Gut microbiota modulation of chemotherapy efficacy and toxicity

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Abstract

Evidence is growing that the gut microbiota modulates the host response to chemotherapeutic drugs, with three main clinical outcomes: facilitation of drug

efficacy; abrogation & compromise of anti-cancer effects, and mediation of toxicity. The implication is that gut microbiota are critical to the development of personalized cancer treatment strategies and, therefore, a greater insight into prokaryotic co-metabolism of chemotherapeutic drugs is now required. This thinking is based on evidence from human, animal and *in vitro* studies and gut bacteria are intimately linked to the pharmacological effects of chemotherapies (5-fluorouracil, cyclophosphamide, irinotecan, oxaliplatin, gemcitabine, methotrexate) and novel targeted immunotherapies such as anti-PD-L1 and anti-CLTA-4. The gut microbiota modulate these agents through key mechanisms, structured as the '**TIMER**' mechanistic framework: namely **T**ranslocation, **I**mmunomodulation, **M**etabolism, **E**nzymatic degradation, and **R**educed diversity and ecological variation The gut microbiota can now, therefore, be targeted to improve efficacy and reduce the toxicity of current chemotherapy agents. In this Review, we outline the implications of pharmacomicrobiomics in cancer therapeutics and define how the microbiota might be modified in clinical practice to improve efficacy and reduce the toxic burden of these compounds.

Key points

- Evidence is increasing that the gut microbiota modulate the actions of chemotherapeutic drugs used in cancer and other diseases
- We propose the 'TIMER' mechanistic framework to explain how gut bacteria influence chemotherapy effects on the host: Translocation, Immunomodulation, Metabolism, Enzymatic degradation and Reduced diversity and ecological variation

- A number of tools for manipulating the gut microbiota in this context, including dietary modifications, probiotics and synthetically engineered bacteria, are in development
- The gut microbiota will be central to the future of personalized cancer treatment strategies

Author biographies

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Introduction

Cancer survival has dramatically increased in the past half century, driven by a deeper understanding of cancer biology, improved surgical outcomes and increasingly efficacious multimodal chemotherapy and radiotherapy regimens [1]. Cytotoxic drugs continue to be the mainstay of medical treatment for most patients with advanced disease, yet they have an unpredictable treatment response and considerable treatment-related morbidity and mortality[2]. The next generation of personalized cancer therapies are now emerging[3], which exploit advances in molecular and phenotypic heterogeneity[4,5], tumour evolution, immunotherapy and vaccination. However, even targeted therapies, which

have revolutionized outcomes in cancers such as malignant melanoma, suffer from novel problems such as acquired resistance, idiosyncratic adverse effects and high costs[6-8].

Systems medicine science provides multiparametric, time-specific insights into complex biological pathogenic states[9]. A major contribution of systems biology to modern medicine has been the rediscovery of the importance of the gut microbiota to almost all aspects of human health[10]. The major focus however of 'oncomicrobiome' research to date has been on the microbiome's role in the aetiology of cancer and cancer risk[11]. This focus is because the gut microbiota co-develops with the host and it sits at the interface of multiple anti-neoplastic and carcinogenic metabolic, immune and inflammatory pathways[12]. However, the gut microbiota also have a major role in defining the efficacy and toxicity of a broad range of drugs[13,14]. Drug metabolism by intestinal microorganisms has been well recognized since the 1960s[15]; for example, microbiota-driven drug metabolism is essential for the activation of certain azo prodrugs such as prontosil and neoprontosil, and can affect drug disposition[16] and toxicity[17]. However, advances in high-throughput sequencing and other '–omics' platforms, have led to the concept of 'pharmacomicrobiomics', and the importance of the gut microbiota for chemotherapeutic drug modulation and drug discovery is now increasingly recognized[18].

Despite this progress, we are still lacking a complete map of microbiome – host–drug interactions in cancer, and biological complexity remains a considerable obstacle to the development of these 'precision' therapies. For

example, the gut microbiota exert both direct and indirect effects on chemotherapy toxicity and efficacy through a large suite of chemical signalling cascades (figure 1); as a result it seems to exert its influence on almost all classes of chemotherapeutic agents and it achieves this on a moving playing field. Not only are the gut microbiota niche-specific and highly personalized, but their ecology is also dynamic. For example, the majority of cancers strike at extremes of age, in which the structural ecology of the gut might be immature or perturbed through exposure to a lifetime of environmental modifiers[19]. Moreover, pathological disease states or surgical and medical therapies might also create a state of dysbiosis, further exaggerating the influence of deleterious bacteria, reducing efficacy and exacerbating the toxicity of chemotherapy. A paradox also exists, as chemotherapeutics might further exacerbate any dysbiotic state rather than correct it, with potentially serious implications for drug toxicity. The gut microbiota must, therefore, have a critical role in the development of precision treatment strategies for cancer and they are increasingly seen as a target for next-generation cancer therapies.

This Review describes the established mechanisms through which chemotherapy treatment efficacy is facilitated and abrogated by the gut microbiota, through what we have described as the 'TIMER' mechanistic framework — Translocation, Immunomodulation, Metabolism, Enzymatic degradation, and Reduced diversity (Table 1). We review evidence that commensals are implicated in oncological treatment-related toxicity and the role of ecological engineering in modulating chemotherapy efficacy. Finally, we discuss the implications of such discoveries on future treatment for patients with

cancer and outline how the gut microbiota could be modulated for developing precision strategies in the treatment of cancer.

[H1] Translocation and immunomodulation

Almost 1 in 10 patients with cancer will require a hospital visit for an infection during chemotherapy[20]. Translocation describes the process through which commensal or pathogenic bacteria pass across the gut barrier into the systemic milieu, where they can contribute to the morbidity of chemotherapy[21]. Although it was first described in the 1960s[22], and risk factors for its presence were established, the microbial and host mechanisms that drive this event are not fully defined.

However, studies have begun to question whether translocation modifies not only the risk of sepsis, but chemotherapeutic efficacy. Cyclophosphamide exerts its anti-neoplastic effects through a variety of immunological pathways[23-25]. Viaud and colleagues conducted a series of experiments in mouse models, investigating the role of small intestinal commensal microbes in cyclophosphamide treatment[26]. Both cyclophosphamide and doxorubicin cause shortening of intestinal villi, focal accumulation of inflammatory cells and discontinuity of the intestinal barrier, with accompanying translocation of commensal bacteria into secondary lymphoid organs in a mouse model. Although there was no reduction in the total bacterial counts in the small intestine at 7 days of treatment with cyclophosphamide, there was a reduction in the abundance of lactobacilli and enterococci. Furthermore, a specific set of Gram-positive bacteria (segmented filamentous bacteria and *Lactobacillus*

johnsonii, *Lactobacillus murinus* and *Enterococcus hirae*) was necessary to mediate cyclophosphamide-driven accumulation of type 17 T helper (T_H17) and type 1 T helper (T_H1)-cell responses, and long-term treatment with antibiotics reduced the capacity of cyclophosphamide to cure P815 mastocytomas. Pre-conditioning with vancomycin, which is an antibiotic active against Gram-positive microorganisms, and to a lesser extent colistin (active against Gram-negative microorganisms), inhibited the anti-cancer effects of cyclophosphamide in mice inoculated with MCA205 tumours. Indeed, a follow-up study from the same group, in addition to validating the primacy of *E. hirae* in restoring cyclophosphamide-induced anti-tumour effects to microbiota-depleted mice, also identified the colonic Gram-negative microorganism *Barnesiella intestinihominis* as an orchestrator of cyclophosphamide effects[27]. Interestingly, some of the actions of *Barnesiella* in this setting, increasing systemic polyfunctional Tc1 and T_H1 cell responses and re-instating intratumoral IFN-γ-producing $\gamma\delta$ tumour-infiltrating lymphocytes were distinct from those mechanisms previously identified for other microbiota.

Also using mouse models, Iida and co-workers provided further evidence that gut bacteria regulate the immune response to chemotherapy[28]. In mice treated with intra-tumoral CpG oligodeoxynucleotides they showed that the microbiota primes tumour-associated innate myeloid cells for inflammatory cytokine production and that antibiotic treatment or germ-free (sterile) status attenuates this response. Moreover, the ability to eradicate tumours and prolong survival in control mice was markedly reduced with antibiotic treatment, whereas germ-free animals failed to respond. For the platinum-salt oxaliplatin, the antibiotic

effect manifested within 2 days of treatment, implying that the effect in this case was due to direct suppression of early cytotoxicity, rather than as a result of later cell death and subsequent immune responses.

The importance of gut microbial facilitation of immune responses in cancer treatment extends to novel targeted immunotherapies, such as synthetic monoclonal antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and anti-programmed cell death ligand 1 (anti-PD-L1). MCA205 sarcoma progression is controlled by CTLA-4-specific antibodies in specific pathogen-free mice, but not in germ-free mice or those treated with broad-spectrum antibiotics[29]. In the latter groups, there was a decrease in activation of splenic effector $CD4^+$ T cells and tumour-infiltrating lymphocytes. Clinical response to anti-PD-L1, which acts on programmed cell death protein ligand 1, is more frequent in patients who show evidence of T-cell response in the tumour microenvironment[30]. This phenomenon was explored by studying melanoma growth in genetically similar mice from different facilities (JAX and TAC), which differ in the composition of their gut bacteria[31]. Tumours grew more aggressively in TAC mice than JAX mice, but differences in tumour growth and immune responses were ablated by co-housing. Transfer of faecal material from JAX to TAC mice delayed tumour growth and enhanced immune responses in the latter; corresponding transfer in the opposite direction had no effect on JAX mice. Furthermore, combination of JAX microbiota and anti-PD-L1 monoclonal antibody improved tumour control more than individual treatment with either in isolation. Furthermore, 16S ribosomal RNA gene sequencing demonstrated that *Bifidobacterium* spp. showed the largest

increase in relative abundance in JAX -fed TAC mice, and also exhibited the strongest association with anti-tumour T-cell responses. Finally, mice treated with a *Bifidobacterium* commercial cocktail markedly improved tumour control, with induction of tumour specific T cells and increased T cells in the tumour microenvironment. This effect was abrogated by depletion of $CD8^+$ T cells and by heat inactivation of bacteria. The absence of bacteria in secondary lymphoid organs suggests that systemic immunological effects occurred independently of bacterial translocation. Instead, the authors concluded that *Bifidobacterium*-derived signals are mediated through dendritic cell activation, as they demonstrated using genome-wide transcriptional profiling and functional *in vitro* experiments.

CTLA-4 blockade-induced colitis, an unwelcome phenomenon resulting from mucosal immune dysregulation, is seen in approximately one-third of patients receiving ipilimumab treatment against metastatic melanoma[7,32]. Dubin and co-workers have demonstrated that over-representation of the *Bacteroidetes* phylum correlates with resistance to this particular form of colitis, and that a lack of genetic pathways involved in polyamine transport and B vitamin synthesis is associated with increased risk of this type of colitis[33]. In line with these findings, a cocktail of Bacteroidales and Burkholderiales ameliorates CTLA-4-blockade-induced subclinical colitis and colon inflammatory scores in antibiotic-treated mice.[29]

Evidence is also emerging that the interface of innate immunity and the microbiota mediates chemotherapy-induced toxicity. Toll-like receptors (TLRs)

are targets for commensal bacteria, and evidence from IBD models suggests this interaction acts to regulate inflammation and aids healing in the healthy colon[34,35]. Frank and colleagues showed that mice depleted in gut microbes after treatment with broad-spectrum antibiotics were more susceptible to methotrexate-induced small intestinal injury, but this effect was rescued by administration of a TLR2 ligand[36]. Further experiments pointed to myeloid-cell-expressed transmembrane p-glycoprotein (p-gp) as critical in this pathway. The findings were validated *in vitro* using human duodenal epithelial tissue. Conversely, knockout of *Tlr4* in mice is associated with improvement in symptoms of toxicity from irinotecan (CPT-11, a topoisomerase I poison), despite modestly elevated levels of Proteobacteria, which express β-glucuronidases and, therefore, might increase reactivation of SN-38 (the active metabolite of irinotecan)[37].

[H1] Microbial enzymatic degradation and metabolism

Gastrointestinal toxicity in the form of mucositis, causing diarrhoea, pain and weight loss, is a common adverse effect of chemotherapy, resulting in morbidity and mortality[38]. Moreover, it often leads to dose-limitation, which reduces the efficacy of anti-cancer treatment. In addition to the multiple host proinflammatory and apoptotic pathways activated by chemotherapy, the gut microbiota are central to the pathogenesis of mucositis[39] and we draw readers' attention to an excellent systematic review on this topic by Touchfeu and colleagues[40]. However, there remains a paucity of effective interventions to prevent gastrointestinal toxicity or mitigate against its symptoms[41].

Irinotecan causes severe diarrhoea in up to 40% of patients, requiring dose reduction and, in many cases, premature termination of the drug[42]. The effect of CPT-11 on the intestinal microbiota composition has been explored in rats[43]. CPT-11 increased caecal *Clostridium* cluster XI and Enterobacteriaceae, both of which are potentially pathogenic. However, subsequent analysis of the beneficial effects of dietary fibres in this context suggests that dysbiotic changes might not be a major cause of CPT-11 toxicity, pointing instead to the importance of microbial production of the short-chain fatty acid (SCFA) butyrate, which correlates with genetic and phenotypic markers of host health[17]. The implication is that functional, rather than compositional, changes of the microbiota are of greater significance.

Irinotecan's active metabolite, SN-38, is converted in the liver to an inactive glucuronide (SN-38G), which is excreted into the gut via the bile[44]. Wallace and colleagues showed that bacterial β-glucuronidases cleave the glucuronide moiety for use as a carbon source, releasing the active SN-38 metabolite into the bowel lumen, generating diarrhoea[45]. Bacterial β-glucuronidases (or possible candidate structures) are found in 43% of species in The Human Microbiome database (http://hmpdacc.org/). Furthermore, the bacterial enzyme contains a 'bacterial loop' not found in the human form of the enzyme, enabling highly selective inhibitors of the bacterial enzyme to be developed, two of which blocked the active site of the *E. coli* β-glucuronidase, but had no effect on bovine liver glucuronidase. The quinolone antibiotic ciprofloxacin has also been shown to inhibit this enzyme, and low doses of amoxapine, a known inhibitor of bacterial β-glucuronidases, suppressed diarrhoea associated with irinotecan in

a rat model[46,47]. An analysis of crystal structures of representative β-glucuronidases from *Streptococcus agalactiae* and *Clostridium perfringens* and the Proteobacteria *Escherichia coli* and the Bacteroidetes *Bacteroides fragilis* has demonstrated that these enzymes have marked differences in catalytic properties and propensities for inhibition, suggesting that the gut microbiome is able to ensure functional diversity in orthologous enzymes. Small changes in the structure of designed inhibitors can induce major conformational changes in the β-glucuronidase active site[48]. An alternative strategy to reduce irinotecan-related gastrointestinal toxicity which, in contrast, requires active biotransformation via bacterial β-glucuronidase to exert its restorative effect on the intestinal epithelium, is provided by a Chinese herbal medicine (PHY906), which achieves this step through regeneration of stem cells and potentiation of Wnt signalling[49].

An interesting microbiome -driven drug–drug interaction, with potentially serious consequences, including death, has also been seen with the antiviral drug sorivudine (1-β-D-arabinofuranosyl-5-(E)-(2-bromovinyl) uracil) an antiviral that was sometimes co-administered with 5-fluorouracil (5-FU). Biotransformation of sorivudine by gut bacterial phosphorolytic enzymes to (E)-5-(2-bromovinyl) uracil (BVU) occurs (with high hydrolytic activity seen with the *Bacteroides* species, *B. vulgatus*, *B. thetaiotaomicron*, *B. fragilis*, *B. uniformis* and *B. eggerthii*). The effect of the microflora was demonstrated by the fact that administration of ampicillin, metronidazole, or a cocktail of bacitracin, neomycin and streptomycin, reduced the exposure of rats to circulating BVU whilst concentrations were increased after administration of the antibiotic kanamycin

(which is selective for aerobes over anaerobes). BVU inactivates hepatic dihydropyrimidine dehydrogenase, an enzyme that is responsible for the detoxication of 5-FU. The coadministration of sorivudine and 5-FU (or prodrugs thereof) in the presence of an active gut microflora capable of producing BVU produces an increase in systemic concentrations of 5-FU, and this combination was associated with the death of 16 patients in Japan as a consequence[50,51].

The enzymatic functions of specific target bacteria also have a key role in modifying the toxic profile of some chemotherapeutics. For example, *Mycoplasma hyorhinis* has been associated with a range of different cancers, including gastric carcinoma[52]. This bacterium encodes a thymidine phosphorylase that markedly restricts the cytostatic activity of pyrimidine nucleoside analogue compounds such as 5-fluoro-2'-deoxyuridine and 5-trifluorothymidine *in vitro*[53]. However, the opposite is true for the capecitabine metabolite 5-fluoro-5'-deoxyuridine, which is more effective in the presence of *M. hyorhinis* infection, as this pro-drug is activated by the same enzyme. For the cancer drug gemcitabine, *Mycoplasma* pyrimidine nucleoside phosphorylase and cytidine deaminase enzymes have deleterious effects on its therapeutic efficacy[54]. This example illustrates a major challenge for the design of more personalized chemotherapeutic strategies based on microbiota modulation, as the same strain has two contrasting influences on two different targets based on the same enzyme.

[H1] Reduced diversity and ecological variation

Our understanding of novel interactions determining the effects of the microbiota on chemotherapeutics must be further developed to account for reciprocal modification of the microbiota by these agents. Data from a study in rats treated with methotrexate showed that animals that developed mucositis also demonstrated a global reduction in microbial abundance, with an absolute and relative decrease of anaerobes (13-fold) and streptococci (296-fold) with relative increase of *Bacteroides*, and that this change in microbiota composition was associated with diarrhoea and reduced villous length[55]. The structure of the human gut microbiota after treatment with chemotherapy is also characterized by reductions in diversity and richness [56,57]. Importantly, attempts are now being made to understand the functional effects of chemotherapy on the gut microbiota. Studying 28 patients with non-Hodgkin lymphoma undergoing a 5-day myeloablative chemotherapy regimen prior to human stem cell transplantation (HSCT), all of whom developed gastrointestinal-mucositis-related symptoms, Montassier and co-workers saw profound alterations in gut microbial community structure and reduction in diversity[58]. Proteobacteria abundance was increased, with levels of Firmicutes and Actinobacteria decreased. Taxa known to diminish inflammation by modulation of the NF-κB pathway and through production of SCFAs were depleted after chemotherapy. Enterobacteriaciae abundance increased following chemotherapy, showing similarities to the changes observed in an active colitis model[59]. Metabolic pathways associated with intestinal inflammation, including cell motility, glycan metabolism and xenobiotic degradation, were negatively correlated with Firmicutes[58].

[H1] 'Pharmacomicrobiomics' and cancer therapy

The concept that bacteria or their products have a therapeutic part to play in cancer is not novel. In 1891, Coley used the toxins from *Streptococcus erysipelas* and *Bacillus prodigiosus* to treat sarcoma[60], and mycobacteria are still used in the treatment of bladder cancer[61]. Bacteria exert their chemotherapeutic effect by competing for nutrients, secreting toxins and eliciting host immune responses. However, we are now entering a new age in which the entire ecology of the gut could be targeted to influence therapeutic efficacy. But, for this vision to be delivered in practice, several critical unmet needs must be overcome (figure 2).

[H2] Phenome trials and systems oncology

Phenome: the sum of all phenotypes expressed by an organism. Phenomics is the study of the interaction of genes and the environment.

The complexity of the gut microbiome means that novel approaches for studying its role in drug efficacy are required. This complexity necessitates a detailed functional map of the vast number of metabolic and immune functions that these bacteria exert on the host, cancer and the drugs used to treat it. Moreover, it is clear that many cancer therapies will not only influence the metabolism of the host, but also that of the gut bacteria themselves, and the unintended consequences of this function remain unknown. This influence is critical as modern chemotherapeutic regimens are commonly multimodal and the potential for unintended harm to be caused to patients through this route is substantial. Longitudinal drug studies that target host–microbiota interactions using a suite of metabonomic, metagenomic and metatranscriptomic

approaches are required to provide meaningful insights into the mechanisms of the microbiome, to identify novel drug targets and to reduce their lead times into clinical use.[62]

[H2] Gut microbiota as a biomarker for outcomes

Personalized biomarkers are urgently required to help clinicians identify dysbiotic states associated with poor or deleterious outcomes from chemotherapy, and to identify microbial targets suitable for modification. This aim is a major challenge, as these biomarkers must account not only for inter-individual variation, but also variation in the expression of bacteria according to tumour stage and phenotype. For example, the mucosal expression of strains of *Fusobacterium nucleatum* is associated with advanced stage and poor outcome in patients with colorectal cancer[63]. The challenge is further exacerbated by system complexity and co-dependent functions of microbial networks and the very large portfolio of enzymatic functions carried by multiple different species, exemplified by the glucuronidase family.

However, evidence does suggest that the gut microbiota could act as a predictor of patient response to treatment and that this approach is feasible. Montassier and colleagues found that a diverse gut microbiota is associated with protection against chemotherapy-related bloodstream infection[64]. Interestingly, decreases in diversity preceded the start of treatment and the authors suggest a microbiota-based predictive risk index model, which might be used to stratify patients at risk of complications prior to treatment. Correspondingly, Galloway-Pena et al. found that microbial diversity at baseline

was markedly lower in patients with acute myeloid leukaemia who developed infectious complications of induction chemotherapy than those who did not[65]. Furthermore, a deleterious prognostic impact of reduced microbial diversity over the course of treatment was sustained – patients in this category were more likely to suffer an infection within 90 days after their neutrophil levels had recovered.

[H1] Optimizing chemotherapy via microbiota

Current tools designed to modify the gut microbiota remain fairly blunt, and almost all have been aimed at reducing the toxicity of chemotherapeutic agents rather than improving their efficacy.

[H2] Dietary interventions

Much work has been done to determine the influence of dietary–microbiota interactions on human health in IBD, obesity and in cancer aetiology[12]. For example, pro-inflammatory diets high in fat and protein exert their carcinogenic effect through upregulated metabolism of choline, branched-chain amino acids and bile salts and a reduction in SCFA metabolism[66]. Although diet (for example, one high in fibre and low in protein and fat) can be an effective method of chemoprevention for colorectal cancer, its role in the modification of chemotherapeutic efficacy remains poorly understood. This lack of knowledge is in part because the analysis of dietary modification of the gut microbiota during cancer remains challenging, not only because of variability in the adverse effects of chemotherapy (such as nausea and appetite suppression), but also because of nutritional interventions (for example, enteral nutrition) and

surgical diversion (stoma formation). However, animal data is emerging, and mice fed a diet high in protein, L-leucine, fish oil, and specific oligosaccharides demonstrated a reduced incidence and severity of *Pseudomonas aeruginosa* translocation during cyclophosphamide-induced immune suppression when compared with an isoenergetic control diet[67].

Nutritional modulation of cancer metabolism is also an emerging therapeutic approach, with fasting[68,69], carbohydrate restriction[70], amino acid restriction (L-asparaginase[71], methionine[72], serine[73], tyrosine and phenylalanine[74]) and ketogenic diets[75] under investigation. Some evidence suggests that fasting might also minimize the adverse effects of some chemotherapies; for example, it has been shown to reduce the vomiting associated with doxorubicin treatment in dogs[76]. Although these approaches have yet to be extensively studied as diet–microbiota–chemotherapy interactions in cancer, data from human obesity and dietary studies clearly shows that the microbiota has a critical role in the modulation of cellular metabolism and the disease phenotype through both direct and indirect (for example SCFA, branched-chain amino acid metabolism and bile acids). It is almost certain, therefore, that the microbiota are modified by diet and that an altered oral intake during cancer treatment, in turn, is a critical mediator of chemotherapy efficacy. Intriguing evidence from experiments in FabplCre;Apc(15lox/+) mice treated with irinotecan support this hypothesis. Animals fasted for 3 days were protected from the adverse effects of irinotecan, although its efficacy was maintained[77]. Although the influence of the microbiota was not studied in this cohort, it is highly likely that it had a major role, and more research is required.

Specific food supplements have also been shown to be modifiers of microbiota-driven chemotherapy toxicity. Ginseng is a popular herbal medicine that is thought to have anti-neoplastic therapeutic potential[78]. The protopanaxadiol group of ginsenosides are metabolised via the enteric microbiota from compound K[79]. *In vitro* studies suggest an enhanced effect of 5-FU against colorectal cancer cell lines in the presence of ginseng compounds[80,81]. Moreover, protopanaxadiol promotes the anti-neoplastic effects of 5-FU in a cell line model, providing further evidence that the microbial metabolism of nutritional constituents modifies the efficacy of chemotherapeutic targets.

Ellagic acid, a product of dietary polyphenols found in certain fruits, walnuts and wines, is metabolised by the gut microbiota to release urolithins[82]. Urolithin A is purported to have anti-proliferative effects in human colon cancer cells, mediating cell cycle arrest[83], and co-treatment *in vitro* with urolithin A potentiates the effects of 5-FU and its pro-drug intermediate 5'DFUR[84]. Polysaccharides from the ink of a squid *Ommastrephes bartrami* also altered the intestinal microbiota composition after cyclophosphamide administration in mice[85]. Specifically, it resulted in the enrichment of bifidobacteria and a reduction in the levels of *Bacteroidetes*, further suggesting that nutritional modulation of the microbiota during cancer therapy might protect against the adverse effects of chemotherapy.

[H2] Probiotics, prebiotics and synbiotics

Good evidence in both animal and human studies support that probiotics, prebiotics and synbiotics have a role in the prevention of mucositis during

chemotherapy, and that they rarely cause sepsis[86]. Bowen and colleagues found that VSL#3, which contains a mixture of *Streptococcus thermophiles*, *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus paracasei*, *L. delbrueckii* subsp. *Bulgaricus*, *L. acidophilus* and *L. plantarum*, reduced diarrhoea and weight loss in irinotecan-treated rats, with associated increased intestinal crypt proliferation and inhibition of apoptosis[87]. *L. fermentum, L. rhamnosus* and *B. lactis* did not protect against intestinal mucositis in mice delivered intraperitoneal 5-FU[88].

L. casei, *L. rhamnosus* and *B. bifidum*, have all demonstrated that they can attenuate chemotherapy associated diarrhoea in a mouse model through the inhibition of TNF, IL-1β and IL-6 mRNA expression[89]. In a randomized study of 150 patients with colorectal cancer receiving 5-FU-based chemotherapy, *L. rhamnosus* GG supplementation also reduced episodes of severe diarrhoea and abdominal discomfort, compared with guar gum fibre[90]. Motoori and co-workers performed a randomized study comparing a synbiotic containing $1x10^8$ living *B. breve* strain *Yakult* and $1x10^8$ *L. casei* strain *Shirota* per gramme with a control substance containing $1x10^9$ *Streptococcus faecalis* during neoadjuvant chemotherapy treatment for patients with oesophageal cancer[91]. Levels of *Bifidobacterium* and *Lactobacillus* species decreased in the control group compared with the treatment group after chemotherapy, and concentrations of SCFAs were higher in the latter. Modest improvements in rates of severe diarrhoea and febrile neutropenia were observed in the treatment group, but the majority of clinical parameters, including response to

chemotherapy, showed no difference between groups. The primary endpoint of the study was not stated.

A small placebo-controlled trial of *B. breve* strain *Yakult* in children undergoing chemotherapy for a variety of malignancies also demonstrated that the treatment group experienced fewer episodes of fever and the frequency of intravenous antibiotic use was reduced compared with controls[92]. This finding implies that there are multiple probiotic targets with applications in this field; however, there is an unmet need for improved methods for treatment stratification as not all studies have demonstrated clinical benefit. For example, a combination of *Enterococcus faecium* M-74 with selenium did not prevent episodes of febrile neutropenia in patients with leukaemia receiving induction or consolidation chemotherapy[93].

The use of prebiotics in conjunction with cytotoxic drugs (5-FU, doxorubicin, vincristine, cyclophosphamide, methotrexate and cytarabine) has been studied in mice[94]. Dietary supplementation with both oligofructose and inulin potentiated the effect of all six drugs, increasing the life span of animals with the intraperitoneal form of a transplantable liver tumour[95]. Continuing this theme, an inulin–doxorubicin conjugate maintains therapeutic response *in vitro* at lower doses than doxorubicin alone[96].

[H2] Antibiotics, ecology and synthetic engineering

Antibiotic therapy is broadly used as a prophylactic approach for preventing sepsis during chemotherapy. However, almost 27% of bacteria causing

infections during chemotherapy in the USA are resistant to standard prophylactic antibiotics[20] and the efficacy of antibiotic prophylaxis in cancer and especially during neutropenia is arguable. Importantly, antibiotic use in these immunocompromised patients is also an unwanted cause of diarrhoea through the potentiation of *Clostridium difficile*[97]. Conversely, to date, no trials have examined whether antibiotics might preferentially improve chemotherapy efficacy, and such approaches have largely been used as a method for experimental modulation of microbiota–host drug interactions[26,29]. However, there are two interesting concepts that arise from the clinical use of antibiotics in cancer. Firstly, as it is possible that given bacteria might transmit antibiotic resistance through horizontal gene transfer[98], it is equally possible that chemotherapeutic resistance might be transmitted in a similar fashion, for example, through the transfer of β-glucuronidase or pyrimidine phosphatase functions. Bacteria modify chemoresistance through a large number of possible pathways, such as through lipopolysaccharide-modulated upregulation TLR4 receptors[99] and, therefore, this finding suggests that the microbiota is a legitimate target for the treatment of patients with metastatic disease. Secondly, it also suggests that there is a role for selective antibiotic use, and that retired compounds can be repurposed for engineering the microbiota. The precedent has been set with amoxapine, a tetracyclic antidepressant, which was re-purposed as a selective β-glucuronidase inhibitor for alleviating chemotoxicity[47].

A different approach for manipulation of the microbiota is to take advantage of ecological engineering, which is the synthetic manipulation of ecology and

ecosystems to deliver a desired change or function. One such drastic method for modification of the ecology of microbiota is faecal microbiota transplantation[100]. This approach has yet to be used for this purpose, although it is probably only a matter of time as data from *C. difficile* studies suggest they have a potentially valuable role[101]. A note of caution arises from disappointing phase II trial results of Seres's stool-derived bacterial spore treatment to prevent recurrent *C. difficile* infection.[102] Moreover, challenges remain in regulation[103], and extra care will be needed in the oncological setting, for which the consequences for cancer survival of re-setting the microbiota during treatment are not known.

Another approach is the engineering of target bacteria to deliver drugs or pro-drugs within target organs and to serve as vectors for gene therapy. The production of the tumour suppressor, bone morphogenetic protein 2 by transgenic bacteria is one such example[104]. Spores of anaerobic bacteria can also be used for this purpose as they will reach oxygen-depleted regions within cancers and will germinate. Din and colleagues demonstrated an elegant example of how synthetic engineering of bacteria can be used to improve the efficacy of chemotherapeutic drugs[105]. They engineered a synchronized lysis circuit based on the quorum sensing feedback loops in *E. coli*, so that the bacteria would lyse at a threshold population density to release genetically encoded cargo. After quorum lysis, a small number of surviving bacteria reseed the growing population, therefore, leading to pulsatile delivery cycles. When the lysis strain, alone or in combination with 5-FU, was orally administered to a syngeneic mouse transplantation model of hepatic colorectal metastases,

tumour activity was markedly reduced and a survival benefit was obtained. This example shows that synthetic engineering now has a major role in the optimization of standard chemotherapeutic approaches in cancer.

[H1] Conclusions

Mechanistic evidence is now available to support the hypothesis that the gut microbiota plays a major part in defining both the efficacy and toxicity of chemotherapeutic agents. Biological complexity remains a major barrier to the full elucidation of the multiple pathways through which host–microbial interactions modulate clinical outcomes; however, systems medicine provides a tangible methodology for mapping these host oncological–microbiota interactions. The implication is that the gut microbiome represents a notable target for making chemotherapy safer and for further improving rates of cancer survival. Clinicians and translational scientists are likely to make a major contribution to this work and will be integral to delivering future trials that are essential if the potential of the gut microbiota in this field is to be realized.

Figure 1. An overview of the TIMER microbiota–host interactions that modulate chemotherapy efficacy and toxicity. Chemotherapeutics can exert their influence through multiple pathways, and these are outlined here. *Translocation*: cyclophosphamide can cause shortening of the intestinal villi and damage to the mucosal barrier, permitting commensals to cross the intestinal barrier and enter secondary lymphoid organs. *Immunomodulatio*n:

intestinal microbiota facilitate a plethora of chemotherapy-induced immune and inflammatory responses. For example, *Lactobacillus*, segmented filamentous bacteria mediate the accumulation of type 17 T helper (T_H17) and type 1 T helper (T_H1)-cell response[26]. Bifidobacteria also modify tumour-specific T-cell induction and increase T cells in the tumour micro-environment in patients treated with anti-PD-L1 immunomodulator. *Metabolism and Enzymatic Degradation*: Direct and indirect bacterial modification of pharmaceuticals may potentiate desirable effects, abrogate efficacy or liberate toxic compounds. The microbiota harbour a large suite of indirect metabolic processes (such as reduction, hydrolysis, dehydroxylation, dealkylation etc.) which may be used for drug metabolism. These secondary metabolites are secreted into the circulation and excreted by the kidney, which may in turn cause toxicity. *E.coli* possess a beta-glucuronidase which cleaves glucuronide from the inactive metabolite of CPT-11 (irinotecan), releasing active metabolite (SN-38) in the gut causing diarrhoea. *Reduced diversity:* Chemotherapy induces changes in the diversity of the mucosal and faecal microbiota through altered biliary excretion and secondary metabolism or associated antibiotic use and dietary modifications. As a result, pathobionts may predominate, leading to deleterious effects such as diarrhoea and colitis. PRR, pattern recognition receptor; TAMC, tumour-associated myeloid cell; TLR, Toll-like receptor; T_{reg} cell, regulatory T cell.

Figure 2. A model for the future analysis and translation of the oncomicrobiome for improved cancer outcomes. The microbiota is highly personalized and niche specific. However, its structure and function is dynamic and it is influenced by all interventions along the modern multimodal treatment journey. The cancer

patient journey is subject to substantial inter-individual and intra-individual variation; for example, not all patients will undergo surgery. However, sampling methodologies can be standardized, and future microbiome studies should seek to sample not only faeces, but tissue and multiple biofluids so that the broad systemic array of microbiome functions can be analysed once stored in biobanks. Metataxonomic (16s ribosomal RNA (rRNA) gene sequencing) and metagenomics (shotgun sequencing) approaches and other '–omics'-based technologies (such as metabonomics and proteomics) are providing notable insights into the mechanisms through which the microbiome modifies chemotherapy toxicity and efficacy. Given that treatment-induced dysbiosis probably affects the efficacy of second and third-line therapies, longitudinal studies will be critical in fully elucidating the role of the microbiome in this field, and microbiota-augmented clinical trials in oncology are now urgently required. There are multiple tools through which the microbiota might be modulated for improved clinical outcomes, which could be deployed at all stages of the treatment journey. However, evidence to support their use is variable, and trials for more controversial interventions such as faecal microbiome transplantation (FMT) are ongoing. However, the microbiota serves as a rich resource for answering many of the unmet clinical needs in oncology and it represents a major new avenue in drug discovery in cancer.

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Table 1 | A summary of the 'TIMER' effects of the gut microbiota on chemotherapy efficacy and toxicity.

Mechanism	Chemotherapy	Bacteria	Effect
Translocation	Cyclophosphamide, Doxorubicin	Gram-positive microorganisms (*Lactobacillus johnsonii*, *L. murinus* and *Enterococcus hirae*)	Commensal bacteria cross the intestinal barrier to enter secondary lymphoid organs [26,27]

Immunomodulation	Cyclophosphamide	*Lactobacillus*, segmented filamentous bacteria	Gram-positive commensals mediate accumulation of T_H17 & T_H1-cell response[26] TAMC = Tumour associate myeloid cell.
	CpG oligodeoxynucleotides,	*Ruminococcus*, *Alistipes*	Priming of TAMC inflammatory responses[28]
	Oxaliplatin		Mediated by TLR4 and reactive oxygen species production by myeloid cells[28].
	CTLA-4 blockade		Decreased activation of splenic effector $CD4^+$ T cells and tumour-infiltrating lymphocytes[29]
	Anti-PD-L1	*Bifidobacterium*	Tumour-specific T-cell induction and increased T cells in tumour micro-environment[31].
	Methotrexate		Gut microbes regulate against chemotherapy induced small bowel injury via TLR2 signalling and drug transporter p-gp[36].
Metabolism	CPT-11 (Irinotecan)		Streptomycin treatment inhibits absorption of CPT-11 and reduces activity of epithelial carboxylesterase.[106]
	Ipilimumab	*Bacteroidetes*	Bacterially mediated B vitamins production and polyamine transport deficiencies associated with increased risk of CTLA-4 blockade-induced colitis[33]
Enzymatic degradation	5-fluor-2'-deoxyuridine and 5-trifluorothymidine Gemcitabine	*Mycoplasma*	Mycoplasma encoded nucleoside phosphorylases restrict cytostatic activity[53,54]
	CPT-11 (Irinotecan)	*Escherichia coli*	Bacterial β-glucuronidase cleaves glucuronide from inactive metabolite, releasing active metabolite (SN-38) in the gut[45]
Reduced diversity and ecological network function	Carmustine, Etoposide, Aracytine and Melphalan combination	Firmicutes, Actinobacteria, Proteobacteria	Chemotherapy associated with reduction in bacteria that limit inflammation and increase in bacteria associated with colitis[58].
	Methotrexate	Anaerobes, streptococci, Bacteroides	Reduced diversity and shifts in relative abundance associated with chemotherap--induced diarrhoea[55]

TAMC, tumour-associated myeloid cell; T_H, T helper; TLR, Toll-like receptor.