These data suggest that **faecal DNA sequencing prior to therapy**, by quantifying the community richness and the relative proportion of putatively identified "beneficial" or "detrimental" bacteria, may be suggestive of outcome and ultimately help guide treatment decisions. Therefore, besides tumour mutational burden (TMB) and T-cell infiltration, the gut microbiome composition could be considered as a complementary prognostic or predictive biomarker for treatment outcomes.

It is important to note that to date, no clear classification has been established distinguishing "beneficial" from "detrimental" bacterial species. In addition, the identification of these correlation is highly susceptible to the workflow. Therefore, the reported associations should be interpreted with caution and new clinical studies are necessary for validation of these associations.

Therapeutic interventions

Based on the established impact of the microbial composition of the gut on cancer therapy, there is growing interest in targeting these commensal bacteria in the treatment of cancer and other diseases. Therapeutic strategies range from complex bacterial community transfers, such as **faecal microbiota transplantation** (FMT), which may have many effects on the recipient, to delivery of a single microbial metabolite with a specific immune-modulatory effect. Alternatively, a beneficial bacterial composition could be acquired by prebiotic supplements or administration of probiotics. Clinical trials have already been started in different cancer types for FMT[24,25] and probiotics[26–28] to assess their impact on cancer development, response to treatment and impact on treatment–related toxicity.

Besides the modulation of the gut microbiome, another therapeutic strategy relying on the molecular-mimicry concept opens new opportunities in the search for neoantigens for personalised cancer vaccination. **Targeting neoantigens that activate cross-reactive memory T-cells** might result in a broadened T-cell response against cancer cells. However, the identification of neoantigens with a high similarity to microbial epitopes and the assessment of T-cell cross-reactivity is still ongoing research.

To conclude, it can be stated that the gut microbiome has already proven to play an important role in cancer progression and treatment efficacy. Therefore, it will be important to consider the microbiota as one of several parameters that affect cancer therapy. Moreover, further unravelling the biological mechanisms behind this complex interaction may offer new therapeutic strategies in personalised medicine.

About myNEO

myNEO (Ghent, Belgium) developed a platform enabling genomic-informed drug discovery in the key therapeutic areas of oncology and immunology. The data-driven ImmunoEngine identifies the most efficacious targets (epitopes) for each cancer patient, uniquely presented on the tumour cells and capable of redirecting a patient's immune system, leading to elimination of the cancer cells. The discovery platform enables targets to be identified even in hard-to-treat tumours with a cold/lowly mutated profile. Similarly, the company has applied its technology to identify immunogenic sequences in infectious diseases, capable of protecting populations with strong broad immune responses. myNEO is one of the companies that emerged from the Novalis biotech incubator fund at the end of 2018, founded by two leading entrepreneurs already known for several successes in the biotech industry: Wim Van Criekinge, professor of computational biology at Ghent University, and childhood friend Jan Van den Berghe.

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