Host microbiomes in tumor precision medicine: how far are we?

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11   Abstract

12   The human gut microbiome has received *a crescendo* of attention in recent years, due to the countless influences on

13   human pathophysiology, including cancer. Research on cancer and anticancer therapy is constantly looking for new hints

14   to improve the response to therapy while reducing the risk of relapse. In this scenario, the gut microbiome and the plethora

15   of microbial-derived metabolites are considered a new opening in the development of innovative anticancer treatments

16   for a better prognosis. This narrative review summarizes the current knowledge on the role of the gut microbiome in

17   ~~cancer~~ the onset and progression of cancer, as well as in response to chemo-immunotherapy. Recent findings regarding

18   the tumor microbiome and its implications for clinical practice are also commented on. ~~The c~~Current microbiome-based

19   intervention strategies (*i.e.*, prebiotics, probiotics, live biotherapeutics and fecal microbiota transplantation) are then

20   discussed, along with key shortcomings, including ~~the~~ a lack of long-term safety information in patients who are already

21   severely compromised by standard treatments. ~~In this scenario~~Thus, the implementation of bioinformatic tools applied to

22   microbiomics and other omics data, such as machine learning, has an enormous potential to push research in the field,

23   ~~allowing~~ enabling the prediction of health risk and therapeutic outcomes, for a truly personalized precision medicine.

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Graphical Abstract

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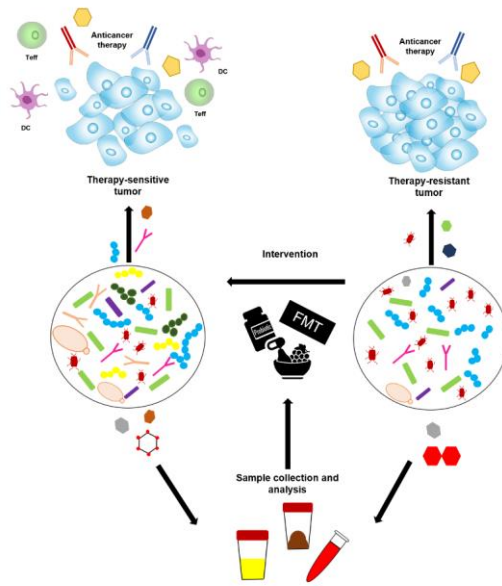
Personalized microbiome-based interventions are critical ~~to~~ ~~for~~ ~~ensure~~ ensuring antitumor immune responses, circumventing resistance to chemo-immunotherapy.

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Keywords: Gut microbiome, Microbial metabolites, Tumor microbiome, Anticancer therapy, Probiotics, Fecal microbiota transplantation, Next-generation probiotics, Machine learning

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1. Introduction

In ~~the last few~~recent years, the microbial community inhabiting the gastrointestinal tract (*i.e.*, the gut microbiome – GM) has received special attention, not only for the well-known supporting functions of host homeostasis [1,2], but also for its involvement in ~~cancer~~ the onset and progression of cancer, as well as in the outcomes of anticancer therapy [3]. Tumor development and patient failure ~~of~~ to ~~patient~~ respons~~ed~~ to anticancer approaches (*i.e.,* chemotherapy, radiotherapy, and immunotherapy) are among the leading causes of death worldwide [4]. For this reason, research is moving towards new fields that could help overcome these obstacles; host-associated microbes, as well as their products, have recently been identified as unexpected key orchestrators in these branches.

Herein, we discuss the role of GM composition and functionality in promoting the development and progression of local and distant tumors, as well as recent evidence on the intratumor microbiome. Particular attention is given to immunological tumors (e.g., leukemia and lymphoma) and to complications related to hematopoietic stem cell transplantation (HSCT), for which an abundant and consistent microbiome-centered literature is available.

In ~~an~~ attempt to fully explore the possibility of using microorganisms/microbiomes as a therapeutic target/tool in the anticancer field, we comment on ~~the~~ microbiome-tailored intervention strategies currently in use, as well as ~~on~~ the most recent clinical trials involving the use of prebiotics, (traditional and next-generation) probiotics and fecal microbiota transplantation (FMT). Finally, we discuss the translational potential of bioinformatics, particularly machine learning, ~~for~~ to stratify~~ing~~ patients, predict~~ing~~ outcomes, and design~~ing~~ personalized precision intervention strategies for a better patient quality of life. See **Figure 1** for a summary of the role of host microbiomes in tumor onset and progression, as well as in response to therapy, and the available microbiome manipulation tools that could help improve patient prognosis.

2. Exploring the human gut microbiome and its functions from eubiosis to dysbiosis

The GM is a key contributor to ~~the~~ maint~~enance of~~aining ~~the~~ host physiological homeostasis [1,2]. While this sentence may seem foregone in 2021, countless studies still attempt to advance our understanding of the close and complex relationship between the GM and the human host. Going back in time, the first signs of the presence of microbes inside the human intestinal tract took place in the late 1800s in Europe, when the German pediatrician Theodor Escherich consolidated the study of ~~the~~ "human gut flora" [5,6]. Indeed, Prof. Escherich discovered what he called "*Bacterium coli commune*" (*i.e.,* currently *Escherichia coli*) in human feces and afterwards "*Bacillus bifidus communis*" (*i.e.,* *Bifidobacterium animalis*) in the gut of newborns and breast-fed infants. For the first time, Escherich and colleagues

spoke of "good bacteria" ~~in a period in which~~at a time when the only link between microorganisms and their host was in the development of ~~pathologies~~ diseases (e.g., cholera, anthrax and tuberculosis) [5]. From then to the present day, GM research has always played an important role in science.

GM is a deeply complex ecosystem that includes not only bacteria, which are the most represented, but also archaebacteria and fungi, along with viruses [7]. Until recently, a frequently repeated slogan was that the human body contained 10 times more microorganisms than human cells. However, this was a rough calculation made more than 40 years ago [8], while today we can state that the ratio of human to microbial cells is likely to be 1:1 with the balance slightly in favor of microbes [9]. As just mentioned, the most examined fraction of GM is the bacterial one, 90% of which belongs to Firmicutes and Bacteroidetes phyla, while the remaining 10% is distributed among the subdominants Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia [2]. So far, it is estimated that the collective genome of GM, known as the microbiome, harbors 150 to 500 times more genes than the host, which are implicated in providing functional traits that complement the human repertoire and ~~that~~ are relevant to our metabolic, immunological and neurological homeostasis [10-12].

In the individual's lifespan, the first microbial stimuli derive from the early moments of the infant's life and are closely linked to the birth mode, the maternal microbiota, antibiotic exposure and early-life feeding practices [13,14]. At this time, when ~~the~~ infant-GM symbiosis is ~~going to~~ being established, GM is featured by low bacterial diversity and functional complexity, as well as a higher degree of interpersonal variation ~~compared to~~than the adult-type GM profile [15,16]. Both structural components of microbes and products of their metabolism have been found to be involved in the development, maturation, and education of the child's immune system [17-19], as well as in the regulation of the endocrine and central nervous systems [20]. It is therefore not surprising that GM disruption (*i.e.*, dysbiosis) ~~during~~in the early- life ~~window~~ ~~can~~ may be associated with several disorders [21,22], such as type 1 diabetes, atopic disease, asthma and childhood obesity [23-25]. At weaning, with the cessation of breastfeeding, there is a rapid increase in the ~~the~~ structural and functional diversity of the infant GM, which progressively evolves towards ~~the~~ a mature adult-like state [26]. The development of the adult GM is regulated by a complex interplay between the host and several environmental factors, such as diet, lifestyle, or the so-called geographical effect [27,28] or, more generally, the exposome (*i.e.*, the totality of internal and external exposures that an individual faces throughout his life) [29]. ~~In~~ Under healthy conditions, all of these factors contribute to shaping the microbial community, selecting a eubiotic GM configuration (*i.e.,* a stable, resistant, and resilient GM, with high diversity and functional redundancy) [30], which provides the functional traits necessary for host homeostasis, such as the barrier effect against infectious threats and the production of several bioactive small molecules that support the GM-host metabolic, immunological and neurological connections (e.g., vitamins, fatty acids, protein metabolites, bile acids, polyamines, etc.) [31-33].

As a matter of fact, GM is dominated by species (mainly from *Ruminococcaceae* and *Lachnospiraceae* families) capable of degrading complex carbohydrates, otherwise indigestible for humans, such as glycans and mucins (called Microbiota-Accessible Carbohydrates) [34]. The end products of this fermentation are short-chain fatty acids (SCFAs) [13], mainly acetate, propionate and butyrate, which are indisputably beneficial to health, acting as local (butyrate) and peripheral (acetate and propionate) energy sources, inflammation modulators, vasodilators and regulators of gut motility, wound healing, metabolism and epigenetics [35]. SCFAs also influence the proliferation and differentiation of colonic epithelial cells, including through the modulation of gene expression, and contribute to the protection against pathogens, promoting the integrity of the epithelial barrier, acidifying the intestinal milieu and stimulating the production of bacteriophages [36-39]. At the systemic levelSystemically, SCFAs act as signaling molecules that drive the expansion and function of hematopoietic and non-hematopoietic cell lineages [35]. For example, SCFA-mediated inhibition of histone deacetylase promotes tolerogenic and anti-inflammatory functions that are crucial for the maintenance of immune homeostasis [19,33]. Many other immunoregulatory properties of SCFAs are associated with the activation of G protein-coupled receptors expressed by nearly all types of immune cells, including epithelial cells, neutrophils, monocytes and macrophages [40,41].

Another worthy example of GM-produced metabolites that act as key immunoregulators are polyamines (e.g., putrescine, spermine, and spermidine), which are usually produced by amino acid decarboxylases [42]. These molecules are essential for host cell function, barrier integrity and pathogen defense against pathogens, as well as for local and systemic adaptive immunity [43-46]. Alterations in polyamine metabolism with higher levels of these compounds have been shown to be associated with cell growth bugs, and acute and chronic inflammation up to carcinogenesis [47]. Indeed, highly proliferative cells, such as tumor cells, require polyamines, among others, to support rapid growth. For example, increased circulating and urinary levels of polyamines have been observed in patients with colorectal cancer (CRC), as well as with skin and hormone-related (*i.e.*, breast and prostate) tumors cancers [49-50]. As recently demonstrated, a "polyamine blocking therapy", based on the reduction of intratumoral polyamine availability, could therefore have an antiproliferative effect but also reverse immunosuppression in the tumor microenvironment and heighten antitumor immune responses [51,52]. On the other hand, it should be mentioned that in autophagy-competent tumors, treatment with spermidine (as an autophagy-inducing caloric restriction mimetic) improved the efficacy of anticancer chemotherapy and enhanced immunosurveillance [53].

Not only the metabolites produced or contributed by GM but also the structural components of the microbes are able to influence the host immunological landscape. Since birth, the human immune system coexists with a plethora of microorganisms and develops pattern recognition receptors (PRRs), capable of detecting microbe-associated molecular patterns (MAMPs) (e.g., lipopolysaccharide (LPS), peptidoglycan, flagellin and unmethylated bacterial DNA CpG

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143 motifs) [54]. This intimate GM-immune system crosstalk is strategic for maintaining the delicate balance between

144 tolerance towards commensal microbes and recognition and attack towards pathogens or pathobionts [32,55]. Upon PRR-

145 mediated response activation, a complex signaling cascade is initiated ~~that~~ lead~~s~~ing to the release of host immune system

146 effectors, such as cytokines, chemokines, and acute phase proteins [56,57]. Furthermore, MAMPs are involved in the

147 modulation of immune cell function, such as neutrophil migration and function [58] and differentiation of T cell

148 populations into helper cells (T$_H$) (*i.e.,* T$_H$1, T$_H$2 or T$_H$17) or T regulatory cells (T$_{reg}$) [54]. For example, in preclinical

149 models, *Bacteroides fragilis*-specific structural polysaccharide (PSA) has been shown to restore a T$_H$1/T$_H$2 imbalance

150 through stimulation of Toll-like receptor (TLR) 2 signaling and interleukin (IL)-12 production by dendritic cells, and

151 suppress inflammation by driving IL-10 production [59,60].

152 As mentioned above, during human lifespan, GM is able to respond and adapt to changes in endogenous and exogenous

153 variables, such as diet, lifestyle and geography. This is made possible by the great GM plasticity, *i.e.*, the ability to oscillate

154 between different healthy states, without losing diversity, stability, as well as ~~and~~ microbe-microbe and microbe-host

155 interactions [30]. However, it is also well known that ~~in~~ under certain conditions, such as intake of antibiotics or other

156 drugs, pathogen infection and inflammation, just to name a few, stability is compromised and unbalanced (dysbiotic)

157 states are established, which can be resilient and explain the onset and progression of diseases, as well as resistance to the

158 ~~efficacy~~ effectiveness of treatments [12]. Although the exact boundaries of a healthy GM are still ~~lacking~~ missing [61],

159 disease-associated GM profiles are typically featured by less biodiversity and distinct compositional alterations, which

160 fall~~ing~~ into the following categories: selective suppression of certain health-associated members (generally SCFA-

161 producing, oxidative stress-sensitive *Lachnospiraceae* and *Ruminococcaceae* taxa) and/or burst of subdominant taxa,

162 including overt pathogens or pathobionts [62]. These alterations are generally reflected in an inappropriate pattern of

163 metabolites, which can improperly regulate human biology, with even deleterious consequences for health [63,33].

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165 **3. The gut microbiome in cancer development and progression**

166 *3.1 The role of the gut microbiome ~~role~~ in ~~the~~ tumor onset: CRC and beyond*

167 ~~Until now,~~So far unhealthy states of GM have been found in the context of multiple intestinal but also metabolic,

168 immunological, hepatic, respiratory, cardiovascular, neurological, psychiatric, and oncological disorders [3]. In most

169 cases, it is still impossible to define whether or not GM has a causative role ~~or not~~, even if some hypotheses supporting

170 ~~causality~~ causation have been advanced, especially in obesity and related complications [64]. Notwithstanding this, it

171 must be said that for some disorders, the related literature is already quite substantial (e.g., for inflammatory bowel disease

172 (IBD) and CRC) [65-67], while for others, such as different tumor types, there is still a long way to go to understand the

GM-disease relationship. As expected, the loss of intestinal homeostasis has been linked to both local (*i.e.*, CRC and gastric cancer) [68,69] and distant tumors, such as pancreatic [70], laryngeal [71] and gallbladder [72] carcinomas.

To date, microbial pathogens are known to drive 20% of carcinogenesis [73,74]. Carcinogenesis is a multistep process, whose the progression of which is characterized by the gradual accumulation of slow and random genetic and epigenetic mutations that can take more than 10 years depending on the frequency [75]. In particular, as far as the GM field is concerned, the most frequent (and obvious) connection has been made with CRC. CRC is sporadic for in approximately 90% of cases and develops gradually from normal epithelium to adenomatous polyps until the settlement of an invasive carcinoma [76]. In addition to genetic predispositions that can increase the risk of developing CRC (e.g., adenomatous polyposis coli gene mutation), leading to the development of hundreds to thousands of polyps, several environmental factors have been shown to be involved in CRC onset, including diet, chronic inflammation (e.g., IBD) and GM [77]. As for the latter, several studies have shown highlighted a CRC-associated GM profile enriched with opportunistic pathogens, such as *Fusobacterium*, several members of the *Enterococcaceae* family and *Campylobacter*, as well as other pro-inflammatory taxa*, i.e.,* *Erysipelotrichaceae* and *Collinsella* (recently proposed as a potential marker of metabolic disorders) [78-81]. In parallel, a reduction of in health-associated microbial partners, including the butyrate producers *Faecalibacterium* and *Roseburia*, is frequently observed [82]. However, all of these studies, although milestones in the CRC-associated GM literature, are purely observational and have therefore have not explored the mechanisms by which GM members can influence CRC or, more importantly, the triggers that shift the GM profile towards a tumor-associated one. Most of these questions were answered by coupling next-generation sequencing-based approaches with animal models, which helped to better outline the role of GM microbes in tumor onset. Research conducted in recent years has highlighted the fairness of the bacterial driver-passenger model developed in 2012 from by Tjalsma et al. [83] Briefly, several environmental (e.g., diet, pathogen infection) and genetic (e.g., chronic inflammation, mutations) factors can push the GM homeostatic profile towards a dysbiotic, pro-inflammatory one that settles in the gastrointestinal tract. CRC can therefore be promoted by commensal bacteria with pro-carcinogenic features (known as bacterial drivers) that drive the DNA damage of the colon epithelium, leading to CRC development. Afterwards, the local microenvironment is altered as a result of ongoing inflammation and carcinogenesis, which paves the way for bacterial passengers, *i.e.*, microorganisms that show a competitive advantage in the tumor microenvironment and allow for cancer progression [83]. Therefore, inflammation is a trigger for initiating the GM-dependent pro-inflammatory cycle, which is detrimental to the host health [69]. The bacterial drivers identified so far are mostly subdominant components of GM, capable of inducing a harmful inflammatory loop, synthesizing genotoxins and other toxic molecules that can directly damage host cells, and activating dietary heterocyclic amines to pro-carcinogens [69]. Some eExamples are include superoxide-producing *Enterococcus faecalis* strains [84,85], toxigenic strains belonging to the *B. fragilis* species [86,87], and genotoxin-

producing *Salmonella enterica* and *E. coli* strains [88-91]. As for bacterial passengers, again ~~they~~ these are usually subdominant GM commensals, which may however show either tumor-promoting or tumor-suppressive properties, depending on the microorganism type. Indeed, ~~the~~ tumor tissue is selectively colonized by opportunistic pathogens, such as *Clostridium septicum* [92], *Fusobacterium nucleatum* [93], *Streptococcus gallolyticus* [94], and several *Enterobacteriaceae* members [83], but sometimes also ~~sometimes~~ enriched in health-associated bacteria, such as *Roseburia* and *Faecalibacterium*, for which a possible protective role as CRC quenchers has been advanced [95].

It is clearly very simple to explain the relationship ~~of~~ between GM ~~to~~ and CRC, but the pro-carcinogenic role of commensal microbes extends far beyond the gastrointestinal tract [74,96]. Due to its physical proximity and close physiological links, the liver is one of the organs most affected by GM. The development of hepatocellular carcinoma (HCC) may be related to various GM-derived functions and metabolites, including LPS, ~~whose~~ the presence of which potentiates HCC tumorigenesis through the activation of innate immune system effectors, such as TLR4 [97]. Moreover, some GM taxa play the role of oncogenic drivers by producing secondary bile acids (*i.e.,* deoxycholic acid, DCA), deriving from ~~the~~ GM-mediated deconjugation and metabolism of primary bile acids [98]. Once absorbed from the gastrointestinal tract, DCA can reach the liver through the portal circulation, where it can exert tumorigenic functions by inducing DNA damage and senescence on hepatocytes, with the establishment of a pro-inflammatory liver environment [99,100]. Consistently, in murine models fed high-fat diets, ~~it has been observed that the~~ enrichment of GM species belonging to the *Clostridium* genus, including *C. scindens*, *C. hiranonis*, *C. hylemonae* and *C. sordelli*, capable of producing DCA [101], led to progression from non-alcoholic steatohepatitis to HCC [99]. The same tumor-driving actions by GM members have recently also been reported in esophageal tumors [100]. Finally, it is worth mentioning that GM dysbiosis and the consequent dysregulation of metabolite production have been shown to be involved in the development of breast cancer [102,103]. In particular, gut microbes are able to metabolize liver-derived estrogens through beta-glucuronidase and beta-glucosidase activities in the gastrointestinal tract (the so-called estrobolome, *i.e.*, "the aggregate of enteric bacterial genes whose products are capable of metabolizing estrogens") [104]. This GM role in modulating the systemic ~~estrogen~~ pool of estrogens could affect their enterohepatic circulation and reabsorption, thus contributing to an increased risk of hormonal cancers, such as breast cancer [105-107].

3.2 Gut microbiome middles with anticancer therapies

A new frontier of research is the understanding of the bidirectional relationship between GM and drugs (*i.e.*, pharmacomicrobiomics) [108]. GM can in fact modulate the host response to therapies through several mechanisms, including immune system interactions and drug metabolism [109], and, in turn, drugs can affect the GM structure and thereby its mutualistic relationship with the host [110]. Identifying the pivots of this relationship can therefore be crucial

for improving patients' clinical outcomes, as it can inform the development of novel, evidence-based intervention strategies, aimed at manipulating GM to enhance therapeutic efficacy, reduce toxicity and possibly, also the risk of relapse [111,112].

The first research on GM in anticancer therapies dates back to 1890, when two heat-inactivated microbes belonging to the *Streptococcus* genus were injected intratumorally as an attempt to cure cancer in humans. In those years, Dr. Coley thought that a local bacterial infection could boost the patient immune response against inoperable tumors. For more than 40 years, more than 1000 patients were injected intratumorally with microbes or microbial products, with excellent results mostly in inoperable bone and soft-tissue sarcomas. From this moment on, albeit rudimental, this approach was called Coley's Toxins and defined as the "father of immunotherapy" [113,114]. Several years later, the same approach was used to treat patients with bladder cancer, in whom *Mycobacterium bovis* was intratumorally injected right after tumor resection, resulting in reduced tumor recurrence through activation of local immune responses [115]. Furthermore, oral administration of the well-known probiotic species *Lactobacillus casei* has been associated with the decrease in recurrence of superficial bladder cancer [116]. Later on, all these studies confirmed the intuition of those researchers: the antitumor responses were stimulated by the microbial activation of two important effectors of the immune system: natural killer cells and macrophages [117]. Although these are rudimentary and "seasoned" studies, they have paved the way for many clinical trials, some still ongoing, using attenuated GM members to aid cancer treatments [118]. Recently, ~~it has been observed that~~ *Mycobacterium obuense* [119,120] and genetically modified *Salmonella* Typhimurium [121,122] have been shown to promote anticancer responses in several refractory solid tumors (e.g., pancreatic, melanoma), by activating the host immune system and exerting a cytotoxic effect on tumor cells. While very promising, many studies are still needed to refine microbial therapies before they can be used in clinical routine. Still today, the action and toxicity of microbes inside the tumor are very hazy and mainly correlated to the long microbial half-life with the possibility of antibiotic resistance accumulation, as well as the onset of mutations reverting the attenuated bacterial phenotype [123].

In addition to the intratumoral effect of individual microbes, the GM has recently been associated with the therapeutic outcome of anticancer treatments [124]. Since the discovery of the cytotoxic effects of mustard gas during the Second World War, cytotoxic chemotherapeutic ~~cytotoxic~~ agents (*i.e.*, alkylating agents, platinum-based drugs and cytotoxic antibiotics) have been developed and are still the major staple of anticancer approaches [125]. However, some ~~tumors~~ cancers fail to respond to treatment and/or ~~tumor~~ the cancer relapse ~~oc~~recurs. To overcome these hurdles, novel anticancer approaches are constantly in progress [126]. The first advancement in this field was the development of targeted immunotherapy [127,128] and, of course, research focusing on the relationship between GM~~-~~ and anticancer therapy has ~~relationship~~ followed the same trend. Gut microbes have been shown to influence drug pharmacokinetics, anticancer activity and toxicity of chemo-immunotherapy treatments to varying degrees [110,129]. A striking example of a GM-

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drug interaction is represented by irinotecan, a chemotherapy drug administered parenterally in an inactive form to patients with CRC, which is activated by host enzymes, detoxified in the liver and subsequently excreted in the intestinal lumen via the bile [130]. Here, GM members can reverse the detoxification process through bacterial beta-glucuronidases, which catalyze drug deconjugation and reactivation, resulting in intestinal toxicity [131]. In this regard, the use of specific enzyme inhibitors has been shown to prevent irinotecan-induced diarrhea while maintaining its efficacy in animal models [132]. As for the GM influence on anticancer activity, one of the first milestone studies in the field showed that the antitumor effect of oxaliplatin or cisplatin treatment on subcutaneous transplantable tumors was dramatically drastically reduced in germ-free or GM-depleted mice by broad-spectrum antibiotics [133]. The so-called platinum resistance of these models has recently been linked to the role of GM members in promoting oxidative stress and, subsequently, apoptosis of tumor cancer cell apoptosis. Consistently, mice with lung tumors treated with antibiotic-coupled cisplatin therapy have been shown to have reduced long-term survival and developed even larger tumors [134]. On the other hand, when cisplatin was combined with the administration of probiotics, like such as *Lactobacillus acidophilus,* the same animal models showed improved response to chemotherapy, through the activation of pro-apoptotic genes and effectors within the tumor aggregate and the promotion of a proper tumor-specific immune response. Similar to platinum-derived compounds, chemotherapy treatments based on the alkylating agent cyclophosphamide coupled with the oral administration of microbes (*i.e.*, *Lactobacillus johnsonii* and *Enterococcus hirae*) have been shown to promote the conversion of T cells from naïve to pro-inflammatory T_H17, with the final outcome of improved therapeutic efficacy in tumor-bearing mice [135-137]. These findings were also confirmed in advanced lung or ovarian cancer studies, in which patients with GM enrichment of *E. hirae* and *Barnesiella intestinihominis* showed a more favorable prognosis after chemo-immunotherapy [138].

With specific regard to immunotherapy, different studies have highlighted that the administration of CpG oligodeoxynucleotides (*i.e.,* synthetic molecules mimicking microbial DNA) strongly stimulated the host immune system, pushing endogenous anticancer activity in several types of cancer [139]. After *in vivo* intratumoral injection of CpG oligodeoxynucleotides coupled with anti-IL-10 receptor antibody, the host immune cells were activated near the tumor site to produce pro-inflammatory tumor necrosis factor (TNF), leading to reduced tumor growth due to hemorrhagic necrosis. With By a similar mechanism, the administration of *Alistipes shahii* and *Ruminococcus* in antibiotic-treated mice stimulated the production of TNF with a notable improvement of the anticancer therapeutic outcomes [133]. As the literature currently stands, GM members are involved in the intrinsic efficacy of another class of immunotherapy drugs known as immune checkpoint inhibitors, which are commonly used to treat different types of solid tumors. These molecules are capable of blocking immune-inhibitory pathways, thus modulating the activation of T cells against the targeted tumor cells [140-142]. Currently, the checkpoint inhibitors put in place are monoclonal antibodies that target

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cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and programmed cell death 1 (PD1) located on T cell surfaces, as well as its ligand (*i.e.,* programmed cell death ligand 1, PD-L1) [143]. The mechanisms of action are both T cell-specific but while anti-CTLA4 therapy is able to regulate T cell proliferation early during the immune response within the lymph nodes, anti-PD1 suppresses T cell activation later in the body periphery [144]. In this scenario, a landmark study by Vétizou and colleagues [145] showed that antibiotic-treated or germ-free mice with subcutaneous tumors treated with anti-CTLA4 responded poorly to therapy, but the response was significantly increased when GM was enriched in *Bacteroides fragilis* and *Burkholderia cepacia* [145]. Furthermore, oral feeding of GM-depleted mice with different *Bacteroides* species (*i.e.*, *B. thetaiotaomicron* or *B. fragilis*) restored the therapeutic response to anti-CTLA4 by inducing immune cell response in tumor-draining lymph nodes. When *B. fragilis* and *B. cepacia* were administered together in the same murine models, *B. fragilis and B. cepacia were administered together*, the restoration of the anti-CTLA4 response was confirmed but, unlike the administration of single *Bacteroides* taxa, therapy-related side effects, such as intestinal damages and colitis, were also significantly reduced. These findings were confirmed upheld in melanoma patients treated with anti-CTLA4, where the abundance of the Bacteroidetes phylum was positively correlated with the reduction in of therapy-associated colitis. In particular, the GM profiling of these patients revealed three different configurations: one was dominated by *Prevotella* spp., whereas the other two were mostly characterized by the presence of various *Bacteroides* spp. Subsequently, these different GM configurations were used to perform FMT to on germ-free mice. Only the GM profile enriched in *B. thetaiotaomicron* or *B. fragilis* resulted in high responsiveness to anti-CTLA4 treatment in non-responder mice. Taken together, these observations are extremely relevant as they suggest not only that some GM members may affect immunotherapy responses but also that GM manipulation may favor antitumor activity in non-responders. On the same line of as anti-CTLA-4 therapy, Sivan et al. found that in melanoma-bearing mice, the efficacy of PD-L1-targeted antibodies was enhanced in the presence of a GM ecosystem enriched with different *Bifidobacterium* spp., including *B. breve*, *B longum* and *B. adolescentis* [146]. Oral administration with a commercially available probiotic cocktail (*i.e.*, with *B. breve* and *B. longum*) during anti-PD-L1 therapy, was able to activate the immune T cell response and hold tumor growth, while the combined treatment (bifidobacteria and anti-PD-L1) nearly abolished tumor outgrowth. From this moment onwards, multiple translational works have been carried out. Among the most relevant, it is certainly noteworthy that of Routy et al., [140] who found that patients with melanoma treated with antibiotics during anti-PD-L1 immunotherapy showed a lower survival rate [140]. By comparing the GM of responders vs non-responders, the authors were able to identify the GM compositional signatures of response to therapy, which consisted of an enrichment in *Akkermansia* and *Alistipes*. Again, they performed FMT from patients to germ-free mice and found that *Akkermansia muciniphila*, alone or in combination with *E. hirae*, increased intratumoral cytotoxic T cell infiltrates, favoring the PD-1 blockade response. In parallel, similar compositional differences between responders and non-responders to anti-PD-L1

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therapy were found out by Gopalakrishnan et al. [141]. Notably, responders to melanoma-targeted therapy were characterized by higher microbial diversity, as well as increased relative abundance of *Ruminococcaceae* and *Faecalibacterium*, both associated with improved effector T cell function in the peripheral and intratumoral environment. On the other hand, patients showing poor immunotherapeutic response possessed lower microbial diversity and higher relative abundance of Bacteroidales, which ~~was~~ correlated with reduced systemic and antitumor immune responses. Another GM metagenomic characterization in patients with melanoma treated with immune checkpoint inhibitors further corroborated the above findings (*i.e.*, that responders have a distinct GM profile from non-responders), although in this case the efficacy of anti-PD-L1 therapy was associated with *B. longum*, *E. faecium* and *Collinsella aerofaciens* [142].

It should be noted that, despite the huge number of microbial species inhabiting the gastrointestinal tract, to date only a few of them have been suggested to play a role in anticancer responses and only a handful of strains have shown the potential to manipulate ~~the~~ host physiological functions *in vivo* [147,148]. For example, Tanoue and colleagues [149] isolated a consortium of 11 microbial strains (mainly belonging to *Bacteroides* spp.) from feces of healthy human donors, which are able to robustly induce T cell activation within the intestine. *In vivo* colonization with the 11-strain mixture enhanced the therapeutic efficacy of immune checkpoint inhibitors in syngeneic tumor models, confirming the great potential of these microbes as widely effective biotherapeutics in anticancer approaches. Furthermore, three strains belonging to *B. pseudolongum*, *L. johnsonii*, and *Olsenella* have recently been tested in tumor-bearing mice, where they significantly increased the efficacy of immune checkpoint inhibitors [150]. In particular, *B. pseudolongum* enhanced the immunotherapy response through the production of the metabolite inosine, which was able to systemically translocate due to immunotherapy-induced decrease in gut barrier function, and thus activate antitumor T cells. Several questions about the safety of administering live microbes to very often immunocompromised patients during anticancer therapy have been raised over the years and are still largely unanswered. In this context, the usage of prebiotics could be a valid alternative, as discussed below (see the paragraph "Modulation hypothesis: prebiotics, probiotics, live biotherapeutics and FMT, as adjuvant cancer therapy"). As an example, prebiotics (*i.e.,* mucin and inulin) have been shown ~~in syngeneic mouse models~~ to induce antitumor immunity and concomitantly control tumor growth in syngeneic mouse models [151].

3.3 The gut microbiome and hematological malignancies: a focus on hematopoietic stem cell transplantation and related complications

As discussed above, most studies on the role of GM in influencing therapies have focused on solid tumors, particularly melanoma. In parallel, another popular line of research has dealt with profiling the GM of patients suffering from hematological neoplasms (e.g., acute leukemia, lymphoma, and myeloma), with particular regard to the patients' clinical outcomes during and after ~~the~~ chemo-immunotherapeutic treatment. For patients with various blood tumors, first-line and

12

359 life-saving therapy is considered HSCT, a combination of stem cell therapy, conventional treatments (*i.e.*, chemotherapy

360 and radiation) and immune therapy [152]. Unfortunately, HSCT can lead to life-threating complications, such as graft-

361 versus-host disease (GvHD, *i.e.,* when alloreactive donor T cells attack host organs, such as skin, liver and gut), and local

362 and systemic infections, and ~~tumor~~ cancer may relapse~~s may occur~~ [153]. In this context, recent studies in mice and

363 humans suggest important links between GM and clinical outcomes, as well as a role of GM in immunological

364 reconstruction in HSCT recipients [154-156]. Indeed, HSCT practices significantly affect GM balance with a reduction

365 in diversity and sometimes monodominance by Proteobacteria members, *Enterococcus* or *Streptococcus* [157]. In a

366 retrospective study, the reduction of GM diversity in patients undergoing HSCT was associated with a significant increase

367 in mortality (*i.e.*, 52%) compared to patients with a high-diversity GM profile (*i.e.*, 8%) [158]. In addition, during the

368 post-transplant period, antimicrobial treatments are commonly used to treat febrile neutropenia with the ultimate

369 consequence of affecting the GM structure, which can result in increased susceptibility to bacterial infections [159]. For

370 example, an increase in the level of *Enterococcus* spp. after antibiotic exposure correlated with an increased risk of

371 developing bacteremia [157]. Similarly, enrichment of *Enterobacteriaceae* members and other Gram-negative microbes

372 was associated with increased mortality [158]. Recent studies in animal models have shown that the development of

373 GvHD is also associated with a peculiar GM dysbiosis, featured by increased levels of *Enterobacteriaceae* and a reduced

374 amount of obligate anaerobes, mostly belonging to the Clostridiales order [160,161]. These findings were then confirmed

375 in several clinical studies on both adult and pediatric patients [159, 162-165]. By reconstructing the GM dynamics across

376 HSCT, some of these studies have suggested that the so-called "anti-inflammatory Clostridia", *i.e.*, members of the

377 families *Clostridiaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Eubacteriaceae* (the main producers of SCFAs in the

378 intestine), might exert a counteracting effect on GvHD onset and progression [166,167]. As further confirmation, a study

379 on a large cohort of adults showed that the relative abundance of *Blautia*, a well-known health-associated SCFA-

380 producing microorganism belonging to the *Lachnospiraceae* family [168], was correlated with reduced mortality from

381 GvHD [169]. Increased proportions of *Blautia*, along with increased SCFA production, have recently been demonstrated

382 in pediatric patients receiving post-transplant enteral rather than parenteral nutrition [170]. Interestingly, none of the

383 enterally fed patients showed evidence of bloodstream infections, stressing the importance of maintaining a eubiotic GM

384 configuration, capable of producing health-promoting metabolites, to reduce the risk of HSCT-related complications

385 [170,171]. Bacterial-derived SCFAs, especially butyrate, have also been shown in a mouse model to improve the

386 junctional integrity of intestinal epithelial cells, reduce apoptosis, and mitigate GvHD severity [166].

387

388 **4. The big issue of the tumor microbiome**

Since the work by Geller et al. [172] on pancreatic ductal adenocarcinoma, accumulating and robust evidence has confirmed the existence of intratumoral microbes, which can act as intrinsic and essential components of the tumor microenvironment, thus influencing cancer and cancer therapy. These microbes do not necessarily include only "oncomicrobes", *i.e.*, microbes that are known to initiate cancer through genotoxin-mediated mutagenesis (such as fragilisin- and reactive oxygen species-producing *B. fragilis* or colibactin-producing *E. coli*) [173,174] or by interfering with important pathways involved in differentiation and morphogenesis (such as *F. nucleatum* expressing FadA that binds to E-cadherin and activates Wnt-beta-catenin signaling) [175]. Indeed, it has recently emerged that many other microorganisms may not be causative but rather complicit, acting mainly ~~acting~~ through ~~the~~ modulation of the host immune system (immunosuppression or immunogenicity) or molecular mimicry [176].

To date, traces of bacterial DNA have been identified using next-generation sequencing approaches in at least 30 types of cancer, including pancreatic, bile duct, lung, breast, ovarian, cervical, uterine, testicular, prostate, bladder, melanoma, thyroid, kidney, leukemic, bone and brain cancers [177,178]. Notably, most major cancer types appeared to be featured by unique microbial signatures, not only at the tissue level but also in the blood, thus paving the way for the use of plasma-derived cell-free microbial DNA in novel microbiome-based diagnostic tools [178]. In particular, the ratio between taxa belonging to the phylum Proteobacteria and those of Firmicutes varied in the different types of cancer, with the breast cancer microbiome being the richest and most diverse of those analyzed to date [177]. Alongside the compositional traits, it is worth noting that a tissue-specific enrichment of some bacterial functions has been hypothesized, which could be related to the clinical features of the different tumor types [177]. For example, bacterial degradation of hydroxyproline, deriving from bone collagen and particularly high in bone diseases, including cancer, was overrepresented in bone tumors. Likewise, the pathways involved in the degradation of chemicals in cigarette smoke were enriched in lung cancer. While these findings are expected, as they may be the result of host-driven or top-down selection, it should be pointed out that they were generated through inferred metagenomics, with obvious interpretative limitations. The presence of microbial components within some tumor tissues was also investigated by immunohistochemistry, which allowed to confirm that not only nucleic acids but also structural components can be found, such as LPS from Gram-negative bacteria and lipoteichoic acid from Gram-positive bacteria, ~~can be found~~ [177]. However, while LPS was frequently detected, lipoteichoic acid was ~~mainly~~ found mainly in melanoma. These data were apparently in contrast to ~~with those of~~ ~~sequencing~~ sequencing-derived ones, according to which many Gram-positive bacteria were represented in any tumor type, but they may reflect an altered cell morphology (with lack of cell wall), as also hypothesized based on transmission electron microscopy imaging and previous literature [179]. Based on the staining patterns, intratumor bacteria were mostly localized in the cytoplasm and nucleus of cancer cells, as well as in immune cells, *i.e.*, in leukocytes and especially in macrophages [177]. As for their number, in pancreatic ductal adenocarcinomas, an average of one bacterium per about

150 human cells has been estimated [172]. According to tumor mapping by Nejman et al. [177], the percentage of tumors positive for bacterial DNA ranged ~~between~~ from 14.3% in melanoma to >60% in breast, pancreatic and bone tumors. Based on these estimates, Sepich-Poore et al. [176] ~~came to the conclusion of~~concluded about 10^5 to 10^6 bacteria per palpable 1-cm^3 tumor, or about 34 bacteria per mm^2. The source of these microorganisms/microbial components is not yet clear, but they could likely be part of other host-associated ecosystems, such as GM, translocate ~~through~~ across compromised mucosal barriers (e.g., leaky gut), and then reach tumor masses, facilitated by their disorganized and leaky vasculature. For example, this has been strongly suggested for CRC liver metastases, where >99.9% nucleotide identity was found for *Fusobacterium* isolates from the primary tumor and metastatic site, although tissue collection occurred months if not years later [81]. Once in the bloodstream, *F. nucleatum* has been hypothesized to translocate also ~~to~~ in breast cancers, colonization of which is made possible by the binding of its lectin Fap2 to galactose and N-acetyl-d-galactosamine residues, abundantly expressed on cancer cells [180]. Obviously, work ~~is~~ still ~~needed~~ needs to be done to confirm all of these findings, including the dead/alive issue. Similarly, ~~it will have to be determined~~ whether these bacteria are actually involved in tumor pathogenesis or are mere opportunistic residents/passengers, which take advantage of a nutrient-rich and immunosuppressed environment, should be determined. Regardless, they may play a critical role in promoting tumor growth and/or mediating chemoresistance, thus affecting patient response and survival. This has been seen for example for Gammaproteobacteria that can inactivate the chemotherapeutic drug gemcitabine, ~~by~~ through the expression of a long isoform of the bacterial enzyme cytidine deaminase [172], and for *F. nucleatum*, which activates the autophagy pathway (by targeting TLR4 and MYD88 innate immune signaling and specific microRNAs), thus preventing chemotherapy-induced apoptosis [181]. *F. nucleatum* may also accelerate tumor growth by inducing apoptosis in lymphocytes, as suggested by the reduced levels of CD4+ and CD8+ T cells in breast cancers [180]. Notably, the administration of antibiotics (*i.e.*, metronidazole) inhibited *F. nucleatum*-induced tumor enlargement, stressing once again how microbial manipulation can have significant repercussions on clinical outcomes. Finally, it is worth mentioning that greater intratumoral diversity has been correlated with long-term survival in patients with pancreatic adenocarcinoma [182]. Long-term survivors also showed potentially favorable intratumoral microbial signatures (*i.e.*, *Saccharopolyspora*, *Pseudoxanthomonas*, *Streptomyces* and *Bacillus clausii*), which could promote the recruitment and activation of CD8+ T cells, with overproduction of interferon (IFN)-gamma, thus contributing to the antitumor immune response and influencing the natural history of the disease. Although the data are still preliminary, using FMT from long-term survivors with no evidence of disease in tumor-bearing mice, the authors observed immune system activation and antitumor response, thus opening the way to immense microbiome-based therapeutic opportunities. What is certain is, therefore, that future therapeutic strategies ~~can~~ will no longer be able to ignore the presence of intratumoral microbes, rather they

450 could be improved through integration with precision microbiome manipulation tools, targeting microbes and/or the
451 mechanisms in which they are involved.
452
453 **5. Modulation hypothesis: prebiotics, probiotics, live biotherapeutics and FMT, as adjuvant cancer therapy**
454 In recent times, enormous strides have been made in improving anticancer therapies, expanding the plethora of treatments
455 available and significantly reducing side effects, while paying attention to patient compliance [183]. As discussed above,
456 multiple lines of evidence have placed an increasing emphasis on how microbiome modulation may represent be a crucial
457 adjunct to current anticancer therapies [141,184-186]. Microbiome-tailored precision medicine is based on the use of
458 prebiotics, (traditional and next-generation) probiotics and FMT, to be personalized for the best efficacy and safety
459 according to the patient's microbiome configuration and other host metadata, for the best efficacy and safety. Herein, we
460 will discuss the most recent and relevant clinical trials that have been planned (some still ongoing) to shed light on the
461 therapeutic potential of GM manipulation in cancer patients (using the tools mentioned above), in terms of improved
462 response and mitigation of adverse events. See **Table 1** for a summary of the clinical trials that have been registered in
463 the last two years.
464
465 *5.1 Prebiotics*
466 Prebiotics are typically referred to as "a substrate that is selectively utilized by host microorganisms conferring a health
467 benefit" [187]. In particular, they exert their beneficial effects by promoting the expansion and/or metabolic activity of
468 specific groups of commensals, including keystone taxa. Other induced effects at the microbial ecosystem level include
469 growth promotion through cross-feeding interactions and inhibitory effects against pathogens or pathobionts, through
470 displacement, production of antimicrobial metabolites or other changes in microbial fitness [185,188,189]. Evidences
471 gathered from in *in vitro* and *in vivo* studies suggestss that administering prebiotics is a promising and safe therapeutic
472 strategy in different clinical settings [151,185]. A recent study has also demonstrated that discrete dietary fiber structures
473 (*i.e.*, chemically modified resistant starches with small structural differences) are able to induce divergent and highly
474 specific effects on GM, which directly changess in SCFA production, thus paving the way for precision manipulation of
475 the GM through *ad hoc* designed carbohydrates [190]. With specific regard to cancer, however, only a limited number of
476 interventional studies in humans are available to date, with sometimes conflicting results. For example, oral consumption
477 of amylase-resistant starch as a prebiotic formulation, administered in combination with chemotherapy, was not
478 conclusive in the prevention of acute radiation proctitis in patients with cervical cancer [191]. Conversely, a prebiotic
479 regimen based on fructooligosaccharides, xylooligosaccharides, polydextrose, and resistant dextrin, administered 7 days
480 prior to surgery for CRC surgery, improved serum immunological markers, reversing the surgical stress-induced surge of

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opportunistic pathogens in GM [192]. The interventional clinical trial conducted by Garcia-Peris and colleagues ([193]; NCT01549782) on 40 women undergoing radiotherapy for endometrial neoplasms confirmed the hypothesis that a mixture of fructooligosaccharides and inulin modulates the representation of *Lactobacillus* and *Bifidobacterium* within the GM community, while reducing tissue damage at the enterocyte level. As briefly mentioned above, in a mouse melanoma model, inulin and mucin also stimulated *Bifidobacterium* spp. and *A. muciniphila* [151], both previously identified as beneficial GM components, capable of eliciting effective antitumor immunity [140,146]. Regarding clinical trials in cancer patients still ongoing and started in the last two years (**Table 1**), one study foresees the enrolment of 120 participants with gastrointestinal cancer and chemotherapy-related diarrhea (NCT04447443) and focuses on a 2-week supplementation with prebiotic fiber ~~supplement~~ along with loperamide hydrochloride ~~administration~~. Longitudinal monitoring of the GM configuration and subsequent comparison with the results obtained from the administration of placebo (*i.e.*, maltodextrin) and loperamide will allow researchers to dissect the effects specifically induced by prebiotics. A second study (NCT04624568) aims to compare the regression rate of cervical intraepithelial lesions in 150 women after 6-month administration of a vaginal gel composed of hyaluronic acid and prebiotic extract of *Coriolus versicolor*, which improves the re-epithelialization of the uterine cervix [194]. Creating a protective biofilm on the cervix would help restore a niche environment conducive to regression of intraepithelial lesions and human papillomavirus clearance. Although the aforementioned studies are still in their infancy, they have the potential to provide valuable insights into how prebiotic administration modulates the microbiome of cancer patients, while influencing disease markers and clinical outcomes.

5.2 Probiotics and live biotherapeutics

Probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" [195], are a GM manipulation tool with a long history of use. Those currently most used and studied are certainly bifidobacteria and lactobacilli, but also, to a lesser extent, strains of *Lactococcus* spp., *Streptococcus thermophilus*, *Saccharomyces boulardii* and *E. coli* Nissle 1917. Clinical efficacy, mechanisms of action and caveats in the field are admirably discussed elsewhere (see for example Suez et al.~~,~~ [196]). In the context of cancer patients, strains of *Lactobacillus* and *Bifidobacterium* have stood out for their ability to delay tumor formation, inhibit tumor cell proliferation and prevent life-threatening side effects associated with chemotherapy treatments, in addition to ~~the~~ binding and degradation of carcinogenic compounds, ~~the~~ inhibition of carcinogen-producing enzymes, and immunomodulating and anti-inflammatory properties [197-201]. It is also worth noting that probiotic strains of *Bifidobacterium* and *Clostridium*, when administered intravenously, have been shown to colonize hypoxic tumors, preferably thriving in solid malignancies [202]. However, despite the encouraging results, most probiotic therapies in oncology are still in the preclinical stage and very few studies have ~~have~~ reported the effects of probiotics in humans [201,203] (see also **Table**

17

1). Among them, a clinical trial in CRC patients showed that probiotic (*Bifidobacterium lactis* Bl-04 and *L. acidophilus* NCFM) administration promoted the expansion of beneficial butyrate-producing microbes in both mucosa and feces, and tended to reduce *Fusobacterium* proportions (NCT03072641; Hibberd et al., [204]). Furthermore, preoperative probiotic (*S. boulardii*) therapy resulted in reduced levels of IL-1beta, IL-10, and IL-23 mRNA within the colonic mucosa of CRC patients following resection, when compared to controls who received the anticancer treatment alone (NCT01895530) [205]. As for other cancer types, improved relapse-free survival was observed after administering an oral preparation of *L. casei* for one year to patients with superficial bladder cancer, after completion of transurethral resection therapy followed by intravesical administration of epirubicin, although no difference in overall survival was observed compared to the control group [206]. Beneficial effects on GM are also expected in an ongoing clinical trial, which involves a short course of probiotic therapy (with the following 13 species: *S. boulardii*, *Lactobacillus plantarum*, *Bacillus subtilis*, *B. lactis*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *B. breve*, *L. casei*, *Lactobacillus salivarius*, *L. acidophilus*, *Lactobacillus brevis*, *B. longum* and *Lactobacillus paracasei*) in patients with operable breast cancer before surgery (NCT03358511). Interestingly, variations within the tumor microenvironment are also expected, with in particular an increase in the resident CD8+ T cell subpopulation. In another clinical trial, ~~the~~ researchers aim to evaluate the efficacy of the probiotics administered (no information is available on the strains or species used) prior to surgery in 40 patients with breast and lung cancer, while assessing systemic and intratumoral immunomodulatory effects (NCT04857697). The enrollment of 40 patients with potential/resectable non-small cell lung cancer is instead planned to evaluate the safety and effect of neoadjuvant chemotherapy and immunotherapy combined with probiotics (again, no compositional information is available) (NCT04699721). Within the Thoracic POISE project [207], the efficacy of a probiotic blend of *Lactobacillus* and *Bifidobacterium* spp. (*i.e.*, Pro 12) will be assessed in reducing surgical adverse events, prolonging overall survival and pioneering integrative care delivery in 40 patients with esophageal cancer (NCT04871412).

As next-generation probiotics, live biotherapeutics, defined as "live organisms designed and developed to treat, cure, or prevent a disease or condition in humans" [208], have the potential to ~~represent~~ be a decisive tool for the improvement of current anticancer therapies [209]. This category encompasses GM members that have emerged thanks to advances in massive sequencing technologies but also engineered microbes, *i.e.*, GRAS (generally recognized as safe) organisms or commensals, which are used as a delivery vehicle for a bioactive molecule or to express certain functionalities [210]. The former includes, for example, *A. muciniphila* (identified as a predictor of response in melanoma) [140], as well as *Bacteroides ovatus* and *Bacteroides xylanisolvens*, both of which have been associated with enhanced cancer immune surveillance [211,212]. As for recombinant bacterial therapeutics, although they are currently being tested in clinical trials, none of them have so far been approved for use in humans. Among these, *B. longum* expressing the pro-inflammatory IL-12 transgene (bacTRL-IL-12) was selected to evaluate the beneficial effects on solid tumors, in terms

18

543 of stimulation of the local and systemic anticancer immune response (phase I, Symvivo). The clinical trial focusing on

544 the safety, tolerability and preliminary evaluation of the anticancer efficacy of bacTRL-IL-12 following intravenous

545 infusion was recently conducted on 5 participants (NCT04025307). *E. coli* Nissle 1917, engineered to produce cyclic

546 adenosine diphosphate, a stimulator of the STING (STimulator of INterferon Genes) pathway (SYNB1891; phase I

547 Synlogic), has also been identified as a promising live biotherapeutic agent for the adjuvant treatment of solid tumors but,

548 to date, clinical trials are still ongoing. In particular, the study NCT04167137 involves intratumoral injection of

549 SYNB1891 in patients with diagnosed advanced/metastatic solid tumors and lymphomas, undergoing imaging to assess

550 tumor response and safety monitoring. After determination of dose-limiting toxicity, SYNB1891 will be administered in

551 combination with immunotherapy treatment (*i.e.*, atezolizumab). It is worth noting that *E. coli* Nissle 1917 has also been

552 engineered to bind to the surface of CRC cells and secrete myrosinase, an enzyme capable of converting glucosinolates

553 in cruciferous plants into isothiocyanates such as sulforaphane, a small molecule with known anticancer activities [213].

554 In murine models of CRC , fed with engineered microbes and a cruciferous vegetable diet, the authors observed significant

555 tumor regression and reduced tumor recurrence. Finally, a double-blind, randomized interventional study on 100 women

556 with breast cancer has been planned to evaluate the effect of an investigational product (a probiotic from BIOHM Health

557 LLC, engineered to address the key role of fungi in digestive health) administered in combination with standard anticancer

558 therapy , on the breast cancer microbiome and GM (NCT04362826).

559 On the other side of the coin, some studies have shown deleterious effects for probiotics in cancer patients, even using

560 the same strains, such as an increase in tumor penetrance and multiplicity [214]. Discordant and heterogeneous evidence

561 of efficacy strongly underscores the need for a precision tailored approach, which takes into accountconsiders the

562 individual microbiome configuration (in terms of composition and functionality), host metadata (e.g., genetics,

563 anthropometrics and immune profiling) and varying environmental exposures [196,215]. However, it remains undeniable

564 that extreme caution should be taken when administering live microbes to individuals who are very often

565 immunocompromised, due to primary disease and/or therapeutic treatments [196,216].

566

567 *5.3 FMT*

568 As already defined, FMT is the therapeutic procedure that involves the transfer of microbes from healthy individuals to

569 recipients hosting a dysbiotic GM layout, with the aim of normalizing its structure and functionality towards an eubiotic

570 state [186, 217-219]. Since 2018, increasing attention has been paid to the manipulation of GM through FMT in the field

571 of oncology, with particular regard to immune checkpoint blockade [141,220]. From the perspective of microbiome-based

572 medicine, FMT could be administered as a drastic tool for cancer patients who are unresponsive to therapies, to improve

573 systemic and antitumor immune responses. As discussed above, in a pioneering study in an animal model study, FMT

574 from non-responding cancer patients to tumor-bearing mice conferred the resistance phenotype to the recipient, while

575 infusions from responding patients restored reactivity to PD-1 blockade [140]. Subsequently, clinical benefits were

576 obtained as a result of FMT in patients with immunotherapy-resistant metastatic melanoma. In particular, the

577 administration of anti-PD1 in combination with FMT, performed every 14 days for up to 90 days, induced objective or

578 complete responses in three out of five patients, crossing the 6-month progression-free survival landmark [221]. The shifts

579 induced in the GM composition after treatment included the expansion of bacterial species potentially favorable to

580 immunotherapy, *i.e.*, *Ruminococcus* spp. (*R. gnavus* and *R. callidus*) and *B. adolescentis*. In parallel, the tumor

581 microenviroment also underwent a reprogramming, consisting in an upregulation of the IFN-gamma-mediated signaling

582 pathway, together with effector T functions. In a second recent study on 16 patients with advanced melanoma, a single

583 FMT from seven different donors was administered in combination with PD-1 blockade [222]. PD-1 refractory patients

584 exhibited a shift towards donor GM composition, along with significant metabolic changes and reprogramming of the

585 tumor microenvironment, thereby overcoming primary resistance to immunotherapy. Taken together, these pivotal studies

586 led to the first proof of concept that FMT transfers clinical benefits to patients with immunotherapy-resistant metastatic

587 melanoma, by shifting their GM towards a donor-like profile associated with immune activation, mitigation of anti-

588 inflammatory tone and modification of host metabolism.

589 Among the clinical trials on FMT in cancer patients, registered in the past two years and currently underway (**Table 1**),

590 only two studies are in the active recruitment phase. The first trial (NCT04721041) involves the enrollment of 40

591 participants and focuses on the treatment of oncotherapy-related intestinal complications by evaluating the efficacy of

592 washed microbiota transplantation (WMT), a new stage of FMT. Consisting of sequential microfiltration and

593 centrifugation steps, WMT has been shown to reduce the rate of adverse events potentially associated with classic

594 microbiome-based treatment (e.g., fever, diarrhea, abdominal pain, nausea and vomiting), without compromising the

595 effectiveness of the procedure [223,224]. The second randomized controlled clinical trial (NCT04758507) aims to

596 evaluate the efficacy of FMT in improving response rates to immune checkpoint inhibitors in 50 patients with advanced

597 renal cell carcinoma, by selecting donors who respond to therapy. Recipient patients will receive the first infusion by via

598 colonoscopy, while frozen fecal capsules at three and six months after the first treatment. Four of the remaining five

599 clinical trials listed in **Table 1** concern the evaluation of the safety and efficacy of FMT, as well as the enhancement of

600 immunotherapy treatment in 20 patients with advanced lung cancer (NCT04924374), 15 participants with metastatic CRC

601 (NCT04729322), 50 patients with metastatic or inoperable melanoma or non-small cell lung cancer (NCT04521075), and

602 60 patients with malignant melanoma prior refractory to immune checkpoint inhibitors (NCT04577729). Finally, the

603 efficacy of FMT will be assessed in the prevention of allogenic HSCT complications, particularly GvHD, in a prospective

604 multi-center randomized phase II clinical trial on 150 participants (NCT04935684).

While it is proving to be a valid and promising tool for modulating GM, as expected, the safety of FMT is still debated, especially as large cohort studies on long-term safety are currently lacking [225]. The risk of adverse events potentially caused by FMT treatment suggests that great caution should be taken in choosing the most suitable microbiome-tailored treatment, especially in cancer patients already severely compromised by standard chemo-immunotherapy treatments.

6. Application of novel microbiome-based approaches in cancer medicine: machine learning as the~~a~~ key ~~for~~ ~~to~~ patient stratification and outcome prediction

6.1 The promise of machine learning

As discussed in the previous paragraphs, research in recent decades has highlighted the dramatic impact of GM on multiple aspects of human pathophysiology, including the development and progression of cancer. This was possible thanks to 'omics' techniques (*i.e.*, 16S rRNA gene sequencing, shotgun metagenomics and metatranscriptomics, metaproteomics and metabolomics), which led to a paradigm shift in the field of microbiology, moving from the study of single microbial colonies to a high-resolution taxonomic and functional profiling of microbial communities. However, it is undeniable that we are still far from a full understanding of the terms of the GM-host interaction, a *sine qua non* for the development of truly effective preventive and intervention strategies. In parallel, enormous progresses has haves been made in the field of analytical approaches to data, whose collection, organization and mining are fundamental steps for the analysis of complex interaction networks. Overall, technological advances in molecular biology and computer science are driving medical science towards big data. This large amount of data can potentially be explored via artificial intelligence methods, such as machine learning (ML) approaches that can handle large-scale datasets. ML is a data-driven approach capable of mining complex data, discovering informative patterns. In a nutshell, ML identifies algorithms capable of learning patterns from data in a self-manner, which enables the machine to solve a specific task, and deal with invisible data without explicit programming. In principle, the more heterogeneous data are used to train the model, the better the algorithm can generalize the problem when dealing with new data. Within ML, deep learning algorithms can better handle complex, multi-modal data. The premise of the emergence of ML techniques in various scientific fields, including healthcare, is the possibility of automating certain repetitive tasks, with the aim of achieving greater accuracy than that achievable by human experts, with also the possibility of estimating and predicting parameters, such as health risk factors. ML methods can adopt and combine different sources of health-related data, leveraging the tasks of diagnosing, prognosis, disease risk and potential treatments, with the aim of progressing towards a treatment tailored to the patient profile. ML algorithms can be supervised, *i.e.*, we know *a priori*, based on manual curation, how the samples are tagged. Supervised techniques are adopted to answer specific problems, by training the algorithm to recognize distinct features of the dataset. Halfway between supervised and unsupervised learning, semi-supervised learning can be applied

when we have incompletely labelled datasets, as it can be a real-word scenario [226]. With unsupervised algorithms, common features from input data are extracted, for instance, by grouping the samples based on the metagenomic profile. Unsupervised tasks are implemented through clustering (e.g., k-means, hierarchical) and dimensionality reduction algorithms, helping to explore and visualize similarities between samples. Overall, non-linear dimensionality reduction approaches (PCoA, UMAP), and autoencoders are adopted for microbial data, as these techniques are suitable for handling sparse data. Different types of algorithms can be used both for both classifications, identifying a sample as healthy or diseased based on the metagenomics profile, and for regression, for instance determining what the expression value would be for a given bacterial species upon treatment. Furthermore, ensemble strategies combine multiple models, to obtain for more robust and accurate results. When building a new model, the crucial steps are training and testing the model. To validate its performance, the dataset on which the model is trained is divided into training and testing subsets. The more data we can start with, the higher the are the chances that the algorithm can be better trained. The training set will be used to train the model, while the testing set must be a dataset not previously seen by the model during the training phase, in order to evaluate retrospectively and in an unbiased manner its performance. Shortcomings related to the model performance include overfitting, when the model is well trained on the dataset used for training, leading to high accuracy when applied to test data from the same dataset, but poor results when dealing with new datasets (*i.e.*, the model has little power to generalize the problem). On the other hand, the model may be underfitted to the data and not be able to generate predictions with sufficient accuracy even on the testing dataset. Very important components of model training in supervised tasks are the dataset annotation and the degree of curation of the data. In this regard, a certain level of expertise on the data based on the application task and data type, would be another important component of the ML workflow. The performance of an ML model is also subject to the computational power at disposal, which plays an important role in model training, especially when dealing with deep learning models. Furthermore, reproducibility, pipeline standardization and data accessibility are other major challenges. All this is even more true in the field of GM, whose complexity and high inter-individual and temporal variability stress the need for standards and cross-study validation of models. In this regard, the COST Action CA18131 "ML4Microbiome" project aims to tackle the issues related to the advent of ML in the microbiome field [227].

6.2 Machine learning in clinical oncology practice

Following the trend of increasing data collection, ML algorithms have been successfully applied to various problems, predicting human faces, targeting consumer behaviors, and also in relation to protein structure and function [228], drug discovery [229], and cancer detection [230]. ML models and algorithms are highly flexible between different scientific fields. However, data filtering and preparation require some knowledge. In oncology, ML approaches have already some

22

applications. Just to name a few based on imaging data, ML models have been used for breast density assessment [231], and for the detection of malignant lung nodules [232]. Furthermore, CURATE.AI is an artificial intelligence platform that has been trained on prostate cancer patient's health records and used by doctors to choose the optimal dose of drugs [233]. While for model training on imaging data, the detection accuracy is comparable to that of radiologists, or in some cases even better performing, ML models based on health records still need further evaluation. In any case, the model results require a review by the physician, as in some cases they may go against clinical guidelines. One major limitation is the inability to benchmark these tools towards a larger real-world dataset. In order to deploy ML tools in real clinical practice, several aspects must be taken into consideration and continuous and extensive collaboration between clinicians and informaticians is required, as well as data curation and longitudinal studies to monitor clinical outcomes [234].

6.3 Machine learning application in oncology with omics data

Nowadays, the boost of microbiome studies in the microbiome field and the availability of large datasets in public repositories are enabling the application of ML to metagenomics, which could lead to the identification of microbial species or other biomarkers, such as genes/enzymes and metabolites, for cancer diagnosis and prognosis [235]. Microbiome data combined with patient genetic information, but also with other types of omics data (transcriptomics, proteomics, metabolomics), could therefore return a comprehensive picture of the biological complexity of the disease and play a leading role in defining a personalized medicine approach. For example, predictions over microbiome-drug interactions could be the key to guiding precision therapeutic solutions. Recently, metagenomic data from CRC patients and healthy subjects have beenwere used to train a random forest classifier [236]. The model identified six key microbial species, *i.e.*, *Porphyromonas asaccharolytica*, *Peptostreptococcus stomatis*, *Fusobacterium* spp., *Parvimonas* spp., *Streptococcus vestibularis* and *Flavonifractor plautii*, which discriminated between controls and patients. Another work based on a random forest classifier and CRC, was trained on the metagenomic and metabolomics data from an Indian cohort of 30 patients and 30 healthy subjects. This work identified *F. plautii* as a cancer biomarker, and also found discriminating microbial genes for CRC. Interestingly, the authors hypothesized that flavonoid degradation by *Flavonifractor* is a key component for cancer progression [237]. More recently, Jang et al. [238] applied a Bayesian network model to find out species signatures in patients responding to chemotherapy treatment, while Kharrat et al. [239] adopted an ensemble method, including a Bayesian network model, to identify CRC-related microbial species. We would also like to mention the recently established Gut OncoMicrobiome Signatures project, which aims to identify microbial signatures of cancer progression and response to therapy [235]. These applications have defined or aim to define marker microbial species that could help better stratify patients, as well as guide GM remodeling via microbiome-based strategies, as outlined above. In this regard, ML could be used to screen large datasets in order to find potential new probiotics,

23

698 which will then have to be experimentally validated, or novel compounds to be evaluated for their therapeutic potential,

699 as well as to refine/personalize drug therapies. A rRecent work [240] based on unsupervised ML techniques, has identified

700 structural similarities between drugs that can be metabolized by bacteria, with important implications in pharmacological

701 research. For example, it has been shown that chemical groups such as the amide and ester groups can be hydrolyzed by

702 Bacteroidetes members. A similar application led to the implementation of the Drugbug database, a resource that collects

703 data on the bacterial metabolism of drugs. These data were used to train a random forest model that allowed for the

704 classification of compounds based on microbial metabolism [241]. By exploiting different types of omics data (from

705 metagenomics to metabolomics), ML approaches could therefore help to delineate microbiome-drug interactions, with

706 the possibility of predicting drug response and related toxicity. On the other hand, it should be remembered that certain

707 drugs, other than antibiotics, can shape the GM structure, eventually leading to a dysbiotic state. New studies are

708 investigating this aspect [240,242,243].

709 In summary, ML can set the direction of personalized precision medicine, helping to overcome the barrier of huge volume

710 of data analysis, with the ability to perform classification and prediction tasks. ML can be adopted to stratify patients

711 based on individual characteristics, including microbiome profile, and predict clinical outcomes (including response to

712 therapy), as well as identify novel health- or disease-promoting taxa/compounds, determine microbiome-drug interactions

713 and therefore guide the design of microbiome-targeted strategies to prevent/fight cancer, ensuring a long-term positive

714 response. ML methods applied to the microbiome/cancer fields offer a valuable bench-free way to sift through possible

715 solutions, which will then need to be validated by experimenters and clinicians. To speed up research in this field,

716 collaborations between among the clinical, biotechnological and bioinformatic parties for data model evaluation and

717 results interpretation will be mandatory.

718

719 **7. Conclusions and perspectives**

720 The gut microbiome has recently taken a leading role in research focused on maintaining the physiological wellbeing of

721 the host. On the other hand, evidence of a direct relationship between certain microbes and cancer development, as well

722 as the recent involvement of microbiomes in the outcomes of anticancer therapy, have left the door open to a new frontier

723 in microbiome-based research in these fields. In this scenario, the introduction of multi-omics approaches and novel

724 bioinformatic tools are helping to understand the role of microbial ecosystems in these unimaginableed lapels of the

725 relationship with the host. However, there are still few studies in large cohorts and many knowledge gaps to be filled,

726 especially in terms of underlying mechanisms and the development of safe and effective intervention strategies. Only

727 through transdisciplinary collaborations, it will be possible to move forward with the development of personalized

728 microbiome-based interventions, to overcome resistance to anticancer treatments and reduce the risk of relapse.

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References

1. Gilbert, J.A.; Blaser, M.J.; Caporaso, J.G.; Jansson, J.K.; Lynch, S.V.; Knight, R. Current understanding of the human microbiome. *Nat. Med.,* **2018**, *24*, 392-400.

2. Turnbaugh, P.J.; Ley, R.E.; Hamady, M.; Fraser-Liggett, C.M.; Knight, R.; Gordon, J.I. The human microbiome project. *Nature*, **2007**, *449*, 804-810.

3. Lynch, S.V.; Pedersen, O. The human intestinal microbiome in health and disease. *N. Engl. J. Med.*, **2016**, *375*, 2369-2379.

4. Oh, C.M.; Lee, D.; Kong, H.J.; Lee, S.; Won, Y.J.; Jung, K.W.; Cho, H. Causes of death among cancer patients in the era of cancer survivorship in Korea: Attention to the suicide and cardiovascular mortality. *Cancer Med.*, **2020**, *9*, 1741-1752.

5. Farré-Maduella, E.; Casals-Pascual, C. The origins of gut microbiome research in Europe: From Escherich to Nissle. *Hum. Microbiome J.*, **2019**, *14*, 100065.

6. Escherich, T. Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung, Stuttgart: Enke Verlag, **1886**.

7. Rajilić-Stojanović, M.; de Vos, V.M. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol. Rev*., **2014**, *38*, 996-1047.

8. Savage, D.C. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.*, **1977**, *31*, 107-133.

9. Sender, R.; Fuchs, S.; Milo, R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.,* **2016**, *14*, e1002533.

10. Neish, A.S. Microbes in gastrointestinal health and disease. *Gastroenterology*, **2009**, *136*, 65-80.

11. Gill, S.R.; Pop, M.; Deboy, R.T.; Eckburg, P.B.; Turnbaugh, P.J.; Samuel, B.S.; Gordon, J.I.; Relman, D.A.; Fraser-Liggett, C.M.; Nelson, K.E. Metagenomic analysis of the human distal gut microbiome. *Science*, **2006**, *312*, 1355-1359.

12. Candela, M.; Biagi, E.; Maccaferri, S.; Turroni, S.; Brigidi P. Intestinal microbiota is a plastic factor responding to environmental changes. *Trends Microbiol*., **2012**, *20*, 385-391.

13. Robertson, R.C.; Manges, A.R.; Finlay, B.B.; Prendergast, A.J. The human microbiome and child growth - first 1000 days and beyond. *Trends Microbiol.*, **2019**, *27*, 131-147.

14. Derrien, M.; Alvarez, A.S.; de Vos, W.M. The gut microbiota in the first decade of life. *Trends Microbiol*., 2019, *27*, 997-1010.

15. Bäckhed, F.; Roswall, J.; Peng, Y.; Feng, Q.; Jia, H.; Kovatcheva-Datchary, P.; Li, Y.; Xia, Y.; Xie, H.; Zhong, H.; Khan, M.T.; Zhang, J.; Li, J.; Xiao, L.; Al-Aama, J.; Zhang, D.; Lee, Y.S.; Kotowska, D.; Colding, C.;

Tremaroli, V.; Yin, Y.; Bergman, S.; Xu, X.; Madsen, L.; Kristiansen, K.; Dahlgren, J.; Wang, J. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe,* **2015**, *17*, 690-703.

16. Avershina, E.; Lundgård, K.; Sekelja, M.; Dotterud, C.; Storrø, O.; Øien, T.; Johnsen, R.; Rudi, K. Transition from infant- to adult-like gut microbiota. *Environ. Microbiol.,* **2016**, *18*, 2226-2236.

17. Pannaraj, P.S.; Li, F.; Cerini, C.; Bender, J.M.; Yang, S.; Rollie, A.; Adisetiyo, H.; Zabih, S.; Lincez, P.J.; Bittinger, K.; Bailey, A.; Bushman, F.D.; Sleasman, J.W.; Aldrovandi, G.M. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatr.,* **2017**, *171*, 647-654.

18. Vangay, P.; Ward, T.; Gerber, J.S.; Knights, D. Antibiotics, pediatric dysbiosis, and disease. *Cell Host Microbe*, **2015**, *17*, 553-564.

19. Rooks, M.G.; Garrett, W.S. Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol*., **2016**, *16*, 341-352.

20. Macpherson, A.J.; de Agüero, M.G.; Ganal-Vonarburg, S.C. How nutrition and the maternal microbiota shape the neonatal immune system. *Nat. Rev. Immunol*., **2017**, *17*, 508-517.

21. Olszak, T.; An, D.; Zeissig, S.; Vera, M.P.; Richter, J.; Franke, A.; Glickman, J.N.; Siebert, R.; Baron, R.M.; Kasper, D.L.; Blumberg, R.S. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science*, **2012**, *336*, 489-493.

22. Cox, L.M.; Yamanishi, S.; Sohn, J.; Alekseyenko, A.V.; Leung, J.M.; Cho, I.; Kim, S.G.; Li, H.; Gao, Z.; Mahana, D.; Zárate Rodriguez, J.G.; Rogers, A.B.; Robine, N.; Loke, P.; Blaser, M.J. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, **2014**, *158*, 705-721.

23. Vatanen, T.; Franzosa, E.A.; Schwager, R.; Tripathi, S.; Arthur, T.D.; Vehik, K.; Lernmark, Å.; Hagopian, W.A.; Rewers, M.J.; She, J.X.; Toppari, J.; Ziegler, A.G.; Akolkar, B.; Krischer, J.P.; Stewart, C.J.; Ajami, N.J.; Petrosino, J.F.; Gevers, D.; Lähdesmäki, H.; Vlamakis, H.; Huttenhower, C.; Xavier, R.J. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature*, **2018**, *562*, 589-594.

24. Chua, H.H.; Chou, H.C.; Tung, Y.L.; Chiang, B.L.; Liao, C.C.; Liu, H.H.; Ni, Y.H. Intestinal dysbiosis featuring abundance of *Ruminococcus gnavus* associates with allergic diseases in infants. *Gastroenterology*, **2018**, *154*, 154-167.

25. Maruvada, P.; Leone, V.; Kaplan, L.M.; Chang, E.B. The human microbiome and obesity: moving beyond associations. *Cell Host Microbe*, **2017**, *22*, 589-599.

802 26. Stewart, C.J.; Ajami, N.J.; O'Brien, J.L.; Hutchinson, D.S.; Smith, D.P.; Wong, M.C.; Ross, M.C.; Lloyd, R.E.;
803 Doddapaneni, H.; Metcalf, G.A.; Muzny, D.; Gibbs, R.A.; Vatanen, T.; Huttenhower, C.; Xavier, R.J.; Rewers,
804 M.; Hagopian, W.; Toppari, J.; Ziegler, A.G.; She, J.X.; Akolkar, B.; Lernmark, A.; Hyoty, H.; Vehik, K.;
805 Krischer, J.P., Petrosino, J.F. Temporal development of the gut microbiome in early childhood from the TEDDY
806 study. *Nature*, **2018**, *562*, 583-588.

807 27. He, Y.; Wu, W.; Zheng, H.M.; Li, P.; McDonald, D.; Sheng, H.F.; Chen, M.X.; Chen, Z.H.; Ji, G.Y.; Zheng,
808 Z.D.; Mujagond, P.; Chen, X.J.; Rong, Z.H.; Chen, P.; Lyu, L.Y.; Wang, X.; Wu, C.B.; Yu, N.; Xu, Y.J.; Yin,
809 J.; Raes, J.; Knight, R.; Ma, W.J.; Zhou, H.W. Regional variation limits applications of healthy gut microbiome
810 reference ranges and disease models. *Nat. Med.*, **2018**, *24*, 1532-1535.

811 28. Rothschild, D.; Weissbrod, O.; Barkan, E.; Kurilshikov, A.; Korem, T.; Zeevi, D.; Costea, P.I.; Godneva, A.;
812 Kalka, I.N.; Bar, N.; Shilo, S.; Lador, D.; Vila, A.V.; Zmora, N.; Pevsner-Fischer, M.; Israeli, D.; Kosower, N.;
813 Malka, G.; Wolf, B.C.; Avnit-Sagi, T.; Lotan-Pompan, M.; Weinberger, A.; Halpern, Z.; Carmi, S.; Fu, J.;
814 Wijmenga, C.; Zhernakova, A.; Elinav, E.; Segal, E. Environment dominates over host genetics in shaping
815 human gut microbiota. *Nature*, **2018**, *555*, 210-215.

816 29. Wild, C.P. Complementing the genome with an "exposome": the outstanding challenge of environmental
817 exposure measurement in molecular epidemiology. *Cancer Epidemiol. Biomarkers Prev.*, **2005**, *14*, 1847-1850.

818 30. Fassarella, M.; Blaak, E.E.; Penders, J.; Nauta, A.; Smidt, H.; Zoetendal, E.G. Gut microbiome stability and
819 resilience: elucidating the response to perturbations in order to modulate gut health. *Gut*, **2021**, *70*, 595-605.

820 31. Iacob, S.; Iacob, D.G.; Luminos, L.M. Intestinal microbiota as a host defense mechanism to infectious threats.
821 *Front. Microbiol.*, **2018**, *9*, 3328.

822 32. Thaiss, C.A.; Zmora, N.; Levy, M.; Elinav, E. The microbiome and innate immunity. *Nature*, **2016**, *535*, 65-74.

823 33. Turroni, S.; Brigidi, P.; Cavalli, A.; Candela, M. Microbiota-host transgenomic metabolism, bioactive molecules
824 from the Inside. *J. Med. Chem.*, **2018**, *61*, 47-61.

825 34. Sonnenburg, E.D.; Sonnenburg, J.L. Starving our microbial self: the deleterious consequences of a diet deficient
826 in microbiota-accessible carbohydrates. *Cell Metab.,* **2014**, *20*, 779-786.

827 35. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From dietary fiber to host physiology: Short-
828 chain fatty acids as key bacterial metabolites. *Cell*, **2016**, *165*, 1332-1345.

829 36. Desai, M.S.; Seekatz, A.M.; Koropatkin, N.M.; Kamada, N.; Hickey, C.A.; Wolter, M.; Pudlo, N.A.; Kitamoto,
830 S.; Terrapon, N.; Muller, A.; Young, V.B.; Henrissat, B.; Wilmes, P.; Stappenbeck, T.S.; Núñez, G.; Martens,
831 E.C. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen
832 susceptibility. *Cell*, **2016**, *167*, 1339-1353.

833   37. Oh, J.H.; Alexander, L.M.; Pan, M.; Schueler, K.L.; Keller, M.P.; Attie, A.D.; Walter, J.; van Pijkeren, J.P.
834   Dietary fructose and microbiota-derived short-chain fatty acids promote bacteriophage production in the gut
835   symbiont *Lactobacillus reuteri*. *Cell Host Microbe*, **2019**, *25*, 273-284.

836   38. Davie, J.R. Inhibition of histone deacetylase activity by butyrate. *J. Nutr.*, **2003**, *133*, 2485S-2493S.

837   39. Fukuda, S.; Toh, H.; Hase, K.; Oshima, K.; Nakanishi, Y.; Yoshimura, K.; Tobe, T.; Clarke, J.M.; Topping,
838   D.L.; Suzuki, T.; Taylor, T.D.; Itoh, K.; Kikuchi, J.; Morita, H.; Hattori, M.; Ohno, H. Bifidobacteria can protect
839   from enteropathogenic infection through production of acetate. *Nature*, **2011**, *469*, 543-547.

840   40. Brown, A.J.; Goldsworthy, S.M.; Barnes, A.A.; Eilert, M.M.; Tcheang, L.; Daniels, D.; Muir, A.I.;
841   Wigglesworth, M.J.; Kinghorn, I.; Fraser, N.J.; Pike, N.B.; Strum, J.C.; Steplewski, K.M.; Murdock, P.R.;
842   Holder, J.C.; Marshall, F.H.; Szekeres, P.G.; Wilson, S.; Ignar, D.M.; Foord, S.M.; Wise, A.; Dowell, S.J. The
843   Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain
844   carboxylic acids. *J. Biol. Chem.*, **2003**, *278*, 11312-11319.

845   41. Kelly, C.J.; Zheng, L.; Campbell, E.L.; Saeedi, B.; Scholz, C.C.; Bayless, A.J.; Wilson, K.E.; Glover, L.E.;
846   Kominsky, D.J.; Magnuson, A.; Weir, T.L.; Ehrentraut, S.F.; Pickel, C.; Kuhn, K.A.; Lanis, J.M.; Nguyen, V.;
847   Taylor, C.T.; Colgan, S.P. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial
848   HIF augments tissue barrier function. *Cell Host Microbe*, **2015**, *17*, 662-671.

849   42. Di Martino, M.L.; Campilongo, R.; Casalino, M.; Micheli, G.; Colonna, B.; Prosseda, G. Polyamines: emerging
850   players in bacteria-host interactions. *Int. J. Med. Microbiol.*, **2013**, *303*, 484-91.

851   43. Chen, J.; Rao, J.N.; Zou, T.; Liu, L.; Marasa, B.S.; Xiao, L.; Zeng, X.; Turner, D.J.; Wang, J.Y. Polyamines are
852   required for expression of Toll-like receptor 2 modulating intestinal epithelial barrier integrity. *Am. J. Physiol.*
853   *Gastrointest. Liver Physiol.*, **2007**, *293*, G568-G576.

854   44. Liu, L.; Rao, J.N.; Zou, T.; Xiao, L.; Wang, P.Y.; Turner, D.J.; Gorospe, M.; Wang, J.Y. Polyamines regulate c-
855   Myc translation through Chk2-dependent HuR phosphorylation. *Mol. Biol. Cell.*, **2009**, *20*, 4885-4898.

856   45. Perez-Cano, F.J.; González-Castro, A.; Castellote, C.; Franch, A.; Castell, M. Influence of breast milk
857   polyamines on suckling rat immune system maturation. *Dev. Comp. Immunol.*, **2010**, *34*, 210-218.

858   46. Ma, L.; Ni, Y.; Wang, Z.; Tu, W.; Ni, L.; Zhuge, F.; Zheng, A.; Hu, L.; Zhao, Y.; Zheng, L.; Fu, Z. Spermidine
859   improves gut barrier integrity and gut microbiota function in diet-induced obese mice. *Gut Microbes*, **2020**, *12*,
860   1-19.

861   47. Miller-Fleming, L.; Olin-Sandoval, V.; Campbell, K.; Ralser, M. Remaining mysteries of molecular biology: the
862   role of polyamines in the cell. *J. Mol. Biol.*, **2015**, *427*, 3389-3406.

863   48. Gerner, E.W.; Meyskens, F.L. Polyamines and cancer: old molecules, new understanding. *Nat. Rev. Cancer*,

864   **2004**, *4*, 781-792.

865   49. Johnson, C.H.; Dejea, C.M.; Edler, D.; Hoang, L.T.; Santidrian, A.F.; Felding, B.H.; Ivanisevic, J.; Cho, K.;

866   Wick, E.C.; Hechenbleikner, E.M.; Uritboonthai, W.; Goetz, L.; Casero, R.A.; Pardoll, D.M.; White, J.R.; Patti,

867   G.J.; Sears, C.L.; Siuzdak, G. Metabolism links bacterial biofilms and colon carcinogenesis. *Cell Metab*., **2015**,

868   *21*, 891-897.

869   50. Liu, R.; Lin, X.; Li, Z.; Li, Q.; Bi, K. Quantitative metabolomics for investigating the value of polyamines in the

870   early diagnosis and therapy of colorectal cancer. *Oncotarget*, **2018**, *9*, 4583-4592.

871   51. Hayes, C.S.; Shicora, A.C.; Keough, M.P.; Snook, A.E.; Burns, M.R.; Gilmour, S.K. Polyamine-blocking

872   therapy reverses immunosuppression in the tumor microenvironment. *Cancer Immunol. Res.*, **2014**, *2*, 274–285.

873   52. Casero, R.A.; Murray Stewart, T.; Pegg, A.E. Polyamine metabolism and cancer: treatments, challenges and

874   opportunities. *Nat. Rev. Cancer,* **2018**, *18*, 681-695.

875   53. Pietrocola, F.; Pol, J.; Vacchelli, E.; Rao, S.; Enot, D.P.; Baracco, E.E.; Levesque, S.; Castoldi, F.; Jacquelot,

876   N.; Yamazaki, T.; Senovilla, L.; Marino, G.; Aranda, F.; Durand, S.; Sica, V.; Chery, A.; Lachkar, S.; Sigl, V.;

877   Bloy, N.; Buque, A.; Falzoni, S.; Ryffel, B.; Apetoh, L.; Di Virgilio, F.; Madeo, F.; Maiuri, M.C.; Zitvogel, L.;

878   Levine, B.; Penninger, J.M.; Kroemer, G. Caloric restriction mimetics enhance anticancer immunosurveillance.

879   *Cancer Cell,* **2016**, *30*, 147-160.

880   54. Francino, M.P. Early development of the gut microbiota and immune health. *Pathogens*, **2014**, *4*, 769-90.

881   55. Nakanishi, Y.; Sato, T.; Ohteki, T. Commensal Gram-positive bacteria initiates colitis by inducing

882   monocyte/macrophage mobilization. *Mucosal Immunol.*, **2015**, *8*, 152-160.

883   56. Thomas, C.M.; Versalovic, J. Probiotics-host communication modulation of signaling pathways in the intestine.

884   *Gut Microbes*, **2010**, *1*, 1-16.

885   57. Belkaid, Y. Role of the microbiota in immunity and inflammation. *Cell*, **2014**, *157*, 121-141.

886   58. Owaga, E.; Hsieh, R.H.; Mugendi, B.; Masuku, S.; Shih, C.K.; Chang, J.S. Th17 cells as potential probiotic

887   therapeutic targets in inflammatory bowel diseases. *Int. J. Mol. Sci*, **2015**, *16*, 20841-20858.

888   59. Mazmanian, S.; Round, J.; Kasper, D. A microbial symbiosis factor prevents intestinal inflammatory disease.

889   *Nature*, **2008**, *453*, 620-625.

890   60. Round, J.L.; Lee, S.M.; Li, J.; Tran, G.; Jabri, B.; Chatila, T.A.; Mazmanian, S.K. The Toll-like receptor 2

891   pathway establishes colonization by a commensal of the human microbiota. *Science*, **2011**, *332*, 974-977.

892   61. Shanahan, F.; Ghosh, T.S.; O'Toole, P.W. What is the definition of a healthy gut microbiome? *Gastroenterology*,

893   **2021**, *160*, 483-494.

894   62. Duvallet, C.; Gibbons, S.M.; Gurry, T.; Irizarry, R.A.; Alm, E.J. Meta-analysis of gut microbiome studies
895   identifies disease-specific and shared responses. *Nat. Commun.*, **2017**, *8*, 1784.

896   63. Kho, Z.Y.; Lal, S.K. The human gut microbiome - a potential controller of wellness and disease. *Front.*
897   *Microbiol.*, **2018**, *9*, 1835.

898   64. Cani, P.D.; Van Hul, M. Gut microbiota and obesity: causally linked? *Expert Rev. Gastroenterol Hepatol.*, **2020**,
899   *14,* 401-403.

900   65. Lloyd-Price, J.; Arze, C.; Ananthakrishnan, A.N.; Schirmer, M.; Avila-Pacheco, J.; Poon, T.W.; Andrews, E.;
901   Ajami, N.J.; Bonham, K.S.; Brislawn, C.J.; Casero, D.; Courtney, H.; Gonzalez, A.; Graeber, T.G.; Hall, A.B.;
902   Lake, K.; Landers, C.J.; Mallick, H.; Plichta, D.R.; Prasad, M.; Rahnavard, G.; Sauk, J.; Shungin, D.; Vázquez-
903   Baeza, Y.; White, R.A.; IBDMDB Investigators; Braun, J.; Denson, L.A.; Jansson, J.K.; Knight, R.; Kugathasan,
904   S.; McGovern, D.P.B.; Petrosino, J.F.; Stappenbeck, T.S.; Winter, H.S.; Clish, C.B.; Franzosa, E.A.; Vlamakis,
905   H.; Xavier, R.J.; Huttenhower, C. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases.
906   *Nature*, **2019**, *569*, 655-662.

907   66. Khan, I.; Ullah, N.; Zha, L.; Bai, Y.; Khan, A.; Zhao, T.; Che, T.; Zhang, C. Alteration of gut Mmicrobiota in
908   inflammatory bowel disease (IBD): cause or consequence? IBD treatment targeting the gut microbiome.
909   *Pathogens*, **2019**, *8*, 126.

910   67. Wong, S.H.; Yu, J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat.*
911   *Rev. Gastroenterol. Hepatol.*, **2019**, *16*, 690-704.

912   68. Sheflin, A.M.; Whitney, A.K.; Weir, T.L. Cancer-promoting effects of microbial dysbiosis. *Curr. Oncol. Rep.*,
913   **2014**, *16*, 406.

914   69. Candela, M.; Turroni, S.; Biagi, E.; Carbonero, F.; Rampelli, S.; Fiorentini, C.; Brigidi, P. Inflammation and
915   colorectal cancer, when microbiota-host mutualism breaks. *World J. Gastroenterol.*, **2014**, *20*, 908-922.

916   70. Farrell, J.J.; Zhang, L.; Zhou, H.; Chia, D.; Elashoff, D.; Akin, D.; Paster, B.J.; Joshipura, K.; Wong, D.T.
917   Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. *Gut*, **2012**, *61*,
918   582-588.

919   71. Gong, H.L.; Shi, Y.; Zhou, L.; Wu, C.P.; Cao, P.Y.; Tao, L.; Xu, C.; Hou, D.S.; Wang, Y.Z. The composition of
920   microbiome in larynx and the throat biodiversity between laryngeal squamous cell carcinoma patients and
921   control population. *PLoS One*, **2013**, *8*, e66476.

922   72. Sharma, V.; Chauhan, V.S.; Nath, G.; Kumar, A.; Shukla, V.K. Role of bile bacteria in gallbladder carcinoma.
923   *Hepato-gastroenterology*, **2007**, *54*, 1622-1625.

924   73. Bhatt, A.P.; Redinbo, M.R.; Bultman, S.J. The role of the microbiome in cancer development and therapy. *CA*
925   *Cancer J. Clin.*, **2017**, *67*, 326-344.

926   74. Goodman, B.; Gardner, H. The microbiome and cancer. *J. Pathol.*, **2018**, *244*, 667-676.

927   75. Chaffer, C.L.; Weinberg, R.A. How does multistep tumorigenesis really proceed? *Cancer Discov.*, **2015**, *5*, 22-
928   24.

929   76. Mármol, I.; Sánchez-de-Diego, C.; Pradilla Dieste, A.; Cerrada, E.; Rodriguez Yoldi, M.J. Colorectal carcinoma:
930   A general overview and future perspectives in colorectal cancer. *Int. J. Mol. Sci.*, **2017**, *18*, 197.

931   77. Zhang, L.; Shay, J.W. Multiple roles of APC and its therapeutic implications in colorectal cancer. *J. Natl. Cancer*
932   *Inst.*, **2017**, *109*, djw332.

933   78. McCoy, A.N.; Araújo-Pérez, F.; Azcárate-Peril, A.; Yeh, J.J.; Sandler, R.S.; Keku, T.O. *Fusobacterium* is
934   associated with colorectal adenomas. *PLoS One*, **2013**, *8*, e53653.

935   79. Castellarin, M.; Warren, R.L.; Freeman, J.D.; Dreolini, L.; Krzywinski, M.; Strauss, J.; Barnes, R.; Watson, P.;
936   Allen-Vercoe, E.; Moore, R.A.; Holt, R.A. *Fusobacterium nucleatum* infection is prevalent in human colorectal
937   carcinoma. *Genome research*, **2012**, *22*, 299-306.

938   80. Zheng, J.; Meng, J.; Zhao, S.; Singh, R.; Song, W. *Campylobacter* induced interleukin-8 secretion in polarized
939   human intestinal epithelial cells requires *Campylobacter*-secreted cytolethal distending toxin- and Toll-like
940   receptor-mediated activation of NF-kB. *Infect. Immun.*, **2008**, *76*, 4498-4508.

941   81. Bullman, S.; Pedamallu, C.S.; Sicinska, E.; Clancy, T.E.; Zhang, X.; Cai, D.; Neuberg, D.; Huang, K.; Guevara,
942   F.; Nelson, T.; Chipashvili, O.; Hagan, T.; Walker, M.; Ramachandran, A.; Diosdado, B.; Serna, G.; Mulet, N.;
943   Landolfi, S.; Ramon, Y.; Cajal, S.; Fasani, R.; Aguirre, A.J.; Ng, K.; Élez, E.; Ogino, S.; Tabernero, J.; Fuchs,
944   C.S.; Hahn, W.C.; Nuciforo, P.; Meyerson, M. Analysis of *Fusobacterium* persistence and antibiotic response
945   in colorectal cancer. *Science*, **2017**, *358*, 1443-1448.

946   82. Louis, P.; Flint, H.J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the
947   human large intestine. *FEMS Microbiol. Lett.*, **2009**, *294*, 1-8.

948   83. Tjalsma, H.; Boleij, A.; Marchesi, J.R.; Dutilh, B.E. A bacterial driver-passenger model for colorectal cancer:
949   beyond the usual suspects. *Nat. Rev. Microbiol.*, **2012**, *10*, 575-582.

950   84. Huycke, M.M.; Abrams, V.; Moore, D.R. *Enterococcus faecalis* produces extracellular superoxide and hydrogen
951   peroxide that damages colonic epithelial cell DNA. *Carcinogenesis*, **2002**, *23*, 529-536.

952   85.   de Almeida, C.V.; Taddei, A.; Amedei, A. The controversial role of *Enterococcus faecalis* in colorectal cancer.
953   *Therap. Adv. Gastroenterol.*, **2018**, *1*, 1756284818783606.

954   86. Toprak, N.U.; Yagci, A.; Gulluoglu, B.M.; Akin, M.L.; Demirkalem, P.; Celenk, T.; Soyletir G. A possible role

955   of *Bacteroides fragilis* enterotoxin in the aetiology of colorectal cancer. *Clin. Microbiol. Infect.*, **2006**, *12*, 782-

956   786.

957   87. Haghi, F.; Goli, E.; Mirzaei, B.; Zeighami, H. The association between fecal enterotoxigenic *B. fragilis* with

958   colorectal cancer. *BMC Cancer*, **2019**, *19*, 879.

959   88. Lara-Tejero, M.; Galán, J.E. Cytolethal distending toxin: limited damage as a strategy to modulate cellular

960   functions. *Trends Microbiol.,* **2002**, *10*, 147-152.

961   89. Ge, Z.; Rogers, A.B.; Feng, Y.; Lee, A.; Xu, S.; Taylor, N.S.; Fox, J.G. Bacterial cytolethal distending toxin

962   promotes the development of dysplasia in a model of microbially induced hepatocarcinogenesis. *Cell.*

963   *Microbiol.,* **2007**, *9*, 2070-2080.

964   90. Guerra, L.; Guidi, R.; Slot, I.; Callegari, S.; Sompallae, R.; Pickett, C.L.; Åström, S.; Eisele, F.; Wolf, D.;

965   Sjögren, C.; Masucci, M.G.; Frisan, T. Bacterial genotoxin triggers FEN1-dependent RhoA activation,

966   cytoskeleton remodeling and cell survival. *J. Cell. Sci.*, **2011**, *124*, 2735-2742.

967   91. Martin, O.C.B.; Bergonzini, A.; D'Amico, F.; Chen, P.; Shay, J.W.; Dupuy, J.; Svensson, M.; Masucci, M.G.;

968   Frisan T. Infection with genotoxin-producing *Salmonella enterica* synergises with loss of the tumour suppressor

969   APC in promoting genomic instability via the PI3K pathway in colonic epithelial cells. *Cell. Microbiol.*, **2019**,

970   *21*, e13099.

971   92. Wentling, G.K.; Metzger, P.P.; Dozois, E.J.; Chua, H.K.; Krishna, M. Unusual bacterial infections and colorectal

972   carcinoma - *Streptococcus bovis* and *Clostridium septicum*: report of three cases. *Dis. Colon Rectum*, **2006**, *49*,

973   1223-1227.

974   93. Kostic, A.D.; Chun, E.; Robertson, L. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and

975   modulates the tumor-immune microenvironment. *Cell Host Microbe*, **2013**, *14*, 207-215.

976   94. Boleij, A.; Tjalsma, H. The itinerary of *Streptococcus gallolyticus* infection in patients with colonic malignant

977   disease. *Lancet Infect. Dis.*, **2013**, *13*, 719-724.

978   95. Marchesi, J.R.; Dutilh, B.E.; Hall, N.; Peters, W.H.; Roelofs, R.; Boleij, A.; Tjalsma, H. Towards the human

979   colorectal cancer microbiome. *PLoS One*, **2011**, *6*, e20447.

980   96. Candela, M.; Guidotti, M.; Fabbri, A.; Brigidi, P.; Franceschi, C.; Fiorentini, C. Human intestinal microbiota:

981   cross-talk with the host and its potential role in colorectal cancer. *Crit. Rev. Microbiol*., **2011**, *37*, 1-14.

982   97. Dapito, D.H.; Mencin, A.; Gwak, G.Y.; Pradere, J.P.; Jang, M.K.; Mederacke, I.; Caviglia, J.M.; Khiabanian,

983   H.; Adeyemi, A.; Bataller, R.; Lefkowitch, J.H.; Bower, M.; Friedman, R.; Sartor, R.B.; Rabadan, R.; Schwabe,

R.F. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell.*, **2012**, 21, 504-516.

98. Tremaroli, V.; Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature*, **2012**, *489*, 242-249.

99. Yoshimoto, S.; Loo, T.M.; Atarashi, K.; Kanda, H.; Sato, S.; Oyadomari, S.; Iwakura, Y.; Oshima, K.; Morita, H.; Hattori, M.; Honda, K.; Ishikawa, Y.; Hara, E.; Ohtani, N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature*, **2013**, *499*, 97-101.

100. Quante, M.; Bhagat, G.; Abrams, J.A.; Marache, F.; Good, P.; Lee, M.D.; Lee, Y.; Friedman, R.; Asfaha, S.; Dubeykovskaya, Z.; Mahmood, U.; Figueiredo, J.L.; Kitajewski, J.; Shawber, C.; Lightdale, C.J.; Rustgi, A.K.; Wang, T.C. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like metaplasia. *Cancer Cell.*, **2012**, *21*, 36-51.

101. Ridlon, J.M.; Kang, D.; Hylemon, P.B. Bile salt biotransformations by human intestinal Bacteria. *J. Lipid Res.*, **2006**, *47*, 241-259.

102. Eslami-S, Z.; Majidzadeh-A, K.; Halvaei, S.; Babapirali, F.; Esmaeili, R. Microbiome and breast cancer: New role for an ancient population. *Front. Oncol.*, **2020**, *10*, 120.

103. Goedert, J.J.; Jones, G.; Hua, X.; Xu, X.; Yu, G.; Flores, R.; Falk, R.T.; Gail, M.H.; Shi, J.; Ravel, J.; Feigelson, H.S. Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women: a population-based case-control pilot study. *J. Natl. Cancer Inst.*, **2015**, *107*, djv147.

104. Plottel, C.S.; Blaser, M.J. Microbiome and malignancy. *Cell Host Microbe*, **2011**, *10*, 324-335.

105. Gloux, K., Berteau, O.; El Oumami, H.; Beguet, F.; Leclerc, M.; Dore, J. A metagenomic beta-glucuronidase uncovers a core adaptive function of the human intestinal microbiome. *Proc. Natl. Acad. Sci. U.S.A.*, **2011**, *108*, 4539-4546.

106. Dabek, M.; McCrae, S.I.; Stevens, V.J.; Duncan, S.H.; Louis, P. Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria. *FEMS Microbiol. Ecol.*, **2008**, *66*, 487-495.

107. McIntosh, F.M.; Maison, N.; Holtrop, G.; Young, P.; Stevens, V.J.; Ince, J.; Johnstone, A.M.; Lobley, G.E.; Flint, H.J.; Louis, P. Phylogenetic distribution of genes encoding beta-glucuronidase activity in human colonic bacteria and the impact of diet on faecal glycosidase activities. *Environ. Microbiol.*, **2012**, *14*, 1876-1887.

108. Rizkallah, M.R.; Saad, R.; Aziz, R.K. The Human Microbiome Project, personalized medicine and the birth of pharmacomicrobiomics. *Curr. Pharmacogenomics Pers. Med.*, **2010**, 8, 182-193.

1014   109. Alexander, J.L.; Wilson, I.D.; Teare, J.; Marchesi, J.R.; Nicholson, J.K.; Kinross, J.M. Gut microbiota

1015   modulation of chemotherapy efficacy and toxicity. *Nat. Rev. Gastroenterol. Hepatol.*, **2017**, *14*, 356-365.

1016   110. Roy, S.; Trinchieri, G. Microbiota: A key orchestrator of cancer therapy. *Nat. Rev. Cancer*, **2017**, *17*, 271-285.

1017   111. Nayak, R.R.; Turnbaugh, P.J. Mirror, mirror on the wall: Which microbiomes will help heal them all? *BMC*

1018   *Med.*, **2016**, *14*, 72.

1019   112. Fessler, J.L.; Gajewski, T.F. The microbiota: A new variable impacting cancer treatment outcomes. *Clin. Cancer*

1020   *Res.*, **2017**, *23*, 3229-3231.

1021   113. Nauts, H.C.; Swift, W.E.; Coley, B.L. The treatment of malignant tumors by bacterial toxins as developed by

1022   the late William B. Coley, M.D., reviewed in the light of modern research. *Cancer Res.*, **1946**, 205-216.

1023   114. McCarthy, E.F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *IOWA*

1024   *Orthop. J.*, **2006**, *26*, 154-158.

1025   115. Zbar, B.; Bernstein, I.; Tanaka, T.; Rapp, H.J. Tumor immunity produced by the intradermal inoculation of

1026   living tumor cells and living *Mycobacterium bovis* (strain BCG). *Science*, **1970**, *170*, 1217-1218.

1027   116. Aso, Y.; Akazan, H. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial

1028   bladder cancer. *BLP Study Group Urol. Int.*, **1992**, *49*, 125-129.

1029   117. Hoesl, C.E.; Altwein, J.E. The probiotic approach: An alternative treatment option in urology. *Eur. Urol.*, **2005**,

1030   *47*, 288-296.

1031   118. Vivarelli, S.; Salemi, R.; Candido, S.; Falzone, L.; Santagati, M.; Stefani, S.; Torino, F.; Banna, G.L.; Tonini,

1032   G.; Libra, M. Gut microbiota and cancer: From pathogenesis to therapy. *Cancers*, **2019**, *11*, 38.

1033   119. Stebbing, J.; Dalgleish, A.; Gifford-Moore, A.; Martin, A.; Gleeson, C.; Wilson, G.; Brunet, L.R.; Grange, J.;

1034   Mudan, S. An intra-patient placebo-controlled phase I trial to evaluate the safety and tolerability of intradermal

1035   IMM-101 in melanoma. *Ann. Oncol.*, **2012**, *23*, 1314-1319.

1036   120. Dalgleish, A.G.; Stebbing, J.; Adamson, D.J.; Arif, S.S.; Bidoli, P.; Chang, D.; Cheeseman, S.; Diaz-Beveridge,

1037   R.; Fernandez-Martos, C.; Glynne-Jones, R.; Granetto, C.; Massuti, B.; McAdam, K.; McDermott, R.; Martín,

1038   A.J.; Papamichael, D.; Pazo-Cid, R.; Vieitez, J.M.; Zaniboni, A.; Carroll, K.J.; Wagle, S.; Gaya, A.; Mudan, S.S.

1039   Randomised, open-label, phase II study of gemcitabine with and without IMM-101 for advanced pancreatic

1040   cancer. *Br. J. Cancer.*, **2016**, *115*, 789-796.

1041   121. Toso, J.F.; Gill, V.J.; Hwu, P.; Marincola, F.M.; Restifo, N.P.; Schwartzentruber, D.J.; Sherry, R.M.; Topalian,

1042   S.L.; Yang, J.C.; Stock, F.; Freezer, L.J.; Morton, K.E.; Seipp, C.; Haworth, L.; Mavroukakis, S.; White, D.;

1043   MacDonald, S.; Mao, J.; Sznol, M.; Rosenberg, S.A. Phase I study of the intravenous administration of attenuated

1044   Salmonella typhimurium to patients with metastatic melanoma. *J. Clin. Oncol.*, **2002**, *20*, 142-152.

1045   122. Nemunaitis, J.; Cunningham, C.; Senzer, N.; Kuhn, J.; Cramm, J.; Litz, C.; Cavagnolo, R.; Cahill, A.; Clairmont,
1046   C.; Sznol, M. Pilot trial of genetically modified, attenuated Salmonella expressing the E. coli cytosine deaminase
1047   gene in refractory cancer patients. *Cancer Gene Ther.*, **2003**, *10*, 737-744.

1048   123. Kramer, M.G.; Masner, M.; Ferreira, F.A.; Hoffman, R.M. Bacterial therapy of cancer: Promises, limitations,
1049   and insights for future directions. *Front. Microbiol.*, **2018**, *9*, 16.

1050   124. Schwabe, R.F.; Jobin, C. The microbiome and cancer. *Nat. Rev. Cancer*, **2013**, *13*, 800-812.

1051   125. DeVita, V.T.; Chu, E. A history of cancer chemotherapy. *Cancer Res.*, **2008**, *68*, 8643-8653.

1052   126. McGranahan, N.; Swanton, C. Biological and therapeutic impact of intratumor heterogeneity in cancer
1053   evolution. *Cancer Cell.*, **2015**, *27*, 15-26.

1054   127. Allison, J.P.; McIntyre, B.W.; Bloch, D. Tumor-specific antigen of murine T-lymphoma defined with
1055   monoclonal antibody. *J. Immunol.*, **1982**, *129*, 2293–300.

1056   128. Toh, H.C. Cancer immunotherapy-the end of the beginning. *Chin. Clin. Oncol.*, **2018**, *7*, 12.

1057   129. Dzutsev, A.; Goldszmid, R.S.; Viaud, S.; Zitvogel, L.; Trinchieri, G. The role of the microbiota in inflammation,
1058   carcinogenesis, and cancer therapy. *Eur. J. Immunol.*, **2015**, *45*, 17-31.

1059   130. Mathijssen, R.H.; van Alphen, R.J.; Verweij, J.; Loos, W.J.; Nooter, K.; Stoter, G.; Sparreboom, A. Clinical
1060   pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin. Cancer Res.*, **2001**, *7*, 2182-94.

1061   131.Takasuna, K.; Hagiwara, T.; Hirohashi, M.; Kato, M.; Nomura, M.; Nagai, E.; Yokoi, T.; Kamataki, T.
1062   Involvement of beta-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor
1063   camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. *Cancer Res.*, **1996**, *56*, 3752-7.

1064   132.Cheng, K.W.; Tseng, C.H.; Tzeng, C.C.; Leu, Y.L.; Cheng, T.C.; Wang, J.Y.; Chang, J.M.; Lu, Y.C.; Cheng,
1065   C.M.; Chen, I.J.; Cheng, Y.A.; Chen, Y.L.; Cheng, T.L. Pharmacological inhibition of bacterial β-glucuronidase
1066   prevents irinotecan-induced diarrhea without impairing its antitumor efficacy in vivo. *Pharmacol. Res.,* **2019**,
1067   *139*, 41-49.

1068   133. Iida, N.; Dzutsev, A.; Stewart, C.A.; Smith, L.; Bouladoux, N.; Weingarten, R.A.; Molina, D.A.; Salcedo, R.;
1069   Back, T.; Cramer, S.; Dai, R.M.; Kiu, H.; Cardone, M., Naik, S.; Patri, A.K.; Wang, E.; Marincola, F.M. Frank,
1070   K.M.; Belkaid, Y.; Trinchieri, G.; Goldszmid, R.S. Commensal bacteria control cancer response to therapy by
1071   modulating the tumor microenvironment. *Science*, **2013**, *342*, 967-970.

1072   134. Gui, Q.F.; Lu, H.F.; Zhang, C.X.; Xu, Z.R.; Yang, Y.H. Well-balanced commensal microbiota contributes to
1073   anti-cancer response in a lung cancer mouse model. *Genet. Mol. Res.*, **2015**, *14*, 5642-5651.

1074   135. Viaud, S.; Saccheri, F.; Mignot, G.; Yamazaki, T.; Daillère, R.; Hannani, D.; Enot, D.P.; Pfirschke, C.; Engblom,
1075   C.; Pittet, M.J.; Schlitzer, A.; Ginhoux, F.; Apetoh, L.; Chachaty, E.; Woerther, P.L.; Eberl, G.; Bérard, M.;

1076   Ecobichon, C.; Clermont, D.; Bizet, C.; Gaboriau-Routhia, V.; Cerf-Bensussan, N.; Opolon, P.; Yessaad, N.;

1077   Vivier, E.; Ryffel, B.; Elson, C.O.; Doré, J.; Kroemer, G.; Lepage, P.; Boneca, I.G.; Ghiringhelli, F.; Zitvogel,

1078   L. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science,* **2013**, *342*,

1079   971-976.

1080   136. Zitvogel, L.; Pitt, J.M.; Daillère, R.; Smyth, M.J.; Kroemer, G. Mouse models in oncoimmunology. *Nat. Rev.*

1081   *Cancer.*, **2016**, *16*, 759-773.

1082   137. Ivanov, I.I.; Frutos, R.; Manel, N.; Yoshinaga, K.; Rifkin, D.B.; Sartor, R.B.; Finlay, B.B.; Littman, D.R.

1083   Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small

1084   intestine. *Cell Host Microbe*, **2008**, 4, 337-349.

1085   138. Daillère, R.; Vétizou, M.; Waldschmitt, N.; Yamazaki, T.; Isnard, C.; Poirier-Colame, V.; Duong, C.P.M.;

1086   Flament, C.; Lepage, P.; Roberti, M.P.; Routy, B.; Jacquelot, N.; Apetoh, L.; Becharef, S.; Rusakiewicz, S.;

1087   Langella, P.; Sokol, H.; Kroemer, G.; Enot, D.; Roux, A.; Eggermont, A.; Tartour, E.; Johannes, L.; Woerther,

1088   P.L.; Chachaty, E.; Soria, J.C., Golden, E.; Formenti, S.; Plebanski, M.; Madondo, M.; Rosenstiel, P.; Raoult,

1089   D.; Cattoir, V.; Boneca, I.G.; Chamaillard, M.; Zitvogel, L. *Enterococcus hirae* and *Barnesiella intestinihominis*

1090   facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. *Immunity*, **2016**, *45*, 931-943.

1091   139. Jahrsdörfer, B.; Weiner, G.J. CpG oligodeoxynucleotides as immunotherapy in cancer. *Update Cancer Ther.,*

1092   **2008**, *3*, 27-32.

1093   140. Routy, B.; Le Chatelier, E.; Derosa, L.; Duong, C.P.M.; Alou, M.T.; Daillère, R.; Fluckiger, A.; Messaoudene,

1094   M.; Rauber, C.; Roberti, M.P.; Fidelle, M.; Flament, C.; Poirier-Colame, V., Opolon, P.; Klein, C.; Iribarren, K.;

1095   Mondragón, L.; Jacquelot, N.; Qu, B.; Ferrere, G.; Clémenson, C.; Mezquita, L.; Masip, J.R.; Naltet, C.;

1096   Brosseau, S.; Kaderbhai, C.; Richard, C.; Rizvi, H.; Levenez, F.; Galleron, N.; Quinquis, B., Pons, N.; Ryffel,

1097   B.; Minard-Colin, V.; Gonin, P.; Soria, J.C.; Deutsch, E.; Loriot, Y.; Ghiringhelli, F.; Zalcman, G.; Goldwasser,

1098   F.; Escudier, B.; Hellmann, M.D.; Eggermont, A.; Raoult, D.; Albiges, L.; Kroemer, G.; Zitvogel, L. Gut

1099   microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*, **2018**, *359*,

1100   91-97.

1101   141. Gopalakrishnan, V.; Spencer, C.N.; Nezi, L.; Reuben, A.; Andrews, M.C.; Karpinets, T.V.; Prieto, P.A.;

1102   Vicente, D.; Hoffman, K.; Wei, S.C.; Cogdill, A.P.; Zhao, L.; Hudgens, C.W.; Hutchinson, D.S.; Manzo, T.;

1103   Petaccia de Macedo, M.; Cotechini, T.; Kumar, T.; Chen, W.S.; Reddy, S.M.; Szczepaniak Sloane, R.; Galloway-

1104   Pena, J.; Jiang, H.; Chen, P.L.; Shpall, E.J.; Rezvani, K.; Alousi, A.M.; Chemaly, R.F.; Shelburne, S.; Vence,

1105   L.M.; Okhuysen, P.C.; Jensen, V.B.; Swennes, A.G.; McAllister, F.; Marcelo Riquelme Sanchez, E.; Zhang, Y.,

1106   Le Chatelier, E.; Zitvogel, L.; Pons, N.; Austin-Breneman, J.L.; Haydu, L.E.; Burton, E.M.; Gardner, J.M.;

1107   Sirmans, E.; Hu, J.; Lazar, A.J.; Tsujikawa, T.; Diab, A.; Tawbi, H.; Glitza, I.C.; Hwu, W.J.; Patel, S.P.;

1108   Woodman, S.E.; Amaria, R.N.; Davies, M.A.; Gershenwald, J.E.; Hwu, P.; Lee, J.E.; Zhang, J.; Coussens, L.M.;

1109   Cooper, Z.A.; Futreal, P.A.; Daniel, C.R.; Ajami, N.J.; Petrosino, J.F.; Tetzlaff, M.T.; Sharma, P.; Allison, J.P.;

1110   Jenq, R.R.; Wargo, J.A. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients.

1111   *Science*, **2018**, *359*, 97-103

1112   142. Matson, V.; Fessler, J.; Bao, R.; Chongsuwat, T.; Zha, Y.; Alegre, M.L.; Luke, J.J.; Gajewski, T.F. The

1113   commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*, **2018**,

1114   *359*, 104-108.

1115   143. Chen, Q.; Wang, C.; Chen, G.; Hu, Q.; Gu, Z. Delivery strategies for immune checkpoint blockade. *Adv.*

1116   *Healthc. Mater.*, **2018**, *7*, e1800424.

1117   144.Buchbinder, E.I.; Desai, A. CTLA-4 and PD-1 pathways: Similarities, differences, and implications of their

1118   inhibition. *Am. J. Clin. Oncol.*, **2016**, *39*, 98-106.

1119   145. Vétizou, M.; Pitt, J.M.; Daillère, R.; Lepage, P.; Waldschmitt, N.; Flament, C.; Rusakiewicz, S.; Routy, B.;

1120   Roberti, M.P.; Duong, C.P.; Poirier-Colame, V.; Roux, A.; Becharef, S.; Formenti, S.; Golden, E.; Cording, S.;

1121   Eberl, G.; Schlitzer, A.; Ginhoux, F.; Mani, S.; Yamazaki, T.; Jacquelot, N.; Enot, D.P.; Bérard, M.; Nigou, J.;

1122   Opolon, P.; Eggermont, A.; Woerther, P.L.; Chachaty, E.; Chaput, N.; Robert, C.; Mateus, C.; Kroemer, G.;

1123   Raoult, D.; Boneca, I.G.; Carbonnel, F.; Chamaillard, M.; Zitvogel, L. Anticancer immunotherapy by CTLA-4

1124   blockade relies on the gut microbiota. *Science*, **2015**, *350*, 1079-1084.

1125   146. Sivan, A.; Corrales, L.; Hubert, N.; Williams, J.B.; Aquino-Michaels, K.; Earley, Z.M.; Benyamin, F.W.; Lei,

1126   Y.M.; Jabri, B.; Alegre, M.L.; Chang, E.B.; Gajewski, T.F. Commensal *Bifidobacterium* promotes antitumor

1127   immunity and facilitates anti-PD-L1 efficacy. *Science*, **2015**, *350*, 1084-1089.

1128   147. Gharaibeh, R.Z.; Jobin, C. Microbiota and cancer immunotherapy: in search of microbial signals. *Gut*, **2019**,

1129   *68*, 385-388.

1130   148. Limeta, A.; Ji, B.; Levin, M.; Gatto, F.; Nielsen, J. Meta-analysis of the gut microbiota in predicting response

1131   to cancer immunotherapy in metastatic melanoma. *JCI Insight*, **2020**, *5*, e140940.

1132   149. Tanoue, T.; Morita, S.; Plichta, D.R.; Skelly, A.N.; Suda, W.; Sugiura, Y.; Narushima, S.; Vlamakis, H.; Motoo,

1133   I.; Sugita, K.; Shiota, A.; Takeshita, K.; Yasuma-Mitobe, K.; Riethmacher, D.; Kaisho, T.; Norman, J.M.;

1134   Mucida, D.; Suematsu, M.; Yaguchi, T.; Bucci, V.; Inoue, T.; Kawakami, Y.; Olle, B.; Roberts, B.; Hattori, M.;

1135   Xavier, R.J.; Atarashi, K.; Honda, K. A defined commensal consortium elicits CD8 T cells and anti-cancer

1136   immunity. *Nature*, **2019**, *565*, 600-605.

1137 150. Mager, L.F.; Burkhard, R.; Pett, N.; Cooke, N.C.A.; Brown, K.; Ramay, H.; Paik, S.; Stagg, J.; Groves, R.A.;
1138 Gallo, M.; Lewis, I.A.; Geuking, M.B.; McCoy, K.D. Microbiome-derived inosine modulates response to
1139 checkpoint inhibitor immunotherapy. *Science*, **2020**, *369*, 1481-1489.

1140 151. Li, Y.; Elmén, L.; Segota, I.; Xian, Y.; Tinoco, R.; Feng, Y.; Fujita, Y.; Segura Muñoz, R.R.; Schmaltz, R.;
1141 Bradley, L.M.; Ramer-Tait, A.; Zarecki, R.; Long, T.; Peterson, S.N.; Ronai, Z.A. Prebiotic-induced anti-tumor
1142 immunity attenuates tumor growth. *Cell Rep.*, **2020**, *30*, 1753-1766.e6.

1143 152. Jenq, R.R.; van den Brink, M.R. Allogeneic haematopoietic stem cell transplantation: individualized stem cell
1144 and immune therapy of cancer. *Nat. Rev. Cancer*, **2010**, *10*, 213-221.

1145 153. Zeiser R, Blazar BR. Acute graft-versus-host disease - biologic process, prevention, and therapy. *N. Engl. J.
1146 Med.,* **2017**, *377*, 2167-2179.

1147 154.Peled, J.U.; Gomes, A.L.C.; Devlin, S.M.; Littmann, E.R.; Taur, Y.; Sung, A.D.; Weber, D.; Hashimoto, D.;
1148 Slingerland, A.E.; Slingerland, J.B.; Maloy, M.; Clurman, A.G.; Stein-Thoeringer, C.K.; Markey, K.A.;
1149 Docampo, M.D.; Burgos da Silva, M.; Khan, N.; Gessner, A.; Messina, J.A.; Romero, K.; Lew, M.V.; Bush, A.;
1150 Bohannon, L.; Brereton, D.G.; Fontana, E.; Amoretti, L.A.; Wright, R.J.; Armijo, G.K.; Shono, Y.; Sanchez-
1151 Escamilla, M.; Castillo Flores, N.; Alarcon Tomas, A.; Lin, R.J.; Yáñez San Segundo, L.; Shah, G.L.; Cho, C.;
1152 Scordo, M.; Politikos, I.; Hayasaka, K.; Hasegawa, Y.; Gyurkocza, B.; Ponce, D.M.; Barker, J.N.; Perales, M.A.;
1153 Giralt, S.A.; Jenq, R.R.; Teshima, T.; Chao, N.J.; Holler, E.; Xavier, J.B.; Pamer, E.G.; van den Brink, M.R.M.
1154 Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. *N. Engl. J. Med.*, **2020**,
1155 *382*, 822-834.

1156 155. Ingham, A.C.; Kielsen, K.; Cilieborg, M.S.; Lund, O.; Holmes, S.; Aarestrup, F.M.; Müller, K.G.; Pamp, S.J.
1157 Specific gut microbiome members are associated with distinct immune markers in pediatric allogeneic
1158 hematopoietic stem cell transplantation. *Microbiome*, **2019**, *7*, 131.

1159 156. Zama, D.; Bossù, G.; Leardini, D.; Muratore, E.; Biagi, E.; Prete, A.; Pession, A.; Masetti, R. Insights into the
1160 role of intestinal microbiota in hematopoietic stem-cell transplantation. *Ther. Adv. Hematol.*, **2020**, *11*,
1161 2040620719896961.

1162 157. Taur, Y.; Xavier, J.B.; Lipuma, L.; Ubeda, C.; Goldberg, J.; Gobourne, A.; Lee, Y.J.; Dubin, K.A.; Socci, N.D.;
1163 Viale, A.; Perales, M.A.; Jenq, R.R.; van den Brink. M.R.; Pamer, E.G. Intestinal domination and the risk of
1164 bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin. Infect. Dis.*, **2012**,
1165 *55*, 905-914.

1166 158. Taur, Y.; Jenq, R.R.; Perales, M.A.; Littmann, E.R.; Morjaria, S.; Ling, L.; No, D.; Gobourne, A.; Viale, A.;
1167 Dahi, P.B.; Ponce, D.M.; Barker, J.N.; Giralt, S.; van den Brink, M.; Pamer, E.G. The effects of intestinal tract

1168   bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood*, **2014**, *124*,

1169   1174-1182.

1170   159. Jenq, R.R.; Ubeda, C.; Taur, Y.; Menezes, C.C.; Khanin, R.; Dudakov, J.A.; Liu, C.; West, M.L.; Singer, N.V.;

1171   Equinda, M.J.; Gobourne, A.; Lipuma, L.; Young, L.F.; Smith, O.M.; Ghosh, A.; Hanash, A.M.; Goldberg, J.

1172   D.; Aoyama, K.; Blazar, B.R.; Pamer, E.G., van den Brink, M.R. Regulation of intestinal inflammation by

1173   microbiota following allogeneic bone marrow transplantation. *J. Exp. Med.*, **2012**, *209*, 903-11.

1174   160. Shono, Y.; Docampo, M.D.; Peled, J.U.; Perobelli, S.M.; Velardi, E.; Tsai, J.J.; Slingerland, A.E.; Smith, O.M.;

1175   Young, L.F.; Gupta, J.; Lieberman, S.R.; Jay, H.V.; Ahr, K.F.; Porosnicu Rodriguez, K.A.; Xu, K.; Calarfiore,

1176   M.; Poeck, H.; Caballero, S.; Devlin, S.M.; Rapaport, F.; Dudakov, J.A.; Hanash, A.M.; Gyurkocza, B.; Murphy,

1177   G.F.; Gomes, C.; Liu, C.; Moss, E.L.; Falconer, S.B.; Bhatt, A.S.; Taur, Y.; Pamer, E.G.; van den Brink, M.R.M.;

1178   Jenq, R.R. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic

1179   stem cell transplantation in human patients and mice. *Sci. Transl. Med.*, **2016**, *8*, 339ra71.

1180   161. Heimesaat, M.M.; Nogai, A.; Bereswill, S.; Plickert, R.; Fischer, A.; Loddenkemper, C.; Steinhoff, U.;

1181   Tchaptchet, S.; Thiel, E.; Freudenberg, M.A.; Göbel, U.B.; Uharek, L. MyD88/TLR9 mediated

1182   immunopathology and gut microbiota dynamics in a novel murine model of intestinal graft-versus-host disease.

1183   *Gut*, **2010**, *59*, 1079-1087.

1184   162. Eriguchi, Y.; Takashima, S.; Oka, H.; Shimoji, S.; Nakamura, K.; Uryu, H.; Shimoda, S.; Iwasaki, H.; Shimono,

1185   N.; Ayabe, T.; Akashi, K.; Teshima, T. Graft-versus-host disease disrupts intestinal microbial ecology by

1186   inhibiting Paneth cell production of alpha-defensins. *Blood*, **2012**, *120*, 223-231.

1187   163. Biagi, E.; Zama, D.; Nastasi, C.; Consolandi, C.; Fiori, J.; Rampelli, S.; Turroni, S.; Centanni, M.; Severgnini,

1188   M.; Peano, C.; de Bellis, G.; Basaglia, G.; Gotti, R.; Masetti, R.; Pession, A.; Brigidi, P.; Candela, M. Gut

1189   microbiota trajectory in pediatric patients undergoing hematopoietic SCT. *Bone Marrow Transplant.*, **2015**, *50*,

1190   992-998.

1191   164. Biagi, E.; Zama, D.; Rampelli, S.; Turroni, S.; Brigidi, P.; Consolandi, C.; Severgnini, M.; Picotti, E.; Gasperini,

1192   P.; Merli, P.; Decembrino, N.; Zecca, M.; Cesaro, S.; Faraci, M.; Prete, A.; Locatelli, F.; Pession, A.; Candela,

1193   M.; Masetti, R. Early gut microbiota signature of aGvHD in children given allogeneic hematopoietic cell

1194   transplantation for hematological disorders. *BMC Med. Genomics*, **2019**, *12*, 49.

1195   165. Han, L.; Zhang, H.; Chen, S.; Zhou, L.; Li, Y.; Zhao, K.; Huang, F.; Fan, Z.; Xuan, L.; Zhang, X.; Dai, M.; Lin,

1196   Q.; Jiang, Z.; Peng, J.; Jin, H.; Liu, Q. Intestinal microbiota can predict acute graft-versus-host disease following

1197   allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transpl.*, **2019**, *25*, 1944-1955.

1198   166. Mathewson, N.D.; Jenq, R.; Mathew, A.V.; Koenigsknecht, M.; Hanash, A.; Toubai, T.; Oravecz-Wilson, K.;
1199   Wu, S.R.; Sun, Y.; Rossi, C.; Fujiwara, H.; Byun, J.; Shono, Y.; Lindemans, C.; Calafiore, M.; Schmidt, T.M.;
1200   Honda, K.; Young, V.B.; Pennathur, S.; van den Brink, M.; Reddy, P. Gut microbiome-derived metabolites
1201   modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat. Immunol.*, **2016**, *17*, 505-
1202   513.

1203   167. Atarashi, K.; Tanoue, T.; Oshima, K.; Suda, W.; Nagano, Y.; Nishikawa, H.; Fukuda, S.; Saito, T.; Narushima,
1204   S.; Hase, K.; Kim, S.; Fritz, J.V.; Wilmes, P.; Ueha, S.; Matsushima, K.; Ohno, H.; Olle, B.; Sakaguchi, S.;
1205   Taniguchi, T.; Morita, H.; Hattori, M.; Honda, K. Treg induction by a rationally selected mixture of clostridia
1206   strains from the human microbiota. *Nature*, **2013**, *500*, 232-236.

1207   168. Liu, X.; Mao, B.; Gu, J.; Wu, J.; Cui, S.; Wang, G.; Zhao, J.; Zhang, H.; Chen, W. Blautia-a new functional
1208   genus with potential probiotic properties? *Gut Microbes*, **2021**, *13*, 1-21.

1209   169. Jenq, R.R.; Taur, Y.; Devlin, S.M.; Ponce, D.M.; Goldberg, J.D.; Ahr, K.F.; Littmann, E.R.; Ling, L.; Gobourne,
1210   A.C.; Miller, L.C.; Docampo, M.D.; Peled, J.U.; Arpaia, N.; Cross, J.R.; Peets, T.K.; Lumish, M.A.; Shono, Y.;
1211   Dudakov, J.A.; Poeck, H.; Hanash, A.M.; Barker, J.M.; Perales, M.A.; Giralt, S.A.; Pamer, E.G.; van den Brink,
1212   M.R. Intestinal *Blautia* is associated with reduced death from graft-versus-host disease. *Biol. Blood Marrow*
1213   *Transplant.*, **2015**, *21*, 1373-1383.

1214   170. D'Amico, F.; Biagi, E.; Rampelli, S.; Fiori, J.; Zama, D.; Soverini, M.; Barone, M.; Leardini, D.; Muratore, E.;
1215   Prete, A.; Gotti, R.; Pession, A.; Masetti, R.; Brigidi, P.; Turroni, S.; Candela, M. Enteral nutrition in pediatric
1216   patients undergoing hematopoietic SCT promotes the recovery of gut microbiome homeostasis. *Nutrients*, **2019**,
1217   *11*, 2958.

1218   171. Staffas, A.; Burgos da Silva, M.; Slingerland, A.E.; Lazrak, A.; Bare, C.J.; Holman, C.D.; Docampo, M.D.;
1219   Shono, Y.; Durham, B.; Pickard, A.J.; Cross, J.R.; Stein-Thoeringer, C.; Velardi, E.; Tsai, J.J.; Jahn, L.; Jay, H.;
1220   Lieberman, S.; Smith, O.M.; Pamer, E.G.; Peled, J.U.; Cohen, D.E.; Jenq, R.R.; van den Brink, M.R.M.
1221   Nutritional support from the intestinal microbiota improves hematopoietic reconstitution after bone marrow
1222   transplantation in Mice. *Cell Host Microbe*, **2018**, *23*, 447-457.

1223   172. Geller, L.T.; Barzily-Rokni, M.; Danino, T.; Jonas, O.H.; Shental, N.; Nejman, D.; Gavert, N.; Zwang, Y.;
1224   Cooper, Z.A.; Shee, K.; Thaiss, C.A.; Reuben, A.; Livny, J.; Avraham, R.; Frederick, D.T.; Ligorio, M.;
1225   Chatman, K.; Johnston, S.E.; Mosher, C.M.; Brandis, A.; Fuks, G.; Gurbatri, C.; Gopalakrishnan, V.; Kim, M.;
1226   Hurd, M.W.; Katz, M.; Fleming, J.; Maitra, A.; Smith, D.A.; Skalak, M.; Bu, J.; Michaud, M.; Trauger, S.A.;
1227   Barshack, I.; Golan, T.; Sandbank, J.; Flaherty, K.T.; Mandinova, A.; Garrett, W.S.; Thayer, S.P.; Ferrone, C.R.;
1228   Huttenhower, C.; Bhatia, S.N.; Gevers, D.; Wargo, J.A.; Golub, T.R.; Straussman, R. Potential role of intratumor

1229   bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science*, **2017**, *357*, 1156-

1230   1160.

1231   173. Barrett, M.; Hand, C.K.; Shanahan, F.; Murphy, T.; O'Toole, P.W. Mutagenesis by microbe: the role of the

1232   microbiota in shaping the cancer genome. *Trends Cancer*, **2020**, *6*, 277-287.

1233   174. Pleguezuelos-Manzano, C.; Puschhof, J.; Rosendahl Huber, A.; van Hoeck, A.; Wood, H.M.; Nomburg, J.;

1234   Gurjao, C.; Manders, F.; Dalmasso, G.; Stege, P.B.; Paganelli, F.L.; Geurts, M.H.; Beumer, J.; Mizutani, T.;

1235   Miao, Y.; van der Linden, R.; van der Elst, S.; Genomics England Research Consortium, Garcia, K.C.; Top, J.;

1236   Willems, R.J.L.; Giannakis, M.; Bonnet, R.; Quirke, P.; Meyerson, M.; Cuppen, E.; van Boxtel, R.; Clevers, H.

1237   Mutational signature in colorectal cancer caused by genotoxic pks+ *E. coli*. *Nature*, **2020**, *580*, 269-273.

1238   175. Brennan, C.A.; Garrett, W.S. *Fusobacterium nucleatum* - symbiont, opportunist and oncobacterium. *Nat. Rev.*

1239   *Microbiol.*, **2019**, *17*, 156-166.

1240   176. Sepich-Poore, G.D.; Zitvogel, L.; Straussman, R.; Hasty, J.; Wargo, J.A.; Knight, R. The microbiome and

1241   human cancer. *Science*, **2021**, *71*, eabc4552.

1242   177. Nejman, D.; Livyatan, I.; Fuks, G.; Gavert, N.; Zwang, Y.; Geller, L.T.; Rotter-Maskowitz, A.; Weiser, R.;

1243   Mallel, G.; Gigi, E.; Meltser, A.; Douglas, G.M.; Kamer, I.; Gopalakrishnan, V.; Dadosh, T.; Levin-Zaidman,

1244   S.; Avnet, S.; Atlan, T.; Cooper, Z.A.; Arora, R.; Cogdill, A.P.; Khan, M.A.W.; Ologun, G.; Bussi, Y.;

1245   Weinberger, A.; Lotan-Pompan, M.; Golani, O.; Perry, G.; Rokah, M.; Bahar-Shany, K.; Rozeman, E.A.; Blank,

1246   C.U.; Ronai, A.; Shaoul, R.; Amit, A.; Dorfman, T.; Kremer, R.; Cohen, Z.R.; Harnof, S.; Siegal, T.; Yehuda-

1247   Shnaidman, E.; Gal-Yam, E.N.; Shapira, H.; Baldini, N.; Langille, M.G.I.; Ben-Nun, A.; Kaufman, B.; Nissan,

1248   A.; Golan, T.; Dadiani, M.; Levanon, K.; Bar, J.; Yust-Katz, S.; Barshack, I.; Peeper, D.S.; Raz, D.J.; Segal, E.;

1249   Wargo, J.A.; Sandbank, J.; Shental, N.; Straussman, R. The human tumor microbiome is composed of tumor

1250   type-specific intracellular bacteria. *Science*, **2020**, *368*, 973-980.

1251   178. Poore, G.D.; Kopylova, E.; Zhu, Q.; Carpenter, C.; Fraraccio, S.; Wandro, S.; Kosciolek, T.; Janssen, S.;

1252   Metcalf, J.; Song, S.J.; Kanbar, J.; Miller-Montgomery, S.; Heaton, R.; Mckay, R.; Patel, S.P.; Swafford, A.D.;

1253   Knight, R. Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature*, **2020**, *579*,

1254   567-574.

1255   179. Errington, J. Cell wall-deficient, L-form bacteria in the 21st century: a personal perspective. *Biochem. Soc.*

1256   *Trans.*, **2017**, *45*, 287-295.

1257   180. Parhi, L.; Alon-Maimon, T.; Sol, A.; Nejman, D.; Shhadeh, A.; Fainsod-Levi, T.; Yajuk, O.; Isaacson, B.; Abed,

1258   J.; Maalouf, N.; Nissan, A.; Sandbank, J.; Yehuda-Shnaidman, E.; Ponath, F.; Vogel, J.; Mandelboim, O.;

Granot, Z.; Straussman, R.; Bachrach, G. Breast cancer colonization by *Fusobacterium nucleatum* accelerates tumor growth and metastatic progression. *Nat. Commun.*, **2020**, *11*, 3259.

181. Yu, T.; Guo, F.; Yu, Y.; Sun, T.; Ma, D.; Han, J.; Qian, Y.; Kryczek, I.; Sun, D.; Nagarsheth, N.; Chen, Y.; Chen, H.; Hong, J.; Zou, W.; Fang, J.Y. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell*, **2017**, *170*, 548-563.

182. Riquelme, E.; Zhang, Y.; Zhang, L.; Montiel, M.; Zoltan, M.; Dong, W.; Quesada, P.; Sahin, I.; Chandra, V.; San Lucas, A.; Scheet, P.; Xu, H.; Hanash, S.M.; Feng, L.; Burks, J.K.; Do, K.A.; Peterson, C.B.; Nejman, D.; Tzeng, C.D.; Kim, M.P.; Sears, C.L.; Ajami, N.; Petrosino, J.; Wood, L.D.; Maitra, A.; Straussman, R.; Katz, M.; White, J.R.; Jenq, R.; Wargo, J.; McAllister, F. Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes. *Cell*, **2019**, *178*, 795-806.

183. Falzone, L.; Salomone, S.; Libra, M. Evolution of cancer pharmacological treatments at the turn of the third millennium. *Front. Pharmacol.*, **2018**, *9*, 1300.

184. Helmink, B.A.; Khan, M.A.W.; Hermann, A.; Gopalakrishnan, V.; Wargo, J.A. The microbiome, cancer, and cancer therapy. *Nat. Med.*, **2019**, *25*, 377-388.

185. Parida, S.; Sharma, D. The microbiome and cancer: Creating friendly neighborhoods and removing the foes within. *Cancer Res.*, **2021**, *81*, 790-800.

186. Kaźmierczak-Siedlecka, K.; Daca, A.; Fic, M.; van de Wetering, T.; Folwarski, M.; Makarewicz, W. Therapeutic methods of gut microbiota modification in colorectal cancer management - fecal microbiota transplantation, prebiotics, probiotics, and synbiotics. *Gut Microbes*, **2020**, *11*, 1518-1530.

187. Gibson, G.R.; Hutkins, R.; Sanders, M.E.; Prescott, S.L.; Reimer, R.A.; Salminen, S.J.; Scott, K.; Stanton, C.; Swanson, K.S.; Cani, P.D.; Verbeke, K.; Reid, G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.*, **2017**, *14*, 491-502.

188. Vandeputte, D.; Falony, G.; Vieira-Silva, S.; Wang, J.; Sailer, M.; Theis, S.; Verbeke, K.; Raes, J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut*, **2017**, *66*, 1968-1974.

189. Cunningham, M.; Azcarate-Peril, M.A.; Barnard, A.; Benoit, V.; Grimaldi, R.; Guyonnet, D.; Holscher, H.D.; Hunter, K.; Manurung, S.; Obis, D.; Petrova, M.I.; Steinert, R.E.; Swanson, K.S.; van Sinderen, D.; Vulevic, J.; Gibson, G.R. Shaping the future of probiotics and prebiotics. *Trends Microbiol.*, **2021**, S0966-842X(21)00005-6. [online ahead of print]. doi: 10.1016/j.tim.2021.01.003.

1288   190. Deehan, E.C.; Yang, C.; Perez-Muñoz, M.E.; Nguyen, N.K.; Cheng, C.C.; Triador, L.; Zhang, Z.; Bakal, J.A.;
1289   Walter, J. Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid
1290   production. *Cell Host Microbe*, **2020**, *27*, 389-404.

1291   191.Sasidharan, B.K.; Ramadass, B.; Viswanathan, P.N.; Samuel, P.; Gowri, M.; Pugazhendhi, S.; Ramakrishna,
1292   B.S. A phase 2 randomized controlled trial of oral resistant starch supplements in the prevention of acute
1293   radiation proctitis in patients treated for cervical cancer. *J. Cancer Res. Ther.*, **2019**, *15*, 1383-1391.

1294   192. Xie, X.; He, Y.; Li, H.; Yu, D.; Na, L.; Sun, T.; Zhang, D.; Shi, X.; Xia, Y.; Jiang, T.; Rong, S.; Yang, S.; Ma,
1295   X.; Xu, G. Effects of prebiotics on immunologic indicators and intestinal microbiota structure in perioperative
1296   colorectal cancer patients. *Nutrition*, **2019**, *61*, 132-142.

1297   193. García-Peris, P.; Velasco, C.; Lozano, M.A.; Moreno, Y.; Paron, L.; de la Cuerda, C.; Bretón, I.; Camblor, M.;
1298   García-Hernández, J.; Guarner, F.; Hernández, M. Effect of a mixture of inulin and fructo-oligosaccharide on
1299   *Lactobacillus* and *Bifidobacterium* intestinal microbiota of patients receiving radiotherapy: a randomised,
1300   double-blind, placebo-controlled trial. *Nutr. Hosp.*, **2012**, *27*, 1908-1915.

1301   194. Criscuolo, A.A.; Sesti, F.; Piccione, E.; Mancino, P.; Belloni, E.; Gullo, C.; Ciotti, M. Therapeutic efficacy of
1302   a *Coriolus versicolor*-based vaginal gel in women with cervical uterine high-risk HPV Infection: A retrospective
1303   observational study. *Adv. Ther.*, **2021**, *38*, 1202-1211.

1304   195. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.;
1305   Salminen, S.; Calder, P.C.; Sanders, M.E. Expert consensus document. The International Scientific Association
1306   for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat.*
1307   *Rev. Gastroenterol. Hepatol.*, **2014**, *11*, 506-514.

1308   196. Suez, J.; Zmora, N.; Segal, E.; Elinav, E. The pros, cons, and many unknowns of probiotics. *Nat. Med.*, **2019**,
1309   *25*, 716-729.

1310   197. Liu, C.T.; Chu, F.J.; Chou, C.C.; Yu, R.C. Antiproliferative and anticytotoxic effects of cell fractions and
1311   exopolysaccharides from *Lactobacillus casei* 01. *Mutat. Res.*, **2011**, *721*, 157-62.

1312   198. Reis, S.A.D.; da Conceição, L.L.; Peluzio, M.D.C.G. Intestinal microbiota and colorectal cancer: changes in the
1313   intestinal microenvironment and their relation to the disease. J. Med. Microbiol., 2019, 68, 1391-1407.

1314   199. Molska, M.; Reguła, J. Potential mechanisms of probiotics action in the prevention and treatment of colorectal
1315   cancer. *Nutrients*, **2019**, *11*, 2453.

1316   200. Lu, K.; Dong, S.; Wu, X.; Jin, R.; Chen, H. Probiotics in cancer. *Front. Oncol.*, **2021**, *11*, 638148.

1317   201. Hassan, Z. Anti-cancer and biotherapeutic potentials of probiotic bacteria. *J. Cancer Sci. Ther.*, **2019**, *11*, 9-13.

202. Singh, B.; Mal, G.; Marotta, F. Designer probiotics: Paving the way to living therapeutics. *Trends Biotechnol.*, **2017**, *35*, 679-682.

203. Bedada, T.L.; Feto, T.K.; Awoke, K.S.; Garedew, A.D.; Yifat, F.T.; Birri, D.J. Probiotics for cancer alternative prevention and treatment. *Biomed. Pharmacother.*, **2020**, *129*, 110409.

204. Hibberd, A.A.; Lyra, A.; Ouwehand, A.C.; Rolny, P.; Lindegren, H.; Cedgård, L.; Wettergren, Y. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open Gastroenterol.*, **2017**, *4*, e000145.

205. Consoli, M.L.; da Silva, R.S.; Nicoli, J.R.; Bruña-Romero, O.; da Silva, R.G.; de Vasconcelos Generoso, S.; Correia, M.I. Randomized clinical trial: impact of oral administration of *Saccharomyces boulardii* on gene expression of intestinal cytokines in patients undergoing colon resection. *JPEN J Parenter. Enteral Nutr.*, **2016**, *40*, 1114-1121.

206. Naito, S.; Koga, H.; Yamaguchi, A.; Fujimoto, N.; Hasui, Y.; Kuramoto, H.; Iguchi, A.; Kinukawa, N.; Kyushu University Urological Oncology Group. Prevention of recurrence with epirubicin and lactobacillus casei after transurethral resection of bladder cancer. *J. Urol.*, **2008**, *179*, 485-490.

207. Seely, D.; Ennis, J.E.; McDonell, E.; Fazekas, A.; Zhao, L.; Asmis, T.; Auer, R.C.; Fergusson, D.; Kanji, S.; Maziak, D.E.; Ramsay, T.; Chamberland, P.; Spooner, C.; Threader, J.; Seely, A. Intervention development process for a pragmatic randomized controlled trial: The thoracic peri-operative integrative surgical care evaluation trial. *J. Altern. Complement. Med.*, **2019**, *25*, S112-S123.

208. U.S. Department of Health and Human Services, Food and Drug Administration Guidance for Industry. Early clinical trials with live biotherapeutic products: Chemistry, manufacturing, and control information. www.fda.gov/downloads/Biologi%E2%80%A6/UCM292704.pdf , **2016**.

209. Charbonneau, M.R.; Isabella, V.M.; Li, N.; Kurtz, C.B. Developing a new class of engineered live bacterial therapeutics to treat human diseases. *Nat. Commun.*, **2020**, *11*, 1738.

210. O'Toole, P.W.; Marchesi, J.R.; Hill, C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.*, **2017**, *2*, 17057.

211. Ulsemer, P.; Henderson, G.; Toutounian, K.; Löffler, A.; Schmidt, J.; Karsten, U.; Blaut, M.; Goletz, S. Specific humoral immune response to the Thomsen-Friedenreich tumor antigen (CD176) in mice after vaccination with the commensal bacterium *Bacteroides ovatus* D-6. *Cancer Immunol. Immunother.*, **2013**, *62*, 875-887.

212. Ulsemer, P.; Toutounian, K.; Kressel, G.; Goletz, C.; Schmidt, J.; Karsten, U.; Hahn, A.; Goletz, S. Impact of oral consumption of heat-treated *Bacteroides xylanisolvens* DSM 23964 on the level of natural TFα-specific antibodies in human adults. *Benef. Microbes.*, **2016**, *7*, 485-500.

Codice campo modificato

1349   213. Ho, C.L.; Tan, H.Q.; Chua, K.J.; Kang, A.; Lim, K.H.; Ling, K.L.; Yew, W.S.; Lee, Y.S.; Thiery, J.P.; Chang,

1350   M.W. Engineered commensal microbes for diet-mediated colorectal-cancer chemoprevention. *Nat. Biomed.*

1351   *Eng.,* **2018**, *2*, 27-37.

1352   214. Arthur, J.C.; Gharaibeh, R.Z.; Uronis, J.M.; Perez-Chanona, E.; Sha, W.; Tomkovich, S.; Mühlbauer, M.; Fodor,

1353   A.A.; Jobin, C. VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated

1354   colorectal cancer. *Sci. Rep.*, **2013**, *3*, 2868.

1355   215. Veiga, P.; Suez, J.; Derrien, M.; Elinav, E. Moving from probiotics to precision probiotics. *Nat. Microbiol.*,

1356   **2020**, *5*, 878-880.

1357   216. Sotoudegan, F.; Daniali, M.; Hassani, S.; Nikfar, S.; Abdollahi, M. Reappraisal of probiotics' safety in human.

1358   *Food Chem. Toxicol.*, **2019**, *129*, 22-29.

1359   217. Cammarota, G.; Ianiro, G.; Tilg, H.; Rajilić-Stojanović, M.; Kump, P.; Satokari, R.; Sokol, H.; Arkkila, P.;

1360   Pintus, C.; Hart, A.; Segal, J.; Aloi, M.; Masucci, L.; Molinaro, A.; Scaldaferri, F.; Gasbarrini, G.; Lopez-

1361   Sanroman, A.; Link, A.; de Groot, P.; de Vos, W.M.; Högenauer, C.; Malfertheiner, P.; Mattila, E.;

1362   Milosavljević, T.; Nieuwdorp, M.; Sanguinetti, M.; Simren, M.; Gasbarrini, A. European consensus conference

1363   on faecal microbiota transplantation in clinical practice. *Gut*, **2017**, *66*, 569.

1364   218. Ianiro, G.; Mullish, B.H.; Kelly, C.R.; Kassam, Z.; Kuijper, E.J.; Ng, S.C.; Iqbal, T.H.; Allegretti, J.R.; Bibbò,

1365   S.; Sokol, H.; Zhang, F.; Fischer, M.; Costello, S.P.; Keller, J.J.; Masucci, L.; van Prehn, J.; Quaranta, G.;

1366   Quraishi, M.N.; Segal, J.; Kao, D.; Satokari, R.; Sanguinetti, M.; Tilg, H.; Gasbarrini, A.; Cammarota, G.

1367   Reorganisation of faecal microbiota transplant services during the COVID-19 pandemic. *Gut*, **2020**, *69*, 1555.

1368   219. Gupta, A.; Saha, S.; Khanna, S. Therapies to modulate gut microbiota: Past, present and future. *World J.*

1369   *Gastroenterol.*, **2020**, *26*, 777-788.

1370   220. Rezasoltani, S; Yadegar, A.; Asadzadeh Aghdaei, H.; Reza Zali, M. Modulatory effects of gut microbiome in

1371   cancer immunotherapy: A novel paradigm for blockade of immune checkpoint inhibitors. *Cancer Med.,* **2021**,

1372   *10*, 1141-1154.

1373   221. Baruch, E.N.; Youngster, I.; Ben-Betzalel, G.; Ortenberg, R.; Lahat, A.; Katz, L.; Adler, K.; Dick-Necula, D.;

1374   Raskin, S.; Bloch, N.; Rotin, D.; Anafi, L.; Avivi, C.; Melnichenko, J.; Steinberg-Silman, Y.; Mamtani, R.;

1375   Harati, H.; Asher, N.; Shapira-Frommer, R.; Brosh-Nissimov, T.; Eshet, Y.; Ben-Simon, S.; Ziv, O.; Khan,

1376   M.A.W.; Amit, M.; Ajami, N.J.; Barshack, I.; Schachter, J.; Wargo, J.A.; Koren, O.; Markel, G.; Boursi, B. Fecal

1377   microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science*, **2021**, *371*,

1378   602-609.

1379   222. Davar, D.; Dzutsev, A.K.; McCulloch, J.A.; Rodrigues, R.R.; Chauvin, J.M.; Morrison, R.M.; Deblasio, R.N.;
1380   Menna, C.; Ding, Q.; Pagliano, O.; Zidi, B.; Zhang, S.; Badger, J.H.; Vetizou, M.; Cole, A.M.; Fernandes, M.R.;
1381   Prescott, S.; Costa, R.G.F.; Balaji, A.K.; Morgun, A.; Vujkovic-Cvijin, I.; Wang, H; Borhani, A.A.; Schwartz,
1382   M.B.; Dubner, H.M.; Ernst, S.J.; Rose, A.; Najjar, Y.G.; Belkaid, Y.; Kirkwood, J.M.; Trinchieri, G.; Zarour,
1383   H.M. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*,
1384   **2021**, *371*, 595-602.

1385   223. Zhang, T.; Lu, G.; Zhao, Z.; Liu, Y.; Shen, Q.; Li, P.; Chen, Y.; Yin, H.; Wang, H.; Marcella, C.; Cui, B.; Cheng,
1386   L.; Ji, G.; Zhang, F. Washed microbiota transplantation vs. manual fecal microbiota transplantation: clinical
1387   findings, animal studies and in vitro screening. *Protein Cell.*, **2020**, *11*, 251-266.

1388   224. Zhang, T.; Ding, X.; Dai, M.; Zhang, H.; Xiao, F.; He, X.; Zhang, F.; Zhang, X. Washed microbiota
1389   transplantation in patients with respiratory spreading diseases: Practice recommendations. *Med. Microecol.*,
1390   **2021**, *7*, 100024.

1391   225. Chen, D.; Wu, J.; Jin, D.; Wang, B.; Cao, H. Fecal microbiota transplantation in cancer management: Current
1392   status and perspectives. *Int. J. Cancer.*, **2019**, *145*, 2021-2031.

1393   226. Zhu, X.; Goldberg, A. Introduction to semi-supervised learning. *Morgan Claypool Publ.*,
1394   https://doi.org/10.2200/S00196ED1V01Y200906AIM006 , **2009**.

1395   227. Moreno-Indias, I.; Lahti, L.; Nedyalkova, M.; Elbere, I.; Roshchupkin, G.; Adilovic, M.; Aydemir, O.; Bakir-
1396   Gungor, B.; Santa Pau, E.C.; D'Elia, D.; Desai, M.S.; Falquet, L.; Gundogdu, A.; Hron, K.; Klammsteiner, T.;
1397   Lopes, M.B.; Marcos-Zambrano, L.J.; Marques, C.; Mason, M.; May, P.; Pašić, L.; Pio, G.; Pongor, S.;
1398   Promponas, V.J.; Przymus, P.; Saez-Rodriguez, J.; Sampri, A.; Shigdel, R.; Stres, B.; Suharoschi, R.; Truu, J.;
1399   Truică, C.O.; Vilne, B.; Vlachakis, D.; Yilmaz, E.; Zeller, G.; Zomer, A.L.; Gómez-Cabrero, D.; Claesson, M.J.
1400   Statistical and machine learning techniques in human microbiome studies: Contemporary challenges and
1401   solutions. *Front. Microbiol.*, **2021**, *12*, 635781.

1402   228. Bonetta, R.; Valentino, G. Machine learning techniques for protein function prediction. *Proteins*, **2020**, *88*, 397-
1403   413.

1404   229. Vamathevan, J.; Clark, D.; Czodrowski, P.; Dunham, I.; Ferran, E.; Lee, G.; Li, B.; Madabhushi, A.; Shah, P.;
1405   Spitzer, M.; Zhao, S. Applications of machine learning in drug discovery and development. *Nat. Rev. Drug.
1406   Discov.,* **2019**, *18*, 463-477.

1407   230. McCarthy, J.F.; Marx, K.A.; Hoffman, P.E.; Gee, A.G.; O'Neil, P.; Ujwal, M.L.; Hotchkiss, J. Applications of
1408   machine learning and high-dimensional visualization in cancer detection, diagnosis, and management. *Ann. N.
1409   Y. Acad. Sci.*, **2004**, *1020*, 239-362.

231. Kerlikowske, K.; Scott, C.G.; Mahmoudzadeh, A.P.; Ma, L.; Winham, S.; Jensen, M.R.; Wu, F.F.; Malkov, S.; Pankratz, V.S.; Cummings, S.R.; Shepherd, J.A.; Brandt, K.R.; Miglioretti, D.L.; Vachon, C.M. Automated and clinical breast imaging reporting and data system density measures predict risk for screen-detected and interval cancers: A case-control study. *Ann. Intern. Med.,* **2018**, *168*, 757-765.

232. Nam, J.G.; Park, S.; Hwang, E.J.; Lee, J.H.; Jin, K.N.; Lim, K.Y.; Vu, T.H.; Sohn, J.H.; Hwang, S.; Goo, J.M.; Park, C.M. Development and validation of deep learning-based automatic detection algorithm for malignant pulmonary nodules on chest radiographs. *Radiology*, **2019**, *290*, 218-228.

233. Pantuck, A.J.; Lee, D.K.; Kee, T.; Wang, P.; Lakhotia, S.; Silverman, M.H.; Mathis, C.; Drakaki, A.; Belldegrun, A.S.; Ho, C.M.; Ho, D. Modulating BET bromodomain inhibitor ZEN-3694 and enzalutamide combination dosing in a metastatic prostate cancer patient using CURATE.AI, an artificial intelligence platform. *Adv. Therap.*, **2018**, *1*, 1800104.

234. Ngiam, K.Y.; Khor, I.W. Big data and machine learning algorithms for health-care delivery. *Lancet Oncol.*, **2019**, *20*, e262-e273.

235. Marcos-Zambrano, L.J.; Karaduzovic-Hadziabdic, K.; Loncar Turukalo, T.; Przymus, P.; Trajkovik, V.; Aasmets, O.; Berland, M.; Gruca, A.; Hasic, J.; Hron, K.; Klammsteiner, T.; Kolev, M.; Lahti, L.; Lopes, M.B., Moreno, V.; Naskinova, I.; Org, E.; Paciência, I.; Papoutsoglou, G.; Shigdel, R.; Stres, B.; Vilne, B.; Yousef, M.; Zdravevski, E.; Tsamardinos, I.; Carrillo de Santa Pau, E.; Claesson, M.J.; Moreno-Indias, I.; Truu, J. Applications of machine learning in human microbiome studies: A review on feature selection, biomarker identification, disease prediction and treatment. *Front. Microbiol.,* **2021**, *12*, 634511.

236. Ai, D.; Pan, H.; Han, R.; Li, X.; Liu, G.; Xia, L.C. Using decision tree aggregation with random forest model to identify gut microbes associated with colorectal cancer. *Genes (Basel)*. 2019, 10, 112.

237. Gupta, A.; Dhakan, D.B.; Maji, A.; Saxena, R.; P K, V.P.; Mahajan, S.; Pulikkan, J.; Kurian, J.; Gomez, A.M.; Scaria, J.; Amato, K.R.; Sharma, A.K.; Sharma, V.K. Association of *Flavonifractor plautii*, a Flavonoid-Degrading Bacterium, with the gut microbiome of colorectal cancer patients in India. *mSystems*, **2019**, 4, e00438-19.

238. Jang, B.S.; Chang, J.H.; Chie, E.K.; Kim, K.; Park, J.W.; Kim, M.J.; Song, E.J.; Nam, Y.D.; Kang, S.W.; Jeong, S.Y.; Kim, H.J. Gut microbiome composition is associated with a pathologic response after preoperative chemoradiation in patients with rectal cancer. *Int. J. Radiat. Oncol. Biol. Phys.*, **2020**, 107, 736-746.

239. Kharrat, N.; Assidi, M.; Abu-Elmagd, M.; Pushparaj, P.N.; Alkhaldy, A.; Arfaoui, L.; Naseer, M.I.; El Omri, A.; Messaoudi, S.; Buhmeida, A.; Rebai, A. Data mining analysis of human gut microbiota links *Fusobacterium* spp. with colorectal cancer onset. *Bioinformation*, **2019**, *15*, 372-379.

1441   240. Zimmermann, M.; Zimmermann-Kogadeeva, M.; Wegmann, R.; Goodman, A.L. Mapping human microbiome
1442   drug metabolism by gut bacteria and their genes. *Nature*, **2019**, *570*, 462-467.

1443   241. Sharma, A.K.; Jaiswal, S.K.; Chaudhary, N.; Sharma, V.K. A novel approach for the prediction of species-
1444   specific biotransformation of xenobiotic/drug molecules by the human gut microbiota. *Sci. Rep.*, **2017**, *7*, 9751.

1445   242. Maier, L.; Pruteanu, M.; Kuhn, M.; Zeller, G.; Telzerow, A.; Anderson, E.E.; Brochado, A.R.; Fernandez, K.C.;
1446   Dose, H.; Mori, H.; Patil, K.R.; Bork, P.; Typas, A. Extensive impact of non-antibiotic drugs on human gut
1447   bacteria. *Nature*, **2018**, *555*, 623-628.

1448   243. Zimmermann, M.; Patil, K.R.; Typas, A.; Maier, L. Towards a mechanistic understanding of reciprocal drug-
1449   microbiome interactions. *Mol. Syst. Biol.*, **2021**, *17*, e10116.

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Figures/Illustrations
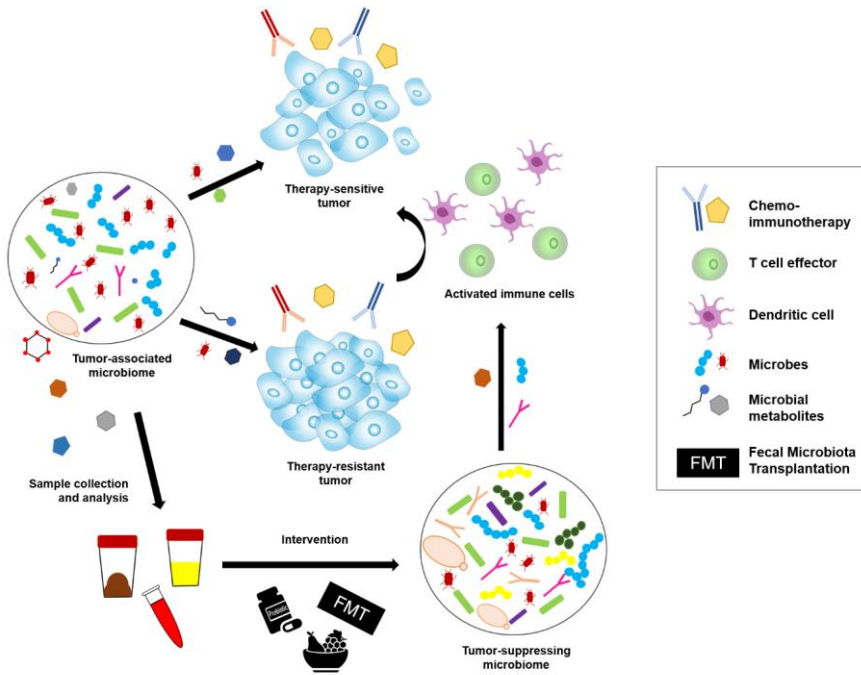
1454 **Figure 1. Characterization of the gut microbiome in cancer onset and response to anticancer therapies in order to**

1455 **develop novel personalized microbiome-based intervention strategies**. An unbalanced gut microbial ecosystem and

1456 its metabolites may be involved in the development of cancer, as well as in a poor response to anticancer therapy. The

1457 collection and analysis of biological samples (e.g., feces, blood and urine) through novel sequencing-based and

1458 metabolomic approaches, as well as bioinformatic tools, are needed to gain knowledge about the microbiome, cancer and

1459 anticancer therapies. In particular, machine learning approaches have great potential in enabling the development of

1460 personalized microbiome-based interventions (*i.e.,* prebiotics, probiotics and FMT), which, through the activation of the

1461 host immune system, could favor the response to therapy and tumor clearance.

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1463 **Tables:**

1464 **Table 1**. Clinical trials registered in the last two years on ClinicalTrials.gov (as accessed on July 2021) concerning the application of prebiotics, probiotics, and fecal microbiota
1465 transplantation (FMT) as adjuvant therapy in cancer patients. Search terms included "cancer", in combination with "prebiotics", "probiotics" or "FMT".

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	Title	Status	Results	Condition	Intervention	Location	URL
Prebiotics	Impact of Dietary Fiber as Prebiotics on Chemotherapy-related Diarrhea in Patients With Gastrointestinal Tumors	Recruiting	Not available	Chemotherapy-related Diarrhea	Dietary supplement with prebiotic fiber + loperamide hydrochloride vs. maltodextrin + loperamide hydrochloride	China	https://ClinicalTrials.gov/show/NCT04447443
	Papilocare®: Effects on Regression of Histologically Confirmed Cervical Intraepithelial Lesions 1 and Tolerance	Recruiting	Not available	Squamous Intraepithelial Lesions of the Cervix, Human Papilloma Virus Infection, Cervix Lesion	PAPILOCARE® device	France	https://ClinicalTrials.gov/show/NCT04624568
Probiotics	Study to Investigate Efficacy of a Novel Probiotic on the Bacteriome and Mycobiome of Breast Cancer	Not yet recruiting	Not available	Breast Cancer	Novel probiotic vs. placebo	United States	https://ClinicalTrials.gov/show/NCT04362826
	Effects of Probiotics on the Gut Microbiome and Immune System in Operable Stage I-III Breast or Lung Cancer	Not yet recruiting	Not available	Anatomic Stage I, IA, IB, II, IIA, IIB, III, IIIA, IIIB, IIIC Breast Cancer AJCC v8	Dietary supplement with probiotic	United States	https://ClinicalTrials.gov/show/NCT04857697
	Clinical Study of Neoadjuvant Chemotherapy and Immunotherapy Combined With Probiotics in Patients With Potential/Resectable NSCLC	Recruiting	Not available	Non-small Cell Lung Cancer Stage III	dietary supplementation with probiotics, nivolumab +paclitaxel (albumin-bound type) + carboplatin AUC5	China	https://ClinicalTrials.gov/show/NCT04699721
	The Thoracic Peri-Operative Integrative Surgical Care Evaluation Trial - Stage II	Not yet recruiting	Not available	Lung Cancer, Gastric Cancer, Esophageal Cancer	Dietary supplement with probiotic (Pro12)	Canada	https://ClinicalTrials.gov/show/NCT04871412
FMT	Fecal Microbiota Transplant and Re-introduction of Anti-PD-1 Therapy (Pembrolizumab or Nivolumab) for the Treatment of Metastatic Colorectal Cancer in Anti-PD-1 Non-responders	Not yet recruiting	Not available	Metastatic Colorectal Adenocarcinoma, Metastatic Small Intestinal Adenocarcinoma, Stage IV, IVA, IVB, IVC Colorectal Cancer AJCC v8, Stage IV Small Intestinal Adenocarcinoma AJCC v8	FMT + nivolumab, FMT + pembrolizumab	United States	https://ClinicalTrials.gov/show/NCT04729322

Microbiota Transplant in Advanced Lung Cancer Treated With Immunotherapy	Active, not recruiting	Not available	Lung Cancer	FMT + anti-PD1 therapy vs. anti-PD-1 therapy	Spain	https://ClinicalTrials.gov/show/NCT04924374
Washed Microbiota Transplantation for The Treatment of Oncotherapy-Related Intestinal Complications	Recruiting	Not available	Intestinal Complications, Cancer	Washed Microbiota Transplantation (WMT)	China	https://ClinicalTrials.gov/show/NCT04721041
A Phase Ib Trial to Evaluate the Safety and Efficacy of FMT and Nivolumab in Subjects With Metastatic Melanoma or NSCLC	Not yet recruiting	Available	Melanoma Stage IV, Unresectable Melanoma, NSCLC Stage IV	FMT	Israel	https://ClinicalTrials.gov/show/NCT04521075
Faecal Microbiota Transplantation After Allogeneic Stem Cell Transplantation	Not yet recruiting	Not available	Acute Leukemia in Remission, Myelodysplastic Syndromes, Myeloproliferative Syndrome, Hodgkin Lymphoma, Lymphoma, Non-Hodgkin, Myeloma, Chronic Lymphocytic Leukemia	FMT	France	https://ClinicalTrials.gov/show/NCT04935684
The IRMI-FMT Trial	Not yet recruiting	Not available	Malignant Melanoma Stage III, Malignant Melanoma Stage IV	Allogenic FMT vs. autologous FMT	Austria	https://ClinicalTrials.gov/show/NCT04577729
Fecal Microbiota Transplantation to Improve Efficacy of Immune Checkpoint Inhibitors in Renal Cell Carcinoma	Recruiting	Not available	Renal Cell Carcinoma	FMT vs. placebo	Italy	https://ClinicalTrials.gov/show/NCT04758507

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