



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

## ARCHIVIO ISTITUZIONALE DELLA RICERCA

### Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

D'Amico, F., Barone, M., Tavella, T., Rampelli, S., Brigidi, P., Turrone, S. (2022). Host microbiomes in tumor precision medicine: how far are we?. *CURRENT MEDICINAL CHEMISTRY*, 29(18), 3202-3230 [10.2174/0929867329666220105121754].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/860105> since: 2022-02-17

*Published:*

DOI: <http://doi.org/10.2174/0929867329666220105121754>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

**D'Amico F, Barone M, Tavella T, Rampelli S, Brigidi P, Turrone S. Host Microbiomes in Tumor Precision Medicine: How far are we? Curr Med Chem. 2022;29(18):3202-3230. doi: 10.2174/0929867329666220105121754. PMID: 34986765.**

The final published version is available online at:  
<https://doi.org/10.2174/0929867329666220105121754>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

*This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)*

***When citing, please refer to the published version.***

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

2 Federica D'Amico<sup>1,2</sup>, Monica Barone<sup>1,2</sup>, Teresa Tavella<sup>2</sup>, Simone Rampelli<sup>2</sup>, Patrizia Brigidi<sup>1</sup> and Silvia Turroni<sup>2,\*</sup>

3

4 <sup>1</sup> Microbiome Unit, Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy

5 <sup>2</sup> Unit of Microbiome Science and Biotechnology, Department of Pharmacy and Biotechnology, University of Bologna,

6 Bologna 40126, Italy

7 \* Corresponding author: Silvia Turroni, PhD, Senior Assistant Professor, Unit of Microbiome Science and Biotechnology,

8 Department of Pharmacy and Biotechnology, University of Bologna, Bologna 40126, Italy. e-mail:

9 silvia.turroni@unibo.it

10

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 e~~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.

24

25

26

27

28

29

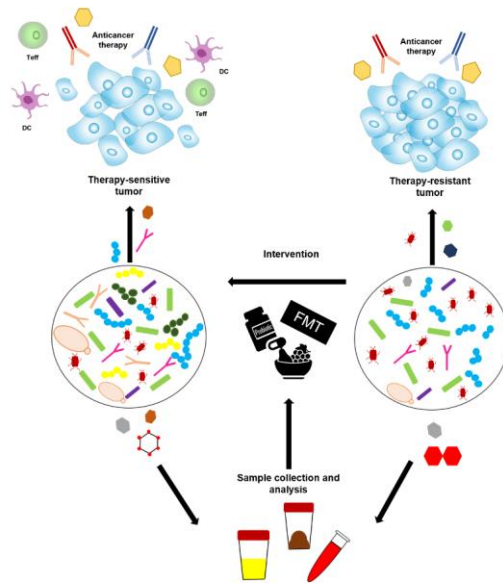
30

31

32

33

34 **Graphical Abstract**



35

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

38

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

41

42

43

44

45

46

47

48

49

50

51

## 52 1. Introduction

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

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

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

71

72

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

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

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

84 GM is a deeply complex ecosystem that includes not only bacteria, which are the most represented, but also archaeobacteria  
85 and fungi, along with viruses [7]. Until recently, a frequently repeated slogan was that the human body contained 10 times  
86 more microorganisms than human cells. However, this was a rough calculation made more than 40 years ago [8], while  
87 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  
88 microbes [9]. As just mentioned, the most examined fraction of GM is the bacterial one, 90% of which belongs to  
89 Firmicutes and Bacteroidetes phyla, while the remaining 10% is distributed among the subdominant Actinobacteria,  
90 Proteobacteria, Fusobacteria and Verrucomicrobia [2]. So far, it is estimated that the collective genome of GM, known  
91 as the microbiome, harbors 150 to 500 times more genes than the host, which are implicated in providing functional traits  
92 that complement the human repertoire and ~~that~~ are relevant to our metabolic, immunological and neurological homeostasis  
93 [10-12].

94 In the individual’s lifespan, the first microbial stimuli derive from the early moments of the infant’s life and are closely  
95 linked to the birth mode, the maternal microbiota, antibiotic exposure and early-life feeding practices [13,14]. At this  
96 time, when ~~the~~ infant-GM symbiosis is ~~going to being~~ established, GM is featured by low bacterial diversity and  
97 functional complexity, as well as a higher degree of interpersonal variation ~~compared to than~~ the adult-type GM profile  
98 [15,16]. Both structural components of microbes and products of their metabolism have been found to be involved in the  
99 development, maturation, and education of the child’s immune system [17-19], as well as in the regulation of the  
100 endocrine and central nervous systems [20]. It is therefore not surprising that GM disruption (*i.e.*, dysbiosis) ~~during in the~~  
101 ~~early-life window can may~~ be associated with several disorders [21,22], such as type 1 diabetes, atopic disease, asthma  
102 and childhood obesity [23-25]. At weaning, with the cessation of breastfeeding, there is a rapid increase in ~~the the~~  
103 structural and functional diversity of the infant GM, which progressively evolves towards ~~the a~~ mature adult-like state  
104 [26]. The development of the adult GM is regulated by a complex interplay between the host and several environmental  
105 factors, such as diet, lifestyle, or the so-called geographical effect [27,28] or, more generally, the exposome (*i.e.*, the  
106 totality of internal and external exposures that an individual faces throughout his life) [29]. ~~In-Under~~ healthy conditions,  
107 all ~~of~~ these factors contribute to shaping the microbial community, selecting a eubiotic GM configuration (*i.e.*, a stable,  
108 resistant, and resilient GM, with high diversity and functional redundancy) [30], which provides the functional traits  
109 necessary for host homeostasis, such as the barrier effect against infectious threats and the production of several bioactive  
110 small molecules that support ~~the~~ GM-host metabolic, immunological and neurological connections (e.g., vitamins, fatty  
111 acids, protein metabolites, bile acids, polyamines, etc.) [31-33].

112 As a matter of fact, GM is dominated by species (mainly from *Ruminococcaceae* and *Lachnospiraceae* families) capable  
113 of degrading complex carbohydrates, otherwise indigestible for humans, such as glycans and mucins (called Microbiota-  
114 Accessible Carbohydrates) [34]. The end products of [this](#) fermentation are short-chain fatty acids (SCFAs) [13], mainly  
115 acetate, propionate and butyrate, which are indisputably beneficial to health, acting as local (butyrate) and peripheral  
116 (acetate and propionate) energy sources, inflammation modulators, vasodilators and regulators of gut motility, wound  
117 healing, metabolism and epigenetics [35]. SCFAs also influence the proliferation and differentiation of colonic epithelial  
118 cells, including through the modulation of gene expression, and contribute to the protection against pathogens, promoting  
119 the integrity of the epithelial barrier, acidifying the intestinal milieu and stimulating the production of bacteriophages [36-  
120 39]. ~~At the systemic level~~[Systemically](#), SCFAs act as signaling molecules that drive the expansion and function of  
121 hematopoietic and non-hematopoietic cell lineages [35]. For example, SCFA-mediated inhibition of histone deacetylase  
122 promotes tolerogenic and anti-inflammatory functions that are crucial for the maintenance of immune homeostasis  
123 [19,33]. Many other immunoregulatory properties of SCFAs are associated with the activation of G protein-coupled  
124 receptors expressed by nearly all types of immune cells, including epithelial cells, neutrophils, monocytes and  
125 macrophages [40,41].

126 Another worthy example of GM-produced metabolites that act as key immunoregulators are polyamines (e.g., putrescine,  
127 spermine, and spermidine), which are usually produced by amino acid decarboxylases [42]. These molecules are essential  
128 for host cell function, barrier integrity and ~~pathogen~~ defense [against pathogens](#), as well as for local and systemic adaptive  
129 immunity [43-46]. Alterations in polyamine metabolism with higher levels of these compounds have been [shown to be](#)  
130 associated with cell growth bugs, and acute and chronic inflammation up to carcinogenesis [47]. Indeed, highly  
131 proliferative cells, such as tumor cells, require polyamines, among others, to support rapid growth. For example, increased  
132 circulating and urinary levels of polyamines have been observed in patients with colorectal cancer (CRC), as well as ~~with~~  
133 skin and hormone-related (*i.e.*, breast and prostate) ~~tumors~~ [cancers](#) [49-50]. As recently demonstrated, a “polyamine  
134 blocking therapy”, based on the reduction of intratumoral polyamine availability, could therefore have an antiproliferative  
135 effect but also reverse immunosuppression in the tumor microenvironment and heighten antitumor immune responses  
136 [51,52]. On the other hand, it should be mentioned that in autophagy-competent tumors, treatment with spermidine (as an  
137 autophagy-inducing caloric restriction mimetic) improved the efficacy of anticancer chemotherapy and enhanced  
138 immunosurveillance [53].

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

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 leads~~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<sub>H</sub>) (*i.e.*, T<sub>H</sub>1, T<sub>H</sub>2 or T<sub>H</sub>17) or T regulatory cells (T<sub>reg</sub>) [54]. For example, in preclinical  
149 models, *Bacteroides fragilis*-specific structural polysaccharide (PSA) has been shown to restore a T<sub>H</sub>1/T<sub>H</sub>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 ~~falling~~ 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].

164

### 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



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

175 To date, microbial pathogens are known to drive 20% of carcinogenesis [73,74]. Carcinogenesis is a multistep process,  
176 ~~whose the~~ progression ~~of which~~ is characterized by the gradual accumulation of slow and random genetic and epigenetic  
177 mutations that can take more than 10 years depending on ~~the~~ frequency [75]. In particular, as far as the GM field is  
178 concerned, the most frequent (and obvious) connection has been made with CRC. CRC is sporadic ~~for-in~~ approximately  
179 90% of cases and develops gradually from normal epithelium to adenomatous polyps until the settlement of an invasive  
180 carcinoma [76]. In addition to genetic predispositions that can increase the risk of developing CRC (e.g., adenomatous  
181 polyposis coli gene mutation), leading to the development of hundreds to thousands of polyps, several environmental  
182 factors have been shown to be involved in CRC onset, including diet, chronic inflammation (e.g., IBD) and GM [77]. As  
183 for the latter, several studies have ~~shown-highlighted~~ a CRC-associated GM profile enriched with opportunistic pathogens,  
184 such as *Fusobacterium*, several members of the *Enterococcaceae* family and *Campylobacter*, as well as other pro-  
185 inflammatory taxa, *i.e.*, *Erysipelotrichaceae* and *Collinsella* (recently proposed as a potential marker of metabolic  
186 disorders) [78-81]. In parallel, a reduction ~~of-in~~ health-associated microbial partners, including ~~the~~ butyrate producers  
187 *Faecalibacterium* and *Roseburia*, is frequently observed [82]. However, all of these studies, although milestones in the  
188 CRC-associated GM literature, are purely observational and ~~have-therefore~~ ~~have~~ not explored the mechanisms by which  
189 GM members can influence CRC or, more importantly, the triggers that shift the GM profile towards a tumor-associated  
190 one. Most of these questions were answered by coupling next-generation sequencing-based approaches with animal  
191 models, which helped to better outline the role of GM microbes in tumor onset. Research conducted in recent years has  
192 highlighted the fairness of the bacterial driver-passenger model developed in 2012 ~~from-by~~ Tjalsma et al. [83] Briefly,  
193 several environmental (e.g., diet, pathogen infection) and genetic (e.g., chronic inflammation, mutations) factors can push  
194 the GM homeostatic profile towards a dysbiotic, pro-inflammatory one that settles in the gastrointestinal tract. CRC can  
195 therefore be promoted by commensal bacteria with pro-carcinogenic features (known as bacterial drivers) that drive the  
196 DNA damage of the colon epithelium, leading to CRC development. Afterwards, the local microenvironment is altered  
197 as a result of ongoing inflammation and carcinogenesis, which paves the way for bacterial passengers, *i.e.*,  
198 microorganisms that show a competitive advantage in the tumor microenvironment and allow for cancer progression [83].  
199 Therefore, inflammation is a trigger for initiating the GM-dependent pro-inflammatory cycle, which is detrimental to the  
200 host health [69]. The bacterial drivers identified so far are mostly subdominant components of GM, capable of inducing  
201 a harmful inflammatory loop, synthesizing genotoxins and other toxic molecules that can directly damage host cells, and  
202 activating dietary heterocyclic amines to pro-carcinogens [69]. ~~Some-e~~Examples ~~are-include~~ superoxide-producing  
203 *Enterococcus faecalis* strains [84,85], toxigenic strains belonging to the *B. fragilis* species [86,87], and genotoxin-

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

210 It is clearly very simple to explain the relationship ~~of-between~~ GM ~~to-and~~ CRC, but the pro-carcinogenic role of  
211 commensal microbes extends far beyond the gastrointestinal tract [74,96]. Due to ~~its~~ physical proximity and close  
212 physiological links, the liver is one of the organs most affected by GM. The development of hepatocellular carcinoma  
213 (HCC) may be related to various GM-derived functions and metabolites, including LPS, ~~whose-the~~ presence ~~of-which~~  
214 potentiates HCC tumorigenesis through the activation of innate immune system effectors, such as TLR4 [97]. Moreover,  
215 some GM taxa play the role of oncogenic drivers by producing secondary bile acids (*i.e.*, deoxycholic acid, DCA),  
216 deriving from ~~the~~-GM-mediated deconjugation and metabolism of primary bile acids [98]. Once absorbed from the  
217 gastrointestinal tract, DCA can reach the liver through the portal circulation, where it can exert tumorigenic functions by  
218 inducing DNA damage and senescence on hepatocytes, with the establishment of a pro-inflammatory liver environment  
219 [99,100]. Consistently, in murine models fed high-fat diets, ~~it-has-been-observed-that-the~~ enrichment of GM species  
220 belonging to the *Clostridium* genus, including *C. scindens*, *C. hiranonis*, *C. hylemonae* and *C. sordelli*, capable of  
221 producing DCA [101], led to progression from non-alcoholic steatohepatitis to HCC [99]. The same tumor-driving actions  
222 by GM members have recently also been reported in esophageal tumors [100]. Finally, it is worth mentioning that GM  
223 dysbiosis and ~~the~~ consequent dysregulation of metabolite production have been shown to be involved in the development  
224 of breast cancer [102,103]. In particular, gut microbes are able to metabolize liver-derived estrogens through beta-  
225 glucuronidase and beta-glucosidase activities in the gastrointestinal tract (the so-called estrobolome, *i.e.*, “the aggregate  
226 of enteric bacterial genes whose products are capable of metabolizing estrogens”) [104]. This GM role in modulating the  
227 systemic ~~estrogen~~-pool ~~of-estrogens~~ could affect their enterohepatic circulation and reabsorption, thus contributing to an  
228 increased risk of hormonal cancers, such as breast cancer [105-107].

229

### 230 3.2 Gut microbiome mingles with anticancer therapies

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

235 for improving patients' clinical outcomes, as it can inform the development of novel, evidence-based intervention  
236 strategies, aimed at manipulating GM to enhance therapeutic efficacy, reduce toxicity and possibly, also the risk of relapse  
237 [111,112].

238 The first research on GM in anticancer therapies dates back to 1890, when two heat-inactivated microbes belonging to  
239 the *Streptococcus* genus were injected intratumorally as an attempt to cure cancer in humans. In those years, Dr. Coley  
240 thought that a local bacterial infection could boost the patient immune response against inoperable tumors. For more than  
241 40 years, more than 1000 patients were injected intratumorally with microbes or microbial products, with excellent results  
242 mostly in inoperable bone and soft-tissue sarcomas. From this moment on, albeit rudimentary, this approach was called  
243 Coley's Toxins and defined as the "father of immunotherapy" [113,114]. Several years later, the same approach was used  
244 to treat patients with bladder cancer, in whom *Mycobacterium bovis* was intratumorally injected right after tumor  
245 resection, resulting in reduced tumor recurrence through activation of local immune responses [115]. Furthermore, oral  
246 administration of the well-known probiotic species *Lactobacillus casei* has been associated with the decrease in recurrence  
247 of superficial bladder cancer [116]. Later on, all these studies confirmed the intuition of those researchers: the antitumor  
248 responses were stimulated by the microbial activation of two important effectors of the immune system: natural killer  
249 cells and macrophages [117]. Although these are rudimentary and "seasoned" studies, they have paved the way for many  
250 clinical trials, some still ongoing, using attenuated GM members to aid cancer treatments [118]. Recently, ~~it has been~~  
251 ~~observed that~~ *Mycobacterium obuense* [119,120] and genetically modified *Salmonella* Typhimurium [121,122] ~~have been~~  
252 ~~shown to~~ promote anticancer responses in several refractory solid tumors (e.g., pancreatic, melanoma), by activating the  
253 host immune system and exerting a cytotoxic effect on tumor cells. While very promising, many studies are still needed  
254 to refine microbial therapies before they can be used in clinical routine. Still today, the action and toxicity of microbes  
255 inside the tumor are very hazy and mainly correlated to the long microbial half-life with the possibility of antibiotic  
256 resistance accumulation, as well as the onset of mutations reverting the attenuated bacterial phenotype [123].

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

266 drug interaction is represented by irinotecan, a chemotherapy drug administered parenterally in an inactive form to  
267 patients with CRC, which is activated by host enzymes, detoxified in the liver and subsequently excreted in the intestinal  
268 lumen via ~~the~~ bile [130]. Here, GM members can reverse the detoxification process through bacterial beta-glucuronidases,  
269 which catalyze drug deconjugation and reactivation, resulting in intestinal toxicity [131]. In this regard, the use of specific  
270 enzyme inhibitors has been shown to prevent irinotecan-induced diarrhea while maintaining its efficacy in animal models  
271 [132]. As for the GM influence on anticancer activity, one of the first milestone studies in the field showed that the  
272 antitumor effect of oxaliplatin or cisplatin treatment on subcutaneous transplantable tumors was ~~dramatically-drastically~~  
273 reduced in germ-free or GM-depleted mice by broad-spectrum antibiotics [133]. The so-called platinum resistance of  
274 these models has recently been linked to the role of GM members in promoting oxidative stress and, subsequently,  
275 ~~apoptosis of tumor-cancer cell-apoptosis~~. Consistently, mice with lung tumors treated with antibiotic-coupled cisplatin  
276 therapy have been shown to have reduced long-term survival and developed even larger tumors [134]. On the other hand,  
277 when cisplatin was combined with ~~the~~ administration of probiotics, ~~like-such as~~ *Lactobacillus acidophilus*, the same  
278 animal models showed improved response to chemotherapy, through the activation of pro-apoptotic genes and effectors  
279 within the tumor aggregate and the promotion of a proper tumor-specific immune response. Similar to platinum-derived  
280 compounds, chemotherapy treatments based on the alkylating agent cyclophosphamide coupled with ~~the~~-oral  
281 administration of microbes (*i.e.*, *Lactobacillus johnsonii* and *Enterococcus hirae*) have been shown to promote the  
282 conversion of T cells from naïve to pro-inflammatory T<sub>H</sub>17, with the final outcome of improved therapeutic efficacy in  
283 tumor-bearing mice [135-137]. These findings were also confirmed in advanced lung or ovarian cancer studies, in which  
284 patients with GM enrichment of *E. hirae* and *Barnesiella intestinihominis* showed a more favorable prognosis after  
285 chemo-immunotherapy [138].

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

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

328 therapy were found out by Gopalakrishnan et al. [141]. Notably, responders to melanoma-targeted therapy were  
329 characterized by higher microbial diversity, as well as increased relative abundance of *Ruminococcaceae* and  
330 *Faecalibacterium*, both associated with improved effector T cell function in the peripheral and intratumoral environment.  
331 On the other hand, patients showing poor immunotherapeutic response possessed lower microbial diversity and higher  
332 relative abundance of Bacteroidales, which ~~was~~-correlated with reduced systemic and antitumor immune responses.  
333 Another GM metagenomic characterization in patients with melanoma treated with immune checkpoint inhibitors further  
334 corroborated the above findings (*i.e.*, that responders have a distinct GM profile from non-responders), although in this  
335 case the efficacy of anti-PD-L1 therapy was associated with *B. longum*, *E. faecium* and *Collinsella aerofaciens* [142].  
336 It should be noted that, despite the huge number of microbial species inhabiting the gastrointestinal tract, to date only a  
337 few of them have been suggested to play a role in anticancer responses and only a handful of strains have shown the  
338 potential to manipulate ~~the~~-host physiological functions *in vivo* [147,148]. For example, Tanoue and colleagues [149]  
339 isolated a consortium of 11 microbial strains (mainly belonging to *Bacteroides* spp.) from feces of healthy human donors,  
340 which are able to robustly induce T cell activation within the intestine. *In vivo* colonization with the 11-strain mixture  
341 enhanced the therapeutic efficacy of immune checkpoint inhibitors in syngeneic tumor models, confirming the great  
342 potential of these microbes as widely effective biotherapeutics in anticancer approaches. Furthermore, three strains  
343 belonging to *B. pseudolongum*, *L. johnsonii*, and *Olsenella* have recently been tested in tumor-bearing mice, where they  
344 significantly increased the efficacy of immune checkpoint inhibitors [150]. In particular, *B. pseudolongum* enhanced the  
345 immunotherapy response through the production of the metabolite inosine, which was able to systemically translocate  
346 due to immunotherapy-induced decrease in gut barrier function, and thus activate antitumor T cells. Several questions  
347 about the safety of administering live microbes to very often immunocompromised patients during anticancer therapy  
348 have been raised over the years and are still largely unanswered. In this context, the usage of prebiotics could be a valid  
349 alternative, as discussed below (see the paragraph “Modulation hypothesis: prebiotics, probiotics, live biotherapeutics  
350 and FMT, as adjuvant cancer therapy”). As an example, prebiotics (*i.e.*, mucin and inulin) have been shown ~~in syngeneic~~  
351 ~~mouse models~~ to induce antitumor immunity and concomitantly control tumor growth in syngeneic mouse models [151].

### 353 3.3 The gut microbiome and hematological malignancies: a focus on hematopoietic stem cell transplantation and related 354 complications

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

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-threatening 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 relapses 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**

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

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



420 150 human cells has been estimated [172]. According to tumor mapping by Nejman et al. [177], the percentage of tumors  
421 positive for bacterial DNA ranged ~~between-from~~ 14.3% in melanoma to >60% in breast, pancreatic and bone tumors.  
422 Based on these estimates, Sepich-Poore et al. [176] ~~came-to-the-conclusion-of~~concluded about 10<sup>5</sup> to 10<sup>6</sup> bacteria per  
423 palpable 1-cm<sup>3</sup> tumor, or about 34 bacteria per mm<sup>2</sup>. The source of these microorganisms/microbial components is not  
424 yet clear, but they could likely be part of other host-associated ecosystems, such as GM, translocate ~~through-across~~  
425 compromised mucosal barriers (e.g., leaky gut), and then reach tumor masses, facilitated by their disorganized and leaky  
426 vasculature. For example, this has been strongly suggested for CRC liver metastases, where >99.9% nucleotide identity  
427 was found for *Fusobacterium* isolates from the primary tumor and metastatic site, although tissue collection occurred  
428 months if not years later [81]. Once in the bloodstream, *F. nucleatum* has been hypothesized to translocate also ~~to-in~~  
429 breast cancers, colonization of which is made possible by the binding of its lectin Fap2 to galactose and N-acetyl-d-  
430 galactosamine residues, abundantly expressed on cancer cells [180]. Obviously, work ~~is-still~~ ~~needed-needs to be done~~ to  
431 confirm all of these findings, including the dead/alive issue. Similarly, ~~it-will have to be determined~~ whether these bacteria  
432 are actually involved in tumor pathogenesis or are mere opportunistic residents/passengers, which take advantage of a  
433 nutrient-rich and immunosuppressed environment, ~~should be determined~~. Regardless, they may play a critical role in  
434 promoting tumor growth and/or mediating chemoresistance, thus affecting patient response and survival. This has been  
435 seen for example for Gammaproteobacteria that can inactivate the chemotherapeutic drug gemcitabine, ~~by-through the~~  
436 expression of a long isoform of the bacterial enzyme cytidine deaminase [172], and for *F. nucleatum*, which activates the  
437 autophagy pathway (by targeting TLR4 and MYD88 innate immune signaling and specific microRNAs), thus preventing  
438 chemotherapy-induced apoptosis [181]. *F. nucleatum* may also accelerate tumor growth by inducing apoptosis in  
439 lymphocytes, as suggested by the reduced levels of CD4+ and CD8+ T cells in breast cancers [180]. Notably, the  
440 administration of antibiotics (*i.e.*, metronidazole) inhibited *F. nucleatum*-induced tumor enlargement, stressing once again  
441 how microbial manipulation can have significant repercussions on clinical outcomes. Finally, it is worth mentioning that  
442 greater intratumoral diversity has been correlated with long-term survival in patients with pancreatic adenocarcinoma  
443 [182]. Long-term survivors also showed potentially favorable intratumoral microbial signatures (*i.e.*, *Saccharopolyspora*,  
444 *Pseudoxanthomonas*, *Streptomyces* and *Bacillus clausii*), which could promote ~~the~~ recruitment and activation of CD8+ T  
445 cells, with overproduction of interferon (IFN)-gamma, thus contributing to the antitumor immune response and  
446 influencing the natural history of the disease. Although the data are still preliminary, using FMT from long-term survivors  
447 with no evidence of disease in tumor-bearing mice, the authors observed immune system activation and antitumor  
448 response, thus opening the way to immense microbiome-based therapeutic opportunities. What is certain is, therefore,  
449 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 vitro and in vivo studies suggest~~[s](#) 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~~[changes](#)[s](#) 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

481 opportunistic pathogens in GM [192]. The interventional clinical trial conducted by Garcia-Peris and colleagues ([193];  
482 NCT01549782) on 40 women undergoing radiotherapy for endometrial neoplasms confirmed the hypothesis that a  
483 mixture of fructooligosaccharides and inulin modulates the representation of *Lactobacillus* and *Bifidobacterium* within  
484 the GM community, while reducing tissue damage at the enterocyte level. As briefly mentioned above, in a mouse  
485 melanoma model, inulin and mucin also stimulated *Bifidobacterium* spp. and *A. muciniphila* [151], both previously  
486 identified as beneficial GM components, capable of eliciting effective antitumor immunity [140,146]. Regarding clinical  
487 trials in cancer patients still ongoing and started in the last two years (**Table 1**), one study foresees the enrolment of 120  
488 participants with gastrointestinal cancer and chemotherapy-related diarrhea (NCT04447443) and focuses on a 2-week  
489 supplementation with prebiotic fiber ~~supplement~~ along with loperamide hydrochloride ~~administration~~. Longitudinal  
490 monitoring of the GM configuration and subsequent comparison with the results obtained from the administration of  
491 placebo (*i.e.*, maltodextrin) and loperamide will allow researchers to dissect the effects specifically induced by prebiotics.  
492 A second study (NCT04624568) aims to compare the regression rate of cervical intraepithelial lesions in 150 women after  
493 6-month administration of a vaginal gel composed of hyaluronic acid and prebiotic extract of *Coriolus versicolor*, which  
494 improves the re-epithelialization of the uterine cervix [194]. Creating a protective biofilm on the cervix would help restore  
495 a niche environment conducive to regression of intraepithelial lesions and human papillomavirus clearance. Although the  
496 aforementioned studies are still in their infancy, they have the potential to provide valuable insights into how prebiotic  
497 administration modulates the microbiome of cancer patients, while influencing disease markers and clinical outcomes.

498

## 499 5.2 Probiotics and live biotherapeutics

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

512 1). Among them, a clinical trial in CRC patients showed that probiotic (*Bifidobacterium lactis* BI-04 and *L. acidophilus*  
513 NCFM) administration promoted the expansion of beneficial butyrate-producing microbes in both mucosa and feces, and  
514 tended to reduce *Fusobacterium* proportions (NCT03072641; Hibberd et al., [204]). Furthermore, preoperative probiotic  
515 (*S. boulardii*) therapy resulted in reduced levels of IL-1beta, IL-10, and IL-23 mRNA within the colonic mucosa of CRC  
516 patients following resection, when compared to controls who received the anticancer treatment alone (NCT01895530)  
517 [205]. As for other cancer types, improved relapse-free survival was observed after administering an oral preparation of  
518 *L. casei* for one year to patients with superficial bladder cancer, after completion of transurethral resection therapy  
519 followed by intravesical administration of epirubicin, although no difference in overall survival was observed compared  
520 to the control group [206]. Beneficial effects on GM are also expected in an ongoing clinical trial, which involves a short  
521 course of probiotic therapy (with the following 13 species: *S. boulardii*, *Lactobacillus plantarum*, *Bacillus subtilis*, *B.*  
522 *lactis*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *B. breve*, *L. casei*, *Lactobacillus salivarius*, *L. acidophilus*,  
523 *Lactobacillus brevis*, *B. longum* and *Lactobacillus paracasei*) in patients with operable breast cancer before surgery  
524 (NCT03358511). Interestingly, variations within the tumor microenvironment are also expected, with in particular an  
525 increase in the resident CD8+ T cell subpopulation. In another clinical trial, the researchers aim to evaluate the efficacy  
526 of the probiotics administered (no information is available on the strains or species used) prior to surgery in 40 patients  
527 with breast and lung cancer, while assessing systemic and intratumoral immunomodulatory effects (NCT04857697). The  
528 enrollment of 40 patients with potential/resectable non-small cell lung cancer is instead planned to evaluate the safety and  
529 effect of neoadjuvant chemotherapy and immunotherapy combined with probiotics (again, no compositional information  
530 is available) (NCT04699721). Within the Thoracic POISE project [207], the efficacy of a probiotic blend of *Lactobacillus*  
531 and *Bifidobacterium* spp. (i.e., Pro 12) will be assessed in reducing surgical adverse events, prolonging overall survival  
532 and pioneering integrative care delivery in 40 patients with esophageal cancer (NCT04871412).

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

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<sub>+</sub>-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<sub>+</sub> 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 account~~ considers  
562 the 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 a 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 microenvironment 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).

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

609

## 610 **6. Application of novel microbiome-based approaches in cancer medicine: machine learning as ~~the~~ key ~~for~~-to** 611 **patient stratification and outcome prediction**

### 612 *6.1 The promise of machine learning*

613 As discussed in the previous paragraphs, research in recent decades has highlighted the dramatic impact of GM on  
614 multiple aspects of human pathophysiology, including the development and progression of cancer. This was possible  
615 thanks to 'omics' techniques (*i.e.*, 16S rRNA gene sequencing, shotgun metagenomics and metatranscriptomics,  
616 metaproteomics and metabolomics), which led to a paradigm shift in the field of microbiology, moving from the study of  
617 single microbial colonies to a high-resolution taxonomic and functional profiling of microbial communities. However, it  
618 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  
619 development of truly effective preventive and intervention strategies. In parallel, enormous ~~progresses~~ ~~has~~-~~haves~~ been  
620 made in the field of analytical approaches to data, whose collection, organization and mining are fundamental steps for  
621 the analysis of complex interaction networks. Overall, technological advances in molecular biology and computer science  
622 are driving medical science towards big data. This large amount of data can potentially be explored via artificial  
623 intelligence methods, such as machine learning (ML) approaches that can handle large-scale datasets. ML is a data-driven  
624 approach capable of mining complex data, discovering informative patterns. In a nutshell, ML identifies algorithms  
625 capable of learning patterns from data in a self-manner, which enables the machine to solve a specific task, and deal with  
626 invisible data without explicit programming. In principle, the more heterogeneous data are used to train the model, the  
627 better the algorithm can generalize the problem when dealing with new data. Within ML, deep learning algorithms can  
628 better handle complex, multi-modal data. The premise of the emergence of ML techniques in various scientific fields,  
629 including healthcare, is the possibility of automating certain repetitive tasks, with the aim of achieving greater accuracy  
630 than that achievable by human experts, with also the possibility of estimating and predicting parameters, such as health  
631 risk factors. ML methods can adopt and combine different sources of health-related data, leveraging the tasks of  
632 diagnosing, prognosis, disease risk and potential treatments, with the aim of progressing towards a treatment tailored to  
633 the patient profile. ML algorithms can be supervised, *i.e.*, we know ~~a~~-*priori*, based on manual curation, how the samples  
634 are tagged. Supervised techniques are adopted to answer specific problems, by training the algorithm to recognize distinct  
635 features of the dataset. Halfway between supervised and unsupervised learning, semi-supervised learning can be applied

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

661

## 662 *6.2 Machine learning in clinical oncology practice*

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



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

676

### 677 6.3 Machine learning application in oncology with omics data

678 Nowadays, the boost of [microbiome](#) studies ~~in the microbiome field~~ and the availability of large datasets in public  
679 repositories are enabling the application of ML to metagenomics, which could lead to the identification of microbial  
680 species or other biomarkers, such as genes/enzymes and metabolites, for cancer diagnosis and prognosis [235].  
681 Microbiome data combined with patient genetic information, but also with other types of omics data (transcriptomics,  
682 proteomics, metabolomics), could therefore return a comprehensive picture of the biological complexity of the disease  
683 and play a leading role in defining a personalized medicine approach. For example, predictions over microbiome-drug  
684 interactions could be the key to guiding precision therapeutic solutions. Recently, metagenomic data from CRC patients  
685 and healthy subjects [have been were](#) used to train a random forest classifier [236]. The model identified six key microbial  
686 species, *i.e.*, *Porphyromonas asaccharolytica*, *Peptostreptococcus stomatis*, *Fusobacterium* spp., *Parvimonas* spp.,  
687 *Streptococcus vestibularis* and *Flavonifractor plautii*, which discriminated between controls and patients. Another work  
688 based on a random forest classifier and CRC<sub>7</sub> was trained on the metagenomic and metabolomics data from an Indian  
689 cohort of 30 patients and 30 healthy subjects. This work identified *F. plautii* as a cancer biomarker, and also found  
690 discriminating microbial genes for CRC. Interestingly, the authors hypothesized that flavonoid degradation by  
691 *Flavonifractor* is a key component for cancer progression [237]. More recently, Jang et al. [238] applied a Bayesian  
692 network model to find out species signatures in patients responding to chemotherapy treatment, while Kharrat et al. [239]  
693 adopted an ensemble method, including a Bayesian network model, to identify CRC-related microbial species. We would  
694 also like to mention the recently established Gut OncoMicrobiome Signatures project, which aims to identify microbial  
695 signatures of cancer progression and response to therapy [235]. These applications have defined or aim to define marker  
696 microbial species that could help better stratify patients, as well as guide GM remodeling via microbiome-based strategies,  
697 as outlined above. In this regard, ML could be used to screen large datasets in order to find potential new probiotics,

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 recent 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 unimaginable ~~ableed~~ ~~lapels~~ of  
725 the 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.

729

730 **Consent for Publication**

731 Not applicable.

732

733 **Funding**

734 This review did not require funding.

735

736 **Conflict of Interest**

737 The authors declare no conflict of interest, financial or otherwise.

738

739 **Acknowledgements**

740 We would like to thank Francesca D'Amico for the support in graphical abstract and figure preparation.

741 **References**

- 742 1. Gilbert, J.A.; Blaser, M.J.; Caporaso, J.G.; Jansson, J.K.; Lynch, S.V.; Knight, R. Current understanding of the  
743 human microbiome. *Nat. Med.*, **2018**, *24*, 392-400.
- 744 2. Turnbaugh, P.J.; Ley, R.E.; Hamady, M.; Fraser-Liggett, C.M.; Knight, R.; Gordon, J.I. The human microbiome  
745 project. *Nature*, **2007**, *449*, 804-810.
- 746 3. Lynch, S.V.; Pedersen, O. The human intestinal microbiome in health and disease. *N. Engl. J. Med.*, **2016**, *375*,  
747 2369-2379.
- 748 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  
749 in the era of cancer survivorship in Korea: Attention to the suicide and cardiovascular mortality. *Cancer Med.*,  
750 **2020**, *9*, 1741-1752.
- 751 5. Farré-Maduella, E.; Casals-Pascual, C. The origins of gut microbiome research in Europe: From Escherich to  
752 Nissle. *Hum. Microbiome J.*, **2019**, *14*, 100065.
- 753 6. Escherich, T. Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung, Stuttgart:  
754 Enke Verlag, **1886**.
- 755 7. Rajilić-Stojanović, M.; de Vos, V.M. The first 1000 cultured species of the human gastrointestinal microbiota.  
756 *FEMS Microbiol. Rev.*, **2014**, *38*, 996-1047.
- 757 8. Savage, D.C. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.*, **1977**, *31*, 107-133.
- 758 9. Sender, R.; Fuchs, S.; Milo, R. Revised estimates for the number of human and bacteria cells in the body. *PLoS*  
759 *Biol.*, **2016**, *14*, e1002533.
- 760 10. Neish, A.S. Microbes in gastrointestinal health and disease. *Gastroenterology*, **2009**, *136*, 65-80.
- 761 11. Gill, S.R.; Pop, M.; Deboy, R.T.; Eckburg, P.B.; Turnbaugh, P.J.; Samuel, B.S.; Gordon, J.I.; Relman, D.A.;  
762 Fraser-Liggett, C.M.; Nelson, K.E. Metagenomic analysis of the human distal gut microbiome. *Science*, **2006**,  
763 *312*, 1355-1359.
- 764 12. Candela, M.; Biagi, E.; Maccaferri, S.; Turrioni, S.; Brigidi P. Intestinal microbiota is a plastic factor responding  
765 to environmental changes. *Trends Microbiol.*, **2012**, *20*, 385-391.
- 766 13. Robertson, R.C.; Manges, A.R.; Finlay, B.B.; Prendergast, A.J. The human microbiome and child growth - first  
767 1000 days and beyond. *Trends Microbiol.*, **2019**, *27*, 131-147.
- 768 14. Derrien, M.; Alvarez, A.S.; de Vos, W.M. The gut microbiota in the first decade of life. *Trends Microbiol.*, 2019,  
769 *27*, 997-1010.
- 770 15. Bäckhed, F.; Roswall, J.; Peng, Y.; Feng, Q.; Jia, H.; Kovatcheva-Datchary, P.; Li, Y.; Xia, Y.; Xie, H.; Zhong,  
771 H.; Khan, M.T.; Zhang, J.; Li, J.; Xiao, L.; Al-Aama, J.; Zhang, D.; Lee, Y.S.; Kotowska, D.; Colding, C.;

- 772 Tremaroli, V.; Yin, Y.; Bergman, S.; Xu, X.; Madsen, L.; Kristiansen, K.; Dahlgren, J.; Wang, J. Dynamics and  
773 stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe*, **2015**, *17*, 690-703.
- 774 16. Avershina, E.; Lundgård, K.; Sekelja, M.; Dotterud, C.; Storrø, O.; Øien, T.; Johnsen, R.; Rudi, K. Transition  
775 from infant- to adult-like gut microbiota. *Environ. Microbiol.*, **2016**, *18*, 2226-2236.
- 776 17. Pannaraj, P.S.; Li, F.; Cerini, C.; Bender, J.M.; Yang, S.; Rollie, A.; Adisetiyo, H.; Zabih, S.; Lincez, P.J.;  
777 Bittinger, K.; Bailey, A.; Bushman, F.D.; Sleasman, J.W.; Aldrovandi, G.M. Association between breast milk  
778 bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatr.*, **2017**,  
779 *171*, 647-654.
- 780 18. Vangay, P.; Ward, T.; Gerber, J.S.; Knights, D. Antibiotics, pediatric dysbiosis, and disease. *Cell Host Microbe*,  
781 **2015**, *17*, 553-564.
- 782 19. Rooks, M.G.; Garrett, W.S. Gut microbiota, metabolites and host immunity. *Nat. Rev. Immunol.*, **2016**, *16*, 341-  
783 352.
- 784 20. Macpherson, A.J.; de Agüero, M.G.; Ganai-Vonarburg, S.C. How nutrition and the maternal microbiota shape  
785 the neonatal immune system. *Nat. Rev. Immunol.*, **2017**, *17*, 508-517.
- 786 21. Olszak, T.; An, D.; Zeissig, S.; Vera, M.P.; Richter, J.; Franke, A.; Glickman, J.N.; Siebert, R.; Baron, R.M.;  
787 Kasper, D.L.; Blumberg, R.S. Microbial exposure during early life has persistent effects on natural killer T cell  
788 function. *Science*, **2012**, *336*, 489-493.
- 789 22. Cox, L.M.; Yamanishi, S.; Sohn, J.; Alekseyenko, A.V.; Leung, J.M.; Cho, I.; Kim, S.G.; Li, H.; Gao, Z.;  
790 Mahana, D.; Zárate Rodríguez, J.G.; Rogers, A.B.; Robine, N.; Loke, P.; Blaser, M.J. Altering the intestinal  
791 microbiota during a critical developmental window has lasting metabolic consequences. *Cell*, **2014**, *158*, 705-  
792 721.
- 793 23. Vatanen, T.; Franzosa, E.A.; Schwager, R.; Tripathi, S.; Arthur, T.D.; Vehik, K.; Lernmark, Å.; Hagopian, W.A.;  
794 Rewers, M.J.; She, J.X.; Toppari, J.; Ziegler, A.G.; Akolkar, B.; Krischer, J.P.; Stewart, C.J.; Ajami, N.J.;  
795 Petrosino, J.F.; Gevers, D.; Lähdesmäki, H.; Vlamakis, H.; Huttenhower, C.; Xavier, R.J. The human gut  
796 microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature*, **2018**, *562*, 589-594.
- 797 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  
798 abundance of *Ruminococcus gnavus* associates with allergic diseases in infants. *Gastroenterology*, **2018**, *154*,  
799 154-167.
- 800 25. Maruvada, P.; Leone, V.; Kaplan, L.M.; Chang, E.B. The human microbiome and obesity: moving beyond  
801 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.; Zernakova, 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. Thaïss, 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.; Stepleski, 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.; Ehrentaut, 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.; Jacquilot,  
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.; Jshipura, 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.; Rodríguez 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-κB. *Infect. Immun.*, **2008**, *76*, 4498-4508.
- 941 81. Bullman, S.; Pedamallu, C.S.; Sicinska, E.; Clancy, T.E.; Zhang, X.; Cai, D.; Neubergh, 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.; Taberner, 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.; Khiabani,  
983 H.; Adeyemi, A.; Bataller, R.; Lefkowitz, J.H.; Bower, M.; Friedman, R.; Sartor, R.B.; Rabadan, R.; Schwabe,

- 984 R.F. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell.*, **2012**, 21, 504-  
985 516.
- 986 98. Tremaroli, V.; Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature*,  
987 **2012**, 489, 242-249.
- 988 99. Yoshimoto, S.; Loo, T.M.; Atarashi, K.; Kanda, H.; Sato, S.; Oyadomari, S.; Iwakura, Y.; Oshima, K.; Morita,  
989 H.; Hattori, M.; Honda, K.; Ishikawa, Y.; Hara, E.; Ohtani, N. Obesity-induced gut microbial metabolite  
990 promotes liver cancer through senescence secretome. *Nature*, **2013**, 499, 97-101.
- 991 100. Quante, M.; Bhagat, G.; Abrams, J.A.; Marache, F.; Good, P.; Lee, M.D.; Lee, Y.; Friedman, R.; Asfaha, S.;  
992 Dubeykovskaya, Z.; Mahmood, U.; Figueiredo, J.L.; Kitajewski, J.; Shawber, C.; Lightdale, C.J.; Rustgi, A.K.;  
993 Wang, T.C. Bile acid and inflammation activate gastric cardia stem cells in a mouse model of Barrett-like  
994 metaplasia. *Cancer Cell.*, **2012**, 21, 36-51.
- 995 101. Ridlon, J.M.; Kang, D.; Hylemon, P.B. Bile salt biotransformations by human intestinal Bacteria. *J. Lipid Res.*,  
996 **2006**, 47, 241-259.
- 997 102. Eslami-S, Z.; Majidzadeh-A, K.; Halvaei, S.; Babapirali, F.; Esmaili, R. Microbiome and breast cancer: New  
998 role for an ancient population. *Front. Oncol.*, **2020**, 10, 120.
- 999 103. Goedert, J.J.; Jones, G.; Hua, X.; Xu, X.; Yu, G.; Flores, R.; Falk, R.T.; Gail, M.H.; Shi, J.; Ravel, J.; Feigelson,  
1000 H.S. Investigation of the association between the fecal microbiota and breast cancer in postmenopausal women:  
1001 a population-based case-control pilot study. *J. Natl. Cancer Inst.*, **2015**, 107, djv147.
- 1002 104. Plottel, C.S.; Blaser, M.J. Microbiome and malignancy. *Cell Host Microbe*, **2011**, 10, 324-335.
- 1003 105. Gloux, K.; Berteau, O.; El Oumami, H.; Beguet, F.; Leclerc, M.; Dore, J. A metagenomic beta-glucuronidase  
1004 uncovers a core adaptive function of the human intestinal microbiome. *Proc. Natl. Acad. Sci. U.S.A.*, **2011**, 108,  
1005 4539-4546.
- 1006 106. Dabek, M.; McCrae, S.I.; Stevens, V.J.; Duncan, S.H.; Louis, P. Distribution of beta-glucosidase and beta-  
1007 glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria. *FEMS Microbiol. Ecol.*,  
1008 **2008**, 66, 487-495.
- 1009 107. McIntosh, F.M.; Maison, N.; Holtrop, G.; Young, P.; Stevens, V.J.; Ince, J.; Johnstone, A.M.; Loble, G.E.;  
1010 Flint, H.J.; Louis, P. Phylogenetic distribution of genes encoding beta-glucuronidase activity in human colonic  
1011 bacteria and the impact of diet on faecal glycosidase activities. *Environ. Microbiol.*, **2012**, 14, 1876-1887.
- 1012 108. Rizkallah, M.R.; Saad, R.; Aziz, R.K. The Human Microbiome Project, personalized medicine and the birth of  
1013 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. Hoessl, 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.; Dalglish, 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. Dalglish, A.G.; Stebbing, J.; Adamson, D.J.; Arif, S.S.; Bidoli, P.; Chang, D.; Cheeseman, S.; Diaz-Beveridge,  
1037 R.; Fernandez-Martos, C.; Glynn-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  $\beta$ -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. Jahrdsdörfer, 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.; Karpnits, 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.; Breteron, 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.; Gouborne, 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.; Turrioni, 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.; Turrioni, 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.; Koenigsnecht, M.; Hanash, A.; Toubai, T.; Oravec-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.; Turrone, 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.; Thaïss, 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.; Dalmaso, 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.; Kosciolk, 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.;

- 1259 Granot, Z.; Straussman, R.; Bachrach, G. Breast cancer colonization by *Fusobacterium nucleatum* accelerates  
1260 tumor growth and metastatic progression. *Nat. Commun.*, **2020**, *11*, 3259.
- 1261 181. Yu, T.; Guo, F.; Yu, Y.; Sun, T.; Ma, D.; Han, J.; Qian, Y.; Kryczek, I.; Sun, D.; Nagarsheth, N.; Chen, Y.;  
1262 Chen, H.; Hong, J.; Zou, W.; Fang, J.Y. *Fusobacterium nucleatum* promotes chemoresistance to colorectal  
1263 cancer by modulating autophagy. *Cell*, **2017**, *170*, 548-563.
- 1264 182. Riquelme, E.; Zhang, Y.; Zhang, L.; Montiel, M.; Zoltan, M.; Dong, W.; Quesada, P.; Sahin, I.; Chandra, V.;  
1265 San Lucas, A.; Scheet, P.; Xu, H.; Hanash, S.M.; Feng, L.; Burks, J.K.; Do, K.A.; Peterson, C.B.; Nejman, D.;  
1266 Tzeng, C.D.; Kim, M.P.; Sears, C.L.; Ajami, N.; Petrosino, J.; Wood, L.D.; Maitra, A.; Straussman, R.; Katz,  
1267 M.; White, J.R.; Jenq, R.; Wargo, J.; McAllister, F. Tumor Microbiome Diversity and Composition Influence  
1268 Pancreatic Cancer Outcomes. *Cell*, **2019**, *178*, 795-806.
- 1269 183. Falzone, L.; Salomone, S.; Libra, M. Evolution of cancer pharmacological treatments at the turn of the third  
1270 millennium. *Front. Pharmacol.*, **2018**, *9*, 1300.
- 1271 184. Helmkink, B.A.; Khan, M.A.W.; Hermann, A.; Gopalakrishnan, V.; Wargo, J.A. The microbiome, cancer, and  
1272 cancer therapy. *Nat. Med.*, **2019**, *25*, 377-388.
- 1273 185. Parida, S.; Sharma, D. The microbiome and cancer: Creating friendly neighborhoods and removing the foes  
1274 within. *Cancer Res.*, **2021**, *81*, 790-800.
- 1275 186. Kaźmierczak-Siedlecka, K.; Daca, A.; Fic, M.; van de Wetering, T.; Folwarski, M.; Makarewicz, W.  
1276 Therapeutic methods of gut microbiota modification in colorectal cancer management - fecal microbiota  
1277 transplantation, prebiotics, probiotics, and synbiotics. *Gut Microbes*, **2020**, *11*, 1518-1530.
- 1278 187. Gibson, G.R.; Hutkins, R.; Sanders, M.E.; Prescott, S.L.; Reimer, R.A.; Salminen, S.J.; Scott, K.; Stanton, C.;  
1279 Swanson, K.S.; Cani, P.D.; Verbeke, K.; Reid, G. Expert consensus document: The International Scientific  
1280 Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics.  
1281 *Nat. Rev. Gastroenterol. Hepatol.*, **2017**, *14*, 491-502.
- 1282 188. Vandeputte, D.; Falony, G.; Vieira-Silva, S.; Wang, J.; Sailer, M.; Theis, S.; Verbeke, K.; Raes, J. Prebiotic  
1283 inulin-type fructans induce specific changes in the human gut microbiota. *Gut*, **2017**, *66*, 1968-1974.
- 1284 189. Cunningham, M.; Azcarate-Peril, M.A.; Barnard, A.; Benoit, V.; Grimaldi, R.; Guyonnet, D.; Holscher, H.D.;  
1285 Hunter, K.; Manurung, S.; Obis, D.; Petrova, M.I.; Steinert, R.E.; Swanson, K.S.; van Sinderen, D.; Vulevic, J.;  
1286 Gibson, G.R. Shaping the future of probiotics and prebiotics. *Trends Microbiol.*, **2021**, S0966-842X(21)00005-  
1287 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.; Cambor, 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. Crisuolo, A.A.; Sesti, F.; Piccione, E.; Mancino, P.; Belloni, E.; Gullo, C.; Ciotti, M. Therapeutic efficacy of  
1302 a *Coriolor 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.

- 1318 202. Singh, B.; Mal, G.; Marotta, F. Designer probiotics: Paving the way to living therapeutics. *Trends Biotechnol.*,  
1319 **2017**, *35*, 679-682.
- 1320 203. Bedada, T.L.; Feto, T.K.; Awoke, K.S.; Garede, A.D.; Yifat, F.T.; Birri, D.J. Probiotics for cancer alternative  
1321 prevention and treatment. *Biomed. Pharmacother.*, **2020**, *129*, 110409.
- 1322 204. Hibberd, A.A.; Lyra, A.; Ouwehand, A.C.; Rolny, P.; Lindegren, H.; Cedgård, L.; Wettergren, Y. Intestinal  
1323 microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open*  
1324 *Gastroenterol.*, **2017**, *4*, e000145.
- 1325 205. Consoli, M.L.; da Silva, R.S.; Nicoli, J.R.; Bruña-Romero, O.; da Silva, R.G.; de Vasconcelos Generoso, S.;  
1326 Correia, M.I. Randomized clinical trial: impact of oral administration of *Saccharomyces boulardii* on gene  
1327 expression of intestinal cytokines in patients undergoing colon resection. *JPEN J Parenter. Enteral Nutr.*, **2016**,  
1328 *40*, 1114-1121.
- 1329 206. Naito, S.; Koga, H.; Yamaguchi, A.; Fujimoto, N.; Hasui, Y.; Kuramoto, H.; Iguchi, A.; Kinukawa, N.; Kyushu  
1330 University Urological Oncology Group. Prevention of recurrence with epirubicin and lactobacillus casei after  
1331 transurethral resection of bladder cancer. *J. Urol.*, **2008**, *179*, 485-490.
- 1332 207. Seely, D.; Ennis, J.E.; McDonnell, E.; Fazekas, A.; Zhao, L.; Asmis, T.; Auer, R.C.; Fergusson, D.; Kanji, S.;  
1333 Maziak, D.E.; Ramsay, T.; Chamberland, P.; Spooner, C.; Threader, J.; Seely, A. Intervention development  
1334 process for a pragmatic randomized controlled trial: The thoracic peri-operative integrative surgical care  
1335 evaluation trial. *J. Altern. Complement. Med.*, **2019**, *25*, S112-S123.
- 1336 208. U.S. Department of Health and Human Services, Food and Drug Administration Guidance for Industry. Early  
1337 clinical trials with live biotherapeutic products: Chemistry, manufacturing, and control information.  
1338 [www.fda.gov/downloads/Biologics%20and%20Biologics/UCM292704.pdf](http://www.fda.gov/downloads/Biologics%20and%20Biologics/UCM292704.pdf) , **2016**.
- 1339 209. Charbonneau, M.R.; Isabella, V.M.; Li, N.; Kurtz, C.B. Developing a new class of engineered live bacterial  
1340 therapeutics to treat human diseases. *Nat. Commun.*, **2020**, *11*, 1738.
- 1341 210. O'Toole, P.W.; Marchesi, J.R.; Hill, C. Next-generation probiotics: the spectrum from probiotics to live  
1342 biotherapeutics. *Nat. Microbiol.*, **2017**, *2*, 17057.
- 1343 211. Ulsemer, P.; Henderson, G.; Toutounian, K.; Löffler, A.; Schmidt, J.; Karsten, U.; Blaut, M.; Goletz, S. Specific  
1344 humoral immune response to the Thomsen-Friedenreich tumor antigen (CD176) in mice after vaccination with  
1345 the commensal bacterium *Bacteroides ovatus* D-6. *Cancer Immunol. Immunother.*, **2013**, *62*, 875-887.
- 1346 212. Ulsemer, P.; Toutounian, K.; Kressel, G.; Goletz, C.; Schmidt, J.; Karsten, U.; Hahn, A.; Goletz, S. Impact of  
1347 oral consumption of heat-treated *Bacteroides xylanisolvens* DSM 23964 on the level of natural TFA-specific  
1348 antibodies in human adults. *Benef. Microbes.*, **2016**, *7*, 485-500.

Codice campo modificato

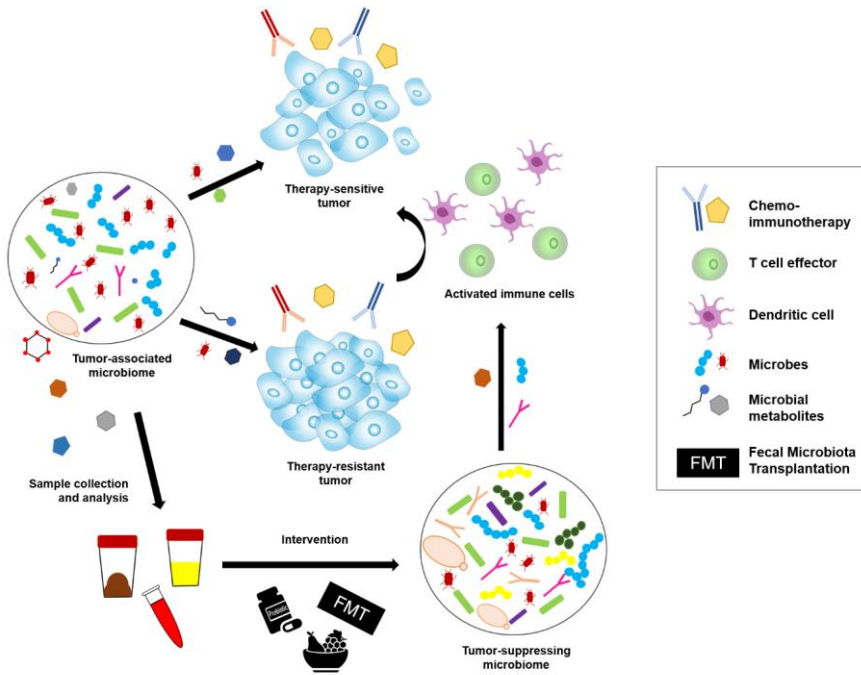
- 1349 213. Ho, C.L.; Tan, H.Q.; Chua, K.J.; Kang, A.; Lim, K.H.; Ling, K.L.; Yew, W.S.; Lee, Y.S.; Thiery, J.P.; Chang,  
1350 M.W. Engineered commensal microbes for diet-mediated colorectal-cancer chemoprevention. *Nat. Biomed.*  
1351 *Eng.*, **2018**, *2*, 27-37.
- 1352 214. Arthur, J.C.; Gharaibeh, 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.; Aloï, M.; Masucci, L.; Molinaro, A.; Scaldaferrì, 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 Aghdai, 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.; Mantani, 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.

- 1410 231. Kerlikowske, K.; Scott, C.G.; Mahmoudzadeh, A.P.; Ma, L.; Winham, S.; Jensen, M.R.; Wu, F.F.; Malkov, S.;  
1411 Pankratz, V.S.; Cummings, S.R.; Shepherd, J.A.; Brandt, K.R.; Miglioretti, D.L.; Vachon, C.M. Automated and  
1412 clinical breast imaging reporting and data system density measures predict risk for screen-detected and interval  
1413 cancers: A case-control study. *Ann. Intern. Med.*, **2018**, *168*, 757-765.
- 1414 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.;  
1415 Park, C.M. Development and validation of deep learning-based automatic detection algorithm for malignant  
1416 pulmonary nodules on chest radiographs. *Radiology*, **2019**, *290*, 218-228.
- 1417 233. Pantuck, A.J.; Lee, D.K.; Kee, T.; Wang, P.; Lakhota, S.; Silverman, M.H.; Mathis, C.; Drakaki, A.; Beldegrun,  
1418 A.S.; Ho, C.M.; Ho, D. Modulating BET bromodomain inhibitor ZEN-3694 and enzalutamide combination  
1419 dosing in a metastatic prostate cancer patient using CURATE.AI, an artificial intelligence platform. *Adv.*  
1420 *Therap.*, **2018**, *1*, 1800104.
- 1421 234. Ngiam, K.Y.; Khor, I.W. Big data and machine learning algorithms for health-care delivery. *Lancet Oncol.*,  
1422 **2019**, *20*, e262-e273.
- 1423 235. Marcos-Zambrano, L.J.; Karadzovic-Hadziabdic, K.; Loncar Turukalo, T.; Przymus, P.; Trajkovic, V.;  
1424 Aasmets, O.; Berland, M.; Gruca, A.; Hasic, J.; Hron, K.; Klammsteiner, T.; Kolev, M.; Lahti, L.; Lopes, M.B.,  
1425 Moreno, V.; Naskinova, I.; Org, E.; Paciência, I.; Papoutsoglou, G.; Shigdel, R.; Stres, B.; Vilne, B.; Yousef,  
1426 M.; Zdravevski, E.; Tsamardinos, I.; Carrillo de Santa Pau, E.; Claesson, M.J.; Moreno-Indias, I.; Truu, J.  
1427 Applications of machine learning in human microbiome studies: A review on feature selection, biomarker  
1428 identification, disease prediction and treatment. *Front. Microbiol.*, **2021**, *12*, 634511.
- 1429 236. Ai, D.; Pan, H.; Han, R.; Li, X.; Liu, G.; Xia, L.C. Using decision tree aggregation with random forest model to  
1430 identify gut microbes associated with colorectal cancer. *Genes (Basel)*. 2019, *10*, 112.
- 1431 237. Gupta, A.; Dhakan, D.B.; Maji, A.; Saxena, R.; P K, V.P.; Mahajan, S.; Pulikkan, J.; Kurian, J.; Gomez, A.M.;  
1432 Scaria, J.; Amato, K.R.; Sharma, A.K.; Sharma, V.K. Association of *Flavonifractor plautii*, a Flavonoid-  
1433 Degrading Bacterium, with the gut microbiome of colorectal cancer patients in India. *mSystems*, **2019**, *4*, e00438-  
1434 19.
- 1435 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,  
1436 S.Y.; Kim, H.J. Gut microbiome composition is associated with a pathologic response after preoperative  
1437 chemoradiation in patients with rectal cancer. *Int. J. Radiat. Oncol. Biol. Phys.*, **2020**, *107*, 736-746.
- 1438 239. Kharrat, N.; Assidi, M.; Abu-Elmagd, M.; Pushparaj, P.N.; Alkhalidy, A.; Arfaoui, L.; Naseer, M.I.; El Omri,  
1439 A.; Messaoudi, S.; Buhmeida, A.; Rebai, A. Data mining analysis of human gut microbiota links *Fusobacterium*  
1440 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.
- 1450
- 1451



1453  
 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.

1462

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”.

1466

	Title	Status	Results	Condition	Intervention	Location	URL
<b>Prebiotics</b>	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	<a href="https://ClinicalTrials.gov/show/NCT04447443">https://ClinicalTrials.gov/show/NCT04447443</a>
	Papilocare®: Effects on Regression of Histologically Confirmed Cervical Intraepithelial Lesions I and Tolerance	Recruiting	Not available	Squamous Intraepithelial Lesions of the Cervix, Human Papilloma Virus Infection, Cervix Lesion	PAPILOCARE® device	France	<a href="https://ClinicalTrials.gov/show/NCT04624568">https://ClinicalTrials.gov/show/NCT04624568</a>
<b>Probiotics</b>	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	<a href="https://ClinicalTrials.gov/show/NCT04362826">https://ClinicalTrials.gov/show/NCT04362826</a>
	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	<a href="https://ClinicalTrials.gov/show/NCT04857697">https://ClinicalTrials.gov/show/NCT04857697</a>
	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	<a href="https://ClinicalTrials.gov/show/NCT04699721">https://ClinicalTrials.gov/show/NCT04699721</a>
	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	<a href="https://ClinicalTrials.gov/show/NCT04871412">https://ClinicalTrials.gov/show/NCT04871412</a>
<b>FMT</b>	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	<a href="https://ClinicalTrials.gov/show/NCT04729322">https://ClinicalTrials.gov/show/NCT04729322</a>

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	<a href="https://ClinicalTrials.gov/show/NCT04924374">https://ClinicalTrials.gov/show/NCT04924374</a>
Washed Microbiota Transplantation for The Treatment of Oncotherapy-Related Intestinal Complications	Recruiting	Not available	Intestinal Complications, Cancer	Washed Microbiota Transplantation (WMT)	China	<a href="https://ClinicalTrials.gov/show/NCT04721041">https://ClinicalTrials.gov/show/NCT04721041</a>
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	<a href="https://ClinicalTrials.gov/show/NCT04521075">https://ClinicalTrials.gov/show/NCT04521075</a>
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	<a href="https://ClinicalTrials.gov/show/NCT04935684">https://ClinicalTrials.gov/show/NCT04935684</a>
The IRMI-FMT Trial	Not yet recruiting	Not available	Malignant Melanoma Stage III, Malignant Melanoma Stage IV	Allogenic FMT vs. autologous FMT	Austria	<a href="https://ClinicalTrials.gov/show/NCT04577729">https://ClinicalTrials.gov/show/NCT04577729</a>
Fecal Microbiota Transplantation to Improve Efficacy of Immune Checkpoint Inhibitors in Renal Cell Carcinoma	Recruiting	Not available	Renal Cell Carcinoma	FMT vs. placebo	Italy	<a href="https://ClinicalTrials.gov/show/NCT04758507">https://ClinicalTrials.gov/show/NCT04758507</a>