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Gut microbiota-drug interactions in cancer pharmacotherapies: implications for efficacy and adverse effects

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Abstract

Introduction: The gut microbiota is involved in host physiology and health. Reciprocal microbiota-drug interactions are increasingly recognized as underlying some individual differences in therapy response and adverse events. Cancer pharmacotherapies are characterized by a high degree of interpatient variability in efficacy and side effect profile and recently, the microbiota has emerged as a factor that may underlie these differences.

Areas covered: The effects of cancer pharmacotherapy on microbiota composition and function are reviewed with consideration of the relationship between baseline microbiota composition, microbiota modification, antibiotics exposure and cancer therapy efficacy. We assess the evidence implicating the microbiota in cancer therapy-related adverse events including impaired gut function, cognition and pain perception. Finally, potential mechanisms underlying microbiota-cancer drug interactions are described, including direct microbial metabolism, and microbial modulation of liver metabolism and immune function. This review focused on preclinical and clinical studies conducted in the last 5 years.

Expert opinion: Preclinical and clinical research supports a role for baseline microbiota in cancer therapy efficacy, with emerging evidence that the microbiota modification may assist in side effect management. Future efforts should focus on exploiting this knowledge towards the development of microbiota-targeted therapies. Finally, a focus on specific drug-microbiota-cancer interactions is warranted.

Keywords: cancer therapy, chemotherapy, gut microbiome, gut-brain axis, immunotherapy, pharmacomicrobiomics, tyrosine kinase inhibitors

Article highlights:

- Gut microbiota-drug interactions are involved in cancer therapy efficacy and related adverse events.
- Chemotherapy may transiently shift microbiota composition and short-chain fatty acid production.
- Baseline microbiota composition and antibiotic exposure determine immunotherapy efficacy, and may also contribute to chemotherapy efficacy.
- Supplementation with particular *Bifidobacterium* strains and *Akkermansia muciniphila* augment immunotherapy efficacy in preclinical models.
- The gut microbiota is associated with cancer therapy-associated infection risk, and gastrointestinal and neurological adverse events.
- Cancer therapies may interact with the gut microbiota through direct microbial metabolism, or indirectly through microbial effects on liver metabolism and the immune system.

1. Introduction

The human gastrointestinal tract is populated by a complex community of bacteria, archaea and eukarya, collectively known as the gut microbiota. These microbes have co-evolved with host species throughout evolution to produce an elaborate, symbiotic relationship [1]. The gut microbiota confers a wide range of benefits to the host, including improved energy harvest, strengthening gut integrity and barrier function, protecting against infection, and immune modulation [2]. The influence of the gut microbiota on cancer development is an important consideration with specific bacteria and general compositional alterations reported [3]. Microbial metabolism of the gastrointestinal contents is a key component of the symbiotic relationship between the gut microbiota and host. The substances consumed by the host organism, or released into the gut lumen by the host, provide essential nourishment for the microbes, shaping gut microbiota composition, and the products of microbial enzymes can then interact with host tissues, either locally at the gut epithelium or following absorption [4, 5]. In order to take full advantage of the vast range of substances that make their way into the gastrointestinal tract, a healthy gut microbiota exhibits a substantial enzymatic repertoire [6] capable of impacting the fate and activity of several drugs.

The emerging field of pharmacomicrobiomics investigates reciprocal drug-microbiota interactions, including how the microbiota impacts a drug's pharmacokinetic, pharmacodynamic and toxicity profile [7]. Microbial interactions with drugs occur via multiple routes (summarized in Figure 1), and are thought to influence at least some of the interpatient variability in drug treatment efficacy and side effect profile [8]. Orally-administered therapies that are not readily absorbed in the upper gastrointestinal tract are more likely to induce localized microbial effects in the intestine due to the relatively high local drug concentration in the lumen. These compounds can also undergo direct transformation by bacteria prior to absorption and first-pass liver metabolism. Contrastingly, drugs administered both orally or parenterally may interact with the gut microbiota during excretion if the compound or associated metabolites are excreted via the biliary duct back into the gut lumen. These microbial metabolites may be reabsorbed into host circulation or can have direct effects on host gut physiology [9, 10]. Furthermore, a wide range of drugs are known to affect microbiota composition, although the effects of these compositional changes on host health and function, as well as drug pharmacokinetics and pharmacodynamics, are understudied [7]. These compositional changes may lead to functional differences in the gut microbiota, which could in turn indirectly impact a drug's action and fate by modifying hepatic and gut first-pass metabolism, immune function and the repertoire of metabolites available to host tissues.

Cancer pharmacotherapies – a rapidly growing group of drugs that include traditional cytotoxic chemotherapy, immunotherapy, hormonal therapy and targeted therapies (for a complete updated list see [11] - are associated with a high degree of interpatient variability in efficacy and toxicity. While some of the variability between responders and non-responders to cancer therapy can be explained by tumor heterogeneity and host genetics, emerging evidence supports a role of the gut microbiota in these differences, particularly in the case of cytotoxic chemotherapy and immunotherapy [3, 12]. Furthermore, drug-

microbiota interactions may underlie some of the adverse effects associated with these therapies [13].

In this review, we move beyond a focus on the influence of the gut microbiota on cancer development *per se* to focus attention on the gut microbiota and cancer therapy. We reviewed literature from both preclinical and clinical studies assessing the role of the gut microbiota in cancer therapy efficacy and side effect profile, focusing on studies published in the last 5 years. Extensive searches were conducted both on Pubmed and google scholar between April and July 2021 using relevant search terms ("[onco* OR cancer] AND [chemo* OR immun* OR anti-cancer OR targeted OR "cancer therapy" OR "tyrosine kinase inhibitor"] AND [microbi* OR probiotic OR prebiotic OR bacteria]"), and the reference lists were examined for missing papers from all relevant papers and literature reviews identified in the initial search. Interactions between cancer therapies and the gut microbiota were first observed in colorectal cancer and have since formed the basis for investigations into cancer therapy and associated microbial interactions. Further, mounting evidence implicates the gut microbiota in cancer therapy efficacy and toxicity in cancers outside of the gastrointestinal tract which forms the basis of this review. Here we synthesize the available literature surrounding gut microbiota taxonomic and functional changes in response to cancer therapy, the impact of baseline microbiota composition and antibiotics on cancer therapy response, and the role of the gut microbiota in associated adverse events affecting the gastrointestinal tract, central and peripheral nervous systems and infection. Finally, we present the available evidence addressing the mechanisms by which the gut microbiota and cancer therapies may interact.

2. Cancer therapies can alter gut microbiota composition and metabolite production

The gut microbiota, the ecosystem of microorganisms including bacteria, viruses, archaea and fungi that inhabit the mammalian gastrointestinal tract, are critically involved in many processes essential to host health and function. The gut microbiota modulates gut function through fermentation of dietary fiber [14], synthesis of vitamins [15] and biosynthesis of molecules [16], as well as affecting gut motility and transit and conferring protection against pathogens [17]. Additionally the gut microbiota plays an important role in immune education[18, 19], host metabolism and circadian rhythm control [20, 21]. In line with its important role in host physiology and health, the gut microbiota has been implicated in wide range of disease, including inflammatory and autoimmune [22, 23], cardiometabolic [24] and neuropsychiatric [25, 26] conditions. Microbiota composition is typically analyzed using sequencing marker genes such as the 16S rRNA gene in bacteria or the internal transcribed spacer (ITS) in fungi. These genes, with both conserved and variable regions, allow for phylogenetic description of the gut microbiota, usually at a genus and sometimes a species level but are susceptible to bias [27], give only relative abundance measures [28] and provide no direct functional information. Increasingly, whole metagenome sequencing approaches are being employed, providing both high-resolution taxonomic and functional characterization [29]. Following sequence processing, the diversity of microbiota samples are assessed using measures of alpha diversity, the microbial diversity within a sample, and beta diversity, the microbial diversity between samples, as well as differential abundance analyses for bacterial taxa of interest.

The bulk of research investigating the role of the gut microbiota in cancer therapy response has focused on colorectal cancer, as it is associated with substantial changes in gut microbiota composition [30] and occurs in the region of the gastrointestinal tract with the highest gut microbiota density and diversity [2] . Several bacteria, including *Fusobacterium nucleatum* [31], colibactin-associated *Escherichia coli* [32] and enterotoxigenic *Bacteroides fragilis* [33], have been associated with poorer outcomes in colorectal cancer. The role of these bacterial strains in interpatient variability in therapy response and resistance, as well as the relationship between several drugs not traditionally used for cancer prevention and colorectal cancer risk (such as metformin [34] and aspirin [35, 36]), underscores the importance of the gut microbiota in colorectal cancer prevention and therapy. The interaction between specific gut microbes, therapy response, and colorectal cancer risk and progression has been studied extensively (discussed in recent reviews [30, 37]) and this research has improved our understanding of the prevention and management of colorectal and other gastrointestinal cancers.

In a recent reanalysis of multiple cohorts relative to healthy controls, patients with non-gastrointestinal cancers exhibit altered gut microbiota composition as well as reduced alpha diversity [38]. While these effects may be partially explained by systemic physiological changes associated with cancer, multiple longitudinal studies indicate that the initiation of cancer therapies can have effects both on microbiota composition, and the fecal and serum metabolome in patients with cancer (summarized in Table 1). This is in line with *in vitro* work, which has shown that chemotherapeutic agents and other antimetabolic drugs inhibit the growth of representative human gut bacterial strains, affecting key species related to healthy status and short-chain fatty acid (SCFA) production [39]. SCFAs are key microbial metabolites that have been implicated in gastrointestinal health and function, immune regulation and host metabolism [40], and may reduce carcinogenesis and tumor progression in some cancers [41].

The bulk of available clinical literature focuses on chemotherapeutic agents which induce changes in gut microbiota composition in the short-term, with less consistent evidence of long-term microbiota alterations sustained after treatment completion. Recent work indicates that chemotherapy is associated with reduced fecal SCFA acid content [42, 43], as shown in *in vitro* models [39]. While these studies provide interesting preliminary evidence that chemotherapies transiently alter gut microbiota composition, small sample sizes, sample heterogeneity and difficulties in the interpretation of fecal SCFA data [44] limit the conclusions that can be drawn. Additional longitudinal studies with larger sample sizes investigating the effects of specific drugs on gut microbiota composition and function are needed to determine which chemotherapeutic drugs modify the gut microbiota. Future research should focus on identifying the mechanisms underlying chemotherapy-induced gut microbiota alterations, which likely differ based on route of drug administration (while most chemotherapeutic drugs are administered intravenously an increasing number can be administered orally), specific drug pharmacokinetics, and excretion. Interestingly, a recent study demonstrated that some chemotherapeutic agents may exert antimicrobial effects through prophage induction [45] although further research is required to determine the generalizability of these results.

Only a small number of studies have examined the effects of immunotherapies on gut microbiota composition. Compared to healthy controls, patients treated with immune checkpoint inhibitors (ICIs) exhibit reduced abundance of healthy commensal species [46] as well as fecal SCFA content [47], but the one study that has examined changes in gut microbiota composition in cancer patients before and after ICIs (combined with chemotherapy) reported no significant alterations [46]. These taxonomic differences may be due to the tumor itself rather than the subsequent therapy. Examination of serum metabolomic profile in patients treated with ICIs for non-small cell lung cancer found that acetate and 3-hydroxybutyrate were enriched in responders [48]. Additional studies examining the effects of ICIs alone on gut microbiota composition and function are required to confirm these findings, especially since other immunomodulatory drugs are typically associated with changes in gut microbiota composition [49]. Moreover, programmed cell death protein 1 (PD-1), a key target of ICIs, is known to play a role in host immune regulation of the gut microbiota [50].

Small-molecule inhibitors of indoleamine 2,3-dioxygenase (IDO1) have also been explored in recent clinical trials as an adjunctive immunomodulatory therapy in some cancers. IDO1 can be induced in subsets of antigen-presenting cells promoting immune tolerance to tumor antigens and in preclinical models, IDO1 inhibition promotes an anti-tumor immune response [51]. Further, IDO inhibitors have been shown to accentuate the effects of chemotherapy and ICIs, although results are not consistent across different cancers and clinical trials [52, 53]. Although many questions remain, IDO1 inhibitors are likely to interact with the gut microbiota as they mostly comprise tryptophan mimetics. Several bacterial taxa metabolize tryptophan, altering host tryptophan availability and metabolism, through the expression of tryptophanase and other bacterial enzymes [54]. 1-methyl-tryptophan, one of the first IDO inhibitors assessed for anticancer effects, has been shown to impair the antimicrobial and immunoregulatory effects of IDO1 *in vitro* [55], and may therefore increase the risk of infection. While most IDO1 inhibitors cannot be processed by tryptophanase, some microbes sense these mimetics as an amino acid, inducing tryptophanase expression [56], which many deplete tryptophan available in the gut. Some of these IDO1 inhibitors are metabolized by the gut microbiota; for example, *in vitro* modelling predicted that epacadostat treatment would produce one primary drug metabolite, but three have been detected in patient plasma [57]. Preclinical and human fecal incubation experiments have identified one of these metabolites as microbially dependent [58]. Further work is required to determine how IDO inhibitors may affect gut homeostasis and microbiota composition.

Other cancer therapies, including tyrosine kinase inhibitors and hormone therapies, may also impact microbiota composition. Tyrosine kinase inhibitors are small molecule drugs administered orally with highly variable bioavailability as absorption can be modified by fatty food consumption and drugs that alter gastrointestinal pH, such as proton pump inhibitors [59]. Osimertinib, an epidermal growth factor receptor-tyrosine kinase inhibitor (TKI), has been shown to alter microbiota composition in patients with non-small cell lung cancer [60]. This is in line with preclinical work that has shown multiple TKIs alter microbiota composition in mice [61]. Androgen deprivation therapy for the treatment of prostate cancer may also alter microbiota composition [62] and alter serum levels of 3-formyl indole [63], a microbially-derived metabolite involved in intestinal homeostasis and

mucosal immune reactivity. The effect of hormone-based therapies on the microbiota is not surprising, as the microbiota can modify host neuroendocrine function [64] and gut microbiota composition exhibits subtle sex differences [65]. However, compositional or functional alterations of the gut microbiota has currently not been reported following hormone therapy for the treatment of breast cancer.

Overall, while there is no indication of a common signature of cancer therapies on the gut microbiota, there is evidence that some cancer therapies can shape both microbiota composition and function. Furthermore, there is emerging evidence that cancer is associated with an altered microbiota composition prior to any therapy initiation and further research is required to determine whether this baseline gut microbiota is cancer-promoting or the consequence of altered host physiology. This is important as differences in microbiota composition can impact treatment efficacy and alter the risk of adverse events, discussed in more detail below. These therapy-induced differences in gut microbiota composition are likely through direct interactions between drug compounds or metabolites and bacterial strains, either through compounds exerting specific antimicrobial effects or through bacterial drug metabolism. Additionally, gut microbiota composition and function may be indirectly modulated by cancer therapies via their direct effects on immune signaling. Patients who are exposed to multiple therapies over the course of their experience with cancer may respond differently depending on the order in which they are exposed to different drug classes and the recovery time between trialing different therapies. Furthermore cancer patients, especially older patients with comorbidities and those with advanced and intractable cancers, will often experience polypharmacy [66] which may further complicate the interaction between the gut microbiota and therapy efficacy. Additional research into the effects of specific cancer drugs on patient gut microbiota composition and function are needed to fully evaluate the extent of these effects, the mechanisms underlying them, as well as their consequences for patient health and treatment outcomes.

3. Baseline microbiota composition affects cancer therapy efficacy

One of the greatest concerns for modern cancer research is addressing the high interpatient variability in response to cancer therapies. While host genetics [67] and tumor heterogeneity [68] have been shown to underlie some of the differences between responders and non-responders to various cancer therapies, there is substantial variance that remains unexplained. Differences in microbiota composition have been identified as an important predictor of patients' response to therapy, particularly in the case of immunotherapy.

3.1. Immunotherapy

Multiple recent systematic reviews indicate that gut microbiota composition and function differs between responders and non-responders to ICIs. A systematic review of studies assessing 16S microbiota composition concluded that while no specific commensal bacterium was associated with ICI efficacy, an intact microbiota with high levels of bacterial diversity and high ratios of responder-associated bacterial species is associated with better patient outcomes [69]. A recent meta-analysis of metagenomic studies investigating the

role of the microbiota in ICI response revealed that *Faecalibacterium*, *Barnesiella intestinihomonis* and microbial vitamin B metabolism are enriched in patients who responded well to immune checkpoint inhibition [70]. Similarly, a recent study examining the role of the microbiota in combined ICI therapy response found that overall microbiota composition was similar between responders to ICI monotherapy and combined therapies, with metagenomic analyses identifying *Bacteroides stercoris* and *Parabacteroides distasonis* as associated with treatment response in advanced melanoma patients undergoing combined CTLA-4 and PD-1 blockade [71].

These taxonomic differences may be associated with differences in metabolomic profiles as well: patients with non-small cell lung cancer who responded to nivolumab, an anti-PD-1 monoclonal antibody, exhibited increased fecal SCFAs and terpenes compared to non-responders [72] while a study in metastatic melanoma patients revealed high levels of fecal anacardic acid in ICI responders [73], which inhibits cancer cell growth *in vitro* [74] and has been shown to stimulate neutrophils and macrophages [75].

Two recent phase 1 clinical trials investigating whether fecal microbiota transfer (FMT) can confer ICI responsivity have provided preliminary evidence that microbiota compositional differences may underlie observed interpatient differences. The first induced two partial and one complete clinical response to anti-PD-1 therapy by FMT from two anti-PD-1 responding patients into ten patients with anti-PD-1-refractory metastatic melanoma. FMT was associated with increased immune cell infiltration and favorable changes in gene expression in the tumor microenvironment [76]. The second study also examined the effect of adjunctive FMT from responding patients into 15 patients with anti-PD-1-refractory melanoma and provided clinical benefit to six of the patients. Responders to anti-PD-1 following FMT exhibited increased CD8+ T cell activation and fecal abundance of taxa associated with ICI responders [77]. Recent preclinical work has indicated that FMT from both responders and non-responders is able to modulate the tumor microbiota in the case of pancreatic adenocarcinoma which underlies changes in T cell activation and tumor size reduction [78], but additional studies are required to determine if these effects will generalize to other cancers. There has been increasing interest in the tumor microbiota over the last few years as we have developed the capacity to characterize the bacterial strains present: recent studies indicate that these bacteria may also interact with drugs directly at the tumor site and are associated with therapy response [78, 79, 80]. While most of the clinical data available has focused on PD-1 blockade, similar effects been shown for cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibition in mice. Tumors in germ-free and antibiotic-treated mice are resistant to anti-CTLA-4 therapy, but this resistance can be reversed by supplementation with live *Bacteroides fragilis*, immunization with *Bacteroides fragilis* polysaccharides or adoptive transfer of *Bacteroides fragilis*-specific T cells [81].

Furthermore, there is emerging evidence that previous drug exposure effects on gut microbiota composition may also alter ICI response. Derosa and colleagues showed that exposure to TKIs (most frequently sunitinib or axitinib) prior to immunotherapy initiation shifted microbiota composition, enriching *Alistipes senegalensis* and *Akkermansia muciniphila* relative to healthy controls [61], bacterial strains that have previously been associated with anti-PD-1 therapy responders [82]. They then went on to systematically

assess the effects of TKIs (sunitinib, axitinib and cabozantinib) on microbiota composition in mice, and showed similar increases in *Alistipes senegalensis* and *Akkermansia muciniphila* as those observed in clinical samples [61]. Interestingly, mitogen-activated protein kinase inhibition have been shown to induce immunotherapy resistance in both mice and humans via altering the immune profile of the tumor microenvironment [83], which may be related to gut microbiota changes.

In summary, there is substantial evidence for a relationship between microbiota composition and ICI efficacy and assessment of pre-treatment microbiota composition can be used to predict treatment outcomes. Emerging clinical data support preclinical evidence that show differences in microbiota composition underlie interpatient differences in response to ICIs. The importance of baseline microbiota for ICI efficacy is most likely related to unique contributions of bacterial strains to antitumor immune response, although further work is required to demonstrate this relationship in clinical populations. FMT and probiotic approaches may provide an avenue for reversing ICI resistance and could be used as adjunctive measures to improve responding patients' outcomes and reduce treatment times.

3.2. Chemotherapy

Similar to immunotherapy, recent studies indicate that baseline microbiota composition may also relate to patients' response to chemotherapy. In a study examining patients with multiple cancer types receiving either chemotherapy or chemotherapy combined with ICIs, responders exhibited higher levels of alpha diversity and were enriched for several specific bacterial species that reduced tumor size when provided to mice, including *Bacteroides xylanisolvens*, *Bacteroides ovatus*, *Prevotella copri* and multiple *Alistipes* species, while non-responders were enriched with species from the *Firmicutes* phylum [46]. In another small study, chemotherapy response in patients with advanced lung cancer was associated with enriched *Streptococcus mutans* and *Enterococcus casseliflavus*, while non-responders exhibited increased abundance of *Leuconostoc lactis* and *Eubacterium siraeum* [84]. Furthermore, fecal microbiome composition at diagnosis was associated with prognosis of early breast cancer, and the microbiota of patients with aggressive cancer both before and following chemotherapy was enriched with several species previously associated with primary resistance to ICIs [85]. Similarly, differences in baseline microbiota composition has been observed between responders and non-responders to chemoradiotherapy [86, 87], where increased diversity is also associated with increased tumor infiltration and more favorable responding [86].

This preliminary work suggests that gut microbiota composition may also be an important factor in interpatient variability in chemotherapy efficacy. Additional research identifying the specific taxonomic and functional differences that predict treatment response to each chemotherapeutic agent would provide invaluable insight into the potential for microbiota modification as an adjunctive therapy in patients undergoing chemotherapy. Furthermore, these studies may guide selection of chemotherapeutic agents in the future as cancer therapies become increasingly personalized.

3.3. Concomitant drug therapies: effects of antibiotics and other drugs that modify gut microbiota

Infections are one of the most common complications experienced by cancer patients [88]. Subsequently, many cancer patients will receive either oral or intravenous antibiotics as part of their treatment plan, either prophylactically or in response to infections. Substantial clinical research has examined the effects of antibiotics on ICI efficacy in solid tumors. Two recent meta-analyses examining the effect of antibiotic exposure shortly before or during treatment with ICIs on treatment efficacy concluded that antibiotic treatment was associated with decreased overall survival as well as progression-free survival [89, 90]. Of note, while some cancer therapies are associated with prophylactic antibiotic administration, they are also administered in the context of infection, which may also contribute to reduced overall survival. A recent retrospective study examining the impact of antibiotics on ICI efficacy in patients with advanced non-small cell lung cancer showed that negative outcomes were only associated with antibiotics in patients with high levels of PD-1 expression [91]. This suggests that antibiotic exposure may only impair ICI efficacy in patients with cancers sensitive to ICIs, and that limiting antibiotic use may not overcome primary ICI resistance. Since these interactions are not limited to a specific class of antibiotics, it is likely that antibiotic-induced alterations to the microbiota at least partially explain these effects.

Other microbiota-modifying drugs have been associated less consistently with immunotherapy clinical outcomes. Proton pump inhibitors, commonly prescribed for gastroesophageal reflux, are known to decrease microbial diversity and alter microbiota composition [92], and may therefore interact with ICI efficacy. In a large cohort of patients with non-small cell lung cancer receiving anti-PD1 pembrolizumab, proton pump inhibitors were associated with poor ICI performance and reduced overall survival [93]. However, large-scale retrospective analyses of patients with multiple cancers [94] or hepatocellular carcinoma [95] did not detect interactions between proton pump inhibitors and ICI efficacy. Retrospective assessment of other drugs known to modify the microbiota, including nonsteroidal anti-inflammatory drugs, statins, opioids, anti-vitamin K, levothyroxine, vitamin D3, antiarrhythmics, metformin and phloroglucinol, do not appear to interact with ICI efficacy [94].

The relationship between antibiotic use and chemotherapy efficacy is less clear. Antibiotic treatment increases survival time in patients with advanced cancer [96] and tumor-bearing mice [79] undergoing gemcitabine-containing treatment. Conversely, antibiotic treatment has been associated with reduced progression-free survival and overall survival in both hepatocellular carcinoma [97] and esophageal cancers [98]. Trials comparing the effects of antibiotics on immunotherapy and chemotherapy outcomes have shown that antibiotic-induced reductions in overall survival and progression-free survival appear limited to patients undergoing immunotherapy [99, 100].

There has been less focus on the effect of antibiotics on other cancer therapies. One preclinical study showed that tumor endothelial cells were more sensitive to TKIs foretinib, crizotinib and cabozanitinib when derived from mice treated with antibiotics rather than healthy control mice [101], supporting the idea of a tumor microbiota underlying some of

the effects of antibiotic treatment. Further, the antitumor efficacy of a neoantigen cancer vaccine was amplified when mice underwent prolonged antibiotic exposure [102]. Overall, the current literature supports further investigation into the role of antibiotics in cancer therapy efficacy, and additional work is needed to determine whether the effects due to antibiotic exposure are through changes to gut microbiota composition or drug-drug interactions.

3.4. Effect of prebiotics and probiotics

Preclinical work has provided support for several candidate probiotic strains in improving cancer therapy efficacy, and emerging clinical data reinforces the use some of these strains for improving patient outcomes. So far research in the context of cancers outside of the gastrointestinal tract has focused on particular *Bifidobacterium* strains and *Akkermansia muciniphila*, which are enriched in immunotherapy therapy responders and induce favorable anticancer immune responses in preclinical models [103].

In preclinical rodent models specific *Bifidobacterium* strains have been shown to augment the anti-tumor effects of anti-PD-1 immunotherapy or chemotherapy by enhancing immune cell function and tumor infiltration [104, 105, 106]. Of note, there are no clear genetic differences between *Bifidobacterium* strains that work synergistically with cancer therapies (*Bifidobacterium bifidum* K57, K18 and MG31) and those that do not (*Bifidobacterium bifidum* BO6, R71 and CKDB001), and interaction with immunotherapy appears to be due to specific strain effects on host immune priming [104]. Furthermore, a recent study assessing the benefit of probiotic yoghurt supplemented with *Bifidobacterium animalis* alongside TKI therapy in patients with metastatic renal cell carcinoma found no difference in clinical benefit rate between patients consuming probiotics and controls [107]. Additional work determining which *Bifidobacterium* strains augment specific cancer therapies in preclinical models will provide a better basis for translating these early findings into effective clinical practice.

Akkermansia muciniphila, an intestinal microbe that degrades mucin and improves metabolic and immune health in both rodents and humans [108, 109], has recently been identified as enriched in ICI responders in two studies investigating the role of the microbiota in anti-PD1 efficacy [82, 110]. Furthermore, this bacterium may also be enriched in prostate cancer patients on oral androgen receptor axis-targeted therapies [111]. *Akkermansia muciniphila* supplementation increases intestinal concentrations of multiple metabolites including SCFAs and spermidine which have previously been associated with cancer therapy efficacy [112]. One preclinical study has shown that *Akkermansia muciniphila* potentiated the effects of immunotherapy in mice bearing melanoma and colorectal cancer [113]. While the clinical evidence for a causal relationship between *Akkermansia muciniphila* and ICI efficacy is still lacking, the preliminary evidence available supports further investigation into the benefits of this potential, next-generation probiotic, especially in light of its effectiveness in a range of human conditions associated with dysfunctional metabolism and immune signaling.

Of note, there is emerging evidence that untargeted probiotic use may impair ICI efficacy: conventional probiotics were associated with 70% reduced responding to ICIs in a small

group of melanoma patients [114] and in a larger study of melanoma patients commercial probiotics were associated with reduced microbial species diversity, inferior ICI response and reduced survival [115]. It should be noted that these intriguing results have been presented only at conferences and have not at time of writing undergone peer-review. Conversely, a retrospective analysis of patients with advanced non-small cell lung cancer treated with ICIs indicated that probiotic therapy with *Clostridium butyricum* MIYAIRI 588 increased both progression-free survival and overall survival, even in patients who received antibiotics [116]. Further work is required to determine whether there are specific probiotics that can potentiate cancer therapy efficacy.

4. The gut microbiota is associated with cancer therapy side effects

Despite substantial improvements in clinical efficacy and survival rates, most cancer therapies are associated with wide-ranging side effects impacting nearly all organ systems. Similar to treatment efficacy, these adverse effects exhibit dramatic interpatient variability. The most common adverse effects associated with cancer therapies are gastrointestinal symptoms [117], which are often associated with changes in microbiota composition and sometimes directly the result of microbiota-drug interactions. Furthermore, there is emerging evidence that the microbiota may modulate infection risk [118] as well as behavioral and neurological side effects [119, 120] experienced by cancer patients.

4.1. Gastrointestinal adverse effects and toxicities

The gastrointestinal epithelium is a site of substantial cell turnover and proliferation, and therefore commonly experiences off-target side effects of cancer therapies, many of which aim to slow or halt rapid cell proliferation. However, these drug-related adverse effects can be ameliorated or exacerbated by the resident microbiota. Furthermore, some drug-related gastrointestinal adverse effects are due to microbial transformation of drug metabolites during their excretion into the gastrointestinal tract.

Chemotherapy is commonly associated with nausea, gastrointestinal mucositis, and diarrhea. Chemotherapy-induced mucositis dramatically alters microbiota composition and is hypothesized to result from microbial interactions with drug metabolites, bile acid synthesis and barrier function [121]. For example, irinotecan, a common chemotherapeutic used in colon cancer, is limited in use due to associated severe mucositis and diarrhea, which is largely dependent on bacterial β-glucuronidase activity. Other factors that predict irinotecan toxicity include hereditary mutations in the conjugation pathway such as Gilbert's syndrome [122]. In mice, carboplatin-induced intestinal mucositis was found to be causally related to *Prevotella copri* abundance: targeted antibiotic treatment reducing *P.copri* reduced intestinal mucosal injury while *P.copri* supplementation exacerbated the effects of carboplatin treatment [123].

Probiotic treatments have been shown to reduce chemotherapy-induced intestinal damage for some chemotherapeutic agents in preclinical models. A recent systematic review concluded that probiotics, predominantly comprised of single or multiple strains of Lactobacilli and Bifidobacteria, are effective in the treatment of some common radiotherapy- and chemotherapy-induced gastrointestinal symptoms, with most studies

focusing on diarrhea [124]. Recent preclinical work has shown that intestinal mucositis induced by platinum-based chemotherapeutic agents and associated diarrhea can be ameliorated using prebiotics comprised of multiple bacterial strains (including *Clostridium butyricum*, *Bacillus mesentericus*, *Streptococcus faecalis* and multiple Lactobacilli and Bifidobacteria) [125, 126, 127].

Gastrointestinal adverse events are some of the most common side effects associated with immunotherapy and the most frequent cause of emergency visits for patients receiving ICIs [128]. There is emerging evidence that ICI-induced colitis is associated with baseline microbiota: patients with metastatic melanoma treated with ipilimumab were less likely to develop colitis if their baseline microbiota composition was enriched with bacteria belonging to the Bacteroidetes phylum, and the development of colitis was associated with reduced bacterial genetic pathways associated with polyamine transport and vitamin B biosynthesis [129] and pre-immunotherapy antibiotic exposure was associated with greater incidence of treatment-induced colitis in patients with advanced melanoma [130]. Additionally, a recent case-study where ICI-induced colitis was successfully treated by FMT in two patients [131] has provided further support that the microbiota is involved in ICI-induced gastrointestinal adverse events. Furthermore, ICI-induced mucositis in murine models has been reversed by probiotic supplementation with *Bifidobacterium breve* [132], combined *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium lactis* and *Bifidobacterium breve* [133], and *Lactobacillus reuteri* [134]. A recent study examining the role of the microbiota in combination immunotherapy therapy in advanced melanoma patients identified that *Bacteroides intestinalis* and increased intestinal interleukin 1β expression were associated with ICI-induced toxicity, and in a preclinical mouse model of combined immunotherapy treatment-associated subclinical colitis was reduced by both an IL-1 receptor antagonist and antibiotic treatment, and exacerbated by Bacteroides intestinalis supplementation [71]. Trials combining prophylactic use of anti-TNF agents with ICIs are underway both in an attempt to reduce gut toxicity and to increase antitumor effects [135]. Anti-TNF therapies may reduce gut toxicity via effects on the gut microbiota, as they have been shown to modulate microbiota composition in preclinical studies of autoimmune conditions [136].

Common clinical toxicities associated with TKI therapy include nausea and diarrhea [137]. Small studies have shown that microbiota composition is associated with TKI-induced diarrhea in patients with metastatic renal cell carcinoma [138], advanced hepatocellular carcinoma [139], and breast cancer [140]. Furthermore, in the latter study baseline microbiota could predict TKI-induced diarrhea [140] and a recent clinical trial found that FMT from healthy donors successfully treated TKI-induced diarrhea in patients with metastatic renal cell carcinoma with no serious adverse events observed [141].

In conclusion, there is substantial evidence across a range of cancer therapies with gastrointestinal adverse events are associated with an altered gut microbiota, and therefore suggest that these events may be ameliorated through microbiota modification using FMT or supplementation with some probiotic strains. However, recent meta-analyses of clinical trials using probiotics for cancer therapy-induced diarrhea concluded that there is limited low-certainty evidence for probiotics preventing or reducing the incidence and severity of

diarrhea [142, 143], and additional work is required to determine if the promising effects observed in preclinical models can be translated to patient populations.

4.2. Infection risk

Increased risk of infection is a common complication in cancer, particularly in hematological malignancies where chemotherapy regimens are used to ablate cancer cells in the bone marrow leading to immunocompromise. Across multiple small studies in patients with non-Hodgkin lymphoma [144], acute myelogenous leukemia [145] and myeloid lymphoma [146], lower microbial alpha diversity was associated with increased risk of infection both during chemotherapy regimen and following neutrophil recovery. However, the relationship between microbial alpha diversity and risk of infection is unclear, as patients who developed infections during intensive chemotherapy in the latter two studies were treated with carbapenem which further reduced alpha diversity [145, 146]. A recent study examining the effect of autologous FMT in a small group of acute myeloid leukemia patients receiving both chemotherapy and antibiotics showed that FMT restored both microbiota alpha diversity and several microbial communities depleted by therapy and did not induce infectious complications [147]. Overall, preliminary evidence indicates that the gut microbiota may be involved in infection risk following intensive chemotherapy, although microbial diversity may function as a biomarker of antibiotic use in response to therapy-related infections [145, 146]. Further research is required to determine the therapeutic potential of the gut microbiota for infection management in patients undergoing chemotherapy.

4.3. Central and peripheral neurological and behavioral adverse effects and toxicities

A subset of cancer patients undergoing various cancer therapies report behavioral and neurological impairments that reduce their quality of life, including impaired cognition [148], increased incidence of mood and anxiety disorders [149], and increased pain and fatigue [150]. While most of the findings are associated with more traditional cytotoxic chemotherapy regimens in cancer patients and survivors, there is emerging evidence that more novel immunotherapies and targeted cancer therapies may have similar behavioral effects [151, 152]. The emergence of the FACT-ICM questionnaire may help to standardize patient reported outcomes with immunotherapy including fatigue and could be combined with sequential microbiome sequencing [153]. Chemotherapeutic agents are able to interact directly with the central and peripheral nervous systems, reducing cell division of support cells throughout the nervous system and neuronal stem cell populations in the hippocampus which is important in memory and executive function [154]. Furthermore, these agents are known to activate both peripheral and central inflammation, and are associated with reduced neurotransmitter release and loss of dendrites and dendritic spines [155].

There is increasing evidence for microbiota involvement in several behavioral conditions through modification of neuroactive metabolite availability, as well as actions on the vagus nerve and immune system. The microbiota has been linked to altered cognition, anxiety-like behavior, depression-like behavior, pain behavior and fatigue – all behaviors affected by cancer therapies – in numerous models [156]. Several experts in the field have identified the

microbiota as a potential site for intervention in cancer therapy-induced behavioral impairment [119, 157]. A recent systematic review of the human literature concluded that microbiota composition was associated with fatigue, anxiety, depression, sleep quality, cognitive impairment and peripheral neuropathy in patients undergoing chemotherapy [158]. However, this was based on two cross-sectional and one longitudinal study and additional research is required to confirm these associations.

This is in line with the evidence from preclinical animal models where platinum-containing chemotherapy-induced behavioral impairments and peripheral neuropathy are related to microbiota composition and can be modified through interventions targeting the microbiota. Germ-free or antibiotic-treated mice exhibit reduced mechanical hyperalgesia following treatment with oxaliplatin, and this protection can be reversed by FMT from conventional animals [159]. Paclitaxel-induced peripheral neuropathy was related to gut microbiota composition in mice, and paclitaxel sensitivity and resistance could be transferred to naïve mice by FMT [160]. Cognitive impairments induced by paclitaxel in mice have been shown to be related to microbiota composition and colonic crypt depth [161], and some of the paclitaxel-induced behavioral impairments can be mitigated by co-housing with healthy mice [162]. While antibiotic treatment did not prevent paclitaxel-induced behavioral impairments, FMT from paclitaxel-treated mice into naïve mice reduced locomotion in an open field and increased hippocampal cytokine expression [162]. Furthermore, a multi-strain probiotic SLAB51 (containing *Streptococcus thermophilus*, *Bifidobacterium longum*, *B. breve*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, *L delbrueckii* subsp. *bulgaricus* and *L. brevis*) prevented paclitaxel-induced mechanical and cold hypersensitivity and protected against paclitaxel-induced reduction in nerve fibers in mice [126].

Overall, there is strong preliminary evidence that behavioral and neurological impairments associated with platinum-based chemotherapies may be at least partially explained by chemotherapy-induced changes in the gut microbiota (potential mechanisms illustrated in Figure 2). Further research investigating the role of the gut microbiota in neurological and behavioral impairments associated with other cancer therapies is required.

5. Underlying mechanisms – direct and indirect effects of the gut microbiota on cancer therapies

While there is clear evidence that the gut microbiota plays a role in both cancer therapy efficacy and some adverse events, our understanding of the mechanisms by which microbes exert these effects is less well elaborated. Bacteria can exert their effects on drugs that enter the gut directly, by interactions with the drug or its excreted metabolites. Indirectly, the microbiota both modulates hepatic enzymatic activity and produces microbial metabolites that can modify host physiology. Specific to cancer therapies, some of these metabolites are known to modulate immune function.

5.1. Direct microbiota-drug interactions

The microbiota is collectively able to metabolize and utilize a wide range of substrates consumed by the host. This enzymatic capacity also enables the metabolism and

modification of several drugs and associated metabolites that enter the gastrointestinal tract, either because they were administered orally, or excreted via the biliary tract. Seminal *in vitro* experiments have demonstrated that a wide range of human gut microbes are able to chemically modify medicinal drugs, including agents used in cancer treatment [163].

Glucuronidation by uridine diphosphate-glucuronosyl transferase in the liver is a primary pathway by which a number of xenobiotics are excreted into the gut lumen as waste, by increasing their solubility. Once in the gut bacterial β-glucuronidases remove the conjugated glucuronides, often reactivating the substance, although this enzymatic activity can be modified by a range of host and environmental factors [164]. Of note, a number of anti-cancer agents, including axitinib, epirubicin and irinotecan, are predicted to interact with bacterial β-glucuronidases [165]. The adverse gastrointestinal effects observed in irinotecan treatment depend on microbial β-glucuronidases that reactivate the drug in the gut [166, 167], and bacterial-specific β-glucuronidase inhibition which can prevent intestinal toxicity while maintaining antitumor efficacy in mice, dramatically improving survival rates [168]. Of note, irinotecan does not appear to exert any effects on human microbiota composition when studied independently of the host in *ex vivo* biofermenter experiments [169]. Similarly, abiraterone acetate, an inhibitor of androgen biosynthesis used in the treatment of prostate cancer, has been recently shown to be metabolized by the microbiota in *ex vivo* bioreactor experiments [62], although the specific bacterial strains and enzymes involved remains unknown. Furthermore, there is increasing evidence that the bacteria in the tumor microenvironment may mediate therapy response by metabolizing chemotherapeutic agents that reach the tumor [79].

Studies of fluoropyrimidine chemotherapy toxicity in *Caenorhabiditis elegans* models have implicated their food source, *Escherichia coli*, as a primary mechanism by which chemotherapy toxicity can be modified. *Escherichia coli* rapidly evolve bacterial resistance to fluoropyrimidine chemotherapies, and most loss-of-function mutations reduced drug toxicity observed in the cohabiting *C. elegans* [170]. Increasing dietary serine and thymidine enhances fluoropyrimidine toxicity in *C. elegans* by interactions with *E. coli*: thymidine promotes microbial conversion of prodrug into toxic metabolites while serine alters *E. coli* metabolism, reducing nucleotides available to the host [171]. This latter study indicates that diet-microbiota interactions may shift drug pharmacodynamics without direct drug-microbiota interactions, however further work is needed to determine whether similar effects will occur in more complex host-microbiota interactions.

Investigation into the role of direct microbial metabolism of cancer drugs and their related metabolites has focused predominantly on cytotoxic chemotherapy, with limited investigation into tyrosine kinase inhibitors and other orally administered anti-cancer therapies. Further work is required to determine the relative contribution of direct microbial metabolism in the relationship between the gut microbiota and cancer therapy efficacy, and adverse events for a number of anti-cancer drugs. Furthermore, the presence of microbes in the gastrointestinal tract that can transform and detoxify cancer drugs may play a role in shaping the gut microbiota by protecting more sensitive taxa [172], which may subsequently shape host physiology and therapy response.

5.2. Microbiota interactions with liver metabolism

The liver is a key site for drug metabolism: the portal vein drains from the gastrointestinal tract into the liver, ensuring that orally administered drugs absorbed by the gut are appropriately modified for safe circulation around the body. While parenterally administered drugs avoid this first-pass metabolism, the liver can still exert effects on their bioavailability by second-pass metabolism, as a fraction of venous blood travels through the liver via the portal vein. Liver metabolism can be modified by the microbiota in preclinical models, most likely via modulation of the nuclear receptors (there is evidence for microbial interactions with the farnesoid X receptor [173], pregnane X receptor [174], and aryl hydrocarbon receptor [175]) that regulate the expression of a number of drug-metabolizing enzymes and transporters [176]. Germ-free status is associated with a downregulation of genes related to xenobiotic metabolism with corresponding reductions in protein levels of cytochrome P450 3a [177], a major drug-metabolizing enzyme, resulting in increased drug plasma concentrations and half-life [178]. In a more recent study, recolonization of germ-free mice normalized hepatic cytochrome P450 3a11 and multidrug resistance protein 1 expression to conventional levels [179]. Similarly, genetic deletion of the pregnane X receptor also reduced cytochrome P450 family 3 gene expression and dampened hepatic transcriptome changes in response to microbial depletion with antibiotics [174]. Of note, the role of the gut microbiota in liver metabolism is particularly pronounced in male mice, with limited differences observed in females, suggesting that there may be a role of steroid hormones in some of these sex-specific effects [174, 177].

A recent small clinical study in healthy volunteers indicated that the microbiota may also regulate liver metabolism in humans: 7 days of antibiotics reduced the activity of cytochrome P450 1a2, 2c19 and 3a, and reductions in alpha diversity were associated with increased drug and metabolite content in fecal samples [180]. Overall, while these preliminary studies are promising, further work is required to determine whether the compositional changes in the gut microbiota associated with cancer therapy can then lead to changes in liver metabolism, or if microbiota-dependent changes in liver metabolic activity can alter cancer therapy efficacy or toxicity.

5.3. Indirect modulation by microbial metabolites

One of the primary mechanisms by which the gut microbiota interacts with the host is through the production of metabolites, which can act locally on the gut epithelium or at distal sites and organs if absorbed into the blood. These metabolites then exert their effects in the host either as signaling molecules or metabolic substrates, affecting almost every organ system [181]. While a vast range of microbial metabolites may exert effects on host physiology, the bulk of research has focused on the role of SCFAs and tryptophan metabolism in host response to cancer therapies.

SCFAs are uniquely produced by the gut microbiota via the fermentation of fiber and are involved in a number of important physiological processes in the host, including gut epithelial health and function and immune function. Furthermore, they have been shown to have antitumorigenic effects in a number of gastrointestinal cancers [40]. SCFA content has

been shown to be associated with chemotherapy [42] and immunotherapy [48, 182, 183] response in a number of clinical cohorts. In preclinical models, supplementation with butyrate [184] and acetate [185] have been shown to improve chemotherapy outcomes. Conversely, in a recent study in mice treated with CTLA-4 blockade butyrate worsened therapeutic response [186]. These effects may be due to SCFA effects on regulatory T cells, which suppress the antitumor immune response [187]: both butyrate and propionate supplementation increased generation of extrathymic regulatory T cells in healthy mice [188]. Overall, while there are interesting clinical associations between SCFAs and cancer therapy response, there is limited evidence of causality. Additional interventional studies supplementing SCFAs alongside cancer therapies are required to determine whether these metabolites potentiate or dampen the effects of various cancer therapies.

Tryptophan metabolites are another key group of microbially derived metabolites known to exert effects on host physiology, with actions on gut health and function, and the immune system [189, 190, 191]. Patients with cancer exhibit altered tryptophan metabolism, with relatively high levels of kynurenine [192, 193], and tryptophan metabolites have been shown to exert cytostatic effects on cancer cells *in vitro* [194, 195]. In patients with renal cell cancer and with melanoma changes in the kynurenine/tryptophan ratio predict response to ICIs [196]. While the potential of tryptophan metabolites as adjunctive treatments alongside cancer therapies has not yet been examined, the use of IDO inhibitors alongside ICIs has shown some promise, as discussed above. A recent study has shown another immunomodulatory metabolite, inosine, can enhance ICI efficacy in four different murine cancer models by altering T cell expression [197].

5.4. Microbial modulation of the immune response

Finally, the gut microbiota may interact with cancer therapies through effects of specific bacteria on the immune system. The microbiota modulates the bioactivity of immunomodulators [198], and emerging preclinical evidence indicates that this may be through direct effects of specific taxa on subsets of immune cells. For example, both oxaliplatin and cisplatin are less effective in germ-free and antibiotic-treated mice and this appears due to reduced pro-inflammatory response in the tumor microenvironment [199]. Additionally, oxaliplatin efficacy was potentiated through cotreatment with bacterial ghosts – empty cell walls from Gram-negative bacteria – in a murine model of cancer [200]. Further, the anticancer agent cyclophosphamide stimulates an anticancer immune response by inducing translocation of some species of Gram-positive bacteria into secondary lymphoid tissues, stimulating a T helper 17 cell response that is absent in germ-free and antibiotic-treated mice [201]. These findings provide a mechanistic underpinning for the importance of baseline microbiota composition in immunotherapy response: specific microbial taxa may prime specific immune cell populations to improve or worsen the anticancer immune response.

6. Conclusion

As research into the role of the gut microbiota in cancer carcinogenesis and prognosis continues, increasing preclinical and clinical evidence also demonstrates a strong, reproducible association between gut microbiota composition and function, and cancer

therapy efficacy, toxicity and related adverse events, with recent studies focusing on immunotherapy. The data regarding cancer therapy effects on gut microbiota composition so far is sparse and inconsistent between studies. This is most likely due to small sample sizes, lack of longitudinal assessment, and patient, tumor and therapy heterogeneity. Indeed, baseline microbiota composition is an important predictor of patient response to cancer therapies. Furthermore, the gut microbiota has been implicated in therapy-related adverse effects involving the gut, and central and peripheral nervous systems, as well as infection risk in cancer patients undergoing intensive chemotherapy among others. Currently, it is difficult to discriminate between the toxic effects of cancer drugs alone and toxic effects of drug-microbiota interactions: future research should focus on identifying biomarkers that signal when adverse reactions and toxicities may be managed by microbiota-targeted interventions. While assessment of specific cancer therapy-gut microbiota interactions have identified some of the mechanisms by which the gut microbiota can modify cancer therapy efficacy and toxicity, substantial additional research is required to determine the drug-microbiota interactions for each drug class and to determine whether these interactions are modified by the physiological changes evoked by cancer in the host.

7. Expert Opinion

This literature highlights the strong association between the gut microbiota and cancer therapy efficacy and related adverse events. Most of the research to date has focused on chemotherapeutic and immunotherapeutic drugs, although the microbiota has also been implicated in some of the observed effects of tyrosine kinase inhibitors and adjunctive therapies. While the relationship between the gut microbiota, cancer therapy and tumor response has been well-documented in the case of colorectal and other gastrointestinal cancers, the role of the gut microbiota in cancer therapy more generally is increasingly apparent.

The clinical evidence highlights that baseline gut microbiota composition and function is a key predictor of immunotherapy response, and preclinical experiments have shown that modification of the microbiota with antibiotics or probiotics can both worsen or enhance therapy efficacy. While these findings are promising and imply that the gut microbiota may be modified to augment cancer therapies, the challenge for future research is to develop targeted therapeutics to potentiate therapy response and reduce the risk of gut microbiota-related adverse events. While phase I trials of fecal microbiota transfer (FMT) for ICI-refractory cancers have shown some success in increasing therapy efficacy, and systematic reviews have shown FMT is well-tolerated in other conditions [202, 203], some concerns remain regarding safety in immunocompromised cancer patients. Furthermore, these phase I trials have been conducted using fecal material from ICI responders which may be logistically challenging to procure in sufficient quantities for routine therapy. Therefore, probiotic and symbiotic strategies that aim to enrich specific taxa may provide a more practical avenue for intervention. Furthermore, modification to the gut microbiota may have the potential modulate the tumor microbiota which may enhance treatment efficacy. Novel technologies include using probiotics to deliver ICIs to the tumor microenvironment [204, 205] and engineered bacterial strains that excrete compounds to stimulate an anti-tumor immune response [206, 207]. Further work understanding the relationship between

the tumor microbiota and gut microbiota is required to understand how these communities interact.

It is likely that gut microbiota modification will need to be highly personalized to be effective: gut microbiota composition differs across the lifespan and by sex, diet [208], geography [209] among other host and environmental factors [210]. This will require phenotyping the microbiota to a species or strain level, as bacteria within the same genus can have variable effects on a disease process [12]. In fact in the case of cancer therapy, sequencing alone may not be sufficient: Lee and colleagues recently showed that genetic differences do not underlie differences in the effects of Bifidobacterium strains on immunotherapy response [104], suggesting that metabolomics or other analyses may also be necessary. While sequencing and multi-omics costs have reduced dramatically over the last decade, this remains a key issue for implementation in clinical practice and will limit the utility of microbial modifications [211]. Therefore, the success of these potential therapies will depend on further innovation to reduce the cost of microbiota phenotyping and future research should address how to identify patients who would benefit from specific microbiota modifications quickly and cost-effectively.

Finally, while substantial work has been performed investigating the role of the gut microbiota in cancer therapy efficacy and related adverse events, this field is still in its infancy. So far research has focused on the effects of whole drug classes across multiple cancers. For example, studies to date have examined the interactions between any cytotoxic chemotherapy and the gut microbiota which has yielded inconsistent results. This is unsurprising as cytotoxic chemotherapy comprises several different drug classes or drug combinations with unique pharmacokinetics and pharmacodynamics that are likely to exhibit unique interactions with the gut microbiota. Increasingly patients with lung and upper gastrointestinal cancers are treated with first line combinations of cytotoxic chemotherapy and ICIs and little is known about the microbiome in this setting [212]. Additionally, most cancer pharmacotherapies are used in combination with radiotherapy and surgery which also alter gut microbiota composition and function and it is likely that some differences result from treatment interactions. The heterogeneity of cancers adds another layer of complexity: since the relative contribution of a drug's mechanisms of action are unique for different cancers, it is likely that the relative contribution of the gut microbiota to cancer therapy efficacy will also depend on the specific cancer investigated. Comprehensive assessment of the role of the gut microbiota in the actions of different drugs and drug combinations are essential to advance this field of study.

Taken together with research streams that emphasize the influence of gut microbiota on cancer development and strategies to therapeutically target the gut microbiota of patient with cancer, understanding the complex interactions between the gut microbiota and cancer therapy is an important avenue of research to fully deliver on the promise inherent in this field. It is likely that longitudinal analyses will be needed to fully understand the contribution of the gut microbiome to the variability in cancer development, progression and response to treatment. Additional clinical potential exists in the development of microbiota-directed interventions to support the efficacy or limit the side effects associated with current cancer treatment options, and several trials are underway examining the potential of various probiotics as adjunctive therapies [213]. However, many unanswered

questions remain and a key challenge to delivering on this potential is a superior understanding in granular detail of the mechanisms underpinning these observations and the identity of the key microorganism(s) involved. Translational efforts connecting basic and clinical studies will be essential to fill in these knowledge gaps and expedite the emergence of the gut microbiome as a precision medicine tool to support the optimal clinical management of cancer patients.

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Table 1: Selected clinical studies assessing the associations between gut microbiota and drug-based cancer therapies in cancers outside of the gastrointestinal tract

Study	Malignancy	Therapy	Sample size	Design, Duration	Microbiota methods	Effect of therapy on microbiota
Chemotherapy						
El Alam et al., 2021[214]	Cervical, vaginal or vulvar cancer	Chemoradiotherapy	58	Longitudinal (baseline, at 1, 3 and 5 weeks of therapy, and at 12 weeks (follow-up))	16S rRNA sequencing	Therapy decreased both alpha and beta diversity throughout, which returned to baseline levels at follow-up. Therapy increased *Proteobacteria* and reduced *Clostridiales* with post-treatment increases in *Bacteroides* species.
Papanicolas et al., 2021[215]	Non-haematological malignancies	Multiple chemotherapeutic agents	19	Longitudinal (baseline, 7-12 days following chemotherapy initiation and at the end of the first cycle)	16S rRNA sequencing	Therapy increased alpha diversity 7-12 days after treatment initiation. Therapy increased the variance in microbiome composition observed over time relative to healthy controls.
Zidi et al., 2021[42]	Breast cancer	5-fluorouracil-epiribucine-cyclophosphamide	8	Longitudinal (baseline, and at the end of the second and third cycles)	Untargeted metabolomics	In patients that responded to therapy, chemotherapy increased some amino acids which was not observed in non-responders. In non-responders treatment reduced butyrate levels.
Hueso et al., 2020[43]	Acute myeloid leukemia	Induction chemotherapy (7 days cytarabine and 3 days idarubicin or daunoribucin)	15	Longitudinal (baseline, during aplasia and after haematological recovery)	16S rRNA sequencing	Treatment reduced alpha diversity and beta diversity with no recovery over time. Increased *Enterococcaceae* abundance. Reduced faecal SCFA concentrations following treatment All patients were receiving antibiotics alongside chemotherapy
Patrizz et al., 2020[216]	Glioma	Temozolomide	53	Longitudinal (baseline, following chemoradiation)	16s rRNA sequencing	No therapy effects on alpha or beta diversity. *Verrucomicrobia*, *Akkermansiaceae* and *Akkermansia* abundance trend to decrease following treatment. No effect of *Akkermansia* abundance on progression-free survival
Terrisse et	Breast cancer	Multiple	76	Longitudinal	Metagenomics	Adjuvant chemotherapy modified beta diversity of faecal

al., 2021[85]		chemotherapeutic agents	patients (46 for longitudinal)	(baseline, following chemotherapy)		composition, and increased health-related strains, and reduced some strains associated with poor prognosis. No effect on *Akkermansia muciniphila*. The functional pathways influenced by chemotherapy included increased L-ornithine biosynthesis, glycolytic intermediates, L-glutamate degradation, lipid biosynthesis and ketogenesis.
Tong et al., 2020[217]	Ovarian cancer	Carboplatin and paclitaxel ; cisplatin and paclitaxel	18	Longitudinal (baseline, post-operative and samples after the first to fifth cycle of chemotherapy)	16S rRNA sequencing	Therapy altered gut microbiota composition, reducing abundance of *Enterobacteriaceae*, *Klebsiella* and *Enterobacter* and increasing abundance of *Bacteroides*, *Bilophila*, *Collinsella*, *Faecalibacterium* and *Coprococcus*. Abundance of *Bifidobacterium*, *Akkermansia*, *Desulfovibrio*, *Enterococcus* and *Dorea* were significantly associated with lymph node metastasis
Zwielehner et al., 2011[218]	Multiple cancers	Multiple chemotherapeutic agents	17	Longitudinal (baseline and 5-9 days following chemotherapy initiation)	Targeted PCR and metagenomics	Therapy reduced alpha diversity as well as diversity of *Clostridium* clusters IV and XIVa compared to healthy controls. *C. difficile* colonization was observed in a subset of patients receiving both chemotherapy and antibiotic treatment.
Montassier et al., 2014[219]	Non-Hodgkin's lymphoma	Bone marrow transplant conditioning chemotherapy (high-dose carmustine, etoposide, aracytin and melphalan)	8	Longitudinal (baseline and 7 days following chemotherapy initiation)	16S rRNA sequencing	Therapy reduced alpha diversity and significantly altered microbiota composition. Therapy was associated with reduced *Faecalibacterium* and increased *Escherichia*. All but 1 patient were on prophylactic antibiotics for the duration.
Other therapies						
Chi et al., 2020[63]	Prostate cancer	Androgen deprivation therapy	20	Longitudinal (baseline, and 3 and 6 months after treatment initiation)	Untargeted metabolomics on serum	Therapy reduced 3-hydroxybutyric acid and 3-formyl indole.
Cong et al., 2020[60]	Non-small cell lung cancer	Targeted Therapy: Osimertinib (epidermal growth factor receptor-	8; 21 healthy controls	Longitudinal (baseline, and then approximately	16S rRNA sequencing	Therapy enriched *Sutterella*, *Peptoniphilus* and *Anaeroglobus* and depleted *Clostridium* XIVa. No significant differences in alpha diversity or microbiota composition between patients and healthy controls; no

		tyrosine kinase inhibitor)		every 6 weeks over 1 year)		difference in microbiota composition in patients across 1 year of therapy.
Daisley et al., 2020[62]	Prostate cancer	Androgen deprivation therapy with Abiraterone acetate	68	Cross-sectional	16s rRNA sequencing and PICRUSt	Androgen deprivation therapy depletes Corynebacterium species. Oral abiraterone acetate enriches *Akkermansia muciniphila*. Abiraterone acetate is associated with an increased in inferred bacterial biosynthesis of vitamin K2.
Hesshiki et al., 2020[46]	Multiple cancers	Chemotherapy or combined chemotherapy and immunotherapy	26	Longitudinal (baseline, and after the first and second cycles of chemotherapy)	Shotgun metagenomics	No effect of treatment on alpha or beta diversity. No differentially abundant taxa, functional pathways or modules. Significant differences between cancer patients and healthy individuals from the Human Microbiome Project.
Vernocchi et al., 2020[47]	Non-small cell lung cancer	Anti-PD1	11 (4 non-responders); 8 healthy controls	Cross-sectional	Metagenomics and untargeted metabolomics	SCFAs were enriched in healthy controls. Commensal bacteria including *Akkermansia muciniphila*, *Rikenellaceae*, *Bacteroides*, *Peptostreptococcaceae*, *Mogibacteriaceae* and *Clostridiaceae* were more abundant in healthy controls than cancer patients.

Figure legends:

Figure 1. Microbial contributions to cancer drug metabolism and therapeutic response.

Following oral administration, a drug's pharmacokinetics will determine the potential for direct drug-microbiota interactions. Drugs that are absorbed rapidly by the gastrointestinal tract will undergo first-pass metabolism by the gut epithelium and liver before entering the systemic circulation, while poorly-absorbed drugs may undergo substantial microbial modification prior to absorption and host metabolism. Oral drugs that reach systemic circulation, as well as drugs administered intravenously, can experience second-pass hepatic metabolism where drugs or related metabolites undergo conjugation and subsequently reach the gut lumen via biliary excretion. In the gut lumen, microbes can inactivate or reactivate drugs and their related metabolites, or produce toxic compounds that lead to drug-related adverse events. Additionally, drugs and their metabolites can exert effects of gut microbiota composition and function, enriching or depleting specific taxa and altering community dynamics. This leads to altered host metabolite availability, impacting both hepatic function and host physiology more generally, and can regulate immune function. Overall, diverse microbiota composition is typically associated with cancer therapy responding, while lower diversity and antibiotics use is associated with poor therapy response.

Figure 2. The microbiota-gut-brain axis, behaviour and chemotherapy.

The microbiota-gut-brain axis comprises a complex communication system between the gut and nervous system that regulates brain health and function, and has been implicated in some of the effects of chemotherapy on the brain. Platinum-based and other chemotherapeutic agents are known to induce neurological adverse events, associated with reduced dendritic complexity and neurogenesis in the brain, and increased inflammation and reduced neurotransmitter content across both central and peripheral nervous systems (grey boxes) associated with changes in attention, learning and memory, and feelings of fatigue and malaise. Additionally, chemotherapeutic agents induce nausea, vomiting and diarrhoea and are associated in mucositis in the gastrointestinal tract (green boxes). The gut microbiota metabolises a wide range of substrates in the gut lumen into neuroactive and immunomodulatory metabolites, including neurotransmitters, short-chain fatty acids (SCFA) and tryptophan metabolites that then exert effects throughout the nervous system and may modulate the effects of chemotherapy. Additionally, there is emerging evidence that chemotherapeutic agents act on gut microbiota composition to alter metabolite production that may also impact host physiology. Chemotherapeutic agents may exert their effects on the nervous system through known interactions with key aspects of the microbiota-gut-brain axis.