

AKADÉMIAI KIADÓ

# Modulation of the intestinal microbiota impacts the efficacy of immunotherapy in cancer patients – A recent literature survey

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## REVIEW PAPER



## ABSTRACT

In line with the current development of individualized cancer treatments, targeted and specialized therapeutic regimens such as immunotherapy gain importance and factors improving its efficacy come into the focus of actual research. Given the orchestrated interaction of the intestinal microbiota with host immunity the modulation of the human gut microbiota represents a therapy-enhancing factor. We therefore performed an actual literature survey on the role of the gut microbiota composition and the effects of its modification during immunotherapy of cancer patients. The included 23 studies published in the past 10 years revealed that both, distinct bacterial species and genera including *Faecalibacterium prausnitzii* and *Bifidobacterium*, respectively, enhanced distinct immunotherapy responses following PD-1/PD-L1 and CTLA-4 blockage, for instance, resulting in a better clinical outcome of cancer patients. Conversely, a high intestinal abundance of Bacteroidetes and *Fusobacterium* species correlated with a less efficient immunotherapy resulting in shorter progress-free survival outcomes. In conclusion, modifications of the gut microbiota by fecal microbiota transplantation or application of probiotic compounds represent potential adjunct options for immunotherapy in cancer patients which needs to be further addressed in future trials to provide individually tailored and safe adjuvant therapeutic measures in the combat of cancer.

## KEYWORDS

human gut microbiota composition, treatment efficacy, immunotherapy, cancer therapy, PD-1 blockage, CTLA-4 blockage

## INTRODUCTION

### The human gut microbiota

Human beings are physiologically populated by a plethora of bacteria, most of which live on surface structures such as the skin and mucous membranes, serving as a barrier to the surrounding environment [1–5]. The community of regularly present bacteria are termed the residential flora, while occasionally existent bacteria are referred to as transient flora [5]. The residential bacterial flora of humans forms part of the microbiota, which besides bacteria also contains viruses, yeasts, molds, and parasites. Therefore, the term microbiome comprises the genetic content of the microbiota formed by all microbes including their collective genomes, and in some cases their environmental interactions. The vast majority of the microorganisms colonizing the human body live in symbiosis and do not act as pathogens, thus exerting health-beneficial properties, e.g. by the production of antimicrobial agents, by deprivation of nutrients needed for pathogenic invasion, or by stimulating the immune system [3, 5–9]. The balanced composition of the human gastrointestinal microbiome

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depends on many factors, such as age, diet, genetic and epigenetic factors, geography, morbidities including inflammatory conditions, antibiotic medication, and immune deficiency, among others [1, 6–8, 10]. Moreover, the different compartments of the gastrointestinal tract present varying intraluminal milieus providing both, favorable and unfavorable microbial growth conditions [1, 2, 8, 11]. Given that the majority of the microbiota is formed by bacteria and the viral microbiome awaits further investigations, we will focus on the bacterial part of the microbiota and the microbiome in this review. The composition of the bacterial microbiota alongside the gastrointestinal tract depends on the intraluminal pH changing from acidic pH 1 in the stomach to alkaline pH 8 in the proximal small intestines and to an almost neutral pH in the terminal ileum and colon. The diversity of the bacterial microbiota increases from the proximal to the distal small intestines and the large intestines and shifts from mainly facultative to obligate anaerobic bacterial species [5].

Overall, beside the Gram-negative Bacteroidetes, the Gram-positive Firmicutes represent the most prominent bacteria of the gut microbiota (i.e., approximately 60%) and include bifidobacteria, lactobacilli, enterococci, staphylococci, *Ruminococcus* species and clostridia [2, 5]. *Faecalibacterium prausnitzii* represent approximately 5% of the gut microbiota composition, whereas 10% are represented by Gram-negative obligate anaerobic Bacteroidetes, such as *Bacteroides* species (spp.), *Porphyromonas* spp. and *Eggerthella* spp., for instance. Additional 5–10% are made up by anaerobic Verrucomicrobia and further 10% by Archaeobacteria. Aerobic Gram-negative Proteobacteria such as the *Escherichia coli* bacteria of the Enterobacteriaceae family represent approximately 1–5% of the gut microbiota. Transient gut microbial members are *Proteus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., and fungi such as *Candida* spp. Overall, 300 to 1,000 bacterial species are estimated to form the gut microbiota, 20 of which are considered to represent the most abundant species.

### The relationship between the gut microbiota and the host immune system

The well-orchestrated interplay between the commensal intestinal bacteria and the host immune system is pivotal for homeostasis, whereas imbalances might result in dysbiosis and disease [4, 6–8]. The physiological equilibrium of intestinal immune cells with the microbiota carries great importance for several health preserving functions, namely development, differentiation, maturation, and regulation required for maintenance of both, the innate and adaptive immune system functions, which in turn coordinate essential features of host-microbe symbiosis by production of antimicrobial peptides and antibodies [6–9, 12]. An imbalance in this bidirectional relationship, termed dysbiosis, increases susceptibility towards a multitude of immunopathological including inflammatory conditions, such as allergies, asthma, and chronic inflammatory morbidities affecting the intestinal tract and the joints, but also obesity, metabolic syndrome,

and diabetes mellitus [1, 6, 13–17]. The co-evolution of the immune system and the human microbiota has resulted in an extensive and complex communicatory network involving extracellular receptors, such as Toll-like receptors, NOD-like receptors, certain key players in immunity such as innate lymphoid cells and distinct molecules including antimicrobial peptides, polysaccharide A, and dectin-1 [6–8, 10, 16–18]. The bacterial gut microbiota is further involved in preserving epithelial barrier functions, in stimulating mucosal antimicrobial peptide production, in nutrient utilization and detoxification, in the regulation of intestinal blood flow, and in the modification of pharmaceuticals [6–8, 12]. Furthermore, the microbiota plays an important role in regulating mucosal tissue regeneration, mucus formation, gut motility, and permeability [6]. With respect to metabolism, the bacterial microbiota enhances nutrient accessibility and lessens energy expenditure. Bacterial metabolites such as short-chain fatty acids (SCFAs) including butyrate, propionate, and acetate feed the intestinal enterocytes and support the local host defense by inducing production of secretory IgA and of cytokines including interleukin (IL) -17 and IL-22. Furthermore, SCFAs are known to stimulate distinct immune cell subsets such as Foxp3<sup>+</sup> regulatory T (T<sub>reg</sub>) cells, follicular T cells and T helper (Th) 17 cells in Peyer's patches of the intestine which in turn, support the class switch of B lymphocytes and the generation of secretory IgA contributing to compartmentalization and the bacterial species composition of the commensal gut microbiota [6]. Furthermore, SCFAs stimulate the antimicrobial lectin RegIIIy in mucous membranes [5]. Especially *Faecalibacterium* spp. are known to produce SCFAs and prevent chronic intestinal inflammation, whereas *Bacteroides fragilis* have been shown to initiate CD4<sup>+</sup> T cell differentiation and to keep the balance between Th1 and Th2 populations [3, 6]. Moreover, microbiota-derived SCFAs promote the memory potential of CD8<sup>+</sup> T cells [6]. Of note, the gut microbiota impacts the functions of various extra-intestinal organs, such as skin, liver, lungs, and the brain. Finally, recent research results suggest a deep impact of the gut microbiota on brain functions ("gut-brain axis"). For instance, associations of distinct gut microbiota signatures with autism spectrum disorders such as the ADNP syndrome, with Alzheimer's disease, and with other morbidities of the nervous system have been described [19–21].

**The bidirectional relationship of the gastrointestinal microbiota and cancer.** The microbiota of every individual is considered as a highly dynamic ecosystem since the bacterial composition adapts to both, external and internal factors [1, 8]. Given that dysbiosis can contribute to initiation and progression of distinct morbidities, there is rising interest in the role of the microbiota composition in cancer development and immune surveillance [1, 7, 8, 22]. For instance, the bacterial species *Fusobacterium nucleatum* is considered to play a role in the immunopathogenesis of colonic cancer, given that the bacteria have been shown to inhibit natural killer (NK) cells known to eliminate tumors in the intestinal microenvironment. The anti-NK cell directed effect of



*F. nucleatum* is hypothesized to be mediated by the interaction of the bacterial Fap2 protein with the human T cell Immunoreceptor with Ig and ITIM domains (TIGIT Receptor) [1, 6, 23, 24]. A high abundance of *F. nucleatum* has also been shown to be accompanied by lower CD3<sup>+</sup> T cell numbers, the presence of which is associated with a better clinical outcome [6, 25]. Furthermore, *Fusobacterium* spp., but also *Porphyromonas* spp. and *Peptostreptococcus* spp. could be detected in high numbers in fecal and mucosal samples derived from colorectal cancer patients [1]. The importance of distinct bacteria in cancerogenesis is further supported by the observation that bile acids are utilized by commensal clostridia as messengers to enhance the anti-tumoral effects of hepatic CXR6<sup>+</sup> NK T cells [6, 26].

**Modern concepts of immunotherapy in cancer treatments.** Optimizing cancer treatment and immune surveillance constitute important topics of tumor research worldwide. Besides the established methods of chemotherapy with cytostatic compounds and radiotherapy, the more recent immunotherapeutic approaches have gained increasing importance in oncology and provide more specified and individualized treatment options for cancer patients [27, 28]. The most important target-oriented therapeutic options currently at hand in so called “individualized medicine” are monoclonal antibodies, immune checkpoint inhibitors, treatment vaccines and adoptive T cell transfer therapy, such as tumor-infiltrating lymphocytes or chimeric antigen receptor (CAR) T cell therapy, for instance [23, 27, 28]. Immune-modulating molecules such as activating antibodies, multi-specific antibodies, and cytokines constitute further treatment options [29]. To date, two therapeutic strategies against checkpoint targets are in approved application, namely blocking the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and the programmed death ligand 1 (PD-L1/PD-1) immune checkpoints [23, 27]. Since tumors can hide and escape from immune surveillance by triggering such immunosuppressive receptors, the blockage of these agents can enhance the efficacy of anti-tumor responses [26, 30, 31]. CTLA-4 constitutes an immunosuppressive receptor constitutively expressed on T<sub>reg</sub> and activated T cells. Antigen-presenting cells express ligands called B7-1 (CD80) and B7-2 (CD86), which can bind to these CTLA-4 receptors [28, 30]. The activation of CTLA-4 subsequently leads to an inhibition of T cell proliferation and activity, disturbs immune conjugate formation, promotes recruitment of additional inhibitory effector cells and lessens the activity of transcription factors, such as nuclear factor kappa B (NF-κB), activator protein 1 or nuclear factor of activated T cells [28]. PD-1 has been shown to regulate T cell populations by control of T cell exhaustion, by orchestration of T<sub>reg</sub> cell differentiation and activity, as well as by binding to its ligands, PD-L1 and PD-L2, which are expressed on antigen-presenting cells required for modulation of T cell functions. Excessive levels of PD-L1 or PD-L2 in cancer cells limit the cytotoxic anti-tumoral response of CD8<sup>+</sup> T cells, whereas

its blockage can support tumor cytolysis and reduce metastasis [28]. However, modulating immunotherapeutic responses by alterations in gut microbiota composition, for instance, may result in different efficacy depending on tumor localization, tissue specificity, and growth state.

### Aim of this literary survey

The purpose of this literature survey was to summarize most recent studies for a better understanding of the intertwined relationship between the human bacterial gut microbiota, cancer development, and tumor immunotherapy and to highlight potential implications of these interactions for optimized individualized treatment regimens in cancer patients.

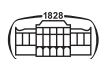
## METHODS

### Search strategy and data collection

A structured literary survey was conducted in the period of June 10th to June 30th, 2022 by applying the meta database “PubMed” of the U.S. National Institute of Health, accessing biomedical and medical studies, including MEDLINE, PubMed Central and MeSH Databases. Firstly, a rather broad search approach was performed in the PubMed data base by the combined term “gastrointestinal microbiome” and then complemented by the combined term “gastrointestinal microbiota”. Further synonyms were recruited, such as “GI microbiome”, “gut microbiome” and “intestinal microbiome”, as well as “GI microbiota”, “gut microbiota” and “intestinal microbiota” using quotation marks in the PubMed Advanced Search Builder. Eventually all terms were connected by the Boolean logic “OR” and restricted to a publication date within the last 10 years. The same procedure was applied with the additional term “human”, leading to search entries for “human GI microbiome”, “human gut microbiome” and “human intestinal microbiome”, as well as “human GI microbiota”, “human gut microbiota” and “human intestinal microbiota”.

Secondly, the database was surveyed for results in “immunotherapy” and “cancer”, united by the Boolean operator “AND”. The concluding MeSH terms for immunotherapy were “immunotherapies” or “immunotherapy’s”, while for “cancer” the results were “cancer’s”, “cancerated”, “canceration”, “cancerization”, “cancerized”, “cancerous”, and “neoplasms”.

Finally, both search histories were intertwined with “AND” and, searching in the Title/Abstract section only, leading to the final entry (“human gastrointestinal microbiome” OR “human gastrointestinal microbiota” OR “human GI microbiome” OR “human GI microbiota” OR “human intestinal microbiome” OR “human intestinal microbiota” OR “human gut microbiome” OR “human gut microbiota”) AND (“gastrointestinal microbiome” OR “gastrointestinal microbiota” OR “GI microbiome” OR “GI microbiota” OR “gut microbiome” OR “gut microbiota” OR “intestinal microbiome” OR “intestinal microbiota”) AND (immunotherapy AND cancer”).



## Inclusion and exclusion criteria

Included were different studies published in English or German within the past 10 years. Study designs of clinical trials, meta-analysis, randomized controlled trial and systematic review were accepted. Due to the limited scope of publications in this regard we decided not to narrow the search results further down with a specific range of age, gender or geographical population. Furthermore, to outline the whole realm of possible interventions and future perspectives in cancer treatment, studies for all types, localizations and stages of cancer were included.

## Data extraction

Collecting results for the survey within the last 10 years showed great recency in research, since most included papers were published within the last 5 years even. In total, the search results on PubMed presented 17 studies with the search history listed above, from which 14 were included. In combination with the snowballing system 6 more studies could be identified, among them clinical trials and two meta-analyses. Two studies on PubMed were excluded due to lack of relevance for the topic and one due to inaccessibility of full text. From the few studies in this topic, the majority of trials focused on immune checkpoint blockade treatments with PD-1/PD-L1 agents, and only two addressed CTLA-4 treatment.

## RESULTS

### The bidirectional relationship of the gut microbiota and immunotherapy

In a very recent investigation, McCulloch and colleagues addressed potential associations of treatment responses in anti-PD-1 therapy of melanoma patients with distinct microbiota signatures [31]. Therefore, stool samples were collected from 94 patients either before treatment, within the first 4 months or after 4–41 months of treatment and subjected to gene sequence analysis for assessing bacterial signatures. The obtained results revealed that members from the Lachnospiraceae and Streptococcaceae families were associated with favorable as well as unfavorable clinical outcomes, while both contributed to immune-related adverse events resulting in poorer progression-free survival. In progressor patients, the Bacteroidetes and Proteobacteria phyla were more abundant, whereas in non-progressor patients the members from the Actinobacteria phylum and the Lachnospiraceae family were enriched. The assessment of the clinical outcomes further revealed that non-progressor patients harboring a “beneficial” microbiota sustained these also during therapy, thereby offering a predictive tool for clinical therapy responses. Furthermore, the authors found a correlation between Gram-negative bacteria and a high neutrophil-to-lymphocyte ratio (NLR), a higher abundance of pro-inflammatory immune cell populations such as neutrophils, macrophages, monocytes, and dendritic cells,

and poor prognosis of the patients. The identified favorable bacterial taxa signatures included higher numbers for genes encoding for molecules involved in iron bioavailability and transport, in production of reactive oxygen species, in synthesis of flavin and riboflavin constituting by-products recognized by mucosal-associated T cells that were shown to be activated in PD-1 responders. Unfavorable taxa signatures contained genes for lipopolysaccharide (LPS) synthesis and mucus degradation, for example alpha-L-fucosidase and alpha-galactosidase. Genes coding for pro-inflammatory mediators such as IL-1beta and CXCL8, superoxide dismutase-2 and distinct transcription factors were elevated in progressors, while genes for mucosal and endotoxin protective membrane mucins including the mucins MUC13 and MUC20 and apolipoproteins were enriched in non-progressors [31].

In another study, both, oral and fecal microbiome analyses were performed in melanoma patients undergoing anti-PD-1 therapy [32]. Therefore, respective samples were collected at the initiation of treatment, followed by 16S rRNA gene sequencing, metagenomic whole genome shotgun sequencing and multi-parameter immunohistochemistry. The results revealed significant increases in alpha-diversity of the gut microbiota in patients responding to therapy (responders) as well as a prolonged to progression-free survival, whereas neither significant changes in oral microbiota composition nor correlations to progression-free survival were detected. Higher abundance of Clostridiales, especially *Faecalibacterium* spp., were found in responders, while *E. coli* and Bacteroidales such as *Bacteroides thetaiotaomicron* were elevated in fecal samples derived from patients who did not respond to the treatment (non-responders), which also held true for the oral microbiota composition. Patients harboring high levels of *Faecalibacterium* spp. in their gut microbiota experienced significantly longer progression-free survival. Conversely, patients with increased fecal abundance of Bacteroidales exhibited shorter progression-free survival. Bacterial signatures influencing anti-PD-1 treatment efficacy included enrichment of genes involved in anabolic functions, such as amino acid biosynthesis, thereby supporting host immunity, that were predominant in responders. Genes involved in catabolic pathways resulting in a different tumor microenvironment dominated in non-responders, however. Furthermore, higher CD8<sup>+</sup> T cell numbers were found in tumor-associated immune infiltrates in samples of responding versus non-responding patients, which were positively correlated with higher *Faecalibacterium* spp. levels in responders and negatively correlated to Bacteroidales in non-responders. Moreover, patients harboring a microbiota enriched in Clostridiales, Ruminococcaceae or *Faecalibacterium* spp. also displayed higher abundances of effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells in systemic compartments with preserved cytokine responses. In contrast, higher levels of T<sub>reg</sub> cells and myeloid derived suppressor cells, as well as weakened cytokine responses, were measured in patients with more Bacteroidales bacteria [32].

In 2021, results from a clinical trial with colorectal cancer patients who were treated with the PD-1/PD-L1 monoclonal





antibodies regorafenib plus toripalimab were published [33]. The 16S rRNA sequencing analyses of fecal samples before initiation of the treatment regimen revealed that non-responders exhibited higher *Fusobacterium* spp. and lower Proteobacteria gene numbers in their feces. Furthermore, four enriched bacterial taxa, namely *Fusobacterium* spp., *Alistipes* spp., *Bilophila* spp. and *Acidaminococcus* spp. were positively correlated with poor treatment efficacy in non-responders. Baseline levels of both, *Fusobacterium* spp. and *Acidaminococcus* spp. were considered as significant risk factors of shortened progression-free survival periods. However, at least a trend towards more beneficial results (i.e., longer progression-free survival periods) were obtained from patients without liver metastasis, albeit no significant correlation. In summary, the authors found a negative correlation of fecal abundance of *Fusobacterium* spp. with response to therapy and survival in colorectal cancer patients treated with the PD-1/PD-L1 monoclonal antibodies regorafenib plus toripalimab [33].

In 2018, Matson and colleagues surveyed the commensal gut microbiota composition in metastatic melanoma patients receiving anti-PD-1 directed treatment [34]. Fecal samples were obtained from 42 patients and analyzed before and after initiation of the anti-PD-1 treatment. In total, 63 operational taxonomic units could be identified that were different in responding and non-responding individuals. Further analyses revealed 8 bacterial species that were more abundant in responders versus non-responders, namely *Bifidobacterium longum*, *Enterococcus faecium*, *Collinsella aerofaciens*, as well as *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, and *Lactobacillus* spp. Two bacterial species, namely *Ruminococcus obeum* and *Roseburia intestinalis*, were enriched in non-responders, however. Further calculations addressing the correlation between a favorable gut microbiota and slower tumor growth were positive, suggesting the microbiota signature as potential biomarker predicting treatment efficacy. The authors then performed fecal microbiota transplantation (FMT) experiments in germ-free mice with fecal material from responding donor patients and obtained inconclusive results. Whereas in some transplanted and engrafted mice an unexpected drift of the microbiota could be observed that was accompanied by a lack of improvement in therapy efficacy, others could be successfully reconstituted and displayed a favorable outcome. Further analyses of the tumor microenvironment revealed an increase of CD8<sup>+</sup> T-cells, of Batf3-lineage dendritic cells and of Th1 cells in responding microbiota-reconstituted mice, indicative for an impact of the gut microbiota composition on anti-tumor immunity. However, almost no anti-tumor effects could be observed upon anti-PD-1 treatment of non-responding mice.

In a study by Bernicker and colleagues 37 Chinese patients suffering from non-small-cell lung cancer (NSCLC) were subjected to anti-PD-1 treatment with nivolumab and subsequent changes in gut microbiota composition, clinical conditions and distinct immune responses were assessed [35]. The results of the trial revealed that microbiota

diversity and stability in therapy responding patients correlated with longer tumor progression-free survival, with enriched fecal abundance of *B. longum*, *Alistipes putredinis* and *Prevotella copri*, as well as with increased frequencies of memory CD8<sup>+</sup> T cells and NK cells. In contrast, non-responders showed lower fecal microbial diversity and higher abundance of *Ruminococcus* spp. The study furthermore showed no statistical differences in microbiota composition in the course of anti-PD-1 treatment of NSCLC patients.

In a clinical trial published in 2018, Routy et al. assessed changes in gut microbiota composition and immune responses following anti-PD-1 treatment of patient cohorts suffering from epithelial tumors such as NSCLC or renal cell carcinoma [36]. To address this, fecal samples from 60 patients diagnosed with NSCLC and 40 with renal cell carcinoma were collected before and during anti-PD-1 treatment and further analyzed. The authors reported that both, classified and unclassified Firmicutes as well as *Akkermansia* spp., especially *Akkermansia muciniphila*, were significantly over-represented in responders with progression-free survival of more than three months when compared to responders with progression-free survival of less than three months and to non-responders. Other commensal bacteria with increased abundance in the former were *Ruminococcus* spp., *Alistipes* spp. and *Eubacterium* spp., while *B. adolescentis*, *B. longum* and *Parabacteroides distasonis* were decreased. Further results indicated an increased treatment efficacy by oral supplementation of *A. muciniphila* and *Enterococcus hirae* through CD4<sup>+</sup> T lymphocyte recruitment and IL-12 secretion by dendritic cells, through reinstating anticancer effects after preceding antibiotic inhibition, and lessened tumor growth [36].

In 2015, Sivan et al., investigated the role of commensal *Bifidobacterium* spp. in anti-PD-L1 treated mice with melanoma [37]. Mice from two different facilities, namely, Jackson Laboratory (JAX) and Taconic Farms (TAC), that represent genetically similar C57BL/6 mice, but differ in their commensal microbiota composition, were compared in this experiment. When compared to TAC animals, JAX mice showed lessened melanoma growth rates, enhanced accumulation of CD8<sup>+</sup> T cells within the tumor and pronounced tumor-specific T cell responses, as well as a more efficient tumor control upon anti-PD-L1 therapy. Co-housing experiments and oral bacterial challenges revealed that the dominant commensal *Bifidobacterium* spp. were most likely responsible for these effects. Further observations, such as prophylactic oral gavage of *Bifidobacterium* species, including *B. longum*, *Bifidobacterium breve* and *B. adolescentis*, before tumor implantation also resulted in the beneficial effects above. Further examinations of tumors established in TAC mice, that were treated with fecal material from JAX mice during an anti-PD-L1 therapy, also resulted in better anti-tumoral and therapeutical responses, as well as slower tumor growth. Pathway analysis of gene transcripts from mice with increased intestinal *Bifidobacterium* loads (either naive or upon FMT) displayed an up-regulation of immune response upstream of T cells at the dendritic cell level, as well as CD8<sup>+</sup> T cell activation and co-

stimulation of MHC-I, CD40, CD70 and ICAM-1. An additional increase of major histocompatibility complex (MHC) -II was observed in tumors from JAX and *Bifidobacterium*-treated TAC mice. Furthermore, these mice experienced an up-regulation of genes responsible for anti-tumor responses, antigen processing and presentation, interferon signaling and chemokine-mediated activity. Moreover, inoculation of B16 parental tumor cells or MB49 bladder cancer cells with *Bifidobacterium* spp. also resulted in a delayed tumor growth. Altogether, the authors observed that distinct microbiota compositions with higher bifidobacterial loads were associated with enhanced anti-tumor directed, T cell mediated immune responses resulting in less pronounced tumor growth and facilitated efficacy of PD-L1 blockage in mice with melanomas [37].

A systematic review from 2021 addressing the impact of the microbiota in solid tumor development revealed, that patients displaying a higher abundance of the Firmicutes and Verrucomicrobia phyla in their feces experienced better responses upon immune checkpoint inhibitor therapy, whereas patients with enriched Proteobacteria experienced less efficient outcomes [38]. The authors further emphasized that results regarding the role of members from the Bacteroidetes family were inconclusive.

The following two studies tested the effect of immunotherapy by CTLA-4 blockade on gut microbiota composition and intestinal integrity. To address this, Vétizou and colleagues compared melanoma development in specific pathogen-free mice and in germ-free mice [39]. The authors found that after a single injection of CTLA-4 antibodies melanoma mice displayed pronounced shifts in their gut microbiota, given decreased abundances of Bacteroidales and Burkholderiales, whereas increases in Clostridiales could be observed. However, a relative increase of distinct Bacteroides, namely *B. thetaiotaomicron* and *B. uniformis*, was detected in the small intestinal mucosa, contrary to the fecal analysis. Oral challenge of germ-free and antibiotic treated tumor-bearing mice with viable *Bacteroides fragilis* plus *Bacteroides thetaiotaomicron* or in combination with *Burkholderia cepacia* again reestablished anti-cancerous effects of CTLA-4 blockade treatment. Additionally, germ-free mice treated with *Bacteroides fragilis* showed increased IL-12 dependent Th1 immune responses in the tumor-surrounding lymph nodes as well as enhanced maturation of intra-tumoral dendritic cells, also supporting anti-CTLA-4 treatment and sparing intestinal stability [39].

In another anti-CTLA-4 treatment study, Chaput et al. tested the efficacy of ipilimumab treatment in metastatic melanoma patients and the risk of subsequent colitis development [40]. Colitis represents the most common immune-related adverse event of CTLA-4 blockage treatment and exhibits immunopathological features of colitis in inflammatory bowel disease including gut microbiota dysbiosis. To address this, fecal and blood samples were collected from 26 patients at the beginning (baseline), before each treatment infusion and at the end of treatment. Results revealed no significant shifts in fecal microbiota composition due to ipilimumab treatment. However, should colitis occur

during treatment, a significant reduction of the Firmicutes phylum, especially of operational taxonomic units related to *F. prausnitzii*, *Butyrate producing bacterium L2-21* and *Gemmiger formicilis*, and of the overall microbiota diversity was observed.

Another outcome of this trial revealed that a high abundance of *Bacteroides* spp. at baseline stool analysis was associated with a rather poor clinical outcome, whereas high fecal *Faecalibacterium* spp. levels could be observed in patients with long-term benefit of treatment. Furthermore, patients experiencing overall survival longer than 18 months displayed higher numbers of bacteria from the Firmicutes phylum. These results carry potential prediction values through individual microbiota for long-term versus poor treatment benefits already before treatment onset. However, increased Firmicutes at the beginning of the survey seemed to correlate with subsequent colitis development and shorter colitis-free cumulative incidence, whereas high baseline levels of Bacteroidetes were associated with a lower risk of colitis development. Additionally, patients with increased fecal levels of *Faecalibacterium* spp. and hence, long-term benefits of ipilimumab, displayed lower percentages of T<sub>reg</sub> cells, as well as CD4<sup>+</sup> and CD8<sup>+</sup> T cells in peripheral blood counts [40].

### Findings from studies addressing potential perspectives in combined therapy models

**Fecal microbiota transplantation.** One very promising way to directly modulate the gastrointestinal microbiota is represented by FMT. The major goals for such a regimen are i.) to implement a more favorable microbiota composition, ii.) to enhance host immune function and in consequence, iii.) to achieve an improved treatment efficacy [36, 39, 41]. To date, results from clinical trials analyzing the influence of FMT on cancer treatments are scarce. Nevertheless, several studies provided evidence for the impact of gut microbiota modifications on immunotherapy in defined murine cancer models.

After the reconstitution of germ-free mice with *F. prausnitzii*, *E. hirae* or *A. muciniphila* a significant reduction of tumor growth, improvement in response to anti-PD-L1 therapy, higher density of CD8<sup>+</sup> and CD45<sup>+</sup> T cells, up-regulation of PD-L1 in the tumor microenvironment and decrease of suppressive myeloid cells in mice receiving FMT from responding donors were observed [32, 36].

Immune checkpoint inhibitors have shown therapeutic efficacy in patients with acute myeloid leukemia and furthermore, in patients with solid tumor malignancies [36, 38]. A clinical trial for metastatic melanoma patients examined safety, feasibility, and potential positive effects of FMT in patients with refractory anti-PD-1 treatment [41]. The trial was based on two FMT donors, who had been treated with anti-PD-1 therapy for metastatic melanoma before and showed complete response for minimum of a year. After initial microbiota depletion upon a 3-day-course of broad-spectrum antibiotic treatment, eligible FMT



recipients, who had been diagnosed with metastatic melanoma and had been treated with anti-PD-1 antibodies beforehand, received FMT followed by re-induction of the anti-PD-1 therapy. Overall, the recipients' post-treatment microbiota composition showed an increased abundance of the immunotherapy-favorable *Veillonellaceae* family, *Ruminococcus bromii* and *B. adolescentis*, and less abundant *Bifidobacterium bifidum*. The results revealed a higher density of CD68<sup>+</sup> cell infiltrating the subepithelial area of the lamina propria from the sigmoid colon in most patients, as well as an increase in intra-tumoral CD8<sup>+</sup> cytotoxic T cell infiltration in metastasis biopsies from the leg or inguinal area, for instance. When compared to non-responders, the post-treatment microbiota of responding patients was characterized by more abundant Enterococcaceae and *Streptococcus australis* and less abundant *Veillonella atypica*. The authors emphasized, however, that no clear correlation between the given changes in microbiota composition and the outcome could be assessed given similar observations in pre-treatment samples. Nevertheless, an up-regulation of antigen presenting cell-related gene sets via the MHC-I and IL-1 signalling cascade was observed in all post-treatment gut samples, as well as of several immune-related gene sets encoding for proteins involved in T cell activation and interferon pathways in tumor samples. The authors concluded that the obtained results provided evidence for the feasibility, efficacy, and safety of FMT during reinduced anti-PD1 therapy in melanoma patients. However, the results from this study did not show the discriminative power and significance levels required for universal statements that could be further translated to clinical practices.

**Nutrition.** The microbiota can be modulated by dietary changes, such as fiber supplementation or polysaccharide inulin probiotics. One study addressing treatment of colorectal cancer patients with probiotics containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* demonstrated an increase of butyrate-producing bacteria such as *Faecalibacterium* spp. and Clostridiales within the tumor as well as its gastrointestinal environment [42]. Another clinical trial revealed changes in expression levels of proinflammatory mediators including IL-23A in patients who had received preoperative probiotic therapy [43]. However, results were rather conflicting since both, pro- and anti-inflammatory cytokines were affected. Nonetheless, gut microbiota changes following application of defined probiotic compounds may exert inflammation-alleviating and health-beneficial effects in defined gastrointestinal morbidities [22].

**Impact of antibiotic use.** Several research studies have investigated the antibiotic use during immunotherapy of cancers and its consequences on the patients' outcomes. Overall, the studies revealed a negative correlation of antibiotic application and clinical effects of immune checkpoint inhibitors in cancer treatment resulting in worse clinical responses and shorter progression-free survival periods [36, 38, 39, 44]. Nonetheless, there were conflicting results given

that other studies showing no obvious impact of antibiotic use on immunotherapy efficacy [38].

## DISCUSSION

### Main findings of the literature survey

Several of the here reviewed studies suggest a significant impact of the gut microbiota in immunotherapy efficacy given enhanced anti-tumor responses in cancer patients upon gut microbiota modification. Various studies comparing treatment responding and non-responding patients revealed distinct microbiota signatures with differences between rather favorable and unfavorable microbiota compositions. However, so far there is no consistent association between specific bacterial taxa and the clinical outcome following immunotherapy. Due to lack of concordance and many yet to be defined influential factors this question of research awaits further investigations.

Overall, high gut microbial diversity, stability and resilience proved to be beneficial factors for immunotherapy treatment efficacy and clinical outcomes. Antibiotic pre-treatment, gastrointestinal and systemic inflammatory conditions characterized by high numbers of pro-inflammatory innate immune cell subsets including neutrophils, macrophages and monocytes, overgrowth with Gram-negative bacterial species and subsequently high LPS concentrations, however, were identified as rather detrimental factors for the clinical outcome characterized by a higher risk for development of metastases and shortened progression-free survival. Notably, anti-PD-1 therapy did not result in pronounced gut microbiota shifts during treatment, as opposed to anti-CTLA-4 therapy or chemotherapy. Furthermore, bacterial taxa belonging to the Actinobacteria phylum including the Firmicutes, *Bifidobacterium*, and *Lachnospiraceae* were associated with treatment response, whereas conversely, Proteobacteria, Bacteroidetes, Prevotellaceae and Fusobacteria were more abundant in the microbiota of non-responding patients, which is in line with results from meta-analyses [31, 45, 46].

### Obstacles and challenges in immune and microbiome studies

Recent research over the last decade provides the basis for a better understanding of the complex and orchestrated interplay between the microbiota and the immune system [6]. The disentangled balance of health and disease relying on these key players is influenced by various genetic, epigenetic, and environmental factors. Due to differences in experimental protocols including analytic approaches and patients' characteristics, direct comparisons of the data sets derived from different studies are difficult if not impossible. Furthermore, it is not yet clear at what time point during (or maybe before) therapy the distinct gut microbiota composition have the biggest impact on the disease course and its outcome. The increasing variability of human microbiota composition and immune responses, rather due to inter-

individuality as sickness, present experimental difficulties but also a wide range of opportunities for personalized microbiota-targeted treatments. Furthermore, most studies in this field of research have been performed in laboratory mice that harbor a species-dependent commensal microbiota and are equipped with a differently stimulated immune repertoire when compared to humans. New approaches with wild and wildling mice might offer a more natural microbiota composition in line with a robust and naturally trained immune system, though not substituting additional research trials in humans in order to provide a more detailed understanding of the host microbiota-immune interactions.

### Limitations of the literature survey

Since PubMed was considered as most important online database for our literature search, we might have missed studies not published within this data source. Our initial search revealed a broad scope of microbiota studies in general; however, only a limited number of studies could be found addressing our research question raised. Therefore, no further search limitations were applied, such as age of participants in trials, sex, geographical location, diet, state of illness or cancer type, although all these factors contribute to individual microbiota constitution. However, these variables were not considered in most of the conducted studies. This consequently leads to weakened precision and specialization of the resulting statements for patients' future treatments also in conclusion of respective papers. The majority of the studies conducted so far have surveyed the microbiota of patient populations from high-income countries, leading to a bias in variation of socio-geographic factors. Another aspect calling for further research is the limited number of clinical trials with patients and relatively small sample and patient sizes enrolled in these trials, making significant prediction of therapy responses impossible. Moreover, only two studies regarding anti-CTLA-4 treatments could be identified, leaving most of the papers focusing on anti-PD1/PD-L1 immunotherapy.

Additionally, most literature address the impact of the gut microbiota on immunotherapy and potential beneficial effects on the treatment outcome. Only little focus is directed, however, towards the opposite question, if and how the immunotherapy impacts the patients' microbiota and its functional consequences. Given these limitations, it was virtually impossible to address our initial question focusing on the bidirectional relationship of immunotherapy and microbiota.

Nevertheless, we aimed to give an outline of the recent status of knowledge in this area and possible future aspects of health improving opportunities in microbiota-targeted therapeutic strategies in immune-mediated diseases, by naming several relevant bacterial taxa with potential influence and addressing a very recent and prospectively important topic. One needs to take into consideration, however, that research mistakes cannot be ruled out given that data collection and analyses were performed by a single investigator and relevant publications might have been missed.

## CONCLUSIONS AND PERSPECTIVES

The manipulation of the human gut microbiota presents a new potential mediator in cancer therapy response and immune surveillance, with promising results in the trials conducted so far. Still, there are many aspects to learn about and investigate further, such as the exact favorable microbiota composition, significant value in therapy response prediction, and machine learning models in trials.

This leaves future potential for more specialization and individual approaches in targeted therapy models, such as the clinical evaluation of the patients' individual gastrointestinal microbiota status, FMT, diet, probiotics, patient microbiota editing, modulation of bioactive metabolites, or the use as potential biomarkers. A successful improvement of understanding and intervention requires more standardized and unbiased preclinical as well as clinical studies, to ensure adequate reliability and significant results of treatment efficacy in the bidirectional relationship of human gut microbiota and immunotherapy in cancer patients.

*Ethics statement:* Not applicable (literature survey).

*Conflict of interests:* SB and MMH are Editorial Board members.

*Authors' contributions:* SZ conceived and designed the survey, wrote the paper. SB provided critical advice in design of the survey, edited the paper. MMH supervised the survey, co-wrote the paper.

## LIST OF ABBREVIATIONS

CTLA-4	Cytotoxic T-lymphocyte-associated Protein-4
FMT	fecal microbiota transplantation
IL	interleukin
JAX	Jackson Laboratory
LPS	lipopolysaccharide
MHC	major histocompatibility complex
NF- $\kappa$ B	nuclear factor kappa B
NK	natural killer
NLR	neutrophil-to-lymphocyte ratio
NSCLC	non-small-cell lung cancer
PD-L1	Programmed Death-Ligand 1
SCFA	short-chain fatty acid
spp.	species
Th	T helper
TAC	Taconic Farms
T <sub>reg</sub>	regulatory T cell

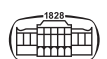
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