OPEN ACCESS

Cancer pharmacomicrobiomics: targeting microbiota to optimise cancer therapy outcomes

Nick Lung-Ngai Ting, Harry Cheuk-Hay Lau, Jun Yu

Institute of Digestive Disease and The Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, CUHK Shenzhen Research Institute, The Chinese University of Hong Kong, Hong Kong SAR, China

Correspondence to
Prof Jun Yu, Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR, China; junyu@cuhk.edu.hk

Received 6 October 2021
Accepted 25 February 2022
Published Online First
11 March 2022

ABSTRACT

Despite the promising advances in novel cancer therapy such as immune checkpoint inhibitors (ICIs), limitations including therapeutic resistance and toxicity remain. In recent years, the relationship between gut microbiota and cancer has been extensively studied. Accumulating evidence reveals the role of microbiota in defining cancer therapeutic efficacy and toxicity. Unlike host genetics, microbiota can be easily modified via multiple strategies, including faecal microbiota transplantation (FMT), probiotics and antibiotics. Preclinical studies have identified the mechanisms on how microbes influence cancer treatment outcomes. Clinical trials have also demonstrated the potential of microbiota modulation in cancer treatments. Herein, we review the mechanistic insights of gut microbial interactions with chemotherapy and ICIs, particularly focusing on the interplay between gut bacteria and the pharmacokinetics (eg, metabolism, enzymatic degradation) or pharmacodynamics (eg, immunomodulation) of cancer treatment. The translational potential of basic findings in clinical settings is then explored, including using microbes as predictive biomarkers and microbial modulation by antibiotics, probiotics, prebiotics, dietary modulations and FMT. We further discuss the current limitations of gut microbiota modulation in patients with cancer and suggest essential directions for future study. In the era of personalised medicine, it is crucial to understand the microbiota and its interactions with cancer. Manipulating the gut microbiota to augment cancer therapeutic responses can provide new insights into cancer treatment.

Key messages

- ⇒ Emerging evidence has shown the critical role of gut microbiota in modulating cancer treatment outcomes especially in chemotherapy and immunotherapy.
- ⇒ The gut microbiota has extremely complex interactions with cancer drugs by means of pharmacokinetics (eg, metabolism, enzymatic degradation) and pharmacodynamics (eg, immunomodulation).
- ⇒ Meanwhile, cancer therapy can alter the microbiota composition, creating bidirectional interactions.
- ⇒ The gut microbiota has the potential to be a predictive biomarker for cancer treatment responses, which can be useful in guiding the selection of appropriate cancer treatments.
- ⇒ Microbiota modulation, including antibiotics, probiotics, prebiotics, dietary modulation and faecal microbiota transplantation, has shown potential in optimising cancer treatment outcomes.
- ⇒ The gut microbiota can be more easily modified compared with host genetics and is expected to play a vital role in next-generation personalised medicine.
- ⇒ Future research should identify a consortium of microbes with remarkable influence on cancer treatments as well as an optimised approach for microbiota modulation.

INTRODUCTION

Human microbiota is a dynamic collection of 40 trillion microbes, with 3000 species encompassing bacteria, fungi and viruses. They mainly inhabit the epithelial surfaces especially the GI tract.[1 2] The gut microbiota orchestrates broad aspects of physiological functions, including nutritional responses and intestinal and immune system homeostasis.[3–5] Dysbiosis, the microbiota compositional shift with disrupted homeostasis, is associated with diseases including GI, neurological and metabolic disorders.[5 6] Specific gut microbes have also been linked to the pathogenesis of cancers, particularly GI malignancies.[7 8] For instance, the *Fusobacterium nucleatum* antigen adhesin A (FadA) promotes colorectal cancer (CRC) via E-cadherin-Wnt-β-catenin signalling; genotoxin colibactin from polyketide synthase (PKS-positive *Escherichia coli* enhances colorectal tumourigenesis; and *Bacteroides fragilis* toxin generates reactive oxygen species (ROS), causing DNA damage.[8–11] While these bacteria have direct effects on tumourigenesis, some microbes promote inflammation or weaken immunosurveillance to indirectly facilitate cancer development. These microbial immunomodulatory activities are referred to as the 'immune-oncology-microbiome axis'.[1]

Beyond pathogenesis, the microbiota also modulates cancer treatment responses. Despite the rapid development in cancer therapy, challenges remain—acquired resistance, adverse effects and heterogeneous treatment outcomes. Pharmacogenomics is at the forefront of scientific research studying the impact of genetic variants on individual pharmacological responses.[12] However, pharmacogenomics cannot fully elucidate the interindividual disparity in drug responses, implying the presence of other contributors.[13] Since the gut microbiota is considered as the second genome in humans, the concept of 'pharmacomicrobiomics' has been proposed to explain the unsolved questions of pharmacogenomics. Pharmacomicrobiomics focuses on the

To cite: Ting NL-N, Lau HC-H, Yu J. *Gut* 2022;**71**:1412–1425.

interplay between gut microbiota and drug response through alterations in pharmacokinetics (ie, modification of drug absorption, distribution, metabolism or elimination) or pharmacodynamics (ie, modification of drug targets or biological pathways leading to varying sensitivity to pharmacological effects).[12] Growing evidence has revealed the intimate relationship between the gut microbiota and anticancer treatments, including chemotherapy,[14] radiotherapy,[15] targeted therapy[16] and immunotherapy.[1] Harnessing the microbiota to optimise cancer treatments has become an alternate avenue for personalised medicine.

Here, we review the pharmacomicrobiomic interactions between the bacterial component of microbiota and chemotherapy or immune checkpoint inhibitors (ICIs). We highlight the mechanistic insights of microbial influences on anticancer agents in terms of pharmacokinetics and pharmacodynamics. The translational potential of these basic findings is also explored. We further discuss the current limitations in cancer pharmacomicrobiomics and suggest feasible future directions.

MICROBIOTA AND CHEMOTHERAPY

Alkylating agents, antimetabolites, topoisomerase inhibitors, mitotic inhibitors and cytotoxic antibiotics are chemotherapies with different mechanisms of action. Their anticancer activities rely on disrupting DNA integrity, enzymes for DNA repair and synthesis. However, chemotherapy also damages normal cells due to its non-specificity.[17] Recent findings have suggested that targeting microbiota could be promising to improve chemotherapeutic efficacy and reduce toxicity (table 1). Generally, commensal microbes interact with chemotherapeutics mainly by modulating drug metabolism (ie, pharmacokinetics) and host immunity (ie, pharmacodynamics).

Table 1 Summary of modulation of chemotherapy efficacy and toxicity by microbiota

Chemotherapy	Involved microbes	Mechanisms	Translational potential
Irinotecan (CPT-11)	► β-glucuronidase-expressing bacteria, especially *Clostridium* clusters XIVa and IV, including *Clostridium*, *Eubacterium* and *Ruminococcus* genera.[23 24]	► Bacterial β-glucuronidase reactivated SN-38G to SN-38 in the gut, inducing significant intestinal toxicity and diarrhoea.[22]	► Probiotics could reduce the activity of β-glucuronidase to decrease incidence of irinotecan-induced diarrhoea.[64]
5-Fluorouracil	► *Escherichia coli*.	► Bacterial vitamins B_6 and B_9, ribonucleotide metabolism, and deoxynucleotide imbalance increased the efficacy of 5-FU.[31]	► N/A.
	► *Fusobacterium nucleatum*.	► *F. nucleatum* activated TLR4/MYD88-dependent pathway to switch CRC cells from apoptosis to autophagy and promote chemoresistance.[47]	► *F. nucleatum* alone had higher accuracy than the AJCC staging in predicting CRC recurrence.[47]
Floxuridine	► *E. coli* and *Comamonas*.	► *E. coli* OP50 increased the efficacy of FUDR; *Comamonas* decreased the efficacy of FUDR.[32]	► N/A.
Camptothecin	► *Comamonas*.	► *Comamonas* increased the efficacy of CPT via metabolism-independent mechanism.[32]	► N/A.
Gemcitabine	► *Mycoplasma hyorhinis* and Gammaproteobacteria.	► Bacterial long isoform cytidine deaminase metabolised gemcitabine into its inactive form.[36]	► Intratumoral LPS, a surrogate marker for Gram-negative bacteria, could be used as a negative predictor of gemcitabine efficacy in PDAC.[50] ► Antibiotic use was associated with improved gemcitabine response in patients with pancreatic cancer.[53–55]
Cyclophosphamide	► *Enterococcus hirae*, *Lactobacillus johnsonii*, *L. murinus* and *Barnesiella intestinihominis*.	► *L. johnsonii* and *E. hirae* translocated into mesenteric lymph nodes and spleen to stimulate Th1 and Th17 immune response on CTX treatment.[39] ► *E. hirae* also increased the intratumoral CD8+/Treg ratio.[40] ► *B. intestinihominis* promoted infiltration of IFN-γ-producing γδT cells in cancer lesions on treatment with CTX.[40]	► *E. hirae*-specific and *B. intestinihominis*-specific Th1 cell responses were correlated with longer PFS in chemotherapy patients.[40] ► Patients receiving anti-Gram-positive antibiotics and cyclophosphamide/cisplatin concurrently had significantly lower PFS and OS.[56–58]
Oxaliplatin	► Unspecified.	► Modulation of MYD88-dependent signalling pathway primed intratumoral myeloid cells for ROS production.[42 43]	► N/A.
	► Immunogenic commensals (non-enterotoxigenic *Bacteroides fragilis* and Erysipelotrichaceae family).	► Epithelial cell apoptosis induced by oxaliplatin plus immunogenic commensals stimulated TFH cells to interact with B cells for IgG2b response and enhanced anticancer effector/memory CD8+ T cells.[44]	
	► Butyrate-producing bacteria.	► Butyrate activated CD8+ T cells via ID2-dependent IL-12 signalling to promote anticancer immune response.[46]	
	► Gram-negative bacteria with LPS component.	► Microbial LPS interacted with TLR4 on macrophages causing hyperalgesia.[48]	
Cisplatin	► Unspecified.	► Modulation of MYD88-dependent signalling pathway primed intratumoral myeloid cells for ROS production.[42 43]	► Patients receiving anti-Gram-positive antibiotics and cyclophosphamide/cisplatin concurrently had significantly lower PFS and OS.[56–58]

AJCC, American Joint Committee on Cancer; CPT-11, irinotecan; CPT, camptothecin; CRC, colorectal cancer; CTX, cyclophosphamide; 5-FU, 5-fluorouracil; FUDR, floxuridine; ID2, inhibitor of DNA binding 2; IFN-γ, interferon-γ; IL, interleukin; LPS, lipopolysaccharides; MYD88, myeloid differentiation primary response 88; N/A, not applicable; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; ROS, reactive oxygen species; SN-38, 7-ethyl-10-hydroxycamptothecin; TFH, follicular T helper; Th, T helper; TLR4, toll-like receptor-4; Treg, T regulatory cells.

Mechanistic overview

Gut bacteria and pharmacokinetics of chemotherapy

Having millions of protein-coding genes, gut bacteria can not only process ingested nutrients but also alter drug pharmacokinetics.[18] Microbes-mediated drug metabolism can be divided into direct or indirect interactions.[18] Microbes can directly convert drugs into active, inactive or even toxic metabolites. Alternatively, this process can be indirectly mediated by microbes-derived metabolites.[18] Here, how bacterial metabolism affects chemotherapeutic outcomes is discussed.

Irinotecan (CPT-11), a topoisomerase I inhibitor, is effective against various cancers and has been the first-line treatment for metastatic CRC.[19 20] Mechanistically, tissue and serum carboxylesterase activates intravenous CPT-11 into metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), which then inhibits topoisomerase I to stop DNA replication and transcription of tumour cells.[19] The major downside of CPT-11 is its dose-limiting side effect of severe diarrhoea occurring in up to 40% of patients.[19] SN-38 is detoxified into SN-38G by liver uridine diphosphate–glucuronosyltransferase before being excreted to the intestine,[21] where bacterial β-glucuronidase could convert SN-38G back to cytotoxic SN-38, causing severe diarrhoea[22] (figure 1A). While *Clostridium* clusters XIVa and IV (including *Clostridium*, *Eubacterium* and *Ruminococcus*) are major producers of β-glucuronidase,[23 24] CPT-11 could increase the abundance of *Clostridium* and Enterobacteriaceae in rat, indicating that CPT-11 itself enhances bacterial β-glucuronidase activity.[25] A logical question to ask here is whether β-glucuronidase could be inhibited to reduce CPT-11 toxicity. Selective β-glucuronidase inhibitors have now been developed to prevent CPT-11-induced microbial alteration and epithelial damage.[26] For example, pyrazolo[4,3-c]quinoline derivatives (TCH-3562) and uronic isofagomine derivatives could reduce CPT-11-induced complications without impairing its efficacy.[27–29] Reducing CPT-11 toxicity could in turn increase patients' tolerance to higher dosage, leading to better therapeutic outcomes.[30] This example illustrates the reciprocal interactions between commensal bacteria and chemotherapy, demonstrating the potential of microbiota modulation in reducing therapeutic toxicity and enhancing efficacy.

Beyond drug toxicity, bacterial metabolism is essential to drug effects. Recent studies using *Caenorhabditis elegans* models have identified bacterial genes mediating chemotherapeutic efficacy especially those involved in ribonucleotide and vitamin B_6 and B_9 metabolism.[31 32] Mechanistically, bacterial ribonucleotide metabolism is required to activate 5-fluorouracil (5-FU) into cytotoxic 5-fluorouridine triphosphate for exhibiting its RNA-damaging effects in *C. elegans*[31 32] (figure 1B). Disruption of bacterial vitamin B_6 and B_9 production, which is linked to ribonucleotide metabolism, diminishes 5-FU efficacy, thus implicating the influence of bacterial metabolites on chemotherapeutics.[31] Notably, the antidiabetic drug metformin could inhibit the bacterial one-carbon metabolism which is needed for 5-FU to exhibit its anticancer effects, causing reduction of 5-FU efficacy.[31] Therefore, special caution is necessary when using chemotherapy in patients with cancer with comorbidity.

Microbes are present intratumorally which can modulate chemotherapeutic efficacy via enzymatic reactions[33 34] (figure 1C). Gemcitabine is a nucleoside analogue for treatment of pancreatic ductal adenocarcinoma (PDAC). However, gemcitabine chemoresistance is common in PDAC, which was initially suspected to be caused by the enzyme cytidine deaminase (CDD) of tumour-infecting *Mycoplasma hyorhinis*.[35] Later, scientists discovered that only the long isoform of bacterial CDD (CDD-L) but not the short isoform (CDD-S) could metabolise gemcitabine into inactive metabolite 2',2'-difluorodeoxyuridine. More than 98% of bacterial species with CDD-L are Gammaproteobacteria, which are commonly enriched in PDAC tumour tissues.[36] Yet *M. hyorhinis* carries CDD-S but still confers gemcitabine resistance, suggesting that contributors other than CDD isoforms also cause chemoresistance. Notably, an exciting translational finding is that coadministration of antibiotic ciprofloxacin could reverse gemcitabine chemoresistance in mice.[36] However, current studies mainly focus on the interplay between intratumoral microbiota and chemoresistance, while how gut microbes influence the intratumoral microbiota composition remains elusive.[37 38] It is still uncertain about how these microbes enter tumours and the microbial biomass required to inactivate gemcitabine. More preclinical studies are required before translating mechanistic findings into clinical applications.

Gut bacteria and pharmacodynamics of chemotherapy

Beyond pharmacokinetics, the gut microbiota can alter patients' sensitivity and response to chemotherapy (ie, pharmacodynamics) via immunomodulation. Apart from cytotoxic effects, chemotherapeutics such as cyclophosphamide (CTX) also stimulate anticancer immunity.[17] Here, how the immunomodulation of gut bacteria influences chemotherapeutic efficacy is explored.

CTX, an alkylating chemotherapeutic agent, induces anticancer immunity by depleting immunosuppressive T regulatory cells (Treg) and promoting T helper (Th)-1 cell differentiation.[17] CTX also modifies the gut microbiota to bolster its immunomodulatory effects. It shortens the intestinal villi in mice to increase intestinal permeability for the translocation of *Enterococcus hirae* and *Lactobacillus johnsonii* into secondary lymphoid organs.[39] Such translocation accumulates 'pathogenic' Th17 and memory Th1 cells, which are essential for CTX-induced anticancer immune response.[39] *E. hirae* also increases the intratumoral CD8+ T cells to CD4+ Treg ratio and even reverses CTX chemoresistance in antibiotics-treated mice[40] (figure 1D). In contrast, *Barnesiella intestinihominis* is not translocated but accumulates in the colon to stimulate systemic polyfunctional CD8+ cytotoxic T cells and Th1 cells, promoting intratumoral infiltration of interferon (IFN)-γ-producing γδT cells.[40 41] Notably, these bacterial anticancer effects are limited by the pattern recognition receptor nucleotide-binding oligomerisation domain-containing 2 (NOD2), suggesting the possibility of combining NOD2-targeted therapy with CTX to optimise treatment responses.

Oxaliplatin and cisplatin are platinum-based antineoplastic drugs. Their early cytotoxic effects rely on ROS production, which is dependent on an intact microbiota. Gut microbes especially Gram-positive bacteria modulate myeloid differentiation primary response 88 (MYD88)-dependent signalling pathway to prime intratumoral myeloid cells for ROS generation in response to oxaliplatin[42 43] (figure 1E). Similarly, the gut bacteria also mediate their late immunomodulatory effects. Oxaliplatin-induced anticancer immune response requires both the antigenicity from oxaliplatin-induced apoptosis and immunogenic gut commensals.[44] Immunogenic bacteria, including non-enterotoxigenic *B. fragilis* and Erysipelotrichaceae, stimulate migratory dendritic cells (DCs) to signal follicular T helper (TFH) cells via interleukin (IL) 1β and IL-12. Stimulated TFH cells then interact with B cells to increase IgG2b response, enhancing the anticancer effector/memory CD8+ T cell activity. However, these immune responses are significantly reduced

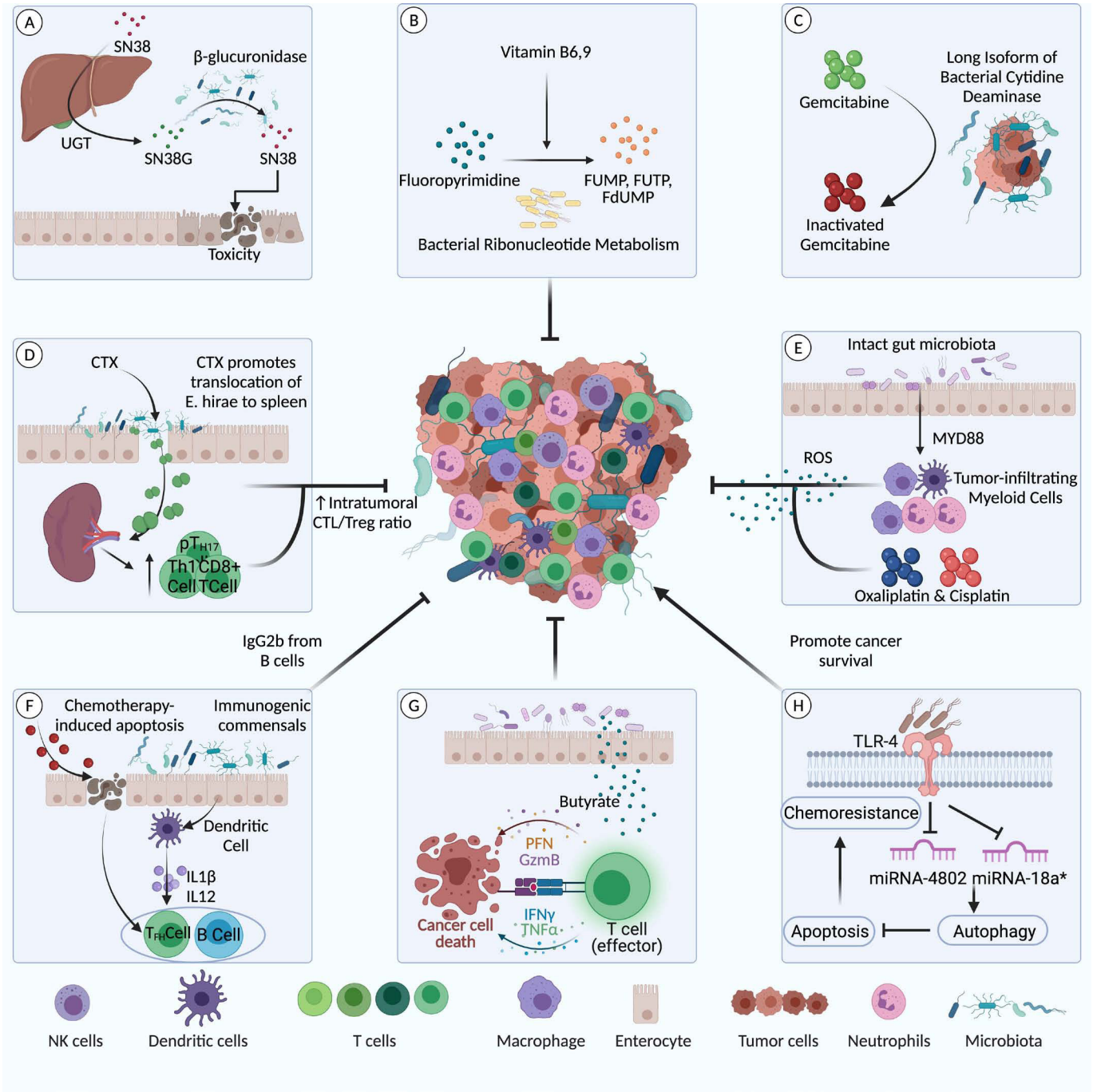

Figure 1 Mechanisms of microbiota modulation on chemotherapy response. (A) Irinotecan (CPT-11) is converted to SN-38 to elicit its cytotoxic effect after injection into the body. SN-38 is then detoxified by UGT in the liver to become SN-38G and excreted into the GI tract. The gut bacteria can reactivate and convert SN-38G back to SN-38, causing toxicity to intestinal cells. (B) Bacterial ribonucleotide metabolism activates fluoropyrimidine prodrugs into activated forms for cytotoxic effects. Vitamin B_6 and B_9 production is required for the metabolism. (C) Intratumoral Gammaproteobacteria with long isoform of cytidine deaminase can inactivate gemcitabine, leading to chemoresistance. (D) CTX increases intestinal permeability to promote *Enterococcus hirae* translocation into the spleen to increase pathogenic Th17 cells and intratumoral CD8+/CD4+ T cells ratio. (E) Gut microbes can prime tumour-infiltrating myeloid cells via MYD88-dependent pathway for ROS production in response to chemotherapeutic drugs. (F) Antigenicity from oxaliplatin-induced apoptosis of epithelial cells together with immunogenic bacteria, including non-enterotoxigenic *Bacteroides fragilis* and Erysipelotrichaceae, can stimulate the differentiation of migratory DCs to TFH cells for B cell activation. (G) Microbial metabolites such as butyrate can activate cytotoxic CD8+ T cells to enhance the efficacy of oxaliplatin. (H) *Fusobacterium nucleatum* can activate TLR4/MYD88-dependent pathway to inhibit certain miRNAs and switch tumour cells from apoptosis to autophagy, leading to chemoresistance. Figure created with BioRender.com. CPT-11, irinotecan; CTL, cytotoxic T lymphocyte; CTX, cyclophosphamide; DCs, dendritic cells; FdUMP, 5-fluorodeoxyuridine 5′-monophosphate; FUMP, 5-fluorouridine 5′-monophosphate; FUTP, 5-fluorouridine 5′-triphosphate; GzmB, Granzyme B; IFN-γ, interferon-γ; IL, interleukin; miRNA, microRNA; MYD88, myeloid differentiation primary response 88; NK, natural killer; PFN, perforin; pTH17 cells, pathogenic T helper 17 cells; ROS, reactive oxygen species; SN-38, 7-ethyl-10-hydroxycamptothecin; TFH, follicular T helper; TLR4, toll-like receptor-4; TNF-α, tumour necrosis factor alpha; Treg, T regulatory cells; UGT, uridine diphosphate glucuronosyltransferase.

in the absence of immunogenic gut commensals[44] (figure 1F). Besides direct interactions with immune cells, anticancer immunity can also be modulated by metabolites.[45 46] For example, short-chain fatty acids (SCFAs), particularly butyrate, could inhibit histone deacetylases to increase expression of the DNA transcription regulator inhibitor of DNA binding 2 (ID2). ID2 then boosts the cytotoxic function of CD8+ T cells via IL-12 signalling to promote oxaliplatin-induced anticancer immune response[46] (figure 1G).

Meanwhile, microbial immunomodulation is also involved in chemoresistance and therapeutic toxicity. *F. nucleatum*, which is highly enriched in patients with CRC, not only promotes colorectal tumourigenesis[11] but also induces chemoresistance to oxaliplatin and 5-FU. It activates toll-like receptor (TLR)-4/MYD88-dependent pathway and inhibits microRNA to switch CRC cells from apoptosis to autophagy. This in turn promotes CRC cell survival under chemotherapy[47] (figure 1H). Moreover, the gut microbiota also contributes to mechanical hyperalgesia, a dose-limiting complication of oxaliplatin. Although the mechanism is not fully characterised, hyperalgesia is in part mediated by the interactions between bacterial lipopolysaccharide and macrophage TLR4.[48]

The host–microbes–drug interactions affecting chemotherapeutic efficacy and toxicity are extremely complex. Even different strains of a bacterial species could have distinct effects, as exemplified by enterotoxigenic and non-enterotoxigenic *B. fragilis*, of which the former promotes CRC while the latter enhances oxaliplatin efficacy.[44 49] Simultaneously, chemotherapy can alter microbiota composition, leading to great disparity in interactions between microbes and drugs among different cancers and individuals. Altogether, these studies have shown the indispensability of gut microbiota to chemotherapy. The impact of concurrent antibiotic use on chemotherapy and the translational potential of these basic findings are worth further investigations.

Bench-to-bedside translation

Predictive biomarkers

Preclinical studies have provided mechanistic basis of how microbes affect chemotherapy. Some of these findings have been validated in human association studies, showing that certain microbial signatures are correlated with treatment responses, prognosis or incidence of adverse effects. These findings suggest the feasibility of using microbes as predictive biomarkers.

The relationship between CDD-L and gemcitabine chemoresistance is well established.[36] As CDD-L is mainly found in Gram-negative bacteria, a clinical study demonstrated the potential of using intratumoral lipopolysaccharide, a cell wall component of Gram-negative bacteria, to be a negative predictor of gemcitabine efficacy in PDAC.[50] Meanwhile, *F. nucleatum*-induced chemoresistance in mice could be conserved in human patients with CRC. Enrichment of intratumoral *F. nucleatum* is associated with shorter recurrence-free survival, and its abundance alone has higher accuracy than the American Joint Committee on Cancer staging in predicting CRC recurrence.[47] In contrast, *E. hirae*-specific and *B. intestinihominis*-specific Th1 immune responses are correlated with improved progression-free survival (PFS) in chemotherapy-treated patients.[40] Currently, the potential use of microbes, metabolites or enzymes in treatment outcome prediction is being extensively investigated, such as examining β-glucuronidase level to predict CPT-11-induced toxicity.[51]

Gut microbiota modulation

Concurrent antibiotic use is commonly applied to prevent opportunistic infection due to chemotherapy-induced immunosuppression.[52] However, antibiotics can change the microbial community, which in turn alters chemotherapeutic effects. As intratumoral bacterial CDD-L confers gemcitabine chemoresistance,[36] several retrospective clinical studies reported that antibiotics targeting the CDD-L-producing bacteria improve gemcitabine response in patients with PDAC.[53–55] Notably, most of these association studies did not involve the collection of tumour samples; thus, how the intratumoral microbiota is altered by antibiotics remains undetermined.

Current studies on antibiotics and chemotherapy have yielded conflicting results. Pflug *et al*[56] found that patients with chronic lymphocytic leukaemia or relapsed lymphoma receiving CTX/cisplatin and antibiotics concurrently have significantly lower PFS and overall survival (OS). Observational studies in patients with head and neck[57] or oesophageal cancer[58] also yielded similar results. In general, antibiotic use in chemotherapy could be a double-edged sword. While it is essential in immunocompromised patients, antibiotics may induce gut dysbiosis, causing unfavourable chemotherapy outcomes. Several important questions should be addressed in future studies concerning antibiotics and chemotherapy: do antibiotics simultaneously remove beneficial microbes, leading to suboptimal responses? how can the risks and benefits of using prophylactic antibiotics in patients with cancer be balanced? is antibiotics a safe and effective way to modulate gut microbiota for optimal treatment response? Alternatively, more differentiated strategies could be applied, such as using selective antibiotics guided by antimicrobial susceptibility testing instead of prescribing broad-spectrum antibiotics.[57]

Another way to modify microbiota is supplementing probiotics, which are live microbes primarily containing *Lactobacillus* and *Bifidobacterium* species. Both preclinical[59 60] and clinical[61 62] data have demonstrated that probiotics could improve chemotherapy outcomes.[63] A randomised controlled trial showed that *Bifidobacterium*-based and *Lactobacillus*-based probiotics could reduce β-glucuronidase activity to decrease the incidence of CPT-11-induced diarrhoea.[64] The probiotic *Clostridium butyricum* could reduce chemotherapy-induced diarrhoea among patients with lung cancer.[65] However, these studies only included small cohorts yielding inconclusive results. Subsequent meta-analyses on clinical trials reported that there is insufficient evidence supporting probiotic use in preventing chemotherapy-induced diarrhoea.[64 66 67] The small cohort size, the short duration of study and the presence of confounders inherent to study design are common limitations in current investigations. Although these studies demonstrated the safety of probiotic use, potential risks including bacteraemia should not be neglected in critically ill patients.[68] Future studies should evaluate the optimal formula of probiotics, ensure their efficacy in large cohorts and standardise research methods to facilitate comparison across clinical trials.

Prebiotics are substrates selectively used by microbes conferring health benefits which also modulate gut microbiota composition.[69] Preclinical studies discovered that prebiotics inulin and oligofructose could potentiate the cytotoxic effects of 5-FU and CTX,[70 71] while oatbase[72] and pectin[73] could reduce methotrexate-induced enterocolitis. Theoretically, prebiotic consumption may selectively enrich beneficial probiotics, including *Bifidobacterium* and *Lactobacillus*, and increase SCFA production. Recent studies confirmed that dietary fibres supplementation increases the abundance of *Bifidobacterium*, *Lactobacillus* and faecal butyrate concentration in healthy adults.[74]

However, the exact mechanisms of how prebiotics augment chemotherapy outcomes remains elusive. The durability of prebiotics should also be explored. Prebiotics usually take 1–2 weeks to alter microbiota composition, but the changes may be short-lived on resumption of normal nutritional intake.[74 75] A clinical trial on patients with gynaecological cancer showed that taking prebiotics 1 week before to 3 weeks after radiotherapy could improve post-treatment stool consistency.[76] However, clinical studies on the effects of prebiotics on chemotherapy are lacking. Another concern to note is the safety issue. Some oligosaccharides could indeed double the β-glucuronidase activity, aggravating CPT-11-induced toxicity in mice.[77] Future investigations are warranted to address the clinical safety of prebiotics.

MICROBIOTA AND ICIS

Resistance and recurrence are common problems of chemotherapy.[78] Since the last decade, the rapid development of immunotherapy has reshaped clinical guidelines in oncology. Generally, tumour cells develop mechanisms to evade immunosurveillance, the host immunity for tumour eradication.[79 80] Tumour cells could express programmed death-ligand 1 (PD-L1) that binds to programmed cell death protein-1 (PD-1) on T cells, causing their inactivation. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is another immune checkpoint on T cells which leads to T cell inactivation on ligand binding.[79 81–83] To reverse immunosuppression, anti-PD-1/PD-L1 and anti-CTLA-4 monoclonal antibodies (mAbs) have been introduced. Promising results from landmark clinical trials[84–86] have led to the United States Food and Drug Administration (FDA) approval of different ICIs for cancer treatment.

Nevertheless, the major challenges facing immunotherapy include the interpatient heterogeneity of ICI responses[87–89] and the immune-related adverse events (irAEs), especially colitis in anti-CTLA-4 mAbs and pneumonitis in anti-PD-1/PD-L1 mAbs.[90–92] Given the intricate interplay between the gut microbiota and host immunity, growing evidence has illustrated the potential application of microbiota modulation in optimising immunotherapy responses (figure 2). Generally, commensal microbes interact with ICIs by altering pharmacodynamics, particularly immunomodulation, rather than pharmacokinetics.

Mechanistic overview

Gut bacteria and anti-CTLA-4 mAbs

Preclinical studies have illustrated the indispensable role of gut microbiota in immunotherapy efficacy[93 94] (table 2). A pioneering study in 2015 demonstrated how gut bacteria influence the anti-CTLA-4 mAbs effects in mice.[93] Anti-CTLA-4 mAbs induce *B. fragilis*, *B. thetaiotaomicron* and *Burkholderia cepacia* to grow in the intestinal mucosa. These bacteria, particularly *B. fragilis*, then produce polysaccharides to stimulate CD11b+ DCs in the lamina propria, improving IL-12-dependent Th1 immune response in tumour-draining lymph nodes (figure 2A). Meanwhile, microbiota-depleted mice have impaired response to anti-CTLA-4 mAbs, signifying the importance of microbiota in immunotherapy. Interestingly, recolonisation of *B. fragilis* and *B. cepacia* in bacteria-depleted mice not only rescues their immunotherapeutic resistance but also reduces the histopathological signs of colitis.[93 95] *Bifidobacterium* administration also minimises anti-CTLA-4 mAbs-induced irAEs without compromising its efficacy. Mechanistically, *Bifidobacterium* enhances the suppressive functions of intestinal Treg through IL-10 mediation.[96 97] *B. bifidum* cell surface β-glucan/galactan polysaccharides also induce Treg by generating regulatory DCs to ameliorate colitis.[98]

Beyond direct interaction with immune cells, indirect immunomodulation via microbes-derived metabolites is also possible (figure 2C). As anti-CTLA-4 mAbs impair intestinal barrier, metabolite inosine derived from *Bifidobacterium pseudolongum* and *Akkermansia muciniphila* could enter the systemic circulation. Inosine then activates Th1 cells via adenosine 2A receptor costimulated by DCs to enhance tumour shrinkage in the presence of ICIs.[99] The anticancer effect of inosine may also be attributed to its role as an alternative fuel to glucose for effector T cells within the tumour microenvironment (TME).[100]

Gut bacteria and anti-PD-1/PD-L1 mAbs

Another pivotal study in 2015 showed that gut bacteria could modulate the efficacy of anti-PD-1/PD-L1 mAbs in mice.[94] *B. breve* and *B. longum* activate DCs for CD8+ T cell priming and infiltration in TME, enhancing the immunotherapeutic effects.[94] *Bifidobacterium* also mediates innate immunity to potentiate anti-PD-1 mAbs efficacy in melanoma mouse models[101] (figure 2B). Mechanically, *Bifidobacterium* secretes metabolite hippurate and inhibits PD-1 expression, which in turn activates natural killer (NK) cells to destroy tumours via perforin and IFN-γ mediation.[101] As *Bifidobacterium* also mitigates irAEs induced by CTLA-4 blockade,[96] it is worth exploring whether *Bifidobacterium* could simultaneously enhance efficacy and reduce toxicity of combined PD-1/PD-L1 and CTLA-4 blockade.

Probiotic bacteria also modulate immunotherapeutic effects. *L. rhamnosus* GG (LGG), a well-studied and commonly used probiotics, could reduce CRC occurrence and impede tumour progression of hepatocellular carcinoma in mice.[102] LGG also improves anti-PD-1 mAbs effects in mice by increasing tumour-infiltrating DCs and T cells. Mechanistically, LGG promotes IFN-β production by activating STING (stimulator of IFN genes) and its secondary messenger cyclic GMP-AMP synthase (cGAS) to induce phosphorylation of interferon regulatory factor 7. The increased IFN-β production via this cGAS/STING-dependent axis eventually enhances cross-priming of anticancer CD8+ T cells.[102] LGG intake also enriches tumour-suppressing *B. uniformis* and *L. murinus* in the intestine, of which *B. uniformis* is associated with the enrichment of IFN-γ+ CD8+ T cells in mesenteric lymph nodes,[103] whereas *L. murinus* is correlated with DC activation in the gut.[104]

Antigen mimicry between commensal bacteria and tumour cells also confers beneficial responses to ICIs (figure 2C). Apart from activating DCs for T cell priming as aforementioned,[94] *B. breve* antigen SVY is homologous to mouse melanoma SIY neoantigen, thereby stimulating cross-reactive T cell response against melanoma cells.[105] Meanwhile, *E. hirae*-infecting bacteriophage expresses tape measure protein (TMP), which stimulates memory CD8+ T cell response to cross-react with cancer antigen proteasome subunit beta type-4 protein. Administrating bacterial strains expressing TMP epitope could improve immunotherapy efficacy in mice.[106] Notably, recent investigations have linked several autoimmune disorders to the molecular mimicry between microbes-derived antigens and self-antigens.[107 108] Considering the diversified proteome in the gut microbiota,[109 110] more microbial antigens with high homology to cancer antigens would likely be discovered.

Epithelial barrier also plays a role in the microbes-mediated immunomodulation (figure 2D). A study reported the enhanced anticancer immunity in *Rnf5*$^{-/-}$ mice with melanoma (RNF5 is a membrane-bound E3 ubiquitin ligase implicated in protein degradation).[111] Mechanistically, *Rnf5*$^{-/-}$ mice had reduced antimicrobial peptides and increased enterocyte apoptosis,

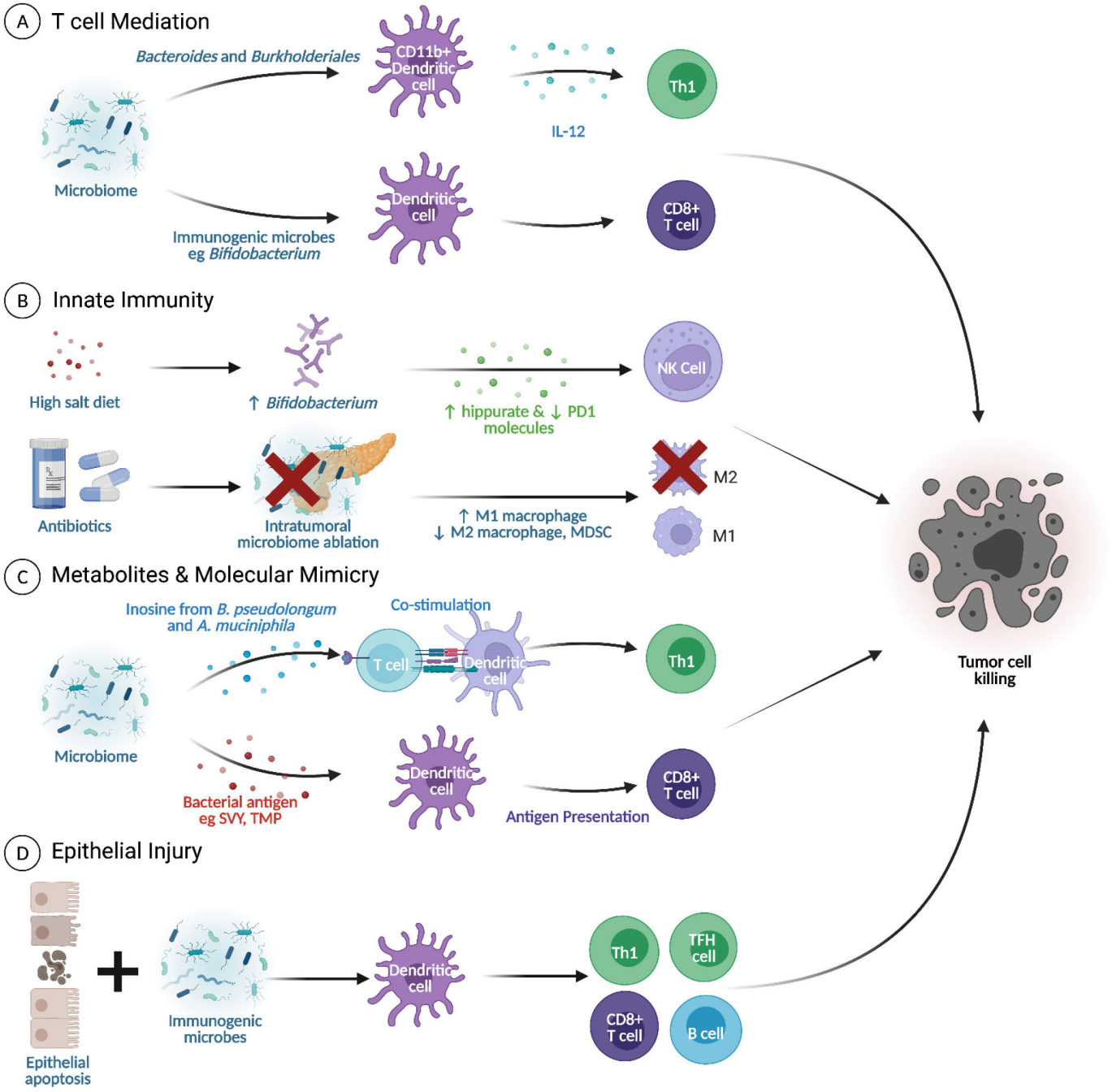

Figure 2 Mechanisms of microbiota modulation on immunotherapy response. Microbes–immunotherapy interactions could be categorised by the 'TIME' mechanistic framework: T cell mediation, Innate immunity, Metabolites, molecular mimicry, and Epithelial injury. (A) Bacteria such as *Bacteroides, Burkholderiales* and *Bifidobacterium* could enhance anticancer T cell immunity mediated by DCs for immunotherapy potentiation. (B) NK cells and proinflammatory M1 macrophages are the main contributors of innate immunity against cancer. *Bifidobacterium* could activate NK cells to combat cancers, while intratumoral microbiota ablation in PDAC could reprogramme M2 macrophages to M1 macrophages and reduce myeloid-derived suppressor cells. Altogether they increase the sensitivity of tumours to immunotherapy. (C) *Bifidobacterium pseudolongum* and *Akkermansia muciniphila* could secrete metabolite inosine. Inosine activates Th1 cells via adenosine 2A receptor costimulated by DCs. Other bacteria could improve ICI anticancer response via molecular mimicry. *Bifidobacterium breve* and *Enterococcus hirae*-infecting bacteriophage have SVY and TMP antigens, respectively, which are highly similar to tumour neoantigens. This leads to cross-reactivity of cytotoxic T cells against tumour cells. (D) Epithelial injury and immunogenic bacteria stimulate DCs for anticancer immunity. Figure created with BioRender.com. DCs, dendritic cells; ICI, immune checkpoint inhibitor; IL, interleukin; MDSC, myeloid-derived suppressor cells; NK, natural killer; PD-1, programmed cell death protein-1; PDAC, pancreatic ductal adenocarcinoma; SVY, SVYRYYGL; TFH, follicular T helper; Th, T helper; TMP, tape measure protein.

causing enrichment of *Bacteroides* and *Parabacteroides*, which together activated DCs to promote intratumoral infiltration of IFN-γ-producing T cells.[111] These findings are indeed similar to the oxaliplatin-induced anticancer immune response.[44] The antigenicity from oxaliplatin-induced apoptosis and immunogenic gut commensals not only mediate the anticancer immunity of oxaliplatin as aforementioned, but also provide synergistic therapeutic effects when combining oxaliplatin with anti-PD-1

Table 2 Mechanistic studies of modulation of immunotherapy effect by microbiota

Immunotherapy	Involved microbes	Mechanisms
Anti-CTLA-4 mAbs	*Bacteroides fragilis, Bacteroides thetaiotaomicron* and *Burkholderia cepacia*	Stimulation of CD11b+ DCs improved IL-12–dependent Th1 immune response for enhanced antitumour immune response.[93]
	Bifidobacterium	*Bifidobacterium* enhanced the suppressive functions of Treg cells to minimise immunopathology induced by treatment.[96 97]
	Bifidobacterium pseudolongum and *Akkermansia muciniphila*	Bacterial-derived inosine acted on adenosine 2A receptors to stimulate Th1 response in the presence of costimulations from DCs and enhanced tumour shrinkage.[99]
Anti-PD-1/PD-L1 mAbs	*Bifidobacterium breve* and *Bifidobacterium longum*	*Bifidobacterium* activated DCs for CD8+ T cell priming and infiltration in the tumour microenvironment to enhance anticancer immune response of treatment.[94] *Bifidobacterium* could also secrete hippurate and reduce PD-1 molecule expression to activate NK cells for anticancer effects.[101]
	Lactobacillus rhamnosus GG	LGG activated DCs via cGAS-STING-TBK1-IRF7-IFN-β cascade to enhance CD8+ T cell activity against tumour cells.[102]
	Bifidobacterium breve	Molecular mimicry between SVY antigen of *B. breve* and SIY neoantigen of mouse melanoma stimulated cross-reactive T cell response against melanoma cells.[105]
	Bacteriophage-infecting *Enterococcus hirae*	TMP of bacteriophage-infecting *E. hirae* stimulated memory CD8+ T cell cross-reaction with cancer antigen PSMB4 protein.[106]

cGAS, cyclic GMP-AMP synthase; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; DCs, dendritic cells; IFN-β, interferon-β; IL, interleukin; IRF7, interferon regulatory factor 7; LGG, *Lactobacillus rhamnosus* GG; mAbs, monoclonal antibodies; NK, natural killer; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; PSMB4, proteasome subunit beta type-4; SIY, SIYRYYGL; STING, stimulator of IFN genes; SVY, SVYRYYGL; TBK1, TANK binding kinase 1; Th, T helper; TMP, tape measure protein; Treg, T regulatory cells.

mAbs. Altogether, epithelial injury and immunogenic bacteria stimulate DCs for helper or cytotoxic T cell immune response against cancers.

The microbial interactions with ICIs are complicated. With reference to a previous review proposing the 'TIMER' (Translocation, Immunomodulation, Metabolism, Enzymatic degradation, and Reduced diversity and ecological variation) mechanistic framework describing the microbes–chemotherapy interactions,[14] we categorise microbes–immunotherapy interactions into a 'TIME' framework: T cell mediation, Innate immunity, Metabolites and molecular mimicry, and Epithelial injury (figure 2). Given the complexity, a mechanistic framework can better illustrate the microbes–immunotherapy interactions, which lays the foundation for translating these basic findings to clinical application.

Bench-to-bedside translation

Predictive biomarkers

Following the preclinical evidence,[93 94] metagenomic studies on patients with melanoma and epithelial tumour in 2018 confirmed the role of gut microbiota in anti-PD-1/PD-L1 mAbs responses[112–114] (table 3). *Faecalibacterium*, *B. longum*, *Collinsella aerofaciens*, *E. faecium*, *A. muciniphila* and *E. hirae*

Table 3 Summary of gut microbes associated with immunotherapy response

Immunotherapy	Patient cohort	Key findings
Anti-PD-1/PD-L1 mAbs	► 43 patients with metastatic melanoma.[114]	► R-enriched: Ruminococcaceae family and *Faecalibacterium* genus (*Faecalibacterium prausnitzii*). ► NR-enriched: Bacteroidales order (*Bacteroides thetaiotaomicron*). ► Elevated abundance of CD8+ T cells in TME.
	► 42 patients with metastatic melanoma.[113]	► R-enriched: *Bifidobacterium longum*, *Collinsella aerofaciens* and *Enterococcus faecium*. ► NR-enriched: *Ruminococcus obeum* and *Roseburia intestinalis*. ► FMT from R to germ-free mice enhances anti-PD-L1 mAbs response with T cell enrichment.
	► 249 patients (140 NSCLC, 67 RCC and 42 urothelial carcinoma).[112] ► 338 patients with NSCLC.[115]	► R-enriched: *Akkermansia muciniphila* and *Enterococcus hirae*.[112] ► Associated with shorter PFS and OS: antibiotic use before or after first injection of ICIs.[112] ► Associated with longer OS and ORR: *Akkermansia muciniphila* (relative abundance <4.799%).[115] ► Akk+ enriched: Ruminococcaceae family, Lachnospiraceae family and others.[115]
Monotherapy or combined immunotherapy	► 27 patients with metastatic melanoma.[116]	► Higher microbial diversity was associated with longer PFS. ► Associated with longer PFS: *Faecalibacterium prausnitzii*, *Coprococcus eutactus*, *Prevotella stercorea*, *Streptococcus sanguinis*, *Streptococcus anginosus* and *Lachnospiraceae bacterium 3 1 46FAA*. ► Associated with shorter PFS: *Bacteroides ovatus*, *Bacteroides dorei*, *Bacteroides massiliensis*, *Ruminococcus gnavus* and *Blautia producta*. ► Risk-associated pathways: L-rhamnose degradation, guanosine nucleotide biosynthesis and B vitamin biosynthesis.
Anti-CTLA-4 mAbs±anti-PD-1	► 39 patients with metastatic melanoma.[117]	► R-enriched (combined anti-CTLA-4/anti-PD-1): *Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron* and *Holdemania filiformis*. ► R-enriched (anti-PD-1): *Dorea formicigenerans*.
Anti-CTLA-4	► 26 patients with metastatic melanoma.[118]	► R-enriched: *Faecalibacterium, Gemmiger* and *Clostridium* XIVa. ► R-depleted: *Bacteroides*.

Akk+, patients with detectable faecal *Akkermansia muciniphila*; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; FMT, faecal microbiota transplantation; ICIs, immune checkpoint inhibitors; mAbs, monoclonal antibodies; NR, non-responders; NSCLC, non-small cell lung carcinoma; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PFS, progression-free survival; R, responders; RCC, renal cell carcinoma; TME, tumour microenvironment.

were enriched in responders of anti-PD-1 mAbs, while *Bacteroides*, *Ruminococcus obeum* and *Roseburia intestinalis* were enriched in non-responders. Consistently, all three studies demonstrated that faecal microbiota transplantation (FMT) from responders to germ-free mice could potentiate the anti-PD-1 mAbs efficacy. However, the microbes identified from these studies are diverse and have little overlap with one another.[112–114] In 2022, a follow-up study was performed to validate the predictive value of *A. muciniphila* in a prospective multicentric cohort of patients with non-small cell lung carcinoma (NSCLC).[115] An interesting distribution was observed: 'normal' abundance of *A. muciniphila* (relative abundance <4.799%) is associated with longer OS, while both the absence and overabundance of *A. muciniphila* (relative abundance >4.799%) are associated with shorter OS.[115] The predictive value of this trichotomic stratification is even more accurate than tumour PD-L1 expression, a clinically used predictive biomarker for ICI response in NSCLC.[115] Compared with tumour cells in patients with undetectable faecal *A. muciniphila*, tumour cells in patients with detectable faecal *A. muciniphila* have higher gene expression related to T cell activation and IFN fingerprint, which are related to improved anti-PD-1 mAb response.[115] These findings are in line with previous mechanistic studies, suggesting that *A. muciniphila* promotes intratumoral infiltration of Th1 cells.[112]

Studies on combined anti-PD-1/anti-CTLA-4 immunotherapy in patients with melanoma were also conducted[116–118] (table 3). *Faecalibacterium prausnitzii*, *Holdemania filiformis* and *B. thetaiotaomicron* were enriched in responders, while *Bacteroides* species were enriched in non-responders.[116 117] Transcriptomic analysis further showed that sugar degradation pathway, vitamin B biosynthesis pathways and guanosine nucleotide biosynthesis pathways are linked to shorter PFS.[116] While Gopalakrishnan *et al*[114] found that amino acid biosynthesis is enriched in responders, Peters *et al* specifically identified that L-isoleucine biosynthesis is linked to longer PFS,[116] which may be related to its immunomodulatory effects.[119]

Currently, consensus in the bacterial species associated with immunotherapeutic responses is lacking even in the same cancer type (eg, metastatic melanoma). Possible reasons include the differences in sample collection, sequencing technology and bioinformatics pipelines among studies.[120 121] A meta-analysis integrated and reanalysed the data from three previous studies,[112–114] concluding that analytical pipelines are not the cause of the interstudy variation.[122] Interestingly, there are more overlaps in the microbial gene content rather than the microbiota composition across studies, implying that the signature microbes identified in different studies could be functionally related. Particularly, microbial gene contents provided a higher predictive value than the microbiota composition for assessing treatment response, with an area under curve >0.7, showing the potential of using microbial gene signatures as predictive biomarkers.[122] A recent prospective multicentric study validated the positive association of *A. muciniphila* with improved ICI effects.[115] This study demonstrated that faecal samples with detectable *A. muciniphila* are enriched with immunogenic bacteria identified across numerous studies,[112 114 116–118 123] including Ruminococcaceae,[114] Lachnospiraceae family[116] and *B. intestinihominis*.[123] To date, different studies have identified a variety of microbial species that are associated with ICI response. However, their interactions with one another and which microbes have a more dominant role in ICIs remain unknown. This study therefore provides initial insights that *A. muciniphila* could be the master regulator of immunogenic bacteria to contribute to the improvement in ICI response.[115]

Altogether, these studies have illustrated that microbial biomarkers may predict cancer treatment outcomes. Some of the bacteria identified from human studies, including *A. muciniphila*,[112] *B. intestinihominis*[123] and *B. thetaiotaomicron*,[117] were also mechanistically shown to improve therapeutic response through immunomodulation,[40 93 99] further confirming their translational potential. However, a major challenge is the lack of consensus on the signature species across studies, making it difficult to establish a well-acknowledged consortium of microbial biomarkers. To reduce interstudy disparity, the methods of sample collection, sequencing and bioinformatics analysis should be standardised.[124] Large cohort studies with multiomics approaches could provide more insights on the correlation between gut microbiota and ICI response. Moreover, further functional investigations and clinical trials are required to explore the translational potential of these observations.

Gut microbiota modulation

Prophylactic antibiotics are commonly used with immunotherapy to prevent life-threatening infections. However, clinical studies observed diminished response to immunotherapy in antibiotics-treated patients,[125–127] in line with evidence from preclinical animal studies.[93 94] Both retrospective[125 127] and prospective[126] studies showed that antibiotic use is associated with lower PFS, OS and response rate. The timing of antibiotic use is also important. A meta-analysis reported that patients without antibiotic use 42 days before ICI initiation have 3.43 times longer OS, while no significant difference in OS was observed between patients with and without antibiotic use 60 days before ICI.[128] These findings are consistent with a study of healthy individuals treated with an antibiotic cocktail (meropenem, gentamicin and vancomycin) for 4 days, of which their microbiota composition recovered to near-baseline within 42 days.[129] Apart from epidemiological observations, recent studies showed that patients with antibiotic use have enriched microbial features associated with poor survival, such as low diversity and enrichment of *C. hathewayi*.[130 131] Antibiotic use should therefore be avoided before ICIs. Alternatively, FMT or probiotics may be plausible option to reverse antibiotics-induced dysbiosis before ICIs. Notably, PDAC could be an exception as preclinical studies have shown that intratumoral bacterial ablation using antibiotics in PDAC improves immunotherapeutic efficacy (figure 2B).[132] Hence, the effects of antibiotics or microbiota on cancer therapy should be carefully studied in distinct cancer types.

Modulating immunotherapy response using probiotics is another plausible method. Many microbes associated with improved immunotherapy response, including *B. longum* and LGG, are indeed commercially available probiotics.[94 102] Preclinical studies showed that probiotics could enhance anticancer immunity by reducing Treg level[133] and enhancing CD8+ T cell activation, CD4+ T cell differentiation and intratumoral infiltration of NK cells.[134] A proof-of-concept study by Tanoue *et al*[103] reported that a defined consortium of 11 bacterial strains (7 *Bacteroidales* and 4 non-*Bacteroidales* species) could enhance ICI efficacy in mice with syngeneic tumours via CD103+ DC-mediated induction of IFN-γ-producing CD8+ T cells. This highlights the feasibility of probiotics as adjuvants to improve immunotherapy outcomes. Clinical trials demonstrated that probiotics *B. lactis* Bl-04 and *L. acidophilus* NCFM could increase the abundance of butyrate-producing species, particularly *Faecalibacterium* and *Clostridiales*, in the gut of patients with CRC.[135] These species were associated with improved immunotherapy response in human patients.[114 116–118] However, current clinical investigations only demonstrate the impact of probiotics on gut microbiota but not their direct causative effects on immunotherapeutic outcomes. A recent clinical study even showed that

Table 4 Current challenges and future directions of microbiota application in clinical settings

Aspects	Challenges	Future directions/potential solutions
Discovery of biomarkers		
Developing predictive biomarkers for treatment response	► Suboptimal sensitivity and specificity.	► Combination of microbial features with other potential biomarkers such as tumour mutational loads.[147]
	► Lack of consensus on the microbial features as predictive biomarkers.	► Standardisation of sample collection, processing and bioinformatics pipelines. ► Large clinical cohorts with different ethnicity.
Modulation of microbiota to optimise cancer treatment outcomes		
Antibiotic use and cancer therapy	► Concurrent use of prophylactic antibiotics and cancer therapy may lead to poor treatment outcomes.	► Use of autologous FMT may restore the dysbiosis induced by antibiotics.[155]
	► Targeting cancer-promoting microbes with antibiotics is non-specific.	► Use of narrow-spectrum antibiotics. ► Development of highly specific approach such as use of phage and engineered microbes.[152–154]
Probiotics use and cancer therapy	► Lack of efficacy of using probiotics to reduce cancer treatment side effects.	► Consider probiotics as an adjuvant therapy to more efficacious treatments. ► Further investigations in multicentre, large-scale, phase III clinical trials with longer duration to confirm the effects of probiotics.
	► Potential risks of infection with probiotics use.	► Avoid the use of probiotics in immunocompromised or critically ill patients.
FMT and cancer therapy	► Potential risk of transferring pathogens from donors to recipients.	► Use of capsules consisting of purified bacterial spores may be safer and have higher consistency than FMT.[156] ► Rigorous and comprehensive donor screening. ► Characterise the baseline microbiota composition that is more likely to respond to FMT.

FMT, faecal microbiota transplantation.

probiotics use is correlated with lower microbial diversity, a common feature in non-responders of immunotherapy.[136] Such finding has been recapitulated in a preclinical mechanistic study showing that mice treated with either probiotic *B. longum* or LGG have worse response to anti-PD-L1 mAbs, with reduced level of intratumoral IFN-γ+ CD8+ T cells.[137] These latest results therefore present an opposite picture compared with previous studies. Over-the-counter probiotics should therefore be discouraged in patients receiving immunotherapy as current understandings are still limited.

Prebiotics and dietary modulation are alternatives to augment ICI responses. Common prebiotics such as inulin and galacto-oligosaccharide could raise the abundance of *Bifidobacterium*, *Lactobacillus* and *Faecalibacterium* species in human gut,[138 139] which are immunogenic bacteria associated with improved anticancer immunity.[93 94 103] However, studies demonstrating the direct effects of prebiotics on ICIs are lacking, which warrant further investigations. On the other hand, dietary modulation was shown to be effective in augmenting therapeutic effects. High-fibre diet in patients with metastatic NSCLC receiving ICIs is associated with enrichment of *Bifidobacterium* species and better clinical outcomes.[140] Patients with melanoma with high-fibre intake also had improved ICI response.[137] This is supported by a parallel mechanistic study demonstrating the increased level of tumour-infiltrating lymphocytes in ICI-treated mice fed with high-fibre diet, of which the enrichment of fibre-fermenting Ruminococcaceae family after fibre supplementation could promote T cell activation and intratumoral infiltration.[137] Another example is that the ketone body 3-hydroxybutyrate produced by ketone diet could enhance ICI efficacy in mice. 3-Hydroxybutyrate increases the expansion of ICI-induced CD8+ T cells and restrains PD-L1 expression to maintain T cell activation for anticancer effects.[141] Ketone diet could enrich *Eisenbergiella massiliensis* in human gut, which is strongly correlated with serum 3-hydroxybutyrate concentration.[141] However, the gut microbiota responds rapidly to dietary changes, implicating the short-lived effects of dietary modulation.[75] Maintaining a new diet is notoriously difficult; hence, how to retain and prolong dietary effects to improve ICI response requires further investigations.

FMT is another clinical approach of microbiota modulation which has received FDA approval for treating recurrent *C. difficile* infection.[142] FMT is the transfer of entire faecal microbial community, including bacteria, viruses, fungi and their metabolites, from a healthy donor into the recipient.[142] Wang *et al*[143] reported the first case of treating immunotherapy-induced colitis using FMT. Normally, ICI-induced colitis is treated with immunosuppressive agents including corticosteroids, which nonetheless have considerable side effects. In this study, the two patients with ICI-induced colitis were refractory to conventional therapy but showed significant improvement after FMT with enrichment of beneficial *Bifidobacterium* species. Nevertheless, future clinical trials are required to validate the translational potential of FMT in immunotherapy due to the pioneering nature of this initial study.

Meanwhile, two recent proof-of-concept clinical trials demonstrated the safety and efficacy of FMT in boosting anti-PD-1 mAbs response in patients with refractory melanoma.[144 145] Baruch *et al*[144] recruited 12 patients with melanoma composing of 10 non-responders and 2 responders to ICIs. The 10 non-responders were treated with antibiotics for microbiota depletion, followed by FMT with stools from the 2 responders and reintroduction of anti-PD-1 mAbs. Two patients showed partial response while one showed complete response, suggesting a true de novo immunotherapeutic response. Similarly, Davar *et al*[145] treated 15 patients with ICI-refractory melanoma with FMT (stools from responders) and anti-PD-1 mAbs. Three patients showed objective response, while another three patients showed stable disease. Both studies have demonstrated the causative effects of FMT on immunotherapy response, implicating the clinical safety and feasibility of FMT in cancer treatment. Unlike other modulation methods, the effects of FMT on gut microbiota could persist for more than 24 weeks with less requirement for frequent interventions.[142] Indeed, in the study by Davar *et al* FMT was performed only once each patient.[145] Additionally, future studies should consider using ICI biomarkers including PD-L1 expression and tumour mutation burden to assess whether a pre-existing adaptive immunity is essential for effective FMT.[146]

FUTURE PERSPECTIVES AND CONCLUSION

Therapeutic resistance and toxicity are major stumbling blocks in cancer therapy. Tremendous efforts have been put to predict

treatment outcomes and optimise treatment response.[147] Gut microbes are undeniably potential candidates for being predictive biomarkers and treatment targets. Despite the current exciting results, the future is not without challenges (table 4). These include inadequate mechanistic understanding of microbiota modulation on therapeutic response, undetermined microbial signatures as biomarkers and the lack of consensus on the optimal microbiota modulation method. Moreover, current studies focus mainly on bacteria, but commensal viruses, fungi and archaea also have unneglectable role in cancers.[148–150] Of note, FMT transfers not only bacteria but also other non-bacterial microbes, yet their effects on recipients are unclear, which raises potential safety concerns.

Concerted efforts are required to overcome challenges lying ahead. First, more functional investigations and prospective longitudinal human studies are needed to dissect the biological complexity of host–microbes–drug interactions. Rigorous identification of the key microbes affecting treatment outcome is the prerequisite for clinical translation. Future studies should go beyond bacteria. A recent study demonstrated that depleting gut bacteria could lead to commensal fungi overgrowth, reducing the radiotherapy-induced anticancer immunity. Interestingly, depleting commensal fungi could enhance radiotherapy efficacy, suggesting the antagonistic roles of gut bacteria and fungi in anticancer immunity. Interkingdom interactions of microbiota in cancer therapy are therefore an important future direction.[15] Second, metagenomic studies should be standardised and integrated with other 'omics', including transcriptomics and metabolomics. Technological advancements such as capsule endoscopy also enable analysis of microbiota along different regions of the gut.[151] Together, these would provide insights on the mechanistic basis of host–microbes–drug interactions. Finally, the best approach of microbiota modulation for augmenting treatment outcomes should be determined. Future clinical trials should assess the efficacy, durability and safety of different methods including probiotics, prebiotics, antibiotics and FMT. Other innovative methods are recently reported, such as using bacteriophages to target specific microbes[152] or engineered microbes for drug delivery and tumour lysis.[153 154] Yet again extensive work is required before clinical translation. It is expected that many more mysteries of human microbiota will be unravelled with the efforts from scientists and clinicians, which will pave the way to next-generation personalised medicine.

Contributors NL-NT collected the data and drafted the manuscript. HCHL revised the manuscript. JY supervised and revised the manuscript.

Funding This study was supported by the National Key R&D Program of China (no: 2020YFA0509200/2020YFA0509203), RGC Theme-based Res Scheme Hong Kong (T21-705/20-N), RGC Collaborative Research Fund (C4039-19GF, C7065-18GF), RGC-GRF Hong Kong (14163817), and Vice-Chancellor's Discretionary Fund Chinese University of Hong Kong.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study does not involve human participants.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Harry Cheuk-Hay Lau http://orcid.org/0000-0003-3581-2909
Jun Yu http://orcid.org/0000-0001-5008-2153

REFERENCES

1 Sepich-Poore GD, Zitvogel L, Straussman R, *et al*. The microbiome and human cancer. *Science* 2021;371:abc4552. doi:10.1126/science.abc4552
2 Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016;14:e1002533.
3 Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol* 2016;16:341–52.
4 Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* 2020;30:492–506.
5 Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* 2021;19:55–71.
6 Zhuang L, Chen H, Zhang S, *et al*. Intestinal microbiota in early life and its implications on childhood health. *Genomics Proteomics Bioinformatics* 2019;17:13–25.
7 Yu L-X, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* 2017;14:527–39.
8 Tilg H, Adolph TE, Gerner RR, *et al*. The intestinal microbiota in colorectal cancer. *Cancer Cell* 2018;33:954–64.
9 Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A, *et al*. Mutational signature in colorectal cancer caused by genotoxic pks+ E. coli. *Nature* 2020;580:269–73.
10 Wilson MR, Jiang Y, Villalta PW, *et al*. The human gut bacterial genotoxin colibactin alkylates DNA. *Science* 2019;363:aar7785. doi:10.1126/science.aar7785
11 Rubinstein MR, Wang X, Liu W, *et al*. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* 2013;14:195–206.
12 Roden DM, McLeod HL, Relling MV, *et al*. Pharmacogenomics. *Lancet* 2019;394:521–32. doi:10.1016/S0140-6736(19)31276-0
13 Doestzada M, Vila AV, Zhernakova A, *et al*. Pharmacomicrobiomics: a novel route towards personalized medicine? *Protein Cell* 2018;9:432–45.
14 Alexander JL, Wilson ID, Teare J, *et al*. Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nat Rev Gastroenterol Hepatol* 2017;14:356–65.
15 Shiao SL, Kershaw KM, Limon JJ, *et al*. Commensal bacteria and fungi differentially regulate tumor responses to radiation therapy. *Cancer Cell* 2021;39:1202–13.
16 Wong CW, Yost SE, Lee JS, *et al*. Analysis of gut microbiome using Explainable machine learning predicts risk of diarrhea associated with tyrosine kinase inhibitor neratinib: a pilot study. *Front Oncol* 2021;11:604584.
17 Galluzzi L, Humeau J, Buqué A, *et al*. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol* 2020;17:725–41.
18 Spanogiannopoulos P, Bess EN, Carmody RN, *et al*. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. *Nat Rev Microbiol* 2016;14:273–87.
19 Vanhoefer U, Harstrick A, Achterrath W, *et al*. Irinotecan in the treatment of colorectal cancer: clinical overview. *J Clin Oncol* 2001;19:1501–18.
20 Bailly C. Irinotecan: 25 years of cancer treatment. *Pharmacol Res* 2019;148:104398.
21 Nagar S, Blanchard RL. Pharmacogenetics of uridine diphosphoglucuronosyltransferase (UGT) 1A family members and its role in patient response to irinotecan. *Drug Metab Rev* 2006;38:393–409.
22 Stringer AM, Gibson RJ, Logan RM, *et al*. Faecal microflora and beta-glucuronidase expression are altered in an irinotecan-induced diarrhea model in rats. *Cancer Biol Ther* 2008;7:1919–25.
23 Dabek M, McCrae SI, Stevens VJ, *et al*. Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene Gus in human colonic bacteria. *FEMS Microbiol Ecol* 2008;66:487–95.
24 McIntosh FM, Maison N, Holtrop G, *et al*. Phylogenetic distribution of genes encoding β-glucuronidase activity in human colonic bacteria and the impact of diet on faecal glycosidase activities. *Environ Microbiol* 2012;14:1876–87.
25 Lin XB, Dieleman LA, Ketabi A, *et al*. Irinotecan (CPT-11) chemotherapy alters intestinal microbiota in tumour bearing rats. *PLoS One* 2012;7:e39764.
26 Wallace BD, Wang H, Lane KT, *et al*. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* 2010;330:831–5.
27 Cheng K-W, Tseng C-H, Tzeng C-C, *et al*. Pharmacological inhibition of bacterial β-glucuronidase prevents irinotecan-induced diarrhea without impairing its antitumor efficacy in vivo. *Pharmacol Res* 2019;139:41–9.
28 Chen Z, Xu X, Piao L, *et al*. Identify old drugs as selective bacterial β-GUS inhibitors by structural-based virtual screening and bio-evaluations. *Chem Biol Drug Des* 2020;95:368–79.
29 Lin H-Y, Chen C-Y, Lin T-C, *et al*. Entropy-driven binding of gut bacterial β-glucuronidase inhibitors ameliorates irinotecan-induced toxicity. *Commun Biol* 2021;4:1–10.
30 Bhatt AP, Pellock SJ, Biernat KA, *et al*. Targeted inhibition of gut bacterial β-glucuronidase activity enhances anticancer drug efficacy. *Proc Natl Acad Sci U S A* 2020;117:7374–81.
31 Scott TA, Quintaneiro LM, Norvaisas P, *et al*. Host-microbe co-metabolism dictates cancer drug efficacy in C. elegans. *Cell* 2017;169:442–56.
32 García-González AP, Ritter AD, Shrestha S, *et al*. Bacterial metabolism affects the C. elegans response to cancer chemotherapeutics. *Cell* 2017;169:431–41.

33 Nejman D, Livyatan I, Fuks G. The human tumor microbiome is composed of tumor type–specific intracellular bacteria. *Science*.
34 Liu W, Zhang X, Xu H, *et al*. Microbial community heterogeneity within colorectal neoplasia and its correlation with colorectal carcinogenesis. *Gastroenterology* 2021;160:2395–408.
35 Vande Voorde J, Sabuncuoğlu S, Noppen S, *et al*. Nucleoside-catabolizing enzymes in mycoplasma-infected tumor cell cultures compromise the cytostatic activity of the anticancer drug gemcitabine. *J Biol Chem* 2014;289:13054–65.
36 Geller LT, Barzily-Rokni M, Danino T, *et al*. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* 2017;357:1156–60.
37 Riquelme E, Zhang Y, Zhang L, *et al*. Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell* 2019;178:795–806.
38 Aykut B, Pushalkar S, Chen R, *et al*. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. *Nature* 2019;574:264–7.
39 Viaud S, Saccheri F, Mignot G, *et al*. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 2013;342:971–6.
40 Daillère R, Vétizou M, Waldschmitt N, *et al*. Enterococcus hirae and Barnesiella intestinihominis facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. *Immunity* 2016;45:931–43.
41 Cheng WY, Wu C-Y, Yu J. The role of gut microbiota in cancer treatment: friend or foe? *Gut* 2020;69:1867–76.
42 Lokody I. Bacterial balance affects cancer treatment. *Nat Rev Cancer* 2014;14:11.
43 Iida N, Dzutsev A, Stewart CA, *et al*. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 2013;342:967–70.
44 Roberti MP, Yonekura S, Duong CPM, *et al*. Chemotherapy-induced ileal crypt apoptosis and the ileal microbiome shape immunosurveillance and prognosis of proximal colon cancer. *Nat Med* 2020;26:919–31.
45 Bachem A, Makhlouf C, Binger KJ, *et al*. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated $CD8^+$ T cells. *Immunity* 2019;51:285–97.
46 He Y, Fu L, Li Y, *et al*. Gut microbial metabolites facilitate anticancer therapy efficacy by modulating cytotoxic $CD8^+$ T cell immunity. *Cell Metab* 2021;33:988–1000.
47 Yu T, Guo F, Yu Y, *et al*. Fusobacterium nucleatum promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell* 2017;170:548–63.
48 Shen S, Lim G, You Z, *et al*. Gut microbiota is critical for the induction of chemotherapy-induced pain. *Nat Neurosci* 2017;20:1213–6.
49 Dejea CM, Fathi P, Craig JM, *et al*. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* 2018;359:592–7.
50 Guenther M, Haas M, Heinemann V, *et al*. Bacterial lipopolysaccharide as negative predictor of gemcitabine efficacy in advanced pancreatic cancer - translational results from the AIO-PK0104 Phase 3 study. *Br J Cancer* 2020;123:1370–6.
51 Chamseddine AN, Ducreux M, Armand J-P, *et al*. Intestinal bacterial β-glucuronidase as a possible predictive biomarker of irinotecan-induced diarrhea severity. *Pharmacol Ther* 2019;199:1–15.
52 Teillant A, Gandra S, Barter D, *et al*. Potential burden of antibiotic resistance on surgery and cancer chemotherapy antibiotic prophylaxis in the USA: a literature review and modelling study. *Lancet Infect Dis* 2015;15:1429–37.
53 Imai H, Saijo K, Komine K, *et al*. Antibiotic therapy augments the efficacy of gemcitabine-containing regimens for advanced cancer: a retrospective study. *Cancer Manag Res* 2019;11:7953–65.
54 Sunakawa Y, Arai H, Izawa N. Antibiotics may enhance the efficacy of gemcitabine treatment for advanced pancreatic cancer. *Ann Oncol* 2018;29:viii251–2.
55 Nakano S, Komatsu Y, Kawamoto Y, *et al*. Association between the use of antibiotics and efficacy of gemcitabine plus nab-paclitaxel in advanced pancreatic cancer. *Medicine* 2020;99:e22250.
56 Pflug N, Kluth S, Vehreschild JJ, *et al*. Efficacy of antineoplastic treatment is associated with the use of antibiotics that modulate intestinal microbiota. *Oncoimmunology* 2016;5:e1150399.
57 Nenclares P, Bhide SA, Sandoval-Insausti H, *et al*. Impact of antibiotic use during curative treatment of locally advanced head and neck cancers with chemotherapy and radiotherapy. *Eur J Cancer* 2020;131:9–15.
58 Wu C, Lai R, Li J, *et al*. Antibiotics modulate chemotherapy efficacy in patients with esophageal cancer. *Cancer Manag Res* 2020;12:4991–7.
59 Zhao L, Xing C, Sun W, *et al*. Lactobacillus supplementation prevents cisplatin-induced cardiotoxicity possibly by inflammation inhibition. *Cancer Chemother Pharmacol* 2018;82:999–1008.
60 do Carmo FLR, Rabah H, Cordeiro BF, *et al*. Probiotic *Propionibacterium freudenreichii* requires SlpB protein to mitigate mucositis induced by chemotherapy. *Oncotarget* 2019;10:7198–219.
61 Xia C, Jiang C, Li W, *et al*. A phase II randomized clinical trial and mechanistic studies using improved probiotics to prevent oral mucositis induced by concurrent radiotherapy and chemotherapy in nasopharyngeal carcinoma. *Front Immunol* 2021;12:618150.
62 Reyna-Figueroa J, Barrón-Calvillo E, García-Parra C, *et al*. Probiotic supplementation decreases chemotherapy-induced gastrointestinal side effects in patients with acute leukemia. *J Pediatr Hematol Oncol* 2019;41:468–72.
63 Badgeley A, Anwar H, Modi K, *et al*. Effect of probiotics and gut microbiota on anti-cancer drugs: mechanistic perspectives. *Biochim Biophys Acta Rev Cancer* 2021;1875:188494.
64 Mego M, Chovanec J, Vochyanova-Andrezalova I, *et al*. Prevention of irinotecan induced diarrhea by probiotics: a randomized double blind, placebo controlled pilot study. *Complement Ther Med* 2015;23:356–62.
65 Tian Y, Li M, Song W, *et al*. Effects of probiotics on chemotherapy in patients with lung cancer. *Oncol Lett* 2019;17:2836–48.
66 Wei D, Heus P, van de Wetering FT. Probiotics for the prevention or treatment of chemotherapy- or radiotherapy-related diarrhoea in people with cancer. *Cochrane Database Syst Rev* 2018;2018:CD008831.
67 Chen H, Xia Y, Shi C. Effects of perioperative probiotics administration on patients with colorectal cancer. *Chin J Clin Nutr* 2014;22:74–81.
68 Yelin I, Flett KB, Merakou C, *et al*. Genomic and epidemiological evidence of bacterial transmission from probiotic capsule to blood in ICU patients. *Nat Med* 2019;25:1728–32.
69 Gibson GR, Hutkins R, Sanders ME, *et al*. Expert consensus document: the International scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 2017;14:491–502.
70 Taper HS, Roberfroid MB. Possible adjuvant cancer therapy by two prebiotics - inulin or oligofructose In Vivo 2005;4.
71 Taper HS, Roberfroid MB. Nontoxic potentiation of cancer chemotherapy by dietary oligofructose or inulin. *Nutr Cancer* 2000;38:1–5.
72 Mao Y, Nobaek S, Kasravi B, *et al*. The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. *Gastroenterology* 1996;111:334–44.
73 Mao Y, Kasravi B, Nobaek S, *et al*. Pectin-supplemented enteral diet reduces the severity of methotrexate induced enterocolitis in rats. *Scand J Gastroenterol* 1996;31:558–67.
74 So D, Whelan K, Rossi M, *et al*. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *Am J Clin Nutr* 2018;107:965–83.
75 David LA, Maurice CF, Carmody RN, *et al*. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559–63.
76 Garcia-Peris P, Velasco C, Hernandez M, *et al*. Effect of inulin and fructo-oligosaccharide on the prevention of acute radiation enteritis in patients with gynecological cancer and impact on quality-of-life: a randomized, double-blind, placebo-controlled trial. *Eur J Clin Nutr* 2016;70:170–4.
77 Xue H, Sawyer MB, Field CJ, *et al*. Nutritional modulation of antitumor efficacy and diarrhea toxicity related to irinotecan chemotherapy in rats bearing the ward colon tumor. *Clin Cancer Res* 2007;13:7146–54.
78 Holohan C, Van Schaeybroeck S, Longley DB, *et al*. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer* 2013;13:714–26.
79 Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020;20:651–68.
80 Decker WK, da Silva RF, Sanabria MH, *et al*. Cancer immunotherapy: historical perspective of a clinical revolution and emerging preclinical animal models. *Front Immunol* 2017;8:829.
81 Lau HCH, Sung JJ-Y, Yu J. Gut microbiota: impacts on gastrointestinal cancer immunotherapy. *Gut Microbes* 2021;13:1–21.
82 Couzin-Frankel J. Breakthrough of the year 2013 cancer immunotherapy. *Science* 2013;342:1432–3.
83 Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* 2017;17:271–85.
84 Hodi FS, O'Day SJ, McDermott DF, *et al*. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711–23.
85 Topalian SL, Hodi FS, Brahmer JR, *et al*. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366:2443–54.
86 Rosenberg JE, Hoffman-Censits J, Powles T, *et al*. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016;387:1909–20.
87 Robert C, Schachter J, Long GV, *et al*. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* 2015;372:2521–32.
88 Robert C, Long GV, Brady B, *et al*. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015;372:320–30.
89 Gide TN, Wilmott JS, Scolyer RA, *et al*. Primary and acquired resistance to immune checkpoint inhibitors in metastatic melanoma. *Clin Cancer Res* 2018;24:1260–70.
90 Martins F, Sofiya L, Sykiotis GP, *et al*. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat Rev Clin Oncol* 2019;16:563–80.
91 Wang DY, Salem J-E, Cohen JV, *et al*. Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis. *JAMA Oncol* 2018;4:1721–8.
92 Michot JM, Bigenwald C, Champiat S, *et al*. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer* 2016;54:139–48.

93 Vétizou M, Pitt JM, Daillère R, *et al*. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 2015;350:1079–84.
94 Sivan A, Corrales L, Hubert N, *et al*. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 2015;350:1084–9.
95 Pitt JM, Vétizou M, Gomperts Boneca I, *et al*. Enhancing the clinical coverage and anticancer efficacy of immune checkpoint blockade through manipulation of the gut microbiota. *Oncoimmunology* 2016;6:e1132137.
96 Wang F, Yin Q, Chen L, *et al*. Bifidobacterium can mitigate intestinal immunopathology in the context of CTLA-4 blockade. *Proc Natl Acad Sci U S A* 2018;115:157–61.
97 Sun S, Luo L, Liang W, *et al*. Bifidobacterium alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. *Proc Natl Acad Sci U S A* 2020;117:27509–15.
98 Verma R, Lee C, Jeun E-J, *et al*. Cell surface polysaccharides of *Bifidobacterium bifidum* induce the generation of Foxp3^{+} regulatory T cells. *Sci Immunol* 2018;3:aat6975. doi:10.1126/sciimmunol.aat6975
99 Mager LF, Burkhard R, Pett N, *et al*. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* 2020;369:1481–9.
100 Wang T, Gnanaprakasam JNR, Chen X, *et al*. Inosine is an alternative carbon source for CD8^{+}-T-cell function under glucose restriction. *Nat Metab* 2020;2:635–47.
101 Rizvi ZA, Dalal R, Sadhu S, *et al*. High-salt diet mediates interplay between NK cells and gut microbiota to induce potent tumor immunity. *Sci Adv* 2021;7:eabg5016. doi:10.1126/sciadv.abg5016
102 Si W, Liang H, Bugno J, *et al*. *Lactobacillus rhamnosus* GG induces cGAS/STING-dependent type I interferon and improves response to immune checkpoint blockade. *Gut* 2022;71:521–33.
103 Tanoue T, Morita S, Plichta DR, *et al*. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* 2019;565:600–5.
104 Huang C-H, Shen C-C, Liang Y-C, *et al*. The probiotic activity of Lactobacillus murinus against food allergy. *J Funct Foods* 2016;25:231–41.
105 Bessell CA, Isser A, Havel JJ, *et al*. Commensal bacteria stimulate antitumor responses via T cell cross-reactivity. *JCI Insight* 2020;5:135597. doi:10.1172/jci.insight.135597
106 Fluckiger A, Daillère R, Sassi M, *et al*. Cross-reactivity between tumor MHC class I-restricted antigens and an enterococcal bacteriophage. *Science* 2020;369:936–42.
107 Gil-Cruz C, Perez-Shibayama C, De Martin A, *et al*. Microbiota-derived peptide mimics drive lethal inflammatory cardiomyopathy. *Science* 2019;366:881–6.
108 Greiling TM, Dehner C, Chen X, *et al*. Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. *Sci Transl Med* 2018;10:aan2306. doi:10.1126/scitranslmed.aan2306
109 Li J, Jia H, Cai X, *et al*. An integrated catalog of reference genes in the human gut microbiome. *Nat Biotechnol* 2014;32:834–41.
110 Almeida A, Nayfach S, Boland M, *et al*. A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nat Biotechnol* 2021;39:105–14.
111 Li Y, Tinoco R, Elmén L, *et al*. Gut microbiota dependent anti-tumor immunity restricts melanoma growth in Rnf5$^{-/-}$ mice. *Nat Commun* 2019;10:1492.
112 Routy B, Le Chatelier E, Derosa L, *et al*. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018;359:91–7.
113 Matson V, Fessler J, Bao R, *et al*. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* 2018;359:104–8.
114 Gopalakrishnan V, Spencer CN, Nezi L, *et al*. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018;359:97–103.
115 Derosa L, Routy B, Thomas AM. Intestinal Akkermansia muciniphila predicts clinical response to PD-1 blockade in patients with advanced non-small-cell lung cancer. *Nat Med* 2022:1–10.
116 Peters BA, Wilson M, Moran U, *et al*. Relating the gut metagenome and metatranscriptome to immunotherapy responses in melanoma patients. *Genome Med* 2019;11:61.
117 Frankel AE, Coughlin LA, Kim J, *et al*. Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. *Neoplasia* 2017;19:848–55.
118 Chaput N, Lepage P, Coutzac C, *et al*. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol* 2017;28:1368–79.
119 Blacher E, Levy M, Tatirovsky E, *et al*. Microbiome-Modulated metabolites at the interface of host immunity. *J Immunol* 2017;198:572–80.
120 Costea PI, Zeller G, Sunagawa S, *et al*. Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol* 2017;35:1069–76.
121 Knight R, Vrbanac A, Taylor BC, *et al*. Best practices for analysing microbiomes. *Nat Rev Microbiol* 2018;16:410–22.
122 Gharaibeh RZ, Jobin C. Microbiota and cancer immunotherapy: in search of microbial signals. *Gut* 2019;68:385–8.
123 Limeta A, Ji B, Levin M, *et al*. Meta-analysis of the gut microbiota in predicting response to cancer immunotherapy in metastatic melanoma. *JCI Insight* 2020;5:140940. doi:10.1172/jci.insight.140940
124 Killock D. Immunotherapy: gut bacteria modulate responses to PD-1 blockade. *Nat Rev Clin Oncol* 2018;15.
125 Derosa L, Hellmann MD, Spaziano M, *et al*. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann Oncol* 2018;29:1437–44.
126 Pinato DJ, Howlett S, Ottaviani D, *et al*. Association of prior antibiotic treatment with survival and response to immune checkpoint inhibitor therapy in patients with cancer. *JAMA Oncol* 2019;5:5.
127 Tinsley N, Zhou C, Tan G, *et al*. Cumulative antibiotic use significantly decreases efficacy of checkpoint inhibitors in patients with advanced cancer. *Oncologist* 2020;25:55–63.
128 Wilson BE, Routy B, Nagrial A, *et al*. The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies. *Cancer Immunol Immunother* 2020;69:343–54.
129 Palleja A, Mikkelsen KH, Forslund SK, *et al*. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat Microbiol* 2018;3:1255–65.
130 Derosa L, Routy B, Fidelle M, *et al*. Gut bacteria composition drives primary resistance to cancer immunotherapy in renal cell carcinoma patients. *Eur Urol* 2020;78:195–206.
131 Hakozaki T, Richard C, Elkrief A, *et al*. The gut microbiome associates with immune checkpoint inhibition outcomes in patients with advanced non-small cell lung cancer. *Cancer Immunol Res* 2020;8:1243–50.
132 Pushalkar S, Hundeyin M, Daley D, *et al*. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov* 2018;8:403–16.
133 Jacouton E, Chain F, Sokol H, *et al*. Probiotic strain *Lactobacillus casei* BL23 prevents colitis-associated colorectal cancer. *Front Immunol* 2017;8:1553.
134 Hu J, Wang C, Ye L, *et al*. Anti-tumour immune effect of oral administration of Lactobacillus plantarum to CT26 tumour-bearing mice. *J Biosci* 2015;40:269–79.
135 Hibberd AA, Lyra A, Ouwehand AC, *et al*. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open Gastroenterol* 2017;4:e000145.
136 Spencer CN, Gopalakrishnan V, McQuade J. Abstract 2838: the gut microbiome (GM) and immunotherapy response are influenced by host lifestyle factors. *Cancer Res* 2019;79:2838.
137 Spencer CN, McQuade JL, Gopalakrishnan V, *et al*. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science* 2021;374:1632–40.
138 Azcarate-Peril MA, Ritter AJ, Savaiano D, *et al*. Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals. *Proc Natl Acad Sci U S A* 2017;114:E367–75.
139 Dewulf EM, Cani PD, Claus SP, *et al*. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112–21.
140 Richard C, Benlaifaoui M, Ouarzadi OE. 679 high fiber diet modifies gut microbiome, propionate production, intratumor immune response and is associated with outcome in patients with lung cancer treated with immune checkpoint inhibitors. *J Immunother Cancer* 2020;8.
141 Ferrere G, Tidjani Alou M, Liu P, *et al*. Ketogenic diet and ketone bodies enhance the anticancer effects of PD-1 blockade. *JCI Insight* 2021;6:145207. doi:10.1172/jci.insight.145207
142 Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. *Nat Rev Gastroenterol Hepatol* 2012;9:88–96.
143 Wang Y, Wiesnoski DH, Helmink BA, *et al*. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med* 2018;24:1804–8.
144 Baruch EN, Youngster I, Ben-Betzalel G, *et al*. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* 2021;371:602–9.
145 Davar D, Dzutsev AK, McCulloch JA, *et al*. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* 2021;371:595–602.
146 Woelk CH, Snyder A. Modulating gut microbiota to treat cancer. *Science* 2021;371:573–4.
147 Jardim DL, Goodman A, de Melo Gagliato D, *et al*. The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell* 2021;39:154–73.
148 Nakatsu G, Zhou H, Wu WKK, *et al*. Alterations in enteric Virome are associated with colorectal cancer and survival outcomes. *Gastroenterology* 2018;155:529–41.
149 Coker OO, Wu WKK, Wong SH, *et al*. Altered gut archaea composition and interaction with bacteria are associated with colorectal cancer. *Gastroenterology* 2020;159:1459–70.
150 Coker OO, Nakatsu G, Dai RZ, *et al*. Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer. *Gut* 2019;68:654–62.
151 Song HJ, Shim K-N. Current status and future perspectives of capsule endoscopy. *Intest Res* 2016;14:21–9.
152 Zheng D-W, Dong X, Pan P, *et al*. Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. *Nat Biomed Eng* 2019;3:717–28.
153 Gurbatri CR, Lia I, Vincent R, *et al*. Engineered probiotics for local tumor delivery of checkpoint blockade nanobodies. *Sci Transl Med* 2020;12. doi:10.1126/scitranslmed.aax0876. [Epub ahead of print: 12 02 2020].
154 Chowdhury S, Castro S, Coker C, *et al*. Programmable bacteria induce durable tumor regression and systemic antitumor immunity. *Nat Med* 2019;25:1057–63.

155 Suez J, Zmora N, Zilberman-Schapira G, *et al*. Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell* 2018;174:1406–23.

156 McGovern BH, Ford CB, Henn MR, *et al*. SER-109, an investigational microbiome drug to reduce recurrence after Clostridioides difficile infection: lessons learned from a phase 2 trial. *Clin Infect Dis* 2021;72:2132–40.