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Advances in research on the relationship between the gut microbiome and cancer

P. SONG[1], Q.-B. WANG[2], B. LIANG[1], S.-J. JIANG[1]

[1]Department of Respiratory Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China
[2]Department of Respiratory Medicine, Jinan Shizhong District People's Hospital, Jinan, China

Abstract. – OBJECTIVE: The objective of this review is to provide currently available information on the relationship between the gut microbiome and cancer.

MATERIALS AND METHODS: In this mini-review, we explored the PubMed, EMBASE, and Google Scholar electronic databases, with regards to the searching terms "gut microbiome, cancer, intestinal flora, immunotherapy, immune checkpoint inhibitor". By reviewing and analyzing the literature, we analyzed how the bacterial microbiome influences the immune system and cancer, as well as how changes in symbiotic flora may be applied to improve the efficacy of cancer immunotherapy.

RESULTS: The microbiota is related to the development of tumors and may promote cancer-ation. In recent years, a number of studies have confirmed the influence of intestinal flora on immune checkpoint inhibitors in cancer patients, and studies have also shown the link between the intestinal microbiome and treatment-related immune toxicity. Antibiotics, proton pump inhibitors, and hormones affect the composition of the gut microbiota.

CONCLUSIONS: Intestinal flora is closely related to cancer. Intestinal flora has a certain impact on cancer occurrence, cancer treatment, cancer immunotherapy efficacy, and side effects.

Key Words:
Gut microbiome, Cancer, Immunotherapy.

Introduction

The mammalian microbiome represents all microorganisms associated with the host. This complex and diverse ecosystem is located at the entrance of all epithelial barriers. It includes bacteria, archaea, virosomes (bacteriophages and eukaryotic viruses), fungi, and fauna (single-celled protozoa and worms)[1], which are obtained through vertical transmission after birth, and it is affected by environmental exposures over the course of the host's lifetime. These microorganisms have coevolved with humans to have a variety of functions that benefit human health, including obtaining unavailable nutrients from certain food items, maintaining the integrity of mucosal barriers, and contributing to the development and stability of the immune system. In the past decade, with the development of high-throughput sequencing methods, our understanding of the microbiome has increased[2,3]. The human gut microbiome contains $1\text{-}3\times10^{13}$ bacteria, most of which are beneficial[4]. From birth onwards, the intestinal flora plays a vital role in innate and acquired immune responses, and it regulates the delicate balance between inflammation, infection, and tolerance to food and food antigens[5,6]. In addition to its effects on intestinal and local immune physiology, the intestinal microbiome has a systemic role in the organism[6]. The microbiome is closely related to human health and disease. Destruction of the gut microbiome (dysbacteriosis) is related to many human diseases, including digestive, nervous, and endocrine system diseases[3]. Furthermore, specific bacterial and viral infections are related to carcinogenesis[7-12], as well as to the efficacy and toxicity of cancer treatment. In recent years, monoclonal antibodies that block the binding of the inhibitory receptor PD-1 to its main ligand PD-L1 have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of more than 10 tumor types. However, primary drug resistance has been observed in 60-70% of cases[13-15]. This resistance may be attributed to the low mutational load and poor inherent

Corresponding Authors: Bin Liang, MD; e-mail: 471613851@qq.com
Shujuan Jiang, MD; e-mail: sfmdsongpeng@sina.com

antigenicity of tumor cells[16,17], loss of potential immunogens due to chemotherapy and radiotherapy[18], defective antigen presentation during the initiation of immune response[19], local immunosuppression of extracellular metabolites, and failure of tumor-infiltrating lymphocytes[20]. The composition of the gut microbiome has profound effects on the immune system and cancer. This review discusses how the bacterial microbiome influences the immune system and cancer, as well as how changes in symbiotic flora may be applied to improve the efficacy of cancer immunotherapy.

The Role of the Gut Microbiota in Cancer Development

The microbiota has long been known to be related to tumor development. Bacterial and viral infections can affect multiple cellular processes, such as metabolism and immune function, and they may also promote cancer. Some bacteria may contribute to the development of malignant tumors of the gastrointestinal system, including gastric cancer (*Helicobacter pylori*)[7] and colorectal cancer (*Fusobacterium nucleatum*)[8]. *H. pylori* has been shown to affect gastric cancer progression through cytotoxins. These toxins can disrupt autophagy and apoptosis pathways and regulate key carcinogenic signaling pathways. In addition to the direct impact of specific microbiota on local tissues, the extensive symbiotic intestinal bacterial community may regulate cancer progression through competitive rejection and other mechanisms. Bacteria involved in the biosynthesis and metabolism of short-chain fatty acids actively participate in maintaining a stable and healthy intestinal community. Research has shown that colorectal cancer patients have low levels of beneficial bacteria that produce short-chain fatty acids. Results from mouse model experiments have shown that dietary fiber prevents colorectal tumors in a microorganism- and butyrate-dependent manner[21,22].

Bacteria are likely to be found in the tumors of patients with pancreatic cancer, and these bacteria are associated with treatment resistance[9]. Some bacteria can enzymatically break down chemotherapeutic agents into inactive metabolites, thereby leading to treatment resistance[23]. In preclinical models, bacteria have also been shown to induce myeloid suppressor cells in the tumor microenvironment and weaken the efficacy of immune checkpoint inhibitors[24,25]. In a colon cancer mouse model, bacteria were found to metabolize gemcitabine into its inactive form, 2'2'-difluorodeoxyuridine[26,27]. This result relies on bacteria having a long-chain form of cytosine deaminase, and any bacteria belonging to the class *Gammaproteobacteria* have this enzyme. *Gammaproteobacteria* is the most diverse class of bacteria; it includes the *Enterobacteriaceae*, *Vibrionaceae*, and *Pseudomonadaceae* families. *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, *Vibrio cholerae*, etc., belong to this class. Because gemcitabine is also a chemotherapeutic agent for lung cancer, gastric cancer, and other tumors, the authors of the aforementioned study suggested that these tumors should be tested to see whether there are bacteria inside and, if so, whether the bacteria are involved in inactivating chemotherapeutic drugs. The clinical use of corresponding antibiotics may improve chemotherapy efficacy. Regarding the source of bacteria in pancreatic tumors, researchers believe that bacteria in the intestine enter the pancreas through the pancreatic duct, and that immune cells in the tumor microenvironment are in a suppressed state[28]. Therefore, once bacteria enter the tumor, it becomes a safe hiding place for them.

The Influence of the Intestinal Microflora on the Efficacy of Immune Checkpoint Inhibitors

Three articles in 2018 confirmed that the intestinal flora affects the efficacy of immune checkpoint inhibitors in cancer patients. Routy et al[29] studied patients with advanced lung cancer, kidney cancer, and urothelial cancer who received anti-PD-1 treatment in Europe and the United States, and they found that patients who received antibiotic treatment before or shortly after anti-PD-1 treatment relapsed earlier. In their study, the patients who received antibiotics had a 50% lower overall survival rate than patients who did not. Also, in that study, the intestinal flora compositions of 100 lung cancer and kidney cancer patients were analyzed by metagenomic sequencing; there were significantly greater *Akkermansia muciniphila* expression levels in the stool samples of lung cancer patients who responded to anti-PD-1 therapy than in the samples of those who did not respond. Further, the frequencies of *Staphylococcus haemolyticus* and *Corynebacterium cerumenum* were higher in the therapy non-responder (NR) patients than in the therapy responder (R) patients, while the frequency of

Enterococcus hirae bacteria was greater in the R patients than in the NR patients. These studies confirmed that memory Th1 and Tc1 responses to *E. hirae* and *Aspergillus mucilus* were associated with good clinical outcomes. In addition, transferring the fecal flora of R patients to sterile or antibiotic-treated mice replicated the donors' ability to respond to immune checkpoint blockade. After administering *A. mucilus* alone or in combination with *E. coli*, mice recolonized with the fecal flora of NR patients developed responsiveness to immune checkpoint blockade[30].

In association with the MD Anderson Cancer Center, Gopalakrishnan et al[31] used 16S rRNA gene sequencing to explore the roles of the oral and gut microbiomes in melanoma patients treated with PD-1. The oral microbiome showed no difference between R and NR patients. However, the stool sample analysis results of 43 patients showed that the alpha diversity in R patients was significantly higher than that in NR patients. NR patients were rich in *Clostridium* species, while R patients were rich in *Ruminococcaceae* and *Faecalibacterium* species. Shotgun metagenomics results confirmed the enrichment of *Feacalibacterium* species in R patients (25 samples from the same cohort). Patients with higher *Faecalibacterium* abundance showed longer progression-free survival (PFS), while the relative abundance of *Bacteroides* species was associated with an increased risk of recurrence. Patients with good intestinal flora compositions at baseline had greater cytotoxic CD8+ T cell infiltration in the tumor bed. After R patient fecal microbiota was transferred to sterile mice, transplanted syngeneic melanoma grew slowly and was infiltrated by a large number of CD8+ T cells.

Matson et al[32] studied pretreatment fecal samples from 38 patients with metastatic melanoma treated with anti-PD-1. The analysis was based on 16S rRNA sequencing data, shotgun metagenomics results, and quantitative polymerase chain reaction results. The researchers identified a variety of bacteria that responded well to immunotherapy (based on RECIST 1.1). In particular, *Bifidobacterium longum* (validating the group's previous murine data[33]), *Enterobacter aerogenes,* and *Enterococcus faecalis* were associated with a better prognosis. The transfer of patients' fecal microbiota to germ-free mice showed that despite the heterogeneity of human bacterial symbiont colonization, tumor control and anti-PD-L1 responses were still observed in the mice that received R patient fecal microbiota.

Jin et al[34] performed shotgun metagenomic sequencing on 25 Chinese patients. Unclassified *Ruminococcus* species were enriched in NR patients, while *Alistipes putredinis*, *Prevotella* species, *Bifidobacterium vulgaris*, *Lachnobacterium* species, *Lachnospiraceae* species, and *Shigella* species were enriched in R patients, and results of dynamic analysis of the intestinal flora showed stable microbial composition. Song et al[35] divided patients into two groups: PFS≥6 months (35 patients) and PFS<6 months (28 patients). Compared with patients in the PFS<6 months group, patients in the PFS≥6 months group had greater gut microbiome β diversity at baseline, and there was a significant statistical difference. There were also differences in composition between the two groups. *Parabacteroides* and *Methanobrevibacter* species were richer in the PFS≥6 months group than in the PFS<6 months group. Bacterial metabolites suggested that potential methane production in the PFS<6 months group was higher than that in the PFS≥6 months group ($p<0.05$) (Table I).

In summary, these studies confirm that the intestinal flora can regulate responses to PD-1/PD-L1 inhibitors. A healthy, highly diverse microbiota and the presence of certain bacterial species are conducive to establishing an anti-tumor immune response at baseline, and anti-PD-1 treatment can enhance this immune response. Changing the balance of the microbiota through antibiotic treatment at the beginning of immune checkpoint inhibitor therapy will reduce its efficacy. These studies identified different bacterial signals (*Akkermansia*, *Faecalibacterium*, *Bifidobacterium*, *Parabacteroides*, and *Methanobrevibacter*) related to PD-1 inhibitor responses. These differences may be related to many confounding factors, such as sampling methods, sample storage methods, DNA extraction techniques, geography, sequencing technologies, and analytical techniques. Alternatively, these microbial signals may be inherent to each cohort and also functionally related, suggesting that function rather than specific species better defines therapeutic efficacy. Finding microbial signals that can be used to predict therapeutic responses requires more in-depth functional studies, as analyzing microbial components alone is unlikely to reveal enough information about complex microbial signals. A deeper understanding of these microbial functions requires RNA sequencing to analyze microorganisms' gene expression levels or metabolomics to identify potential pathways related

Table I. Summary of studies on the influence of intestinal flora on the efficacy of immunotherapy.

Study population	Tumor type	Types of immunotherapy drugs	Dominant flora for immunotherapy	Possible mechanism
American/ European[30]	Lung cancer/ kidney cancer/ urothelial cancer	PD-1 inhibitor	*Akkermansia mucini*, *E. hirae*	Increase the number of CD4+ central memory T cells and promote DCs to secrete more IL-12
American[31]	Melanoma	PPD-1 inhibitor	*Ruminococcaceae*/*Faecali bacterium*	More effector CD4+ T cells and CD8+ T cells
American[32]	Melanoma	PD-1 inhibitor	*Bifidobacterium longum*, *Aerobacter* and *Enterococcus faecalis*	Lead to a decrease in Tregs derived from the periphery
Chinese[34]	Non-small cell lung cancer	PD-1 inhibitor	*Alistipes putredinis*, *Prevotella*, *Bifidobacterium vulgaris*, *Lachnobacterium, Lachnospiraceae*, *Shigella*	Regulate memory T cell response and NK cell function
Chinese[35]	Non-small cell lung cancer	PD-1 inhibitor	*Parabacteroides*, *Methanobrevibacter*	Not given

to therapeutic efficacy. Therefore, the search for microbial signals that determine cancer treatment response is an ongoing work. The next few years are expected to provide exciting new paradigms in cancer research.

The Gut Microbiome and Adverse Reactions Related to Immune Checkpoint Inhibitors

Other preliminary studies have shown a link between the gut microbiome and immunotherapy-related toxicity. These studies have mainly focused on colitis, which is a common, albeit low-frequency, adverse event associated with the use of anti-CTLA-4. In these studies, baseline differences in the gut microbiota were related to the development of colitis in multiple anti-CTLA-4 cohorts. Like the efficacy responses, the specific bacteria found in each cohort were different. Faith et al[36] conducted a prospective study on patients with metastatic melanoma treated with CTLA-4 inhibitors, and they correlated fecal microbiota composition before inflammation with colitis occurrence and development. Patients without colitis were found to be enriched in *Bacteroides* species, which is consistent with the immunomodulatory effect of these symbiotic bacteria. *Bacteroidetes* is one of the main phylum of the human colonic flora, which restricts inflammation through T cell differentiation. Functional analysis results have revealed that riboflavin and pantothenic acid can be used to accurately assess the risk of colitis after CTLA-4 blockade based on the presence of the bacterial polyamine transport system and microbiota-related modules of thiamine biosynthesis. Thiamine and riboflavin concentrations in the blood of patients with Crohn's disease are significantly reduced, and combined pantothenic acid levels in the colonic mucosa decrease with the progression of inflammatory bowel disease[37,38]. The riboflavin metabolite activates a population of innate-like T cells called mucosal-associated invariant T cells *in vitro*[39]. Polyamines are small cationic amines that can be exported from bacterial cells through the spermidine and putrescine transport system. They have an anti-inflammatory effect by promoting colonic epithelial cell proliferation to maintain the epithelial barrier[40]. In patients with active colitis, the levels of ornithine decarboxylase, which is an enzyme involved in polyamine synthesis, are lower than those in control patients, but it is not clear whether reduced polyamine levels contribute to the development or progression of colitis. More research is needed to explore whether the reduced transport capacity of B vitamins and polyamines mediated by microorganisms lowers the threshold for immune-mediated colitis. Other checkpoint blockade therapies, such as anti-PD-1, can also cause adverse gastrointestinal events[41]. With both anti-PD-L1 and anti-CTLA-4 treatments, therapeutic responses to CpG-oligonucleotide immunotherapy and cyclophosphamide are affected by the intestinal flora[42-45]. As with therapeutic effects, the microbiota may play a role in the development of immune-mediated colitis in the context of some

immunotherapies. The identification of biomarkers that can be used to predict the risk of colitis may help identify patients who are particularly vulnerable to inflammation induced by different immunotherapies, such as CTLA-4 blockade, and who may be prophylactically treated before receiving such immunotherapies[46].

Possible Factors Affecting the Composition of the Human Intestinal Flora

To better understand the microbiome and how to best regulate it, it is necessary to understand a series of complex factors that affect the microbiome. Large-scale population-based studies have shown that different microbial communities are largely affected by environmental factors. Studies involving twins have shown that genetic factors account for only 2-8% of the observed variation[47]. Research outside of cancer treatment has shown that microbiomes vary greatly depending on race and geographic location, which may complicate or obscure cross-over studies. In addition, the basic factors driving microbiomes' connections with geography and ethnicity, including diet, cultural traditions, and genetics, may also affect the connections between microbiomes and patients' health, which has important implications for personalized and microbiome-based clinical applications[48]. Given the symbiotic relationship between the gut microbiota and the human host in terms of nutrient digestion, diet is undoubtedly the main determinant of the gut microbiota[49]. Differences in the microbial environment of the gut stem from dietary differences, as seen when comparing those in traditional agricultural communities who tend to consume plant-based diets and those in industrialized countries who tend to be omnivores[50].

Drugs are another key factor in the composition of the gut microbiome, especially antibiotics, which reduce diversity. However, infections are common in cancer patients. Therefore, antibiotics are essential for reducing morbidity and mortality. Other drugs may also affect the gut microbiome, especially proton pump inhibitors[51], metformin[52], antidepressants[53], and even hormones. Prospective studies[54,55] on exogenous and endogenous factors (such as diet, lifestyle, and obesity) and cancer outcomes have shown that continuing to study the influence of the gut microbiota on tumors and immune cells may change treatment outcomes.

Microbial Flora Intervention as a New Method of Cancer Treatment

If a patient lacks the symbiotic microbial community that initiates the immune response to tumor antigens, then it is worth considering fecal microbiota transplantation (FMT) from a microbiologically favorable donor patient. FMT can effectively treat refractory *Clostridium difficile*-associated diarrhea (a previous study found that up to 81% of patients with this condition were effectively treated.)[56]. However, there are many key factors to consider for this method. The most important is the choice of donor. The pass rate of stool donation is less than 3%[57]. In principle, the donor should be an individual with a varied microbial composition, including bacteria associated with good therapeutic effects. One of the most obvious options is to use the stool of a cancer patient with a better clinical response to PD-1 monoclonal antibodies. However, this option has many limitations. The main problem is the transfer of pathogens. Careful screening for bacteria, viruses, and parasites is required. In addition, some bacteria seem to contribute to inflammation-induced cancer. FMT from colorectal cancer patients to sterile mice can induce dysplasia and polyp formation, while transplantation from normal donors does not have this effect[58]. Other variables include fresh or frozen fecal material, optimal storage conditions, single or multiple FMT, and so on[59].

The ideal alternative is to extract beneficial bacteria from cancer immunotherapy responders, to be used alone or in combination. This strategy depends on the ability to identify precise bacterial isolates in the human host that can support improved anti-tumor immunity; culture conditions that can support their *in vitro* expansion; and an encapsulation scheme that can retain their biological activity after oral administration. Current 16S rRNA and shotgun sequencing may preferentially detect the most abundant bacteria associated with favorable clinical outcomes. However, the less abundant bacteria coexisting with more abundant species are also functionally important. Therefore, careful cultivation, isolation, and mechanism testing of rare species (some of which may reside in the small intestine and not be highly abundant in stool samples) need to be considered. Furthermore, bacterial communities, rather than a single major species, may participate in the immune enhancement of gut microbes. Because there are only a few bacterial entities that can be cultured by standard methods, improving the

optimal *in vitro* growth protocol will be a key component of pushing this strategy forward. For example, culture omics can be used to discover new microorganisms related to efficacy.

One finding that distinguishes cancer immunotherapy responders from non-responders is the presumed ratio of favorable to unfavorable bacteria[60]. Therefore, it is conceivable that some symbiotic organisms have a negative impact on the efficacy of immunotherapy. Thus, strategies aimed at eliminating unfavorable bacteria while providing immune enhancement effects should be adopted. Since standard antibiotics may lack specificity and pose risks, more precise strategies are needed. It is worth mentioning that bacteriophages are highly selective for a given bacterial species and have been used in the food industry to eliminate unfavorable bacteria[61].

Laut é-Caly et al[62] proved that *Enterococcus* MRx0518 can effectively stimulate the innate and adaptive immune systems, and they determined that the bacterial flagellin, which is a specific component of MRx0518 bacteria, interacts with host TLR5 receptors as the process intermediary. The TLR5 pathway is involved in the body's response to cancer. The MD Anderson Cancer Center is currently carrying out a phase I/II clinical trial for combined MRx0518 and pembrolizumab treatment, with plans to recruit 132 patients with advanced cancer (including non-small-cell lung cancer, renal cell carcinoma, bladder cancer, etc.) to observe the preliminary efficacy and safety of MRx0518.

Future Research Involving the Intestinal Flora

Like the relationships between the intestinal flora and T cell-targeted therapy and immune checkpoint inhibitors, the relationship between the intestinal flora and cancer management needs to be further studied. For example, understanding the roles of the microbiota in immunotherapies, such as cancer vaccines, oncolytic viruses, and cell-mediated therapies (including chimeric antigen receptor T cell therapy), can help determine which microbiota are associated with specific treatment ingredients. So far, all research efforts have been devoted to linking the efficacy of drugs with bacteria, but the complexity of the microbiota may hide other signals. As mentioned above, fungi and viruses are internal components of the microbiota, and they affect the immune response. All in all, searching for microbial signals related to cancer treatment response is an ongoing work. The next few years are expected to provide exciting new paradigms in cancer research.

Using microorganisms as a class of drugs poses a challenge to traditional treatments. In China and the United States, commercial probiotics can be purchased over the counter. However, they are only administered as food or dietary supplements, not as drugs[63]. If the purpose of probiotics is to produce therapeutic effects, such as cancer immunotherapy, then the FDA allows and supervises the use of microorganisms in drug development and new drug research applications. It should involve clear identification of the genus and species of each probiotic strain, including genome sequencing; laboratory studies pertaining to efficacy and mechanisms; human clinical trials with efficacy endpoints; human safety and adverse event assessments; and notes on the possibility of infection. Multiple cases of transmitted infections have been clearly associated with probiotics, especially in immunosuppressed individuals[64]. The future challenges of integrating microbiology and oncology include developing fast and cost-effective methods for diagnosing intestinal dysfunction, accurately mapping the microbial action mode of each cancer type, and changing the intestinal ecosystem in a repeatable way to improve patient survival rates.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Stappenbeck TS, Virgin HW. Accounting for reciprocal host-microbiome interactions in experimental science. Nature 2016; 534: 191-109.
2) Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486: 207-214.
3) Cho I, Blaser MJ. The Human Microbiome: at the interface of health and disease. Nature Rev Genet 2012; 13: 260-270.
4) Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 2016; 14: e1002533.
5) De Vos WM, De Vos EA. Role of the intestinal microbiome in health and disease: from correlation to causation. Nutr Rev 2012; 70 Suppl 1: S45-S56.
6) Belkaid Y, Naik S. Compartmentalized and systemic control of tissue immunity by commensals. Nat Immunol 2013; 14: 646-653.

7) Peek RM Jr, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002; 2: 28-37.

8) Kosuke M, Yasutaka S, Reiko N, Zhi RQ, Mai Y, Kentaro I, Sun AK, Atsuhiro M, Jonathan A N, Katsuhiko N, Aleksandar DK, Marios G, Hideo W, Susan B, Danny AM, Curtis CH, Edward G, Levi AG, Gordon JF, Glenn D, Andrew TC, Wendy SG, Curtis H, Charles SF, Shuji O. Fusobacterium nucleatum and T cells in colorectal carcinoma. JAMA Oncol 2015; 1: 653-661.

9) Smruti P, Mautin H, Donnele D, Constantinos PZ, Emma K, Ankita M, Navyatha M, Berk A, Mykhaylo U, Luisana ET, Gregor W, Kevin Z, Yuqi G, Qianhao L, Neha A, Sarah L, Benjamin W, Johana G, Juan A, Jeremy WH, Brian D, Alejandro TH, Josh L, Wei W, Pardeep ST, Shivraj S, Malvin J, Anjana S, Xin L, Deirdre C, R Balfour S, Deepak S, George M. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. Cancer Discov 2018; 8: 403-416.

10) Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe 2013; 14: 195-206.

11) Wang F, Meng W, Wang B, Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. Cancer Lett 2014; 345: 196-202.

12) Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer 2010; 10: 878-889.

13) Hossein B, Luis PA, Leora H, David RS, Martin S, Neal ER, Laura QC, Everett EV, Enriqueta F, Esther H, Fabrice B, Martin K, Oscar A, Marco AB, Jérôme F, Hervé L, Elena P, David EG, Scott NG, Charles MR, Naiyer R, Lucio C, George RJ, Scott JA, Cécile D, Christopher TH, Friedrich GF, Julie RB. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015; 373: 1627-1639.

14) Suzanne LT, Hodi FS, Julie RB, Scott NG, David CS, David FM, John DP, Richard DC, Jeffrey AS, Michael BA, Philip DL, David RS, Scott JA, Leora H, Charles GD, Drew MP, Lieping C, William HS, Robert AA, Janis MT, Tracee LM, Xu HY, Alan JK, Maria JK, Shruti A, Daniel M, Georgia DK, Ashok G, Jon MW, Mario S. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443-2454.

15) Robert JM, Bernard E, David FM, Saby G, Hans JH, Sandhya S, Scott ST, Jeffrey AS, Giuseppe P, Elizabeth RP, Daniel C, Toni KC, Howard G, Frede D, Petri B, John W, Thomas CG, Takeshi U, Yoshihiko T, Fabio AS, Christian K, James L, Alain R, Jason SS, Xu LA, Lan MW, Padmanee S. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015; 373: 1803-1813.

16) Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015; 348: 124-128.

17) Nadeem R, Jonathan JH, Sviatoslav MK, Vladimir M, Logan AW, Alexis D, Nils W, Timothy AChan. Recurrent SERPINB3 and SERPINB4 mutations in patients who respond to anti-CTLA4 immunotherapy. Nat Genet 2016; 48: 1327-1329.

18) Matthew DH, Luis PA, Reyes BC, Bogdan Z, Kim SW, Enric CC, Keunchil P, Aurelia A, Lorena L, Emmanuel MJ, Hiroshi S, Istvan A, Alain V, Solange P, Konstantinos S, Fabrice B, Martin R, Hossein B, Julie RB, Kenneth JO, William JG, Prabhu B, Sridhar KR, Ravi SK, Faith EN, Suresh SR. First-line nivolumab in Stage IV or recurrent non-small-cell lung cancer. N Engl J Med 2017; 376: 2415-2426.

19) Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015; 348: 69-74.

20) Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature 2015; 523: 231-235.

21) Dallas RD, Darcy H, Leonard BC, Stephanie AM, Alan CW, Andrew H, Kaitlin PC, Sarah W R, Alicia G, Elizabeth PR, Virginia G, Mark TH, Deborah ST, Anna H, James AS, David WT, Scott JB. A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota–and butyrate-dependent manner. Cancer Discov 2014; 4: 1387-1397.

22) O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, Vipperla K, Naidoo V, Mtshali L, Tims S, Puylaert PG, DeLany J, Krasinskas A, Benefiel AC, Kaseb HO, Newton K, Nicholson JK, de Vos WM, Gaskins HR, Zoetendal EG. Fat, fibre and cancer risk in African Americans and rural Africans. Nat Commun 2015; 6: 6342.

23) Leore TG, Michal BR, Tal D, Oliver HJ, Noam S, Deborah N, Nancy G, Yaara Z, Zachary AC, Kevin S, Christoph AT, Alexandre R, Jonathan L, Roi A, Dennie TF, Matteo L, Kelly C, Stephen E J, Carrie MM, Alexander B, Garold F, Candice G, Vancheswaran G, Michael K, Mark WH, Matthew K, Jason F, Anirban M, David AS, Matt S, Jeffrey B, Monia M, Sunia AT, Iris B, Talia G, Judith S, Keith TF, Anna M, Wendy SG, Sarah PT, Cristina RF, Curtis H, Sangeeta NB, Dirk G, Jennifer AW, Todd RG, Ravid S. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017; 357: 1156-1160.

24) Anshuman P, Janice MM, Kim MH, Greg R, Sherri D, Tracie S, Michael K, Levi S, Mark NS, Elizabeth P, Lorna RR, Ann WS, Joseph A, Nancy C, Jyoti M, Melissa F, Howard LK, Siraj A, Jeffrey SR, Eileen PW, Gyan B, Shridar G. Immune acti-

vation and benefit from avelumab in ebv-positive gastric cancer. J Natl Cancer Inst 2018; 110: 316-320.

25) Smola S. Immunopathogenesis of HPV-associated cancers and prospects for immunotherapy. Viruses 2017; 9: e254.

26) Cogdill AP, Gaudreau PO, Arora R, Gopalakrishnan V, Wargo JA. The impact of intratumoral and gastrointestinal microbiota on systemic cancer therapy. Trends Immunol 2018; 39: 900-920.

27) Thorsten R, Silke T, Tobias JG, Felix M, Busch CJ, Cordula P, Ekkehard D, Malte K. HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. Radiother Oncol 2013; 107: 242-246.

28) Tashiro H, Brenner MK. Immunotherapy against cancer-related viruses. Cell Res 2017; 27: 59-73.

29) Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, Fidelle M, Flament C, Poirier-Colame V, Opolon P, Klein C, Iribarren K, Mondragón L, Jacquelot N, Qu B, Ferrere G, Clémenson C, Mezquita L, Masip JR, Naltet C, Brosseau S, Kaderbhai C, Richard C, Rizvi H, Levenez F, Galleron N, Quinquis B, Pons N, Ryffel B, Minard-Colin V, Gonin P, Soria JC, Deutsch E, Loriot Y, Ghiringhelli F, Zalcman G, Goldwasser F, Escudier B, Hellmann MD, Eggermont A, Raoult D, Albiges L, Kroemer G, Zitvogel L. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018; 359: 91-97.

30) Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350: 1079-1084.

31) Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, Cogdill AP, Zhao L, Hudgens CW, Hutchinson DS, Manzo T, Petaccia de Macedo M, Cotechini T, Kumar T, Chen WS, Reddy SM, Szczepaniak Sloane R, Galloway-Pena J, Jiang H, Chen PL, Shpall EJ, Rezvani K, Alousi AM, Chemaly RF, Shelburne S, Vence LM, Okhuysen PC, Jensen VB, Swennes AG, McAllister F, Marcelo Riquelme Sanchez E, Zhang Y, Le Chatelier E, Zitvogel L, Pons N, Austin-Breneman JL, Haydu LE, Burton EM, Gardner JM, Sirmans E, Hu J, Lazar AJ, Tsujikawa T, Diab A, Tawbi H, Glitza IC, Hwu WJ, Patel SP, Woodman SE, Amaria RN, Davies MA, Gershenwald JE, Hwu P, Lee JE, Zhang J, Coussens LM, Cooper ZA, Futreal PA, Daniel CR, Ajami NJ, Petrosino JF, Tetzlaff MT, Sharma P, Allison JP, Jenq RR, Wargo JA. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018; 359: 97-103.

32) Matson V, Fessler J, Bao R, Chongsuwat T, Zha YY, Alegre ML, Luke JJ, Gajewski TF. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018; 359: 104-108.

33) Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, Chang EB, Gajewski TF. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015; 350: 1084-1089.

34) Jin Y, Dong H, Xia L, Yang Y, Zhu Y, Shen Y, Zheng H, Yao C, Wang Y, Lu S. The diversity of gut microbiome is associated with favorable responses to anti-programmed death 1 immunotherapy in Chinese patients with NSCLC. J Thorac Oncol 2019; 14: 1378-1389.

35) Song P, Yang D, Wang H, Cui X, Si X, Zhang X, Zhang L. Relationship between intestinal flora structure and metabolite analysis and immunotherapy efficacy in Chinese NSCLC patients. Thoracic Cancer 2020; 11: 1621-1632.

36) Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci Transl Med 2014; 6: 220ra11.

37) Kuroki F, Iida M, Tominaga M, Matsumoto T, Hirakawa K, Sugiyama S, Fujishima M. Multiple vitamin status in Crohn's disease. Correlation with disease activity. Dig Dis Sci 1993; 38: 1614-1618.

38) Ellestad-Sayed JJ, Nelson RA, Adson MA, Palmer WM, Soule EH. Pantothenic acid, coenzyme A, and human chronic ulcerative and granulomatous colitis. Am J Clin Nutr 1976; 29: 1333-1338.

39) Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, Bhati M, Chen Z, Kostenko L, Reantragoon R, Williamson NA, Purcell AW, Dudek NL, McConville MJ, O'Hair RA, Khairallah GN, Godfrey DI, Fairlie DP, Rossjohn J, McCluskey J. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 2012; 491: 717-723.

40) Furuchi T, Kashiwagi K, Kobayashi H, Igarashi K. Characteristics of the gene for a spermidine and putrescine transport system that maps at 15 min on the escherichia coli chromosome. J Biol Chem 1991; 266: 20928-20933.

41) Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, Brahmer JR, Lawrence DP, Atkins MB, Powderly JD, Leming PD, Lipson EJ, Puzanov I, Smith DC, Taube JM, Wigginton JM, Kollia GD, Gupta A, Pardoll DM, Sosman JA, Hodi FS. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014; 32: 1020-1030.

42) Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, Benyamin FW, Lei YM, Jabri B, Alegre ML, Chang EB, Gajewski TF. Commensal bifidobacterium promotes antitumor

immunity and facilitates anti-pd-l1 efficacy. Science 2015; 350: 1084-1089.

43) Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350: 1079-1084.

44) Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, Molina DA, Salcedo R, Back T, Cramer S, Dai RM, Kiu H, Cardone M, Naik S, Patri AK, Wang E, Marincola FM, Frank KM, Belkaid Y, Trinchieri G, Goldszmid RS. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013; 342: 967-970.

45) Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, Enot DP, Pfirschke C, Engblom C, Pittet MJ, Schlitzer A, Ginhoux F, Apetoh L, Chachaty E, Woerther PL, Eberl G, Bérard M, Ecobichon C, Clermont D, Bizet C, Gaboriau-Routhiau V, Cerf-Bensussan N, Opolon P, Yessaad N, Vivier E, Ryffel B, Elson CO, Doré J, Kroemer G, Lepage P, Boneca IG, Ghiringhelli F, Zitvogel L. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013; 342: 971-976.

46) Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, No D, Gobourne A, Littmann E, Huttenhower C, Pamer EG, Wolchok JD. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun 2016; 7: 10391.

47) Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486: 207-214.

48) 48 Gaulke CA, Sharpton TJ. The influence of ethnicity and geography on human gut microbiome composition. Nat Med 2018; 24: 1495-1496.

49) McDonald D, Hyde E, Debelius JW. American gut: an open platform for citizen science microbiome research. mSystems 2018; 3: e00031-e00018.

50) Ou J, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, Gaskins HR, O'Keefe SJ. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. Am J Clin Nutr 2013; 98: 111-120.

51) Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, Sutter JL, Welter D, Ley RE, Bell JT, Spector TD, Steves CJ. Proton pump inhibitors alter the composition of the gut microbiota. Gut 2016; 65: 749-756.

52) Forslund K, Hildebrand F, Nielsen T. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 2015; 528: 26.

53) Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, Kurilshikov A, Bonder MJ, Valles-Colomer M, Vandeputte D, Tito RY, Chaffron S, Rymenans L, Verspecht C, De Sutter L, Lima-Mendez G, D'hoe K, Jonckheere K, Homola D, Garcia R, Tigchelaar EF, Eeckhaudt L, Fu J, Henckaerts L, Zhernakova A, Wijmenga C, Raes J. Population-level analysis of gut microbiome variation. Science 2016; 352: 560-564.

54) Ogino S, Nowak JA, Hamada T, Phipps AI, Peters U, Milner DA Jr, Giovannucci EL, Nishihara R, Giannakis M, Garrett WS, Song M. Integrative analysis of exogenous, endogenous, tumour and immune factors for precision medicine. Gut 2018; 67: 1168-1180.

55) Sears CL. The who, where and how of fusobacteria and colon cancer. eLife 2018; 7: e28434.

56) van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013; 368: 407-415.

57) Kassam Z, Dubois N, Ramakrishna B, Ling K, Qazi T, Smith M, Kelly CR, Fischer M, Allegretti JR, Budree S, Panchal P, Kelly CP, Osman M. Donor screening for fecal microbiota transplantation. N Engl J Med 2019; 381: 2070-2072.

58) Wong SH, Zhao L, Zhang X, Nakatsu G, Han J, Xu W, Xiao X, Kwong TNY, Tsoi H, Wu WKK, Zeng B, Chan FKL, Sung JJY, Wei H, Yu J. Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. Gastroenterology 2017; 153: 1621-1633.

59) Moayyedi P, Yuan Y, Baharith H, Ford AC. Faecal microbiota transplantation for Clostridium difficile associated diarrhoea: a systematic review of randomised controlled trials. Med J Aust 2017; 207: 166-172.

60) Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, Luke JJ, Gajewski TF. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018; 359: 104-108.

61) Zhang J, Hong Y, Harman NJ, Das A, Ebner PD. Genome sequence of a salmonella phage used to control salmonella transmission in Swine. Genome Announc 2014; 2: e00521-e00514.

62) Lauté-Caly DL, Raftis EJ, Cowie P, Hennessy E, Holt A, Panzica DA, Sparre C, Minter B, Stroobach E, Mulder IE. The flagellin of candidate live biotherapeutic Enterococcus gallinarum MRx0518 is a potent immunostimulant. Sci Rep 2019; 9: 801.

63) Venugopalan V, Shriner KA, Wong-Beringer A. Regulatory oversight and safety of probiotic use. Emerg Infect Dis 2010; 16: 1661-1665.

64) Enache-Angoulvant A, Hennequin C. Invasive Saccharomyces infection: a comprehensive review. Clin Infect Dis 2005; 41: 1559-1568.