MICROBIOME

Anticancer effects of the microbiome and its products

Laurence Zitvogel[1–4], Romain Daillère[1–3], María Paula Roberti[1–3], Bertrand Routy[1–3] and Guido Kroemer[4–10]

Abstract | The human gut microbiome modulates many host processes, including metabolism, inflammation, and immune and cellular responses. It is becoming increasingly apparent that the microbiome can also influence the development of cancer. In preclinical models, the host response to cancer treatment has been improved by modulating the gut microbiome; this is known to have an altered composition in many diseases, including cancer. In addition, cancer treatment with microbial agents or their products has the potential to shrink tumours. However, the microbiome could also negatively influence cancer prognosis through the production of potentially oncogenic toxins and metabolites by bacteria. Thus, future antineoplastic treatments could combine the modulation of the microbiome and its products with immunotherapeutics and more conventional approaches that directly target malignant cells.

Microbiome
The collective genomes that can be found within a single microbial ecosystem.

Microbiota
The community of microorganisms that exist within a single ecosystem.

Metabolic syndrome
A syndrome characterized by central obesity, dyslipidaemia, increased blood pressure and high blood-sugar levels, increased risk of type 2 diabetes and cardiovascular disease.

Immunosurveillance
A term that is used to describe the processes by which cells of the immune system hunt and target pathogens, such as bacteria and viruses, or pre-cancerous and cancerous host cells.

Correspondence to L.Z. and G.K.
laurence.zitvogel@gustaveroussy.fr; kroemer@orange.fr

doi:10.1038/nrmicro.2017.44
Published online 22 May 2017

The link between cancer and microorganisms is well established, and as much as 20% of the global cancer burden has been estimated to be caused by microbial agents[1]. For example, the pathogens *Helicobacter pylori*, *Fusobacterium nucleatum*, Epstein–Barr virus (EBV) and human papilloma virus (HPV) are all associated with cancer. Humans are also colonized by various commensal microorganisms, which form the microbiome (BOX 1). The microbiota can influence human health by preventing the growth of pathogens, producing beneficial microbial products and metabolizing nutrients and toxins. In the past decade, substantial progress has been made in our understanding of cancer development and the influence that the microbiota has on related host processes. The risk factors for cancer are similar to those for obesity, cardiovascular disease and type 2 diabetes, with the most important factor being ageing[2,3]. Interestingly, these and other diseases have been shown to be markedly influenced by the microbiome[4,5]. In this context, it is also important to note that alterations in the microbiome that favour metabolic syndrome are also risk factors for the development of certain cancers. Although most research has focused on the relationship between the intestinal microbiota and obesity, there is growing awareness that the microbiome influences oncogenesis and tumour progression, in part through inflammatory and immune circuits. The relationship between the gut microbiome (BOX 1) and cancer is multifactorial and most likely bidirectional; cancer-associated changes in the microbiome may occur as a result of the disease but may also contribute to cancer progression.

Cancer development can affect the microbiome through several mechanisms. Cancer can only develop and progress in the context of failed immunosurveillance[6] and is often associated with systemic immunosuppressive effects, which can alter the microbiota. Furthermore, cancer can affect host metabolism, and this also can perturb the gut microbiome, as discussed below. In addition, tumour growth may cause local disruption of barriers, resulting in focal invasion by microorganisms. Conversely, alterations in the microbiome may affect oncogenesis and tumour progression at multiple levels. First, by direct oncogenic effects of microorganisms or their products. Second, by microbiota-mediated alterations in circulating metabolites that favour tumour growth. Third, by favouring the generation of trophic factors, such as growth factors. Fourth, by inducing pro-inflammatory and immunosuppressive effects that may subvert anticancer immunosurveillance. Thus, the microbiota can contribute to the development of malignant disease through several mechanisms.

In this Review, we summarize what is known about the relationship between the microbiome and cancer. We investigate the effect of microbiome manipulation on cancer and then examine the possibility of therapeutically using microbial agents or their products.

The microbiome in cancer

Several studies have reported an altered composition of local microbiota in cancer. For example, oral, bronchial, intestinal and vaginal microbiotas are altered in head and neck, lung, colorectal and cervical carcinomas, respectively[7–9] (TABLE 1).

Pattern recognition receptors
(PRRs). Innate immune components expressed by various cell types to sense infection or tissue damage.

Toll-like receptors
Pattern recognition receptors that mostly recognize bacterial structures.

Immune-checkpoint blockade
A pharmacological intervention whereby monoclonal antibodies neutralize major inhibitory receptors (such as cytotoxic T lymphocyte protein 4 (CTLA4) and programmed cell death 1 (PD1)) expressed by activated lymphocytes to alleviate immune suppression and restore lymphocyte effector functions.

In addition, recent studies demonstrated that anticancer therapies can influence the gut microbiome (BOX 1), which, in turn, affects treatment outcome.

Associations versus cause–effect relationships. One recurrent problem involves understanding the cause–effect relationships (by animal experimentation) of epidemiological associations (which are mere correlations). Possible associations between exposure to antibiotics (which disrupts the microbiome) and an increased risk of cancer have been reported[10]. Furthermore, the advent of next-generation sequencing and modern bioinformatics has led to a large increase in studies on associations between the gut microbiome, cancer development and the response to cancer treatment[5,11] (FIG. 1). However, such associations are difficult to interpret without *in vivo* experimental evidence for several reasons.

First, it is difficult to elucidate whether changes in the microbiota contribute to cancer or vice versa. This is because cancers may disrupt the local microbiome[7–9,12,13], but they could also act at distance through soluble factors, such as CC-chemokine ligand 25 (CCL25)[14], or general metabolic effects[15], to influence the gut microbiome. Indeed, we observed that subcutaneous injection of transplantable cancer cell lines had a profound effect on the gut microbiome in mice (REF. 16; L.Z., R.D., M.P.R., B.R. and G.K., unpublished observations).

Second, it can be difficult to tease apart the relationship between cancer development and alterations in the microbiome because both can be triggered by the same overarching cause. For example, the host immune system or lifestyle choices may influence both the microbiome and cancer development. Genetic variation in pattern recognition receptors (PRRs), such as Toll-like receptors (for example, Toll-like receptor 3 (TLR3), TLR4 and TLR5), can modulate anticancer immunosurveillance and the immune response to the microbiome.

Third, although progress has been made in the deconvolution of deep-sequencing data, such data rarely enable the identification of individual microbial species, strains or strain sequence variants[17]. Consequently, current associations are often limited to correlations between disease parameters and phyla and genera, not individual microbial species. Current analyses may not have high enough resolution to identify oncogenic and oncosuppressive species and subspecies. This obstacle could be overcome by improved isolation and culture methods[18].

Fourth, in the context of cancer, microbial genomes have been detected in tissues and body fluids that are usually sterile (such as plasma or cerebrospinal fluid)[19,20]. It is unclear whether the detection of these genomes is biologically relevant or an issue of contamination.

Despite these uncertainties, multiple associations have been reported between the abundance of specific bacterial phyla and species in distinct cancer-associated locations[8,21,22] (FIG. 1; TABLE 1). However, most of these studies have a single patient cohort, and therefore cannot be used to make general predictions on the relationship between bacteria and cancer development, progression and therapeutic responses.

Treatment-associated shifts in the microbiome. It has been shown that certain anticancer therapies can cause shifts in the gut microbiome, which may then affect treatment outcome. For example, tumour-bearing mice that were treated with the chemotherapeutic cyclophosphamide (CTX) demonstrated the translocation of *Enterococcus hirae* and *Lactobacillus johnsonii* through the intestinal barrier and into lymphoid organs[16,23]. Furthermore, injections of ipilimumab (an immune-checkpoint blockade monoclonal antibody that targets cytotoxic T lymphocyte protein 4 (CTLA4)) led to shifts in the gut microbiome in patients with melanoma. These alterations in the microbiome were associated with increased anti-melanoma immunotherapeutic treatment efficacy but also with gastrointestinal toxicity in patients treated with ipilimumab[24,25].

Importantly, both chemotherapeutics and immunotherapeutics have been shown to lose their capacity to reduce tumour growth in germ-free mice[16,24,26,27]. The negative effects of the removal of the microbiome are as substantial as those of eliminating cytotoxic T lymphocytes (CTLs), which are protective in many types of cancer[16,24,26,27]. Importantly, in germ-free animals, innate and adaptive immune responses (including tumour-specific CTL responses) were reduced compared with littermates reared in specific-pathogen-free conditions. Collectively, these observations suggest that the microbiota is required for a functional host immune system, and that this may be linked to the capacity of the microbiome to determine the activity of chemotherapeutic and immunotherapeutic agents.

Microbiome modifications and cancer

There are known associations between certain microbiome profiles and the development and progression of cancer. Therefore, interventions that change the composition of the microbiome may affect oncogenesis (FIG. 1). The microbiome can remain stable for years but also undergoes permanent changes in response to antibiotic treatment, pathogen exposure, fasting, altered dietary composition and other factors, such as cold stress or perturbations of diurnal rhythms[28–30]. In addition, the microbiome has been reported to affect various traits that range from metabolism to mood[31]. Thus,

Author addresses

[1]Institut de Cancérologie Gustave Roussy Cancer Campus (GRCC), 114 rue Edouard Vaillant, Villejuif 94805, France.
[2]Institut National de la Santé Et de la Recherche Medicale (INSERM), U1015, GRCC, Villejuif 94805, France.
[3]University of Paris-Saclay, Kremlin Bicêtre 94270, France.
[4]Center of Clinical Investigations CIC1428, GRCC, Villejuif 94805, France.
[5]Equipe 11 labelisée par la Ligue Nationale contre le Cancer, INSERM U1138, Centre de Recherche des Cordeliers, Paris 75006, France.
[6]University of Paris Descartes, Sorbonne Paris Cité, Paris 75006, France.
[7]University of Pierre et Marie Curie, Paris 75006, France.
[8]Pôle de Biologie, Hôpital Européen Georges Pompidou, Paris 75015, France.
[9]Metabolomics and Cell Biology Platforms, GRCC, Villejuif 94805, France.
[10]Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm 17176, Sweden.

Box 1 | **The gut microbiome as a complex ecosystem**

The human gut microbiome is a dynamic and complex ecosystem that contains many different types of microorganism. Constant peristalsis moves portions of the microbiome through chemically distinct microenvironments. These range from a low pH environment in the stomach, to the bile salt-enriched jejunum and the alkaline surroundings of the colon. The dynamic and changing nature of the gut leads to variation in the density and composition of the microbiome along longitudinal[117] and transverse gradients. As such, distinct micro-ecosystems reside in different locations of the gut, including the lumen, the mucosa and intestinal crypts[118,119].

Similar to other complex ecosystems, the gut microbiome is characterized by intricate population dynamics. These are shaped by antagonistic relationships between species owing to competition for resources, predator–prey dynamics, chains of infection between bacteriophages, bacteria and eukaryotes, and the secretion of toxins by bacteria to inhibit or kill other bacteria. Secreted toxins are either soluble (and considered to be antibiotics) or are directly injected through secretion systems from one species into another[120]. In addition, distinct microbial species can cooperate with each other for mutual gains. This can occur by different species mediating distinct catabolic reactions required to digest mixtures of nutrients or by cross-feeding (one species produces metabolites that are required by another species). As well as interacting with other microorganisms, commensal bacteria interact with the human host, often by reducing unnecessary immune reactions[52]. To protect itself from systemic invasion by gut microorganisms, the host produces mucus that keeps bacteria at a safe distance from the gut epithelium. This mucus forms a tight, impenetrable structure in the colon and provides a platform for the diffusion of antimicrobial peptides (AMPs) and immunoglobulin A (IgA) in the small intestine[52].

Together, anatomical diversification and complex antagonistic and cooperative population networks create a resilient ecosystem that can remain stable over several years[121]. However, major perturbations, such as treatment with broad-spectrum antibiotics, inflammation, infections and disease, as well as long-lasting changes in diet, may cause permanent changes in the ecosystem.

interventions on the microbiome remain an exciting prospect for many therapeutic areas, including cancer treatment.

Faecal microbiota transplantation. Faecal microbiota transplantation (FMT) is an experimental approach that involves exchanging the gut microbiota between individuals. So far, FMT has been used to treat infection with *Clostridium difficile* and has been tested in the treatment of inflammatory bowel disease and obesity in humans[29,32]. Furthermore, FMT has been successfully used in a clinical pilot study to treat graft-versus-host disease of the gut occurring after allogeneic stem cell transplantation[33]. Preclinical work in mice has suggested that FMT may reduce colorectal carcinogenesis[34]. However, it remains unclear whether FMT could reduce carcinogenesis and tumour progression in humans. Compared with other manipulations of the microbiome, FMT has the advantage of an entire, balanced ecosystem (BOX 1) being transferred from one individual to another, which increases the chance of obtaining a long-term reset of the microbiome.

Antibiotics. Epidemiological studies have suggested that repeated exposure to broad-spectrum antibiotics predisposes humans to develop certain cancers[11]. The underlying mechanisms are unknown; however, they may range from defects in immunosurveillance (caused by irregularities in immunostimulatory bacterial products)[11] to metabolic and endocrine abnormalities[35]. This is evidenced by a study that suggested that antibiotic-induced changes in the microbiome may affect the metabolism of sex hormones, such as oestrogen, thereby influencing the risk of breast cancer[35].

The effects of antibiotic modulation of the microbiome during cancer treatments are complex. Oral treatment with the antibiotic vancomycin, which mostly eliminates Gram-positive bacteria, improves the outcome of cancer immunotherapy that targets CTLA4, presumably by inducing an expansion of immunogenic, Gram-negative bacteria of the order Bacteroidales at the expense of members of the Clostridiales[24]. The expanded population of bacteria in the Bacteroidales triggers type 1 T helper (T_H1) immune responses, which increases the antitumour efficacy of CTLA4 blockade (FIG. 2). Conversely, vancomycin negatively affects the induction of cyclophosphamide-triggered anticancer immune responses in mice[16]. This suggests that antibiotic-mediated effects are context dependent and can be either beneficial or harmful.

Gut-resident bacteria can also produce toxins that have antimicrobial activity, providing them a selective advantage over other species[36]. It is possible that these natural antibiotics, which have narrow activity ranges (contrasting with clinically used broad-spectrum antibiotics), could be used to eliminate harmful microbial species[37]. However, this approach is far from preclinical evaluation.

A different approach may enable the modulation of the carcinogenic potential of the microbiome. One particular strategy may consist of the use of pharmacological agents that block the production of potentially oncogenic bacterial products. For example, small-molecule inhibitors can target the serine protease ClbP, which is required for the secretion of colibactin — a genotoxic, potentially oncogenic compound that is produced by *Escherichia coli*[38,39]. Another strategy involves inhibiting bacterial enzymes that convert anticancer agents into toxic products. For example, inhibiting β-glucuronidases (produced by *E. coli*, *Bacteroides* spp. and *Clostridium perfringens*) prevents the intestinal reactivation of inactive glucuronidated irinotecan metabolites, thereby preventing the generation of a toxic product[40,41].

Therefore, although it seems advisable to avoid repeated exposure to broad-spectrum antibiotics, it may be useful to rationally manipulate the composition of the microbiome using specific antibiotics, or probiotic or prebiotic formulations.

Prebiotics. Prebiotics induce the growth or activity of health-promoting microorganisms in the gut[42]. Most of the literature focuses on natural dietary fibre, but prebiotics can also be administered as chemically defined agents such as *trans*-galactooligosaccharide and inulin.

Non-digestible polysaccharides, which are metabolized by bacteria to short-chain fatty acids (SCFAs), increase the abundance of *Bifidobacterium* spp.[27] that reportedly reduce tumour growth, notably in the context of programmed cell death 1 (PD1) blockade.

Faecal microbiota transplantation
The engraftment of microbiota from a healthy donor into a recipient, which results in the restoration of the normal gut microbial ecosystem.

Graft-versus-host disease
An immune attack of transplanted lymphocytes against host cells, which causes systemic disease following the transfusion of cells from a donor that has distinct histocompatibility antigens.

Probiotic
A live microorganism that can confer a health benefit to the host.

Prebiotic
A non-digestible food ingredient that stimulates the growth and activity of bacteria in the digestive system.

Table 1 | **Epidemiological associations between commensal microorganisms and cancer**

Cancer type	Sample size	Analysed specimen	Bacterial identification	Microbial composition alteration	Association	Refs
HNSCC	19 cases, 25 controls	Saliva and tumour samples	16S rRNA V3–V5	*Streptococcus* spp., *Dialister* spp., *Veillonella* spp., *Neisseria* spp., *Aggregatibacter* spp., *Haemophilus* spp. and *Leptotrichia* spp.	Saliva samples had a higher abundance* of *Streptococcus* spp., *Dialister* spp. and *Veillonella* spp. Tumour samples had a lower abundance‡ of *Neisseria* spp., *Aggregatibacter* spp., *Haemophilus* spp. and *Leptotrichia* spp.	12
ALL	51 cases, 51 controls	Faeces	16S rRNA V1–V3	*Anaerostipes* spp., *Coprococcus* spp., *Roseburia* spp. and *Ruminococcus* spp.	Lower abundance‡ of all of these taxa	123
Pancreatic cancer	361 cases, 371 controls	Oral wash	16S rRNA V3–V4	*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Leptotrichia* spp.	Higher abundance* of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Lower abundance‡ of *Leptotrichia* spp.	124
CCA	28 *Opisthorchis viverrini*-associated cases, 32 *O. viverrini*-non-associated CCA cases	Human bile duct tissue	16S rRNA V3–V6	*Stenotrophomonas* spp., Bifidobacteriaceae, Enterobacteriaceae and Enterococcaceae associated with *O. viverrini* fluke colonization	Higher abundance* of *Stenotrophomonas* spp. in *O. viverrini*-non-associated CCA	125
HPV-associated cervical cancer	340 cases, 90 controls	Cervical mucus	16S rRNA V4	*Lactobacillus iners* and unclassified *Lactobacillus* spp.	Higher abundance* of *L. iners* and unclassified *Lactobacillus* spp.	13
Breast cancer	25 cases, 23 controls	Nipple aspirated fluid	16S rRNA V4	*Alistipes* spp. and Sphingomonadaceae	Higher abundance* of *Alistipes* spp. Lower abundance‡ of an unclassified genus in the Sphingomonadaceae family	21
Biliary tract cancers	64 biliary cancer cases, 122 liver cancer cases, 224 controls	Serum	Serology: multiplex assay against 15 *Helicobacter pylori* proteins	*H. pylori*	Higher seropositivity for *H. pylori* in patients with cancer	126
Urothelial cancer	8 cases, 6 controls	Urine	16S rRNA	*Streptococcus* spp.	Higher abundance* of *Streptococcus* spp. Higher abundance* of *Pseudomonas* spp. or *Anaerococcus* spp. when *Streptococcus* spp. abundance was low	127
Oral cancer	32 cases, 35 controls	Oral cancer swab compared with mouth swab	16 sRNA V4	*Streptococcus* spp. and *Rothia* spp.	Lower abundance‡ of *Streptococcus* spp. and *Rothia* spp.	128
Lung cancer	8 cases, 8 controls	Sputum and buccal samples	16S rRNA V1–V2	*Granulicatella* spp., *Abiotrophia* spp. and *Streptococcus* spp.	Higher abundance* of *Granulicatella* spp., *Abiotrophia* spp. and *Streptococcus* spp.	129

*Higher abundance in cases compared with controls. ‡Lower abundance in cases compared with controls. ALL, acute lymphoblastic leukaemia; CCA, cholangiocarcinoma; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; rRNA, ribosomal RNA; V, variable region.

Importantly, diets that incorporate non-digestible polysaccharides mediate anti-inflammatory effects through SCFAs and reduce the risk of colorectal carcinogenesis[43]. Intriguingly, non-absorbable apple procyanidins decreased the Firmicutes/Bacteroidetes ratio and increased the proportion of *Akkermansia muciniphila,* while inhibiting diet-induced obesity in mice[44]. Whether these effects are linked to the anti-cancer properties of apples remains to be determined[45]. The use of prebiotics (such as oligofructose and inulin) as adjuvants to cytotoxic cancer drugs (such as anthracyclines, antimetabolites and vincaloids) increased the antitumour effects in, and the lifespan of, treated mice[46,47]. Further research is needed to confirm the use of prebiotics to change the microbiome and treat cancer.

The anti-diabetes drug metformin, which could be classed as a prebiotic, has a cancer-preventive effect, which may be linked to its capacity to affect the composition and function of the gut microbiome. Metformin extends the lifespan of *Caenorhabditis elegans* by inhibiting methionine metabolism by commensal *E. coli* and affecting folate metabolism[48]. Furthermore, in mice, metformin can increase the abundance of *A. muciniphila*[49]. Further research is required to confirm whether the anticancer effects of metformin are caused by its ability to modulate the microbiome.

Caloric restriction. Caloric restriction extends the lifespan of model organisms and reduces the incidence of cancer in mice and non-human primates[50]. Caloric restriction

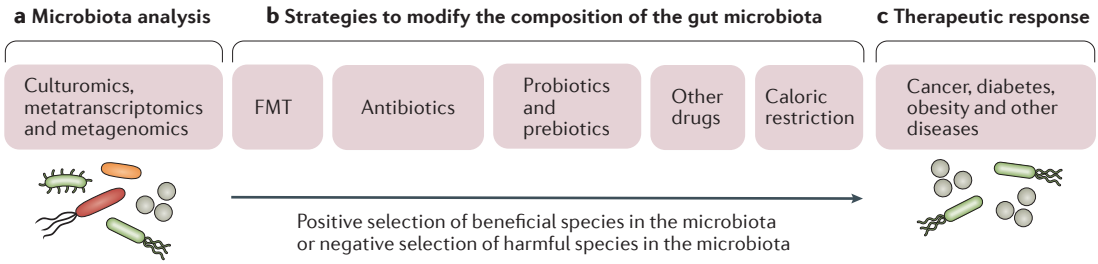

Figure 1 | **Interventions on the microbiota in cancer. a** | Determining the composition of the microbiota in patients with cancer compared with healthy volunteers is becoming feasible with the development of metagenomics, metatranscriptomics and culturomics platforms. Data from these analyses can together build a picture of the microbiota in health and disease, and indicate which bacterial genera or species could be beneficial to patients. **b** | Interventional approaches that could modulate the microbiota in cancer include faecal microbiota transplantation (FMT), antibiotic regimens, prebiotic and/or probiotic formulations, other types of drug (such as the diabetes drug metformin) and dietary-based interventions, such as caloric restriction. **c** | The outcome of microbiota interventions can be evaluated by monitoring the response to standard cancer therapeutics. In addition, microbiota interventions may influence the outcome of other diseases, such as diabetes or obesity.

causes a reduction in the Firmicutes/Bacteroidetes ratio[51], as well as the enrichment of *A. muciniphila* in humans[49]. In mice, starvation favours fucosylation of the intestinal epithelium, thus providing nutrients to commensal bacteria and reducing the probability of pathogenic invasion[52]. Cyclic, short-term fasting also improves anticancer immunosurveillance in mice, presumably through the induction of autophagy in malignant cells and by systemic immunostimulation[53,54]; it is unclear whether starvation-induced changes in the composition or function of the microbiome contribute to these beneficial effects.

Microbial agents for cancer treatment

In 1946, a partially successful anticancer treatment attempt was made using intratumourally injected *Streptococcus pyogenes* and *Serratia marcescens*[55]. Since then, several microbial agents have been tested as cancer therapeutics in human and mouse preclinical models (TABLES 2,3). In 1990, *Mycobacterium bovis* bacille Calmette–Guérin (BCG) was approved for the treatment of superficial bladder cancer. After removal of the tumour, BCG is instilled into the bladder[56], where it induces a local immune response that reduces the probability of relapse[57,58]. No other bacteria have reached clinical approval thus far.

Probiotics. Probiotics are live microorganisms that can confer health benefits. They reinforce natural defences, protect against gastrointestinal disorders and pathogens, and enhance innate and adaptive immunity. Several probiotics may mediate immunomodulatory and anticancer activities in different contexts (FIG. 2). Thus far, it has not been determined whether potentially antineoplastic bacteria may be combined to create an ecosystem with broad antitumour activities.

Lactobacillus spp., which belong to the group of lactic acid bacteria, are prominent probiotic organisms. Numerous reports have shown that different isolates of *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* GG, and *Lactobacillus acidophilus* may mediate anticancer effects through various mechanisms, such as natural killer cell activation, dendritic cell maturation or probiotic-derived ferrichrome (an iron-scavenging peptide) release[59–68]. *L. casei* probiotic-derived ferrichrome may exert a cancer-specific tumour-suppressive effect through the apoptosis-inducing JNK signalling pathway[64]. Furthermore, continuous administration of *Lactobacillus reuteri* strain ATCC-PTA-6475 to tumour-prone mice for several months reduces the frequency of intestinal pre-cancerous lesions. *L. reuteri* also reduces sarcopenia and thymus atrophy in ageing tumour-free mice, which suggests that it has a broad beneficial effect[69].

Moreover, Prohep, a mixture of *L. rhamnosus* GG, *E. coli* Nissle 1917 and heat-inactivated VSL#3, was orally administered to mice and prevented the progression of subcutaneous hepatocellular carcinoma in mice by inducing potent anti-angiogenic effects and reducing inflammation. Prohep shifted the composition of the gut microbial community towards *Prevotella* spp. and *Oscillibacter* spp., specifically enriching *Bacteroides fragilis*, *Alistipes shahii*, *Parabacteroides distasonis* and *A. muciniphila* species. These species were associated with a reduction of pro-inflammatory T_H17 cell polarization and concomitant differentiation of anti-inflammatory T_{reg} cells (regulatory T cells) and/or Tr1 cells (T regulatory type 1 cells) in the gut, as well as the production of anti-inflammatory metabolites[70] (FIG. 2).

Immunotherapy using antibodies against interleukin-10 receptor (anti-IL-10R) with CpG oligodeoxynucleotides was correlated with the overrepresentation of *A. shahii* in the faeces of mice with colon cancer (TABLE 3). Furthermore, the inoculation of antibiotic-pretreated mice with *A. shahii* improved the immunotherapeutic response against subcutaneous colon cancers. In this model, *A. shahii* increased the production of tumour necrosis factor (TNF) by intratumoural myeloid cells, and the neutralization of TNF abolished the therapeutic effect[26]. Thus, *A. shahii* may improve the response of innate immune cells to immunotherapy (FIG. 2).

Enterotoxigenic *B. fragilis* has been shown to elicit pro-inflammatory T_H17 cells, thereby accelerating carcinogenesis in tumour-prone mice[71]. However,

Autophagy
A mechanism of lysosomal degradation that enables the degradation and recycling of cytoplasmic material sequestered in autophagosomes.

Sarcopenia
The degenerative loss of skeletal muscle mass, quality and strength associated with ageing, frailty syndrome and/or cachexia.

Thymus atrophy
An age-dependent reduction in thymic mass that may be accelerated in pathological conditions.

T_H17 cell
(T helper 17 cell). A $CD4^+$ T helper cell induced by the coordinated action of transforming growth factor-β (TGFβ) and interleukin-6 (IL-6), to activate the transcription factor retinoid-related orphan nuclear receptor-γt (RORγt) and to produce IL-17 and IL-22.

Tr1 cells
(T regulatory type 1 cells) $CD4^+$ T regulatory type 1 cells that produce large amounts of interleukin-10 (IL-10) through IL-10R signalling, and induce an anti-inflammatory response.

non-enterotoxin-producing strains of *B. fragilis* may have anticancer properties in the context of anti-CTLA4 immunotherapy[24]. After blockade of this immune checkpoint, *B. fragilis* is overrepresented in the ileum and induces a T cell memory response. Importantly, anti-CTLA4 immunotherapy fails to reduce the growth of subcutaneous sarcomas and colon cancers in germ-free or antibiotic-treated mice. This defect can be overcome by treatment with *B. fragilis* and the adoptive transfer of $CD4^+$ T cells that have been primed (that is, incubated with *B. fragilis*-pulsed dendritic cells). This mechanism could be explained by the observation that *B. fragilis* can stimulate the production of IL-12 by bone marrow-derived dendritic cells *in vitro*[24]. Moreover, neutralization of IL-12 prevented the anticancer effects of *B. fragilis* in the context of CTLA4 blockade *in vivo*. Interestingly, tumours from mice that were exclusively colonized with *B. fragilis* exhibited a more mature dendritic cell phenotype in the tumour infiltrate post-immunotherapy compared with germ-free control mice[24]. The cell wall of *B. fragilis* contains the immunostimulatory polysaccharide A (PSA), which can

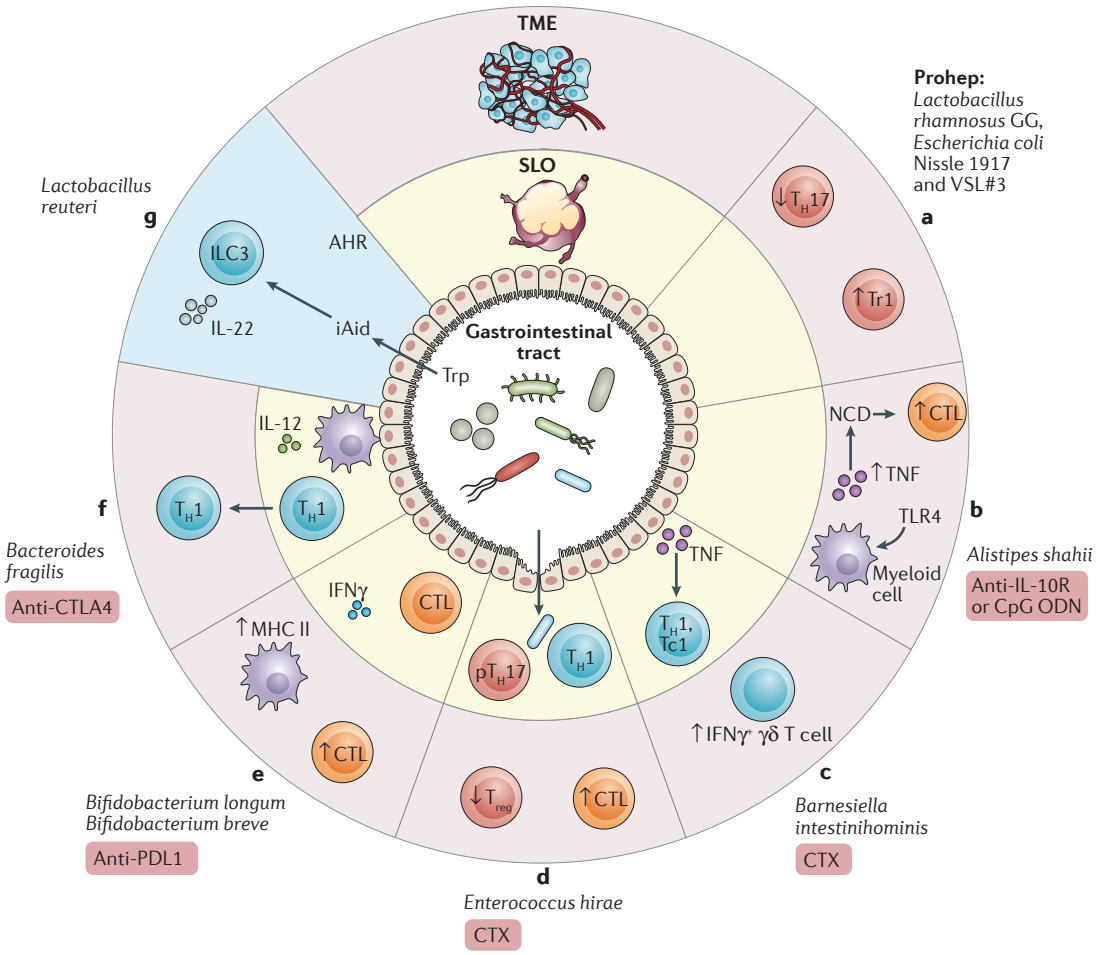

Figure 2 | **Potential immune mechanisms that explain the anticancer effects of probiotics.** Probiotic microorganisms may shape the tumour microenvironment by inducing several effects described here. **a** | Prohep may induce a reduction of pro-inflammatory T helper 17 cells (T_H17 cells) and the differentiation of regulatory T cells (T_{reg} cells) to T regulatory type 1 cells (Tr1 cells). **b** | In antibiotic-pretreated mice, *Alistipes shahii* increases the number of infiltrating innate immune cells against colorectal cancer by triggering tumour necrosis factor (TNF)-mediated necrotic cell death (NCD). **c,d** | Alternatively, microorganisms may act in secondary lymphoid organs, inducing splenic polyfunctional $CD4^+$, $CD8^+$ or γδ T cells, and bacteria-specific $CD4^+$ T_H1 or pathogenic T_H17 (pT_H17) cells[23]. Consequently, they modulate innate and adaptive immune responses in the tumour beds[88]. **e,f** | Following immune checkpoint blockades, *Bifidobacterium* spp. and *Bacteroides fragilis* promote maturing intratumoural dendritic cells and the production of interleukin-12 (IL-12) by bone marrow-derived dendritic cells, respectively, that allow the expansion of anticancer T cells. **g** | *Lactobacillus reuteri* also influences the expression of IL-22 by group 3 innate lymphoid cells (ILC3s) by the immunosuppressive tryptophan catabolite indole-3-aldehyde (iAid)[122]. Globally, these mechanisms enhance cancer antigen-specific cytotoxic T lymphocyte (CTL) responses and cancer immunosurveillance[27]. In this figure, cancer treatments are highlighted in red and cytokines are represented by coloured spheres. The green fields represent secondary lymphoid organs (SLO) and the red fields represent tumour microenvironments (TME). AHR, aryl hydrocarbon receptor; anti-IL-10R, anti-IL-10 receptor; CTLA4, cytotoxic T lymphocyte protein 4; CTX, cyclophosphamide; IFN, interferon; MHC II, major histocompatibility complex class II; ODN, oligodeoxynucleotides; PDL1, programmed cell death 1 ligand 1; Tc1, type 1 $CD8^+$ T; TLR4, Toll-like receptor 4; Trp, tryptophan.

Table 2 | **Bacteria that have putative anticancer properties in humans**

Bacterial species	Cancer type	Interventions and outcomes	Refs
Streptococcus pyogenes and *Serratia marcescens*	Osteosarcoma	Coley's toxins: injection of *S. pyogenes* and *S. marcescens* in patients with sarcoma, with some evidence of objective response	55
Mycobacterium bovis BCG	Urothelial superficial cancers	Intravesical treatment of a live attenuated form of *M. bovis* reduces the risk of short- and long-term relapse	130
Lactobacillus casei str. Shirota (found in the fermented milk product Yakult)	Superficial bladder cancer	Immune-mediated effects (by NK cells and macrophages) and decreased tumour recurrence (except with multiple secondary tumours)	131–133
IMM-101 (heat-killed *Mycobacterium obuense*; NCTC 13365) with gemcitabine	Melanoma and advanced pancreatic ductal adenocarcinoma	Activation of APCs, granulocytes and γδ T cells. Increased survival in metastatic disease in a randomized phase II trial	134,135
Live-attenuated *Listeria monocytogenes* expressing mesothelin (CRS-207) with GVAX-cyclophosphamide	Advanced pancreatic ductal adenocarcinoma	Priming of mesothelin-specific CTLs, loss of regulatory T cells and tertiary lymphoid organ formation, and increased overall survival	136
IL-13–PE: recombinant cytotoxin consisting of human IL-13 and PE	Adrenocortical carcinoma	Majority of patients produce neutralizing antibodies against IL-13–PE within 2–3 weeks	137
IL-4–PE: chimeric fusion protein composed of IL-4 and PE	Astrocytoma	Phase I trial: no systemic complications, median survival of 8.2 months and evidence of necrosis on MRI scans in several patients	138
Attenuated strain of *Salmonella enterica* subsp. *enterica* serovar Typhimurium: VNP20009	Metastatic melanoma and refractory solid tumours	Phase I trial of intravenous infusion of *S.* Typhimurium led to inflammation, DC and T cell activation and evidence of bacterial tumour colonization; however, there was no tumour regression	139,114
TAPET-CD: an attenuated *Salmonella* bacterium that expresses the *Escherichia coli* cytosine deaminase gene	Head and neck squamous cell carcinoma or adenocarcinoma of the oesophagus	Evidence of bacterial colonization and confirmation of the conversion of 5-FC to 5-FU in 2 out of 3 tumours	140
Genetically modified *Corynebacterium diphtheriae*: Tf-CRM107 is a conjugate of transferrin and a point mutant of diphtheria toxin	Malignant brain tumour	MRI scans showed regression of tumour volume in 9 out of 15 patients with no evidence of severe local or systemic complications at low dose	141

5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; APC, antigen presenting cell; BCG, bacille Calmette–Guérin; CTLs, cytotoxic T lymphocytes; DC, dendritic cell; IL, interleukin; MRI, magnetic resonance imaging; NK, natural killer; PE, truncated form of *Pseudomonas* exotoxin A.

activate dendritic cells[72,73]. However, it remains unclear whether PSA alone would be as efficient as *B. fragilis* at stimulating an anticancer response (FIG. 2).

In addition to *B. fragilis*, the *Burkholderia cepacia* population expands in the ileum of anti-CTLA4-treated mice. *B. cepacia* also stimulates IL-12 production by dendritic cells *in vitro*. When combined with *B. fragilis*, *B. cepacia* can mediate an additive anticancer effect in the context of CTLA4 blockade[24].

Barnesiella intestinihominis is preferentially found in the proximal colon and is overrepresented after chemotherapy with CTX. The colonic colonization of *B. intestinihominis* is inhibited by the PRR nucleotide-binding oligomerization domain 2 (NOD2), which recognizes bacterial peptidoglycans. Indeed, CTX is more effective in *Nod1*$^{-/-}$*Nod2*$^{-/-}$ mice, which have a microbiota abundant in members of the Porphyromonadaceae, including *B. intestinihominis*. Furthermore, tumour-bearing wild-type mice that were monoassociated with *B. intestinihominis* had more polyfunctional CD4$^+$, CD8$^+$ or γδ T cells in the spleen and the tumour bed. In addition, these *B. intestinihominis*-fed mice had more interferon-γ (IFNγ)-producing γδ T cells at the expense of immunosuppressive IL-17-producing γδ T cells, thus facilitating anticancer immunity. Finally, CTX in combination with *B. intestinihominis* reduces cancer growth in mice through a pathway that involves T_H1 cells, type 1 CD8$^+$ T cells (Tc1 cells), TNF and IFNγ[23] (FIG. 2).

During CTX-based chemotherapy, *E. hirae* translocates from the proximal small intestine to lymphoid organs; this effect is more pronounced in NOD2-deficient mice[23]. In mice, *E. hirae* induces T_H17 and T_H1 CD4$^+$ T cells[16] and stimulates tumour-specific CD8$^+$ T cells but reduces the numbers of

Table 3 | **Bacteria that have putative anticancer properties in experimental models**

Bacterial species	Cancer type	Interventions and biological effects	Refs
Animal preclinical data			
Clostridium novyi *C. novyi* non-toxic strain spores	Orthotopic F98 rat glioma and dogs with spontaneous solid tumours	Intratumoural injections led to tumour haemorrhagic necrosis, lysis and regression	142
Lactobacillus casei	Orthotopic and transplantable bladder tumours and their metastases	Oral or intravesical injection of dead or alive bacteria increased the levels of IFNγ and the recruitment of neutrophils	143–145
Lactobacillus rhamnosus GG	Bladder tumours	Weekly intravesical instillations directed chemokine and/or cytokine release, recruitment of NK cells and direct cytotoxic effects on cell lines *ex vivo*	146,147
Alistipes shahii	MC38 colon cancer	Gavage after antibiotic treatment increased the production of TNF by intratumoural myeloid cells	26
Bacteroides fragilis and *Burkholderia cepacia*	MCA205 sarcomas and MC38 and CT26 colon cancers	Oral gavage of *B. fragilis* stimulated the production of IL-12 by bone marrow-derived DCs *in vitro*. The mechanism of *B. cepacia* remains unknown	24
Prevotella spp. and *Oscillibacter* spp.	Subcutaneous hepatocellular carcinoma	Oral administration of Prohep, a probiotic mixture, altered the microbiota and reduced tumour growth	70
Enterococcus hirae and *Barnesiella intestinihominis*	Sarcoma	Bacterial translocation: induction of T_H1 cells and pathogenic T_H17 cells, intratumoural regulation of T_{reg} cells and IFNγ-producing γδ T cells, respectively	23
Bifidobacterium longum and *Bifidobacterium breve*	Melanoma	Oral gavage led to the activation of DCs and an increased frequency of tumour-specific CTLs	27
Lactobacillus casei str. Shirota	MCA induced cancer	*L. casei* str. Shirota mixed into mouse diet delayed carcinogenesis through enhancement of NK cell cytotoxicity	148
Lactobacillus casei ATCC334	Colon cancer SW620 cells (Caco2 *in vitro*)	Secretion of ferrichrome, which induces JNK-associated induction of DNA damage-inducible transcript 3. Enhanced apoptosis of colon cancer cells	64
Lactobacillus casei BL23	DMH-associated colorectal cancer	Oral administration of *L. casei* BL23 led to differentiation of T cells towards a T_H17-biased immune response (with the secretion of IL-6, IL-17, IL-10 and TGFβ)	65
Lactobacillus acidophilus	CRC $Apc^{Min/+}$	Daily administration of yogurt formulation decreased overall intestinal inflammation	149,150
Bifidobacterium lactis and RS	Colorectal rat-azoxymethane model	The addition of RS to the diet and bacteria induced apoptosis in tumour cells at the time of cancer initiation	151
Antibiotic-induced loss of members of the Firmicutes and Bacteroidetes phyla; gain of members of the Proteobacteria	LLC and B16F10 lung metastases	Microbiota modifications following antibiotic treatment induced the loss of γδ T cells producing IL-17A	152
Bacillus polyfermenticus and its culture medium	HT-29, DLD-1, Caco2 human colon cancer in mice	Cyclin D1 expression required for ErbB-dependent cell transformation was decreased by culture medium injections near the tumour sites	153
In vitro *studies*			
Propionibacterium freudenreichii	Human colon adenocarcinoma HT-29 cells	Production of SCFAs, which induced pH-dependent differential cell death processes	154
L. acidophilus and *L. casei*	LS513 colorectal cancer cell line	Sensitization of colorectal cancer cells to 5-FU-induced apoptosis	67
Enterococcus faecium RM11 and *Lactobacillus fermentum* RM28	Caco2 cell lines	Antiproliferative effects on CRC cells	155
Lactobacillus delbrueckii CU/22	HT-29 cell line; probiotic supernatant	Apoptosis and necrosis through the production of bacterial hydrogen peroxide and superoxide radicals	156
L. acidophilus 606	HT-29 colon cancer line	Cell-bound exopolysaccharides induced the activation of autophagic cell death promoted directly by the induction of beclin 1 and GRP78	157
B. lactis Bb12 and *L. rhamnosus* GG	Caco2 cancer cell line	Induced apoptosis through the mitochondrial route	158
L. acidophilus and *L. casei*	LS513 colorectal cancer cell line	Sensitized colorectal cancer cells to 5-FU-induced apoptosis	67

5-FU, 5-fluorouracil; *Apc*, adenomatous polyposis coli; CRC, colorectal cancer; CTLs, cytotoxic T lymphocytes; DCs, dendritic cells; DMH, 1,2-dimethylhydrazine; IFNγ, interferon-γ; IL, interleukin; JNK, JUN N-terminal kinase; LLC, Lewis lung carcinoma; *Min*, multiple intestinal neoplasia; NK, natural killer; RS, resistant starch; SCFA, short-chain fatty acid; TGFβ, transforming growth factor-β; T_H, T helper; TNF, tumour necrosis factor; T_{reg}, regulatory T.

immunosuppressive intratumoural T_{reg} cells and IL-17-producing γδ T cells[23]. Mono-association of antibiotic-treated mice with *E. hirae* improved tumour shrinkage induced by CTX; this effect was blocked by treatment with $CD8^+$ T cell-depleting or IFNγ-depleting antibodies, which suggests that it was immune-mediated[23]. At present, it is not known whether *E. hirae* can synergize with *B. intestinihominis* during CTX treatment to enhance treatment efficacy (FIG. 2).

Members of the Bifidobacteriales, which are abundant in some dairy products and are naturally found in the colon, have been associated with immune health in humans. Members of the Bifidobacteriales were abundant in mice that exhibited reduced growth of transplantable melanomas and improved CTL-mediated immunosurveillance[27]. Transfer of *Bifidobacterium breve* or *Bifidobacterium longum* into Bifidobacteriales-free mice was sufficient to reduce melanoma growth and restore anti-melanoma CTL responses. Furthermore, *B. breve* and *B. longum* stimulated dendritic cell maturation, which may enable dendritic cell priming of tumour-specific CTLs. In mice that carried *B. breve* or *B. longum*, CTL-infiltrated tumours responded better to immunotherapy than the tumours of sterile or Bifidobacteriales-free mice[27] (FIG. 2).

Thus, identifying bacterial species that mediate anticancer effects could support the development of wild-type or genetically modified mixtures of probiotics, or pharmacological agents that mimic their presence, for the treatment of cancer.

Genetically modified bacteria. Synthetic engineering of bacteria enhanced the tumoricidal effects of 5-fluorouracil (5-FU) in a model of liver metastases of colorectal cancers. The bacteria were orally delivered to mice and colonized liver metastases. A synchronized lysis cycle of the bacteria based on quorum sensing feedback loops enabled *Salmonella enterica* subsp. *enterica* serovar Typhimurium to deliver 5-FU in pulsatile cycles[74]. Anaerobic bacteria can invade necrotic tumours, which have an anaerobic environment and in which chemotherapy efficacy is limited owing to the low vascular supply. Hence, bacterial engineering can increase the anticancer effect of 5-FU in mouse models. However, complete tumour eradication was not achieved with this strategy, unless combined with immunotherapy or other anticancer agents[74]. Clinical trials are warranted in patients to test this unique way to deliver drugs to avascular sites that are resistant to conventional treatment.

Cancer-modulating microbial products

Bacteria produce various molecules that may affect the survival and growth of cancer cells, or that modulate anticancer immunosurveillance. These include bacterial toxins that have direct anticancer properties, ligands of PRRs that affect the immune response and metabolites that affect host metabolism (FIG. 3). There is no clear distinction between the latter two categories, as some metabolites can act on PRRs; this has been demonstrated for phenazines from *Pseudomonas aeruginosa* and phthiocol from *Mycobacterium tuberculosis*, which act on aryl hydrocarbon receptor (a PRR that functions as a transcription factor)[75], and for *N*-acetylglucosamine (a sugar subunit of bacterial peptidoglycan), which acts on the hexokinase PRR to activate inflammation[76].

Bacterial toxins. Bacteria produce different toxins and antibiotics, which allow them to compete with other microorganisms[52]. Bacterial toxins may have direct anticancer effects, as illustrated for anthracyclines produced by *Streptomyces* spp.[77] (FIG. 3). Indeed, anthracyclines, including doxorubicin, are widely used in anticancer chemotherapy and can induce immunogenic cell death, thereby stimulating anticancer immune responses[78]. However, it remains to be determined whether toxins are produced by intestinal bacteria at doses high enough to mediate such anticancer effects.

Bacterial toxins — including the colicins — are often peptides with amphipathic β-helices that contain cationic charges, which allow the toxin to lyse non-protected bacterial membranes. Importantly, structurally similar oligopeptides, such as LTX-315 (FIG. 3), can be synthesized and used to kill cancer cells by lysing mitochondria[79]. Interestingly, synthetic, bacterial toxin-like mitochondrion-targeted amphipathic peptides can also be fused with motifs that target surface proteins specifically expressed by tumour epithelial and vascular endothelial cell membranes, such as GRP78 and IL-11R. These peptides can mediate antitumour effects in preclinical models and reach their cellular targets in patients with cancer[80,81]. Further research is required to elucidate whether they induce therapeutically relevant anticancer effects through the stimulation of immunogenic cell death.

Ligands of PRRs. PRRs mostly recognize pathogen-associated molecular patterns (PAMPs), although they may also have endogenous ligands. One well-known PAMP is bacterial lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, which interacts with TLR4. LPS can stimulate inflammatory responses when bacteria enter the systemic circulation through breaches in the intestinal barrier. This can occur after cancer treatment with radiation therapy, and may improve the inhibition of tumour growth by activating T cells[82]. TLR4 is also thought to be fundamental for the anticancer effects of BCG[83] (FIG. 3). PAMPs can be used as vaccine adjuvants to elicit an immune response against viruses that can cause cancer. For example, monophosphoryl lipid A (MPL), a derivative of *Salmonella enterica* subsp. *enterica* serovar Minnesota LPS, is approved as an adjuvant in a peptide-based vaccine that is specific for cervical carcinoma-associated strains of human papillomavirus[84]. In addition, synthetic PRR ligands can be produced as immune-system-modulating cancer therapeutics. For example, imiquimod, a synthetic agonist of TLR7 (FIG. 3), is approved for the treatment of actinic keratosis, which is a superficial basal cell carcinoma. However, it is unclear whether imiquimod acts directly through TLR7 or has TLR7-independent effects[85].

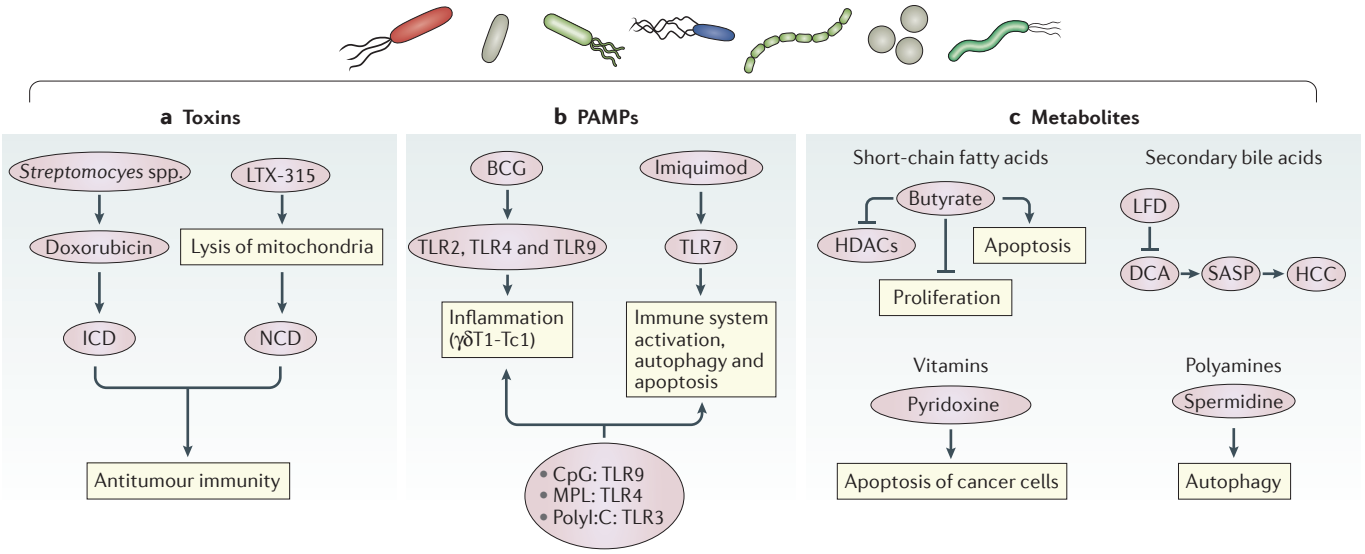

Figure 3 | **Anticancer effects of bacterial products.** Microbial agents can produce several molecules that affect the survival and growth of cancer cells or modulate anticancer immunosurveillance. **a** | Such products include bacterial toxins, such as those produced by *Streptomyces* spp. and LTX-315, which induce immunogenic cell death (ICD) and necrotic cell death (NCD), respectively. **b** | Agonists of Toll-like receptors (TLRs) are already used in the clinic and demonstrate evidence of both immune activity and antitumour effects. Intravesical instillation of bacille Calmette–Guérin (BCG), a ligand of several TLRs, triggers local immune responses. In addition, imiquimod acts on TLR7 and promotes autophagy. **c** | Bacteria-derived metabolites such as butyrate and pyridoxine (also known as vitamin B6) trigger cancer cell apoptosis. Secondary bile acids contribute to hepatocellular carcinoma (HCC) carcinogenesis. Increased intake of polyamines, such as spermidine, increases anticancer immunosurveillance through autophagy. DCA, deoxycholic acid; HDACs, histone deacetylases; LFD, low-fat diet; MPL, monophosphoryl lipid A; PAMPs, pathogen-associated molecular patterns; SASP, senescence-associated secretory phenotype; Tc1, type 1 $CD8^+$ T.

Beyond these approved agents, several PRR ligands have been evaluated in preclinical and clinical trials for their capacity to stimulate the immune system[86] (TABLE 2). It is unclear whether PRR agonists will mediate immunostimulatory anticancer effects with acceptable side effects. However, malignant cells can acquire the ectopic expression of PRRs; for example, breast cancers can express TLR4 or non-small-cell lung cancers can express TLR7 (REFS 87,88). In such cancers, PRR ligands will induce cancer cell proliferation.

Bacterial metabolites. The microbiota has a key role in human metabolism; approximately 50% of metabolites in the plasma are estimated to have a bacterial origin[89]. The gut microbiome synthesizes all SCFAs and secondary bile acids, polyamines and vitamins. Bacterial metabolites may affect cancer development and the efficacy of antineoplastic therapies.

SCFAs, mostly acetate, butyrate and propionate, are produced in the colon from dietary fibre and polysaccharides. Specifically, SCFAs are generated by *Clostridium* clusters IV and XIVa in the Firmicutes phylum, including species in the genera *Eubacterium, Roseburia, Faecalibacterium* and *Coprococcus*. Locally, SCFAs, particularly propionate and butyrate, favour the differentiation and accumulation of T_{reg} cells, thereby mediating anti-inflammatory effects[90–92].

Among the SCFAs, only acetate is present at high concentrations in the systemic circulation of rodents and humans[93]. Importantly, acetate can support the growth of several human cancer types, including glioblastoma, breast, ovarian and lung cancers. Cells from these types of cancer express the nucleocytosolic acetyl-CoA synthetase enzyme, ACSS2, which converts acetate into acetyl-CoA[94–96]. This fuels anabolic reactions, which are favoured by cancer cells. In contrast to acetate, butyrate and propionate inhibit histone deacetylases (HDACs) (FIG. 3) and act as agonists of several G protein-coupled receptors, which has an oncosuppressive effect. Butyrate induces apoptosis in colorectal cancer and lymphoma cells[97,98]. Furthermore, butyrate has negative effects on the proliferative and regenerative potential of colon stem cells, which are located at the bottom of intestinal crypts. Usually, butyrate does not reach these cells because it provides fuel for colonocytes that line the crypt[99]. This suggests that the carcinogenesis-associated disruption of crypt architecture might facilitate butyrate-mediated inhibition of colonic cancer stem cells, which arise from colonic stem cells. By contrast, butyrate causes hyperproliferation of colon epithelial cells in a genetically unstable mouse model[100]. However, in humans, several studies have indicated that patients with colorectal cancer have a decreased abundance of butyrate-producing bacteria compared with healthy controls[101]. Moreover, metabolomic analyses indicate that a diet that decreases the risk of cancer also increases the faecal concentration of butyrate and propionate[102]. In addition, butyrate-producing *Clostridia* strains reduce graft-versus-host disease in the gut induced by allogeneic bone marrow transplantation. This effect has

Ectopic expression
Enforced expression of a gene product, triggered by somatic mutation or genetic manipulation.

Intestinal crypts
Tube-like glands found in the lining of the colon and rectum.

been correlated with improved intestinal epithelial cell junction integrity and reduced apoptosis[103], which may be mediated by butyrate.

Together, these results suggest that acetate is a potential oncometabolite, whereas butyrate may participate in context-dependent tumour suppression. It might be worthwhile to explore measures to increase colonic butyrate (and decrease acetate) production, such as feeding butyrogenic dietary fermentable carbohydrates[102] or providing specific butyrogenic bacteria[104] for the prevention or treatment of cancer.

Bacteria, such as members of the *Clostridium* clusters XI and XIVa, which expand in the context of obesity, can convert primary bile acids (such as chenodeoxycholic acid and cholic acid) into secondary bile acids (such as lithocholic acid and deoxycholic acid); these secondary bile acids have potential DNA-damaging and hence carcinogenic effects. Secondary bile acids also have an increased affinity for bile acid receptors, which can affect host metabolism at multiple levels. In addition, secondary bile acids can affect the composition of the gut microbiota either directly or indirectly through immune activation[105]. Preclinical research has been undertaken to evaluate measures that reduce the production of secondary bile acids by the microbiome for oncosuppressive effects. This can be achieved by either a low-fat diet or by pharmacological inhibition of the microbial conversion of primary to secondary bile acids with difructose anhydride III (FIG. 3). Both interventions prevent hepatic oncogenesis in a mouse model[106], although it remains to be determined whether they have a beneficial effect with non-hepatic cancers. The incidence of overall human mortality can be correlated with the dietary consumption of the polyamines spermidine and spermine[107]; however, a substantial proportion of polyamines are produced by the gut microbiome. In mice, and other model organisms, spermidine can enhance longevity[108], suppress cardiac ageing[107] and augment anticancer immunosurveillance[53]. These effects are mediated by the induction of autophagy in target cells, potentially through inhibition of the EP300 acetyltransferase[53,107] (FIG. 3).

Interestingly, the probiotic strain *Bifidobacterium animalis* subsp. *lactis* LKM512, which increases intestinal luminal polyamine concentrations, can enhance the longevity of mice[109]. This effect was particularly strong if LKM512 was combined with arginine, which is the common precursor of polyamines, which suggests that the effect was mediated by these metabolites[109]. Low expression of enzymes related to polyamine transport has been linked to an increased risk of developing colitis following ipilimumab treatment in patients with melanoma[25]. It will be interesting to further explore specific probiotic and prebiotic interventions on polyamine metabolism with respect to their effects on the development of cancer. Vitamins cannot be synthesized in sufficient quantities by human cells, which means that they must be provided by the diet or synthesized by the gut microbiota. In particular, the human gut microbiome can synthesize at least eight B vitamins: biotin, cobalamin, folate, niacin, pantothenate, pyridoxine (also known as vitamin B6), riboflavin and thiamin[110]. Low expression of enzymes that are involved in the biosynthesis of several B vitamins is associated with colitis following ipilimumab treatment for melanoma[25]. Whether vitamins influence anticancer immunosurveillance has not yet been investigated. However, it is known that pyridoxine stimulates anticancer immunosurveillance in the context of cisplatin-based chemotherapy against non-small-cell lung cancer[111,112] (FIG. 3). Therefore, it may be interesting to explore the therapeutic use of probiotics that produce pyridoxine[113].

In addition, the intestinal microbiota may affect vitamin metabolism in the host. Human ulcerative colitis, ulcerative colitis-associated colorectal cancer and sporadic colorectal cancer are characterized by the increased local expression of enzymes that catabolize all-*trans*-retinoic acid (atRA), which may be associated with microbiota-induced intestinal inflammation[114]. Notably, an external supply of atRA can reduce the tumour burden in colitis-associated colorectal cancer in mice[115]. This suggests that this vitamin A derivate could be useful in the prevention or treatment of colorectal cancer.

Conclusions and outlook

Over the past decade, it has become increasingly clear that most, if not all, major disease categories should be studied in the context of the microbiome. The microbiota can have a major effect on the formation and progression of cancer, and may even influence the outcome of chemotherapies and immunotherapies. Although most of these effects are mediated by indirect effects on immunosurveillance, they also may involve the direct effects of microbial products — such as carcinogens, cytotoxic agents and metabolites — on cancer cells through various processes. These could range from mutagenesis to epigenetic modulation, stimulation of receptors on host cells, and effects on anabolic and catabolic pathways.

This implies that optimal preclinical modelling of oncogenesis, tumour progression and therapeutic responses should include the standardization of the microbiome (rather than the use of mice carrying variable microbiota). Furthermore, mouse 'humanization' with patient-derived cancers and immune cells could be combined with FMT to create a model that unites the patient's neoplastic cells, immune system and microbiome.

It seems plausible that progress in the functional exploration of patient-derived microbiomes, coupled with improved preclinical models will enable the development of four new types of anticancer intervention. Each one of these therapies could be used as a standalone treatment or in combination with other therapeutic measures (such as cytotoxic chemotherapies, targeted therapies or immunotherapies): first, orally administrable microorganisms (probiotics) that can be natural or genetically manipulated[74] given alone, in combination or perhaps even as an entire microbial ecosystem; second, specific dietary or drug-based interventions that favour the expansion of beneficial microorganisms, acting either on endogenous bacteria or on administered

probiotics (thus creating 'synbiotics'); third, drugs that specifically target microbial enzymes that generate harmful toxins and metabolites; and fourth, the administration of microbial products that have anticancer properties.

It should be noted that live microorganisms raise safety concerns, particularly if they are genetically modified. This is because of their potential pathogenicity and the possibility of acquiring antibiotic resistance or enzymatic chemoresistance — that is, resistance to drugs that specifically target microbial enzymes that generate harmful toxins and metabolites[116]. Moreover, live microbial agents are afflicted by complicated regulatory and intellectual property-related issues[37]. Hopefully, the future development of small molecules or chemically defined macromolecules that favourably influence the natural gut microbiome or mimic its beneficial effects will eventually overcome such caveats.

1. Pevsner-Fischer, M. *et al.* Role of the microbiome in non-gastrointestinal cancers. *World J. Clin. Oncol.* **7**, 200–213 (2016).
2. Torre, L. A. *et al.* Global cancer statistics, 2012. *CA Cancer J. Clin.* **65**, 87–108 (2015).
3. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194–1217 (2013).
4. Honda, K. & Littman, D. R. The microbiota in adaptive immune homeostasis and disease. *Nature* **535**, 75–84 (2016).
5. Gilbert, J. A. *et al.* Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature* **535**, 94–103 (2016).
6. Dunn, G. P., Old, L. J. & Schreiber, R. D. The three Es of cancer immunoediting. *Annu. Rev. Immunol.* **22**, 329–360 (2004).
A key paper on the definition of immunosurveillance in oncoimmunology.
7. Nakatsu, G. *et al.* Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat. Commun.* **6**, 8727 (2015).
8. Mitra, A. *et al.* Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Sci. Rep.* **5**, 16865 (2015).
9. Yu, G. *et al.* Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. *Genome Biol.* **17**, 163 (2016).
10. Kilkkinen, A. *et al.* Antibiotic use predicts an increased risk of cancer. *Int. J. Cancer* **123**, 2152–2155 (2008).
This paper provides the first clinical evidence that links modifications of the microbiota to cancer.
11. Zitvogel, L., Ayyoub, M., Routy, B. & Kroemer, G. Microbiome and anticancer immunosurveillance. *Cell* **165**, 276–287 (2016).
12. Guerrero-Preston, R. *et al.* 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment. *Oncotarget* **7**, 51320–51334 (2016).
13. Piyathilake, C. J. *et al.* Cervical microbiota associated with higher grade cervical intraepithelial neoplasia in women infected with high-risk human papillomaviruses. *Cancer Prev. Res. (Phila.)* **9**, 357–366 (2016).
14. Jacquelot, N. *et al.* Chemokine receptor patterns in lymphocytes mirror metastatic spreading in melanoma. *J. Clin. Invest.* **126**, 921–937 (2016).
15. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
16. Viaud, S. *et al.* The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**, 971–976 (2013).
This paper highlights the importance of an intact microbiota for CTX activity, and the pivotal role of *E. hirae* translocation following CTX treatment to induce pathogenic T_H17 cells.
17. Tsilimigras, M. C. B. & Fodor, A. A. Compositional data analysis of the microbiome: fundamentals, tools, and challenges. *Ann. Epidemiol.* **26**, 330–335 (2016).
18. Hugon, P., Lagier, J.-C., Colson, P., Bittar, F. & Raoult, D. Repertoire of human gut microbes. *Microb. Pathog.* http://dx.doi.org/10.1016/j.micpath.2016.06.020 (2016).
19. Hieken, T. J. *et al.* The microbiome of aseptically collected human breast tissue in benign and malignant disease. *Sci. Rep.* **6**, 30751 (2016).
20. Rahbar, A. *et al.* Discordant humoral and cellular immune responses to cytomegalovirus (CMV) in glioblastoma patients whose tumors are positive for CMV. *Oncoimmunology* **4**, e982391 (2015).
21. Chan, A. A. *et al.* Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors. *Sci. Rep.* **6**, 28061 (2016).
22. Drewes, J. L., Housseau, F. & Sears, C. L. Sporadic colorectal cancer: microbial contributors to disease prevention, development and therapy. *Br. J. Cancer* **115**, 273–280 (2016).
23. Daillère, R. *et al. Enterococcus hirae* and *Barnesiella intestinihominis* facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. *Immunity* **45**, 931–943 (2016).
24. Vétizou, M. *et al.* Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079–1084 (2015).
This paper details the mandatory role of the gut microbiota (specifically, *B. fragilis*) in CTLA4-mediated immune anticancer activity, and the protective effect on immune-related colitis.
25. Dubin, K. *et al.* Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat. Commun.* **7**, 10391 (2016).
This paper examines the link between the gut microbiota and immune checkpoint inhibitor blockade, and immune related toxicities in patients with metastatic melanoma.
26. Iida, N. *et al.* Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **342**, 967–970 (2013).
27. Sivan, A. *et al.* Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084–1089 (2015).
This study shows that *Bifidobacterium* spp. promote maturation of intratumoural dendritic cells that allow the expansion of anticancer T cells after programmed cell death 1 ligand 1 (PDL1) treatment in mice.
28. Chevalier, C. *et al.* Gut microbiota orchestrates energy homeostasis during cold. *Cell* **163**, 1360–1374 (2015).
29. McKenney, P. T. & Pamer, E. G. From hype to hope: the gut microbiota in enteric infectious disease. *Cell* **163**, 1326–1332 (2015).
30. Kamdar, K. *et al.* Genetic and metabolic signals during acute enteric bacterial infection alter the microbiota and drive progression to chronic inflammatory disease. *Cell Host Microbe* **19**, 21–31 (2016).
31. Schmidt, C. Mental health: thinking from the gut. *Nature* **518**, S12–S15 (2015).
32. Moayyedi, P. *et al.* Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* **149**, 102–109.e6 (2015).
33. Kakihana, K. *et al.* Fecal microbiota transplantation for patients with steroid-resistant/dependent acute graft-versus-host disease of the gut. *Blood* **128**, 2083–2088 (2016).
This study provides the first clinical demonstration that faecal microbiota transplantation can be safely used and improves outcomes in patients with steroid-refractory gastrointestinal graft-versus-host disease.
34. Bel, S. *et al.* Reprogrammed and transmissible intestinal microbiota confer diminished susceptibility to induced colitis in $TMF^{-/-}$ mice. *Proc. Natl Acad. Sci. USA* **111**, 4964–4969 (2014).
35. Kwa, M., Plottel, C. S., Blaser, M. J. & Adams, S. The intestinal microbiome and estrogen receptor-positive female breast cancer. *J. Natl Cancer Inst.* **108**, djw029 (2016).
36. Dal Peraro, M. & van der Goot, F. G. Pore-forming toxins: ancient, but never really out of fashion. *Nat. Rev. Microbiol.* **14**, 77–92 (2016).
37. Pamer, E. G. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. *Science* **352**, 535–538 (2016).
38. Cougnoux, A. *et al.* Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype. *Gut* **63**, 1932–1942 (2014).
39. Cougnoux, A. *et al.* Small-molecule inhibitors prevent the genotoxic and protumoural effects induced by colibactin-producing bacteria. *Gut* **65**, 278–285 (2016).
40. Wallace, B. D. *et al.* Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* **330**, 831–835 (2010).
41. Wallace, B. D. *et al.* Structure and inhibition of microbiome β-glucuronidases essential to the alleviation of cancer drug toxicity. *Chem. Biol.* **22**, 1238–1249 (2015).
42. Carmody, R. N. & Turnbaugh, P. J. Host-microbial interactions in the metabolism of therapeutic and diet-derived xenobiotics. *J. Clin. Invest.* **124**, 4173–4181 (2014).
43. Hu, Y. *et al.* Manipulation of the gut microbiota using resistant starch is associated with protection against colitis-associated colorectal cancer in rats. *Carcinogenesis* **37**, 366–375 (2016).
44. Masumoto, S. *et al.* Non-absorbable apple procyanidins prevent obesity associated with gut microbial and metabolomic changes. *Sci. Rep.* **6**, 31208 (2016).
45. Gallus, S. *et al.* Does an apple a day keep the oncologist away? *Ann. Oncol.* **16**, 1841–1844 (2005).
46. Taper, H. S. & Roberfroid, M. B. Nontoxic potentiation of cancer chemotherapy by dietary oligofructose or inulin. *Nutr. Cancer* **38**, 1–5 (2000).
47. Schoener, C. A., Carillo-Conde, B., Hutson, H. N. & Peppas, N. A. An inulin and doxorubicin conjugate for improving cancer therapy. *J. Drug Deliv. Sci. Technol.* **23**, 111–118 (2013).
48. Cabreiro, F. *et al.* Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. *Cell* **153**, 228–239 (2013).
49. Dao, M. C. *et al. Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* **65**, 426–436 (2016).
50. Vernieri, C. *et al.* Targeting cancer metabolism: dietary and pharmacologic interventions. *Cancer Discov.* **6**, 1315–1333 (2016).
51. Crawford, P. A. *et al.* Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. *Proc. Natl Acad. Sci. USA* **106**, 11276–11281 (2009).
52. Becattini, S., Taur, Y. & Pamer, E. G. Antibiotic-induced changes in the intestinal microbiota and disease. *Trends Mol. Med.* **22**, 458–478 (2016).
53. Pietrocola, F. *et al.* Caloric restriction mimetics enhance anticancer immunosurveillance. *Cancer Cell* **30**, 147–160 (2016).
This study illustrates the links between caloric restriction mimetics, autophagy and cancer.
54. Di Biase, S. *et al.* Fasting-mimicking diet reduces HO-1 to promote T cell-mediated tumor cytotoxicity. *Cancer Cell* **30**, 136–146 (2016).
55. Nauts, H. C., Swift, W. E. & Coley, B. L. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. *Cancer Res.* **6**, 205–216 (1946).
This paper details the pioneering work of B. L. Coley, who 70 years ago already recognized the anticancer effects of bacteria against sarcoma.
56. Babjuk, M. *et al.* EAU guidelines on non–muscle-invasive urothelial carcinoma of the bladder: update 2013. *Eur. Urol.* **64**, 639–653 (2013).
57. Böhle, A. & Brandau, S. Immune mechanisms in bacillus Calmette–Guerin immunotherapy for superficial bladder cancer. *J. Urol.* **170**, 964–969 (2003).
58. Zbar, B., Bernstein, I., Tanaka, T. & Rapp, H. J. Tumor immunity produced by the intradermal inoculation of living tumor cells and living *Mycobacterium bovis* (strain BCG). *Science* **170**, 1217–1218 (1970).

59. Aragón, F., Carino, S., Perdigón, G. & de Moreno de LeBlanc, A. Inhibition of growth and metastasis of breast cancer in mice by milk fermented with *Lactobacillus casei* CRL 431. *J. Immunother.* **38**, 185–196 (2015).
60. Hu, J. *et al.* Anti-tumour immune effect of oral administration of *Lactobacillus plantarum* to CT26 tumour-bearing mice. *J. Biosci.* **40**, 269–279 (2015).
61. Kato, I., Endo, K. & Yokokura, T. Effects of oral administration of *Lactobacillus casei* on antitumor responses induced by tumor resection in mice. *Int. J. Immunopharmacol.* **16**, 29–36 (1994).
62. Cai, S. *et al. Lactobacillus rhamnosus* GG activation of dendritic cells and neutrophils depends on the dose and time of exposure. *J. Immunol. Res.* **2016**, 7402760 (2016).
63. Khazaie, K. *et al.* Abating colon cancer polyposis by *Lactobacillus acidophilus* deficient in lipoteichoic acid. *Proc. Natl Acad. Sci. USA* **109**, 10462–10467 (2012).
64. Konishi, H. *et al.* Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. *Nat. Commun.* **7**, 12365 (2016).
65. Lenoir, M. *et al. Lactobacillus casei* BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. *J. Gastroenterol.* **51**, 862–873 (2016).
66. Lee, J.-W. *et al.* Immunomodulatory and antitumor effects *in vivo* by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. *J. Vet. Sci.* **5**, 41–48 (2004).
67. Baldwin, C. *et al.* Probiotic *Lactobacillus acidophilus* and *L. casei* mix sensitize colorectal tumoral cells to 5-fluorouracil-induced apoptosis. *Nutr. Cancer* **62**, 371–378 (2010).
68. Takagi, A. *et al.* Relationship between the *in vitro* response of dendritic cells to *Lactobacillus* and prevention of tumorigenesis in the mouse. *J. Gastroenterol.* **43**, 661–669 (2008).
69. Varian, B. J. *et al.* Beneficial bacteria inhibit cachexia. *Oncotarget* **7**, 11803–11816 (2016).
70. Li, J. *et al.* Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proc. Natl Acad. Sci. USA* **113**, E1306–E1315 (2016).
71. Geis, A. L. *et al.* Regulatory T-cell response to enterotoxigenic *Bacteroides fragilis* colonization triggers IL17-dependent colon carcinogenesis. *Cancer Discov.* **5**, 1098–1109 (2015).
72. Wang, Q. *et al.* A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. *J. Exp. Med.* **203**, 2853–2863 (2006).
73. Surana, N. K. & Kasper, D. L. The yin yang of bacterial polysaccharides: lessons learned from *B. fragilis* PSA. *Immunol. Rev.* **245**, 13–26 (2012).
74. Din, M. O. *et al.* Synchronized cycles of bacterial lysis for *in vivo* delivery. *Nature* **536**, 81–85 (2016).
75. Moura-Alves, P. *et al.* AhR sensing of bacterial pigments regulates antibacterial defence. *Nature* **512**, 387–392 (2014).
76. Wolf, A. J. *et al.* Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell* **166**, 624–636 (2016).
77. Malla, S., Niraula, N. P., Singh, B., Liou, K. & Sohng, J. K. Limitations in doxorubicin production from *Streptomyces peucetius*. *Microbiol. Res.* **165**, 427–435 (2010).
78. Kroemer, G., Galluzzi, L., Kepp, O. & Zitvogel, L. Immunogenic cell death in cancer therapy. *Annu. Rev. Immunol.* **31**, 51–72 (2013).
A notable review on the mechanisms and the importance of immunogenic cell death in cancer.
79. Ellerby, H. M. *et al.* Anti-cancer activity of targeted pro-apoptotic peptides. *Nat. Med.* **5**, 1032–1038 (1999).
80. Arap, M. A. *et al.* Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands. *Cancer Cell* **6**, 275–284 (2004).
81. Pasqualini, R. *et al.* Targeting the interleukin-11 receptor α in metastatic prostate cancer: a first-in-man study. *Cancer* **121**, 2411–2421 (2015).
82. Paulos, C. M. *et al.* Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. *J. Clin. Invest.* **117**, 2197–2204 (2007).
83. Galluzzi, L. *et al.* Trial Watch: experimental Toll-like receptor agonists for cancer therapy. *Oncoimmunology* **1**, 699–716 (2012).
84. Paavonen, J. *et al.* Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *Lancet* **374**, 301–314 (2009).
85. Walter, A. *et al.* Aldara activates TLR7-independent immune defence. *Nat. Commun.* **4**, 1560 (2013).
86. Corrales, L. *et al.* Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. *Cell Rep.* **11**, 1018–1030 (2015).
87. Chatterjee, S. *et al.* TLR7 promotes tumor progression, chemotherapy resistance, and poor clinical outcomes in non-small cell lung cancer. *Cancer Res.* **74**, 5008–5018 (2014).
88. Mehmeti, M. *et al.* Expression of functional toll like receptor 4 in estrogen receptor/progesterone receptor-negative breast cancer. *Breast Cancer Res.* **17**, 130 (2015).
89. Martin, F.-P. J. *et al.* A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Mol. Syst. Biol.* **3**, 112 (2007).
90. Arpaia, N. *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**, 451–455 (2013).
91. Furusawa, Y. *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450 (2013).
92. Smith, P. M. *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* **341**, 569–573 (2013).
93. Perry, R. J. *et al.* Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. *Nature* **534**, 213–217 (2016).
94. Comerford, S. A. *et al.* Acetate dependence of tumors. *Cell* **159**, 1591–1602 (2014).
95. Mashimo, T. *et al.* Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. *Cell* **159**, 1603–1614 (2014).
96. Schug, Z. T. *et al.* Acetyl-CoA synthetase 2 promotes acetate utilization and maintains cancer cell growth under metabolic stress. *Cancer Cell* **27**, 57–71 (2015).
97. Jan, G. *et al.* Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. *Cell Death Differ.* **9**, 179–188 (2002).
98. Wei, W., Sun, W., Yu, S., Yang, Y. & Ai, L. Butyrate production from high-fiber diet protects against lymphoma tumor. *Leuk. Lymphoma* **57**, 2401–2408 (2016).
99. Kaiko, G. E. *et al.* The colonic crypt protects stem cells from microbiota-derived metabolites. *Cell* **165**, 1708–1720 (2016).
100. Belcheva, A. *et al.* Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. *Cell* **158**, 288–299 (2014).
101. Bultman, S. J. & Jobin, C. Microbial-derived butyrate: an oncometabolite or tumor-suppressive metabolite? *Cell Host Microbe* **16**, 143–145 (2014).
102. Gutiérrez-Díaz, I., Fernández-Navarro, T., Sánchez, B., Margolles, A. & González, S. Mediterranean diet and faecal microbiota: a transversal study. *Food Funct.* **7**, 2347–2356 (2016).
103. Mathewson, N. D. *et al.* Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat. Immunol.* **17**, 505–513 (2016).
104. Bui, T. P. N. *et al.* Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal. *Nat. Commun.* **6**, 10062 (2015).
105. Wahlström, A., Sayin, S. I., Marschall, H.-U. & Bäckhed, F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab.* **24**, 41–50 (2016).
106. Yoshimoto, S. *et al.* Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* **499**, 97–101 (2013).
107. Eisenberg, T. *et al.* Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat. Med.* 1428–1438 (2016).
108. Eisenberg, T. *et al.* Induction of autophagy by spermidine promotes longevity. *Nat. Cell Biol.* **11**, 1305–1314 (2009).
This paper shows that administration of the natural polyamine spermidine in ageing mice triggers epigenetic deacetylation of histone H3, decreasing oxidative stress and necrosis.
109. Matsumoto, M., Kurihara, S., Kibe, R., Ashida, H. & Benno, Y. Longevity in mice is promoted by probiotic-induced suppression of colonic senescence dependent on upregulation of gut bacterial polyamine production. *PLoS ONE* **6**, e23652 (2011).
110. Magnúsdóttir, S., Ravcheev, D., de Crécy-Lagard, V. & Thiele, I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. *Front. Genet.* **6**, 148 (2015).
111. Galluzzi, L. *et al.* Prognostic impact of vitamin B_6 metabolism in lung cancer. *Cell Rep.* **2**, 257–269 (2012).
112. Aranda, F. *et al.* Immune-dependent antineoplastic effects of cisplatin plus pyridoxine in non-small-cell lung cancer. *Oncogene* **34**, 3053–3062 (2015).
113. Commichau, F. M. *et al.* Engineering *Bacillus subtilis* for the conversion of the antimetabolite 4-hydroxy-l-threonine to pyridoxine. *Metab. Eng.* **29**, 196–207 (2015).
114. Heimann, D. M. & Rosenberg, S. A. Continuous intravenous administration of live genetically modified *Salmonella typhimurium* in patients with metastatic melanoma. *J. Immunother.* **26**, 179–180 (2003).
115. Bhattacharya, N. *et al.* Normalizing microbiota-induced retinoic acid deficiency stimulates protective $CD8^+$ T cell-mediated immunity in colorectal cancer. *Immunity* **45**, 641–655 (2016).
This study shows that, in colon cancer, all-*trans*-retinoic acid supplementation has a key role by enhancing $CD8^+$ T cell migration to the tumour microenvironment.
116. Smith, M. B., Kelly, C. & Alm, E. J. Policy: how to regulate faecal transplants. *Nature* **506**, 290–291 (2014).
117. Sartor, R. B. Microbial influences in inflammatory bowel diseases. *Gastroenterology* **134**, 577–594 (2008).
118. Zoetendal, E. G. *et al.* Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Appl. Environ. Microbiol.* **68**, 3401–3407 (2002).
119. Ringel, Y. *et al.* High throughput sequencing reveals distinct microbial populations within the mucosal and luminal niches in healthy individuals. *Gut Microbes* **6**, 173–181 (2015).
120. Wexler, A. G. *et al.* Human symbionts inject and neutralize antibacterial toxins to persist in the gut. *Proc. Natl Acad. Sci. USA* **113**, 3639–3644 (2016).
121. Faith, J. J. *et al.* The long-term stability of the human gut microbiota. *Science* **341**, 1237439 (2013).
122. Zelante, T. *et al.* Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* **39**, 372–385 (2013).
123. Rajagopala, S. V. *et al.* Gastrointestinal microbial populations can distinguish pediatric and adolescent acute lymphoblastic leukemia (ALL) at the time of disease diagnosis. *BMC Genomics* **17**, 635 (2016).
124. Fan, X. *et al.* Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut* http://dx.doi.org/10.1136/gutjnl-2016-312580 (2016).
125. Chng, K. R. *et al.* Tissue microbiome profiling identifies an enrichment of specific enteric bacteria in *Opisthorchis viverrini* associated cholangiocarcinoma. *EBioMedicine* **8**, 195–202 (2016).
126. Murphy, G. *et al.* Association of seropositivity to *Helicobacter* species and biliary tract cancer in the ATBC study. *Hepatology* **60**, 1963–1971 (2014).
127. Xu, W. *et al.* Mini-review: perspective of the microbiome in the pathogenesis of urothelial carcinoma. *Am. J. Clin. Exp. Urol.* **2**, 57–61 (2014).
128. Schmidt, B. L. *et al.* Changes in abundance of oral microbiota associated with oral cancer. *PLoS ONE* **9**, e98741 (2014).
129. Hosgood, H. D. *et al.* The potential role of lung microbiota in lung cancer attributed to household coal burning exposures. *Environ. Mol. Mutagen.* **55**, 643–651 (2014).
130. Martínez-Piñeiro, J. A. *et al.* Bacillus Calmette–Guerin versus doxorubicin versus thiotepa: a randomized prospective study in 202 patients with superficial bladder cancer. *J. Urol.* **143**, 502–506 (1990).
131. Aso, Y. & Akazan, H. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. BLP Study Group. *Urol. Int.* **49**, 125–129 (1992).
132. Ohashi, Y. *et al.* Habitual intake of lactic acid bacteria and risk reduction of bladder cancer. *Urol. Int.* **68**, 273–280 (2002).
133. Hoesl, C. E. & Altwein, J. E. The probiotic approach: an alternative treatment option in urology. *Eur. Urol.* **47**, 288–296 (2005).
134. Stebbing, J. *et al.* An intra-patient placebo-controlled phase I trial to evaluate the safety and tolerability of intradermal IMM-101 in melanoma. *Ann. Oncol.* **23**, 1314–1319 (2012).

135. Dalgleish, A. G. *et al.* Randomised, open-label, phase II study of gemcitabine with and without IMM-101 for advanced pancreatic cancer. *Br. J. Cancer* **115**, 789–796 (2016).
136. Le, D. T. *et al.* Safety and survival with GVAX pancreas prime and *Listeria monocytogenes*-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J. Clin. Oncol.* **33**, 1325–1333 (2015).
137. Liu-Chittenden, Y. *et al.* Phase I trial of systemic intravenous infusion of interleukin-13-*Pseudomonas* exotoxin in patients with metastatic adrenocortical carcinoma. *Cancer Med.* **4**, 1060–1068 (2015).
138. Weber, F. *et al.* Safety, tolerability, and tumor response of IL4-*Pseudomonas* exotoxin (NBI-3001) in patients with recurrent malignant glioma. *J. Neurooncol.* **64**, 125–137 (2003).
139. Toso, J. F. *et al.* Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *J. Clin. Oncol.* **20**, 142–152 (2002).
This study shows that attenuated *S.* Typhimurium can be safely administered to patients with cancer and is able to colonize some tumours.
140. Nemunaitis, J. *et al.* Pilot trial of genetically modified, attenuated *Salmonella* expressing the *E. coli* cytosine deaminase gene in refractory cancer patients. *Cancer Gene Ther.* **10**, 737–744 (2003).
141. Laske, D. W., Youle, R. J. & Oldfield, E. H. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat. Med.* **3**, 1362–1368 (1997).
142. Roberts, N. J. *et al.* Intratumoral injection of *Clostridium novyi*-NT spores induces antitumor responses. *Sci. Transl Med.* **6**, 249ra111 (2014).
143. Kato, I., Kobayashi, S., Yokokura, T. & Mutai, M. Antitumor activity of *Lactobacillus casei* in mice. *Gan* **72**, 517–523 (1981).
144. Tomita, K. *et al.* Influence of *Lactobacillus casei* on rat bladder carcinogenesis [Japanese]. *Nihon Hinyokika Gakkai Zasshi* **85**, 655–663 (1994).
145. Takahashi, T. *et al.* Antitumor effects of the intravesical instillation of heat killed cells of the *Lactobacillus casei* strain Shirota on the murine orthotopic bladder tumor MBT-2. *J. Urol.* **166**, 2506–2511 (2001).
146. Seow, S. W. *et al. Lactobacillus* species is more cytotoxic to human bladder cancer cells than *Mycobacterium bovis* (bacillus Calmette-Guerin). *J. Urol.* **168**, 2236–2239 (2002).
147. Seow, S. W., Rahmat, J. N., Bay, B. H., Lee, Y. K. & Mahendran, R. Expression of chemokine/cytokine genes and immune cell recruitment following the instillation of *Mycobacterium bovis*, bacillus Calmette-Guérin or *Lactobacillus rhamnosus* strain GG in the healthy murine bladder. *Immunology* **124**, 419–427 (2008).
148. Takagi, A. *et al.* Enhancement of natural killer cytotoxicity delayed murine carcinogenesis by a probiotic microorganism. *Carcinogenesis* **22**, 599–605 (2001).
149. Urbanska, A. M., Bhathena, J., Martoni, C. & Prakash, S. Estimation of the potential antitumor activity of microencapsulated *Lactobacillus acidophilus* yogurt formulation in the attenuation of tumorigenesis in Apc(Min/+) mice. *Dig. Dis. Sci.* **54**, 264–273 (2009).
150. Urbanska, A. M., Bhathena, J., Cherif, S. & Prakash, S. Orally delivered microencapsulated probiotic formulation favorably impacts polyp formation in APC (Min/+) model of intestinal carcinogenesis. *Artif. Cells Nanomed. Biotechnol.* **44**, 1–11 (2016).
151. Le Leu, R. K., Hu, Y., Brown, I. L., Woodman, R. J. & Young, G. P. Synbiotic intervention of *Bifidobacterium lactis* and resistant starch protects against colorectal cancer development in rats. *Carcinogenesis* **31**, 246–251 (2010).
152. Cheng, M. *et al.* Microbiota modulate tumoral immune surveillance in lung through a γδT17 immune cell-dependent mechanism. *Cancer Res.* **74**, 4030–4041 (2014).
This study shows that microbiota modifications in antibiotic-treated mice decrease the induction of γδ T cells and influence immunosurveillance.
153. Ma, E. L. *et al.* The anticancer effect of probiotic *Bacillus polyfermenticus* on human colon cancer cells is mediated through ErbB2 and ErbB3 inhibition. *Int. J. Cancer* **127**, 780–790 (2010).
154. Lan, A., Lagadic-Gossmann, D., Lemaire, C., Brenner, C. & Jan, G. Acidic extracellular pH shifts colorectal cancer cell death from apoptosis to necrosis upon exposure to propionate and acetate, major end-products of the human probiotic propionibacteria. *Apoptosis* **12**, 573–591 (2007).
155. Thirabunyanon, M., Boonprasom, P. & Niamsup, P. Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. *Biotechnol. Lett.* **31**, 571–576 (2009).
156. Strus, M. *et al.* Effect of hydrogen peroxide of bacterial origin on apoptosis and necrosis of gut mucosa epithelial cells as a possible pathomechanism of inflammatory bowel disease and cancer. *J. Physiol. Pharmacol.* **60** (Suppl. 6), 55–60 (2009).
157. Kim, Y., Oh, S., Yun, H. S., Oh, S. & Kim, S. H. Cell-bound exopolysaccharide from probiotic bacteria induces autophagic cell death of tumour cells. *Lett. Appl. Microbiol.* **51**, 123–130 (2010).
158. Altonsy, M. O., Andrews, S. C. & Tuohy, K. M. Differential induction of apoptosis in human colonic carcinoma cells (Caco-2) by Atopobium, and commensal, probiotic and enteropathogenic bacteria: mediation by the mitochondrial pathway. *Int. J. Food Microbiol.* **137**, 190–203 (2010).

Acknowledgements
L.Z. and G.K. are supported by the Institut National Du Cancer (INCA), the Ligue contre le Cancer (équipe labelisée); Agence Nationale de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Institut National du Cancer (INCa); Institut Universitaire de France; Fondation pour la Recherche Médicale (FRM); the European Commission (ArtForce); the European Research Council (ERC); the LabEx Immuno-Oncology; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); the Paris Alliance of Cancer Research Institutes (PACRI); and the PIA2 TORINO-LUMIERE. L.Z. is also supported by the Swiss Institute for Experimental Cancer Research (ISREC), by the Swiss Bridge Foundation, and by IMMUNTRAIN-H2020. G.K. is also supported by the LeDucq Foundation.

Competing interests statement
The authors declare competing interests: see Web version for details.

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