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REVIEW ARTICLES

Role of the microbiome in the development and treatment of gastric cancer: an overview of the biological and clinical landscape

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For decades, the stomach was considered a sterile organ, due to the acid environment. However, starting from the discovery of *Helicobacter pylori*, this concept has progressively refined. By damaging the hydrochloric acid-secreting glands, *H. pylori* infection primes the progression from acute to chronic inflammation in gastric mucosa resulting in atrophic gastritis, intestinal metaplasia, dysplasia and ultimately gastric cancer (GC). Due to the challenging identification of culturing bacteria, the carcinogenic role of gastric microbial community, other than *H. pylori*, remains underestimated. More recently, a growing body of evidence has pointed out the dynamism of gastric microbiota as a crucial step for GC development, besides elucidating some additional activity in modulating the efficacy of cancer treatments. In turn, anticancer therapies can shape gastric microbiota with consequent dysbiosis and a potential correlation with drug-related toxicity. In conclusion, the current review aims to deepen the role of gut microbiota as a key factor in gastric disease at multiple levels, from carcinogenesis to the metastatic phase. It also provides novel insights on gastric microbiota as potential target for tailoring multimodal strategies, either surgical or oncological, to finally provide our patients with more individualized treatment options.

Key words: gastric cancer, microbiota, *Helicobacter pylori*, immunotherapy

INTRODUCTION

Worldwide, gastric cancer (GC) is one of the most common malignancies and a major cause of cancer-related death.[1,2] Despite improvements in the multidisciplinary approach, the prognosis of GC remains poor with a 5-year survival rate of ∼30%-40%.[3] Unfortunately, even in localized disease stages, relapse remains a major challenge.[4] In the advanced setting, due to clinical aggressiveness of the disease and occurrence of drug resistance, median overall survival is low and reaches ∼10-15 months in clinical trials,[5-7] even though real-world data suggest that for the majority of patients median survival is even less.[8] Consequently, a better knowledge of prognostic and predictive factors to tailor anticancer therapies represents an unmet clinical need.

Pre-existing environmental factors may act during carcinogenesis and affect the molecular and immunological mechanisms that drive cancer growth and invasiveness. Robust evidence supports the multifaceted role of human microbiome in the homeostasis of numerous physiological processes including nutrition, metabolism, endocrine functions and modulation of inflammation and immune response.[9] In healthy persons, the human microbiome acts as a symbiont, which presents a barrier to opportunistic pathogens and prevents carcinogenesis.[10] Nevertheless, this commensal microbiome exists in a precarious equilibrium that, if altered (a condition named dysbiosis), can contribute to several pathological conditions including cancer.[11]

Almost four decades ago, Warren and Marshall made a seminal discovery identifying *Helicobacter pylori* as a cause of gastritis.[12,13] So far, it is established that gastric colonization by *H. pylori* can predispose toward GC development by different mechanisms: *H. pylori* infection promotes inflammation of the gastric mucosa, damaging the hydrochloric acid-secreting glands, thus favoring the progression from chronic inflammation to atrophic gastritis intestinal

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metaplasia, dysplasia and finally GC. On the other side, *H. pylori* can have direct effects through the toxic action of virulence factors, mainly cytotoxin-associated gene A (CagA) and vacuolating cytotoxic A (VacA).[14,15] As a result, in 1994 the World Health Organization classified *H. pylori* as a class I carcinogen.[15] Since then, eradication of *H. pylori*, together with changes in food conservation, led to a significant decrease in GC incidence.[16]

In summary, due to the difficulties of identifying and culturing bacteria in gastric juices, the role of microbiota in the development of GC has been underestimated for years. The recent advances in genomic techniques provided a better characterization of the gastric microbiome leading to an increasing quality of the evidence and spurred scientific community to deepen the understanding of gastric dysbiosis and cancerous transformation.[17] Besides summarizing the current knowledge about the role of the microbiome in GC development, this review will also focus on microbiota manipulation as a potential tool to either predict or improve the efficacy of cancer therapies.

IDENTIFICATION AND CHARACTERIZATION OF THE HUMAN MICROBIOTA

The human microbiota describes the microbial taxa associated with humans and consists of as much as 10-100 trillion microbial cells harbored by each person in the different parts of the body. Bacteria comprise the vast majority of the biomass and diversity in the human gut, though small numbers of archaea, viruses and eukaryotes are also present[18] (Figure 1).

The composition of the human microbiota varies depending on different anatomical sites, age, concomitant disease and environmental factors such as diet or antibiotic use.[19] Exploring microbial communities in the human gut requires taxonomic classification and gene functional profiling. Metagenomics is the study of microbial communities in their original living places and refers to sequencing the entire genomes of all microbes present in a sample. When addressing a microbial ecosystem, the presence and abundance of specific bacterial strains are usually weighed by 'alpha diversity' and 'beta diversity'. Alpha diversity is a measure of microbes' variation in the same sample: specifically, the number of distinguishable taxa in a sample is described by species richness, while the species diversity tells us how the microbes are distributed in a sample. The Shannon diversity index combines richness and diversity, by measuring both the number of species and the inequality between species abundances. Beta diversity shows the dissimilarities derived from microbial communities of heterogeneous environments, featuring several profiles in taxonomic abundance in the different samples, and can be quantified by Bray–Curtis dissimilarity, Jaccard distance and UniFrac methods.[20]

COMPOSITION OF NON-CANCEROUS GASTRIC MICROBIOTA

Because of the low pH (median 1.4), the stomach has a lower microbial load [10^2-10^4 colony-forming units (CFU)] compared with the small intestine and the colon (10^{10}-10^{12} CFU)[18,19,21] and—as mentioned above—the composition of commensal microbiota is influenced by any condition that favors increased gastric pH (>4) and bacterial overgrowth (e.g. long-term use of proton pump inhibitors, H2 blockers or chronic gastritis).[22] Besides the underlying gastric condition, microbiota composition also varies with the different type of biosamples: gastric juice, for instance, displays an abundance of Actinobacteria, Bacteroidetes and Firmicutes, whereas mucosal specimens are enriched with *H. pylori* and Proteobacteria.[17,23] Additionally, the traditional study techniques, such as isolation and culture, are not able to identify any more bacteria, since up to 80% of microbes are not cultivable.[24] As reported in the first studies assessing the composition of gastric microbiome in healthy individuals, the only bacteria isolated through culture analysis were *Veillonella* sp., *Lactobacillus* sp. and *Clostridium* sp.[25] Later on, the introduction of genomic techniques (using real-time PCR and next generation sequencing) enabled a better portrait of the gastric microbiota.[26] In 2006, Bik and colleagues measured the alpha diversity of gastric mucosa samples of 23 healthy individuals through a 16S ribosomal DNA (rDNA) clone library approach reporting Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Fusobacteria as the five most dominant phyla in normal conditions.[27] Conversely, the presence of a pathological condition of non-*H. pylori* gastritis, microbiota profiling (16S rRNA) revealed *Streptococcus*, *Prevotella*, *Neisseria*, *Haemophilus* and *Porphyromonas* as the most abundant genera.[28]

Globally, the impact of *H. pylori* infection on the other commensal still remains an open issue, specifically in long-

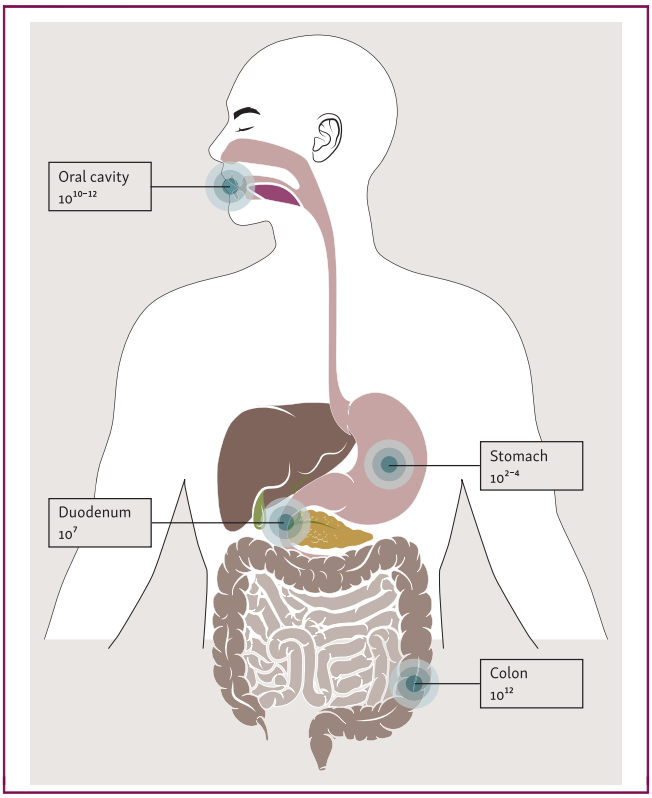

Figure 1. The human gut microbiota.

term infection. In an effort to investigate in this direction, Osaki and colleagues conducted a study in infected Mongolian gerbils, miming a long-term infection by dividing mice into different groups according to whether or not *H. pylori* was cleared at 1 year of infection. Results showed that in the chronically positive group, a relative richness of Proteobacteria, *Spirochetes* and *Acidobacteria* was described along with a decreased abundance of Actinobacteria, Bacteroidetes and Firmicutes.[29] These results were in contrast with other works where no dissimilarities in microbiota composition had been found among the patients, irrespective of *H. pylori* status.[30] Overall, several factors, linked to diet, lifestyles, disease and ethnicity, as well as the consequence of a long-term infection, account for the variable and sometimes contrasting results cited in this section.[31] Therefore, great caution should be applied when interpreting the results from the massive literature production about gastric microbiota.

ROLE OF THE MICROBIOTA IN GASTRIC CANCER DEVELOPMENT

Bacteria can promote the initiation of the gastric carcinogenesis cascade by numerous mechanisms including the induction of an inflammatory environment, DNA damage, dysregulation of cell cycle, tumor growth and immune evasion[24,32] (Figure 2).

GASTRIC DYSBIOSIS: THE UNFOLDING THEORY OF NON-*H. PYLORI* BACTERIA IN PROMOTING CANCER DEVELOPMENT

Despite the infection of *H. pylori* being widely diffused affecting ∼50% of the global population, only 1%-3% of them develop GC.[15] Mounting preclinical evidence supports the pathogenic role of non-*H. pylori* bacteria in GC carcinogenesis.[33] Insulin-gastrin (INS-GAS) transgenic mice represent a unique model for studying the development of GC, since the overexpression of circulating gastrin levels is associated with spontaneous development of atrophic gastritis (AG) and gastric intramucosal neoplasia (GIN), in ∼80% of mice with *H. pylori* infection.[34] Intriguingly, Lofgren and colleagues observed that a less severe gastritis along with a late-onset GIN occurred in *H. pylori*-dominant INS-GAS mice, compared to those mice with a more heterogeneous microbiota composition.[35] In a further study, Lertpiriyapong and colleagues characterized the risk of developing gastric lesions in infected mice carrying different microbiota compositions: germ-free, restricted (with *Lactobacillus*, *Clostridium* and *Bacteroides*) or complex-microbiota mice.[36] Remarkably, in restricted and complex-microbiota INS-GAS mice, a significant expression of inflammatory and cancer-related genes (including *TNF-α*, *Ptger4* and *Tgf-β*) was reported, with a consequent increased risk of gastric disease compared to those germ-free models. Similarly, Li and colleagues had previously demonstrated that anti-inflammatory and antibiotic treatment prevents the progression from severe dysplasia to GC in *H. pylori*-infected INS-GAS mice.[28]

Taken together, these data support the hypothesis of a complicit role of non-*H. pylori* taxa in promoting the malignant alterations.

Later on, clinical studies have better outlined the gastric dysbiosis across the different stages of the carcinogenesis route.[18] In a single-center study population, Ferreira and colleagues profiled patients with either GC or chronic gastritis, describing reduced abundance of *Helicobacter* and *Neisseria* alongside the enrichment of intestinal bacteria (like *Achromobacter*, *Citrobacter*, *Phyllobacterium*, *Clostridium*, *Rhodococcus* and *Lactobacillus*), in those with GC rather than chronic gastritis.[37] Similarly, the 16S ribosomal RNA (rRNA) gene analysis made by Coker and colleagues on a Chinese cohort of 81 patients at different stages of gastric disease (from superficial gastritis/AG to intestinal metaplasia and GC) reported a network centrality in microbiota of patients with GC, with an abundance of *Peptostreptococcus stomatis*, *Streptococcus anginosus*, *Parvimonas micra*, *Slackia exigua* and *Dialister pneumosintes* taxa compared to those patients with superficial gastritis. Interestingly, these results were also confirmed in a validation cohort of 126 Mongolian patients.[38] In a further study, which aimed to characterize gastric microbiota in ∼300 patients with both chronic gastritis and GC, an increased bacterial load in patients with *H. pylori* infection compared with negative cases was established. At the same time, five prevalent genera with potential pro-tumorigenic activity were selectively identified in the microbiota of patients with GC: *Lactobacillus*, *Escherichia—Shigella*, *Nitrospirae*, *Burkholderia fungorum* and *Lachnospiraceae.*[39]

To elucidate the dynamic changes of dysbiosis along the various steps of tumor development, from gastritis to adenoma and early/advanced GC, Park and colleagues characterized the microbiota in the gastric juice of 88 patients,[40] displaying a progressive decrease in the alpha diversity during carcinogenesis route. More precisely, a significant reduction in the abundance of *Akkermansia* and *Lachnospiraceae* NK4A136 was noted in the GC cases alongside an enrichment with *Lactobacillus* and *Veillonella*. Similarly, in a large analysis of 1270 gastric biopsies, pooled by 10 public datasets, and profiled through 16S rRNA sequencing, a reduced diversity in GC samples compared to the other precancerous conditions was reported, and concomitantly led to the identification of four GC-associated bacteria: *Fusobacterium*, *Peptostreptococcus*, *Streptococcus* and *Veillonella*.[41]

To date, the activity of non-*H. pylori* bacteria in GC development has not yet been definitively established. Profiling of GC samples in different studies lighted on the possible carcinogenic role of some oral commensal, such as *Lactococcus* and *Lactobacillus* genera, enabling to acquire virulence when translocating downstream to the gastroenteric tract.[42,43] Although no definitive mechanistic insights have been provided yet, the authors propose that the bacterial-derived lactic acid may represent an energy substrate that favors cancer cell proliferation and tumor progression. Findings from other studies support the role of *N*-nitrosamine compound-generating bacteria (i.e.

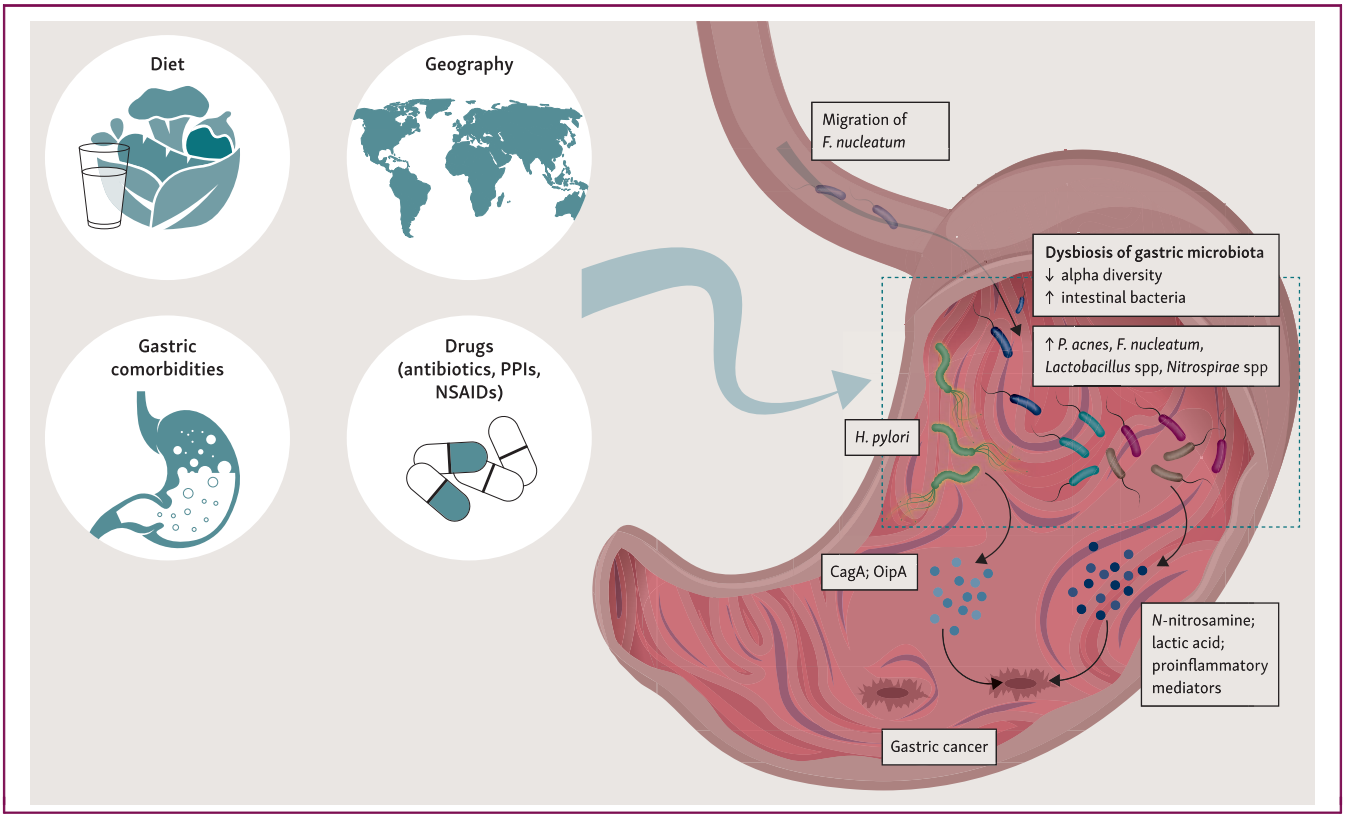

Figure 2. Dysbiosis of gastric microbiota and cancer.
CagA, cytotoxin-associated gene A; NSAIDs, nonsteroidal anti-inflammatory drugs; OipA, outer inflammatory protein A; PPIs, proton pump inhibitors.

Nitrospirae species) in promoting the conversion of nitrite to nitrosamine and enhancing the genotoxic risk.[37,39,44] A further commensal of the oral cavity, which presumably becomes pathogenetic after a downstream migration, is the anaerobic gram-negative *Fusobacterium nucleatum*.[45-48] As seen in colorectal cancers (CRCs), Chen and colleagues reported an abundance of *F. nucleatum* also in GC tissues and in elderly patients, with respect to normal mucosa and younger patients ($P = 0.041$), apart from being associated with tumor lymphocyte infiltration.[48] More in general, despite being associated to a worse prognosis in few cases of diffuse-type GC, the presence of *F. nucleatum* in patients with GC has been linked to contrasting survival outcomes.[49-51]

Finally, although not apparently intuitive, the GC microbiota may be influenced by bacteria residing outside the gastrointestinal (GI) tract. Among different human microhabitats of origin, the skin commensal *Propionibacterium acnes* bacteria are of specific interest, due to their ability to modulate the immune response by producing short-chain fatty acids in patients with lymphocytic gastritis, which seems unrelated to any tumorigenic mechanism.[52] Recently, Park and colleagues found that *Rhizobiales*, well-known plant-root nitrogen-fixing symbionts, were enriched in some cases of intestinal metaplasia with concomitant upregulation of T4SS genes[53] that are involved in the intracellular transport of CagA (one of the main *H. pylori* virulence factors potentially linked to GC development).[54] Conversely, few studies have adequately shed light on the protective role exerted by some specific bacteria. In this respect, emerging data show that *Sphingobium yanoikuyae*, a halotolerant di-*n*-butyl-phthalate-degrading bacterium, seems able to degrade carcinogenic compounds, being also one of the less-represented species among GC-associated microbes.[55]

Comprehensively, an increasing number of studies are providing novel insights about the oncogenic role of microbiota in GC development. On the contrary, less is known about the protective role of some specific bacteria against cancerous initiation, thus envisaging further investigation also in this direction.

MICROBIOTA MAY IMPACT THE EFFICACY OF GASTRIC CANCER TREATMENTS

As a result of a deeper knowledge in tumor biology and due to the availability of more effective treatments, the outcome of resectable GC appreciably improved in the past years. To date, Western guidelines recommend the use of a multimodal strategy in the perioperative setting, encompassing chemotherapy or concurrent chemoradiation as a valid option for preventing disease recurrence.[56,57] In the advanced settings, a combination of chemotherapy plus either checkpoint inhibitors or human epidermal growth factor receptor 2-targeted monoclonal antibodies represents the mainstay of treatment.[6,7] However, sooner or later, the onset of either intrinsic or acquired resistance leads to treatment failure,

thus emphasizing the need for novel biomarkers which enable to tailor the management strategy. And in this scenario, gastric microbiota is emerging as an appealing biomarker at multiple levels.

Microbiota modulation and gastric surgery

The impact of gastric surgery on microbiota composition has been reported in several oncological and non-oncological settings.[58-60] In the cancer care setting, Tseng and colleagues addressed the dynamic changes in gastric microbiota composition in the perioperative period, documenting a prevalence of *Ralstonia* and *Helicobacter* in the cancerous stomach before surgery, and abundance of *Streptococcus* and *Prevotella* genera afterward.[61] Apart from inducing the loss of the gastric barrier and biliary diversion, gastrectomy also increases the oxygenation levels in the bowel with consequent dysbiosis and abundance of typical oral cavity bacteria, aero-tolerant (aerobes/facultative anaerobes) and bile acid-transforming bacteria.[62] Similarly, Erawijantari and colleagues showed that some opportunistic oral bacteria (*Streptococcus*, *Veillonella*, *Prevotella*), aero-tolerant and facultative anaerobic microbes were enriched in the fecal samples of patients with GC who underwent gastrectomy.[63] The long-term consequences on the microbiota population after a sub-total gastrectomy were also addressed in a further study which provided similar results: the microbiota richness and diversity were augmented after tumor excision, with abundance of Firmicutes and aero-tolerant bacteria of Proteobacteria phylum compared with control.[64]

Circumstantially, gastrectomy-induced dysbiosis is related to intestinal inflammation, small intestinal bacterial overgrowth as well as an increased risk of GI malignancies. With regard to the last point, retrospective studies have suggested a causal relationship between surgery for GC and the risk of secondary tumors, both gastric stump cancer and CRC.[65,66] Although the exact mechanisms are not fully understood, a significant enrichment in *F. nucleatum* was found in the stool samples of patients who underwent a gastric surgery,[63] inferring a cause—effect relationship with GI cancer development.[67,68] Finally, metabolomic analysis conducted in patients undergoing gastrectomy supported the relevance of microbiota in increasing the number of biliary acids such as deoxycholic acid that presumably has a pathogenic role in colonic carcinogenesis other than inflammatory bowel disease.[69-71]

Overall, since most of the gastric bacteria are deemed to originate from the oral cavity, the oral microbiota can shape the gastric microbiota composition after gastrectomy. Several studies reported that these changes result with an increasing number of orally derived bacteria in the gut, which in turn may have a clinical impact on patients, both in terms of increased secondary tumors and post-operative complications.

Microbiota modulation and chemotherapy

There is a growing body of evidence that the human microbiome—and even more gut dysbiosis—plays a crucial role in modulating the efficacy and safety of systemic cancer therapies.[72-78] In a Dutch phase II trial, cachectic patients with gastroesophageal cancer receiving capecitabine and oxaliplatin as first-line chemotherapy were randomized to either allogenic or autologous fecal microbiota transplantation (FMT) from healthy obese donor, in order to improve cachexia outcomes. Although allogenic FMT did not improve its primary endpoint, the positive effects were unexpectedly seen on chemotherapy response and outcomes.[79] Supporting this hypothesis, further preclinical evidence reported that chemotherapies alter the host microbiota, with resulting dysbiosis and drug-related toxicity, and vice versa.[73-80] Regarding fluoropyrimidines, the cytostatic activity of the drug is strongly impaired by thymidine phosphorylase-producing bacteria.[75] Even resistance to 5-fluorouracil may be elicited by *F. nucleatum* through the initiation of the Toll-like receptor 4 (TLR4) and MYD88 pathways and autophagy activation.[76] In turn, 5-fluorouracil has been proved to negatively affect the commensal gut microbiota, by promoting the overgrowth of *Lachnospiraceae* NK4A136, *Bacteroides*, *Odoribacter*, *Mucispirillum* and *Blautia* genera.[77,78] The same modulation on drug genotoxicity has also been reported for platinum compounds: gut microbiota disruption may reduce the production of proinflammatory cytokines and reactive oxygen species in the tumor microenvironment (TME), hence impairing the efficacy of oxaliplatin and cisplatin.[80,81] Conversely, butyrate-producing bacteria enhanced the activity of oxaliplatin in some preclinical works by modulating the activation of interleukin (IL)-12 signaling pathway and the induction of cytotoxic CD8+ T-cell response.[82] In other preclinical models, gut microbiota was even responsible for oxaliplatin-induced hyperalgesia as a result of the activation of TLR4 in macrophages.[83] Consistent with this evidence, Ma and colleagues demonstrated that microbiota depletion might prevent the development and persistence of oxaliplatin-induced peripheral neuropathy.[84] Lastly, some reports analyzed the interaction between β-glucuronidase-producing bacteria and irinotecan GI toxicity, showing that bacterial-derived enzymes enhanced the conversion to the active compound SN38, which both mediated the activity of irinotecan and the intestinal mucosal damage.[85-87] Interestingly, in a mouse model, β-glucuronidase inhibition by using antibiotics did not impair irinotecan efficacy but did prevent the onset of diarrhea.[88]

To sum up, although the main evidence about the interaction between microbiota and chemotherapies derives from tumors other than stomach (mainly colorectal), the evidence herein provided can be translated to GC since fluoropyrimidines, irinotecan, platinum and platinum-derived compounds represent the cornerstone treatments for both colorectal and stomach cancers.

Microbiota and immunotherapy

Nowadays, immunotherapy stands out as a breakthrough in cancer treatment.[89] The use of immune checkpoint inhibitors (ICIs), meaning either the anti-programmed death-ligand 1 (PD-L1) nivolumab and pembrolizumab, or the anti-

cytotoxic T-lymphocyte antigen 4 ipilimumab, has become a standard of care in different tumor and line settings, including GC, after having demonstrated both clinical activity and improved survival outcomes.[7,57,58,90-94] The phase III CheckMate 649 study established nivolumab as a new front-line treatment in combination with chemotherapy in patients with metastatic gastroesophageal adenocarcinoma expressing PD-L1 combined positive score $\geq$5.[7] Although deep and durable response to ICIs have been observed in our patients, particularly in microsatellite instable-high subgroup, a significant proportion of patients seem to show an intrinsic resistance, experiencing a rapid disease progression.[7,93,94] Hence, a better comprehension of mechanisms that drive the immune response and cancer cell immune evasion, along with the identification of more efficacious predictive biomarkers, is required. As already addressed in this review, the commensal human microbiota may interplay with immune system, shaping the innate and adaptive immune responses, both in physiological and pathological conditions.[95] Therefore, microbiota modulation appears as an appealing strategy to tailor the efficacy and the tolerability of therapies, in the context of immuno-oncology.

The role of the microbiota as a biomarker of response to ICIs in GC needs an extensive investigation. It has been shown that *H. pylori* could elude the immune response by inducing the up-regulation of PD-L1 in gastric epithelial cells that cause the apoptosis of T cells.[96] In mouse models, the presence of VacA virulence factor could allow *H. pylori* to create an immunosuppressive microenvironment, lastly leading to the inhibition of effector T-cell recruiting and activation.[97] Moreover, *H. pylori* infection could stimulate the production of IL-22 by gastric epithelial cells. The subsequent migration and expansion of myeloid-derived suppressor cells produces proinflammatory cytokines that cause chronic inflammation and decrease the immune response.[98] Recently, the negative link between *H. pylori* infection and response to ICIs has been highlighted, by showing an inhibited CD8+ T-cell infiltration/activation alongside a down-modulation of interferon-γ and IL-6 signaling due to bacterial infection.[99] These data provide the first evidence of gastric microbiota as a biomarker of resistance to ICIs, but novel mechanistic insights are rapidly increasing our knowledge. In their recently published work, Clasen et al. explained that the ability of *H. pylori* to escape the immune system can be ascribed to the presence of a 'silent flagellin'. Flagellin is the protein subunit of bacterium flagellum and is able to tune the innate immune response by stimulating or evading TLR5. Flagellin from *H. pylori* (*Hp*FlaA) fails to bind to TLR5 due to different amino acids in the TLR5 region epitope site.[100] These findings highlight that silent flagellin drives immunotolerance toward commensal bacteria and could represent a promising source of investigation. A further study investigated the relationship between non-*H. pylori* bacteria and immune cell populations in GC microenvironment.[101] Remarkably, intratumoral *Methylobacterium* was correlated with a reduced infiltration of CD3+ and CD8+ T lymphocytes in TME as well as decreased transforming growth factor-β expression and this may be the reason of a shorter relapse-free survival and overall survival reported in this specific subgroup. An investigation led by Kosumi and colleagues evaluated the correlation of *F. nucleatum* and T-cell density in a large cohort of patients with resected esophageal cancer, proving that the abundance of *F. nucleatum* negatively correlated with peritumoral lymphocytic reaction.[102] Finally, preliminary results of the DELIVER study, presented at the American Society of Clinical Oncology (ASCO) GI annual meeting in 2021, demonstrated a role of fecal microbiota as a biomarker of response to nivolumab in patients with advanced GC. Interestingly, metagenomic analyses also showed that *Odoribacter* and *Veillonella* were correlated with response to nivolumab in both training and validation cohorts.[103]

To conclude, although single-center experiences are rapidly emerging with innovative findings, interventional studies in a homogenous population aiming to investigate the potential applicability of microbiome modulation are urgently needed in patients with GC undergoing immunotherapy.

CONCLUSIONS

In the past decade, continuous efforts in translational and clinical investigations have broadened the knowledge about microbiota and GC. Besides the recognized carcinogenic role of *H. pylori*, other microbial species could be involved in the GC pathogenic route, from tumor development to cancer progression. For this reason, the restoration of commensal bacteria homeostasis could play a key role in primary prevention, together with a comprehensive identification of risk factors. Likewise, the identification of biomarkers of response to systemic anticancer treatments provides a compelling line of research that deserves to be further encouraged. Furthermore, appealing therapeutic approaches to improve the efficacy and tolerability of treatments such as the manipulation of human microbiome, through FMT and oral administration of bacterial metabolites, alongside the use of prebiotics and probiotics are constantly emerging. However, although multi-omics studies could offer novel insights and aim to address these open issues, we are still far from applying these innovative strategies in large prospective randomized trials, which should be promptly sponsored.

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REFERENCES

1. Morgan E, Arnold M, Camargo MC, et al. The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: a population-based modelling study. *EClinicalMedicine*. 2022;47:101404.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17-48.
3. Li Y, Feng A, Zheng S, Chen C, Lyu J. Recent estimates and predictions of 5-year survival in patients with gastric cancer: a model-based period analysis. *Cancer Control*. 2022;29:10732748221099227.
4. Petrillo A, Pompella L, Tirino G, et al. Perioperative treatment in resectable gastric cancer: current perspectives and future directions. *Cancers (Basel)*. 2019;11(3):399.
5. De Vita F, Orditura M, Matano E, et al. A phase II study of biweekly oxaliplatin plus infusional 5-fluorouracil and folinic acid (FOLFOX-4) as first-line treatment of advanced gastric cancer patients. *Br J Cancer*. 2005;92(9):1644-1649.
6. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376(9742):687-697 [Published correction appears in *Lancet*. 2010;376(9749):1302].
7. Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet*. 2021;398(10294):27-40.
8. Pape M, Kuijper SC, Vissers PAJ, et al. Beyond median overall survival: estimating trends for multiple survival scenarios in patients with metastatic esophagogastric cancer. *J Natl Compr Canc Netw*. 2022;20(12):1321-1329.e4.
9. Afzaal M, Saeed F, Shah YA, et al. Human gut microbiota in health and disease: unveiling the relationship. *Front Microbiol*. 2022;13:999001.
10. Yang Y, Wang X, Huycke T, Moore DR, Lightfoot SA, Huycke MM. Colon macrophages polarized by commensal bacteria cause colitis and cancer through the bystander effect. *Transl Oncol*. 2013;6(5):596-606.
11. Sheflin AM, Whitney AK, Weir TL. Cancer-promoting effects of microbial dysbiosis. *Curr Oncol Rep*. 2014;16(10):406.
12. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet*. 1983;1(8336):1273-1275.
13. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*. 1984;1(8390):1311-1315.
14. Wroblewski LE, Peek RM Jr, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clin Microbiol Rev*. 2010;23(4):713-739.
15. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med*. 2001;345(11):784-789.
16. Li D, Jiang SF, Lei NY, Shah SC, Corley DA. Effect of Helicobacter pylori eradication therapy on the incidence of noncardia gastric adenocarcinoma in a large diverse population in the United States. *Gastroenterology*. 2023;165(2):391-401.e2.
17. Sung J, Kim N, Kim J, et al. Comparison of gastric microbiota between gastric juice and mucosa by next generation sequencing method. *J Cancer Prev*. 2016;21(1):60-65.
18. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. *Nutr Rev*. 2012;70(suppl 1):S38-S44.
19. Rinninella E, Raoul P, Cintoni M, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*. 2019;7(1):14.
20. Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol*. 2005;71:8228-8235.
21. de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. *Gut*. 2022;71(5):1020-1032.
22. Vesper BJ, Jawdi A, Altman KW, Haines GK 3rd, Tao L, Radosevich JA. The effect of proton pump inhibitors on the human microbiota. *Curr Drug Metab*. 2009;10(1):84-89.
23. Nardone G, Compare D. The human gastric microbiota: is it time to rethink the pathogenesis of stomach diseases? *United European Gastroenterol J*. 2015;3(3):255-260.
24. Yang J, Zhou X, Liu X, Ling Z, Ji F. Role of the gastric microbiome in gastric cancer: from carcinogenesis to treatment. *Front Microbiol*. 2021;12:641322.
25. Zilberstein B, Quintanilha AG, Santos MA, et al. Digestive tract microbiota in healthy volunteers. *Clinics (Sao Paulo)*. 2007;62(1):47-54 [Published correction appears in *Clinics*. 2007;62(2):206].
26. Ianiro G, Molina-Infante J, Gasbarrini A. Gastric microbiota. *Helicobacter*. 2015;20(suppl 1):68-71.
27. Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci U S A*. 2006;103(3):732-737.
28. Li XX, Wong GL, To KF, et al. Bacterial microbiota profiling in gastritis without *Helicobacter pylori* infection or non-steroidal anti-inflammatory drug use. *PLoS One*. 2009;4(11):e7985.
29. Osaki T, Matsuki T, Asahara T, et al. Comparative analysis of gastric bacterial microbiota in Mongolian gerbils after long-term infection with *Helicobacter pylori*. *Microb Pathog*. 2012;53(1):12-18.
30. Khosravi Y, Dieye Y, Poh BH, et al. Culturable bacterial microbiota of the stomach of Helicobacter pylori positive and negative gastric disease patients. *ScientificWorldJournal*. 2014;2014:610421.
31. Maldonado-Contreras A, Goldfarb KC, Godoy-Vitorino F, et al. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. *ISME J*. 2011;5(4):574-579.
32. He Y, Huang J, Li Q, et al. Gut microbiota and tumor immune escape: a new perspective for improving tumor immunotherapy. *Cancers (Basel)*. 2022;14(21):5317.
33. Bessède E, Mégraud F. Microbiota and gastric cancer. *Semin Cancer Biol*. 2022;86(Pt 3):11-17.
34. Wang TC, Koh TJ, Varro A, et al. Processing and proliferative effects of human progastrin in transgenic mice. *J Clin Invest*. 1996;98(8):1918-1929.

35. Lofgren JL, Whary MT, Ge Z, et al. Lack of commensal flora in Helicobacter pylori-infected INS-GAS mice reduce gastritis and delays intraepithelial neoplasia. *Gastroenterology*. 2011;140(1):210-220.
36. Lertpiriyapong K, Whary MT, Muthupalani S, et al. Gastric colonisation with a restricted commensal microbiota replicates the promotion of neoplastic lesions by diverse intestinal microbiota in the Helicobacter pylori INS-GAS mouse model of gastric carcinogenesis. *Gut*. 2014;63(1):54-63.
37. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. *Gut*. 2018;67(2):226-236.
38. Coker OO, Dai Z, Nie Y, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. *Gut*. 2018;67(6):1024-1032.
39. Wang L, Zhou J, Xin Y, et al. Bacterial overgrowth and diversification of microbiota in gastric cancer. *Eur J Gastroenterol Hepatol*. 2016;28(3):261-266.
40. Park JY, Seo H, Kang CS, et al. Dysbiotic change in gastric microbiome and its functional implication in gastric carcinogenesis. *Sci Rep*. 2022;12:4285.
41. Liu D, Zhang R, Chen S, Sun B, Zhang K. Analysis of gastric microbiome reveals three distinctive microbial communities associated with the occurrence of gastric cancer. *BMC Microbiol*. 2022;22(1):184.
42. Stewart OA, Wu F, Chen Y. The role of gastric microbiota in gastric cancer. *Gut Microbes*. 2020;11(5):1220-1230.
43. Castaño-Rodríguez N, Goh KL, Fock KM, Mitchell HM, Kaakoush NO. Dysbiosis of the microbiome in gastric carcinogenesis. *Sci Rep*. 2017;7(1):15957.
44. Tricker AR, Preussmann R. Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. *Mutat Res*. 1991;259(3-4):277-289.
45. De Witte C, Flahou B, Ducatelle R, et al. Detection, isolation and characterization of Fusobacterium gastrosuis sp. nov. colonizing the stomach of pigs. *Syst Appl Microbiol*. 2017;40(1):42-50.
46. Castellarin M, Warren RL, Freeman JD, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res*. 2012;22(2):299-306.
47. Şurlin P, Nicolae FM, Şurlin VM, et al. Could periodontal disease through periopathogen fusobacterium nucleatum be an aggravating factor for gastric cancer? *J Clin Med*. 2020;9(12):3885.
48. Chen XH, Wang A, Chu AN, Gong YH, Yuan Y. Mucosa-associated microbiota in gastric cancer tissues compared with non-cancer tissues. *Front Microbiol*. 2019;10:1261.
49. Nie S, Wang A, Yuan Y. Comparison of clinicopathological parameters, prognosis, micro-ecological environment and metabolic function of gastric cancer with or without Fusobacterium sp. infection. *J Cancer*. 2021;12(4):1023-1032.
50. Boehm ET, Thon C, Kupcinskas J, et al. Fusobacterium nucleatum is associated with worse prognosis in Lauren's diffuse type gastric cancer patients. *Sci Rep*. 2020;10:16240.
51. Liu X, Shao L, Liu X, et al. Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. *EBioMedicine*. 2019;40:336-348.
52. Montalban-Arques A, Wurm P, Trajanoski S, et al. Propionibacterium acnes overabundance and natural killer group 2 member D system activation in corpus-dominant lymphocytic gastritis. *J Pathol*. 2016;240(4):425-436.
53. Park CH, Lee AR, Lee YR, Eun CS, Lee SK, Han DS. Evaluation of gastric microbiome and metagenomic function in patients with intestinal metaplasia using 16S rRNA gene sequencing. *Helicobacter*. 2019;24(1):e12547.
54. Kwok T, Zabler D, Urman S, et al. Helicobacter exploits integrin for type IV secretion and kinase activation. *Nature*. 2007;449(7164):862-866.
55. Hu YL, Pang W, Huang Y, Zhang Y, Zhang CJ. The gastric microbiome is perturbed in advanced gastric adenocarcinoma identified through shotgun metagenomics. *Front Cell Infect Microbiol*. 2018;8:433.
56. Lordick F, Carneiro F, Cascinu S, et al. Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2022;33(10):1005-1020.
57. Ajani JA, D'Amico TA, Bentrem DJ, et al. Gastric cancer, Version 2. 2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2022;20(2):167-192.
58. Smet A, Kupcinskas J, Link A, Hold GL, Bornschein J. The role of microbiota in gastrointestinal cancer and cancer treatment: chance or curse? *Cell Mol Gastroenterol Hepatol*. 2022;13(3):857-874.
59. Tarazi M, Jamel S, Mullish BH, Markar SR, Hanna GB. Impact of gastrointestinal surgery upon the gut microbiome: a systematic review. *Surgery*. 2022;171(5):1331-1340.
60. Quercia I, Dutia R, Kotler DP, Belsley S, Laferrère B. Gastrointestinal changes after bariatric surgery. *Diabetes Metab*. 2014;40(2):87-94.
61. Tseng CH, Lin JT, Ho HJ, et al. Gastric microbiota and predicted gene functions are altered after subtotal gastrectomy in patients with gastric cancer. *Sci Rep*. 2016;6:20701.
62. Maksimaityte V, Bausys A, Kryzauskas M, et al. Gastrectomy impact on the gut microbiome in patients with gastric cancer: a comprehensive review. *World J Gastrointest Surg*. 2021;13(7):678-688.
63. Erawijantari PP, Mizutani S, Shiroma H, et al. Influence of gastrectomy for gastric cancer treatment on faecal microbiome and metabolome profiles. *Gut*. 2020;69(8):1404-1415.
64. Lin XH, Huang KH, Chuang WH, et al. The long-term effect of metabolic profile and microbiota status in early gastric cancer patients after subtotal gastrectomy. *PLoS One*. 2018;13(11):e0206930.
65. Eom BW, Lee HJ, Yoo MW, et al. Synchronous and metachronous cancers in patients with gastric cancer. *J Surg Oncol*. 2008;98(2):106-110.
66. Kim C, Chon HJ, Kang B, et al. Prediction of metachronous multiple primary cancers following the curative resection of gastric cancer. *BMC Cancer*. 2013;13:394.
67. Wu N, Feng YQ, Lyu N, Wang D, Yu WD, Hu YF. Fusobacterium nucleatum promotes colon cancer progression by changing the mucosal microbiota and colon transcriptome in a mouse model. *World J Gastroenterol*. 2022;28(18):1981-1995.
68. Wu J, Li Q, Fu X. Fusobacterium nucleatum contributes to the carcinogenesis of colorectal cancer by inducing inflammation and suppressing host immunity. *Transl Oncol*. 2019;12(6):846-851.
69. Bernstein C, Holubec H, Bhattacharyya AK, et al. Carcinogenicity of deoxycholate, a secondary bile acid. *Arch Toxicol*. 2011;85(8):863-871.
70. Li N, Zhan S, Tian Z, et al. Alterations in bile acid metabolism associated with inflammatory bowel disease. *Inflamm Bowel Dis*. 2021;27(9):1525-1540.
71. Nguyen TT, Ung TT, Kim NH, Jung YD. Role of bile acids in colon carcinogenesis. *World J Clin Cases*. 2018;6(13):577-588.
72. Wallace BD, Wang H, Lane KT, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science*. 2010;330(6005):831-835.
73. Lin XB, Farhangfar A, Valcheva R, et al. The role of intestinal microbiota in development of irinotecan toxicity and in toxicity reduction through dietary fibres in rats. *PLoS One*. 2014;9(1):e83644.
74. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer*. 2003;3(5):330-338.
75. Bronckaers A, Balzarini J, Liekens S. The cytostatic activity of pyrimidine nucleosides is strongly modulated by Mycoplasma hyorhinis infection: implications for cancer therapy. *Biochem Pharmacol*. 2008;76(2):188-197.
76. Yu T, Guo F, Yu Y, et al. Fusobacterium nucleatum promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell*. 2017;170(3):548-563.e16.
77. Yuan L, Zhang S, Li H, et al. The influence of gut microbiota dysbiosis to the efficacy of 5-Fluorouracil treatment on colorectal cancer. *Biomed Pharmacother*. 2018;108:184-193.
78. Sougiannis AT, VanderVeen BN, Enos RT, et al. Impact of 5 fluorouracil chemotherapy on gut inflammation, functional parameters, and gut microbiota. *Brain Behav Immun*. 2019;80:44-55.
79. de Clercq NC, van den Ende T, Prodan A, et al. Fecal microbiota transplantation from overweight or obese donors in cachectic patients with advanced gastroesophageal cancer: a randomized, double-blind, placebo-controlled, phase II study. *Clin Cancer Res*. 2021;27(13):3784-3792.

80. Gui QF, Lu HF, Zhang CX, Xu ZR, Yang YH. Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. *Genet Mol Res*. 2015;14(2):5642-5651.
81. Iida N, Dzutsev A, Stewart CA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science*. 2013;342(6161):967-970.
82. He Y, Fu L, Li Y, et al. Gut microbial metabolites facilitate anticancer therapy efficacy by modulating cytotoxic $CD8^+$ T cell immunity. *Cell Metab*. 2021;33(5):988-1000.e7.
83. Shen S, Lim G, You Z, et al. Gut microbiota is critical for the induction of chemotherapy-induced pain. *Nat Neurosci*. 2017;20:1213-1216.
84. Ma P, Mo R, Liao H, et al. Gut microbiota depletion by antibiotics ameliorates somatic neuropathic pain induced by nerve injury, chemotherapy, and diabetes in mice. *J Neuroinflammation*. 2022;19(1):169.
85. Maugeri-Saccà M, Pizzuti L, Sergi D, et al. FOLFIRI as a second-line therapy in patients with docetaxel-pretreated gastric cancer: a historical cohort. *J Exp Clin Cancer Res*. 2013;32(1):67.
86. Smith NF, Figg WD, Sparreboom A. Pharmacogenetics of irinotecan metabolism and transport: an update. *Toxicol In Vitro*. 2006;20(2):163-175.
87. Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, Keefe DM. Faecal microflora and beta-glucuronidase expression are altered in an irinotecan-induced diarrhea model in rats. *Cancer Biol Ther*. 2008;7(12):1919-1925.
88. Bhatt AP, Pellock SJ, Biernat KA, et al. Targeted inhibition of gut bacterial β-glucuronidase activity enhances anticancer drug efficacy. *Proc Natl Acad Sci U S A*. 2020;117(13):7374-7381.
89. Esfahani K, Roudaia L, Buhlaiga N, Del Rincon SV, Papneja N, Miller WH Jr. A review of cancer immunotherapy: from the past, to the present, to the future. *Curr Oncol*. 2020;27(suppl 2):S87-S97.
90. Smyth EC, Gambardella V, Cervantes A, Fleitas T. Checkpoint inhibitors for gastroesophageal cancers: dissecting heterogeneity to better understand their role in first-line and adjuvant therapy. *Ann Oncol*. 2021;32(5):590-599.
91. Kang YK, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;390(10111):2461-2471.
92. Fuchs CS, Doi T, Jang RW, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. *JAMA Oncol*. 2018;4(5):e180013 [Published correction appears in *JAMA Oncol*. 2019;5(4):579].
93. Shitara K, Ajani JA, Moehler M, et al. Nivolumab plus chemotherapy or ipilimumab in gastro-oesophageal cancer. *Nature*. 2022;603(7903):942-948.
94. Chao J, Fuchs CS, Shitara K, et al. Assessment of pembrolizumab therapy for the treatment of microsatellite instability-high gastric or gastroesophageal junction cancer among patients in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 clinical trials. *JAMA Oncol*. 2021;7(6):895-902.
95. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res*. 2020;30:492-506.
96. Wu YY, Lin CW, Cheng KS, et al. Increased programmed death-ligand-1 expression in human gastric epithelial cells in Helicobacter pylori infection. *Clin Exp Immunol*. 2010;161(3):551-559.
97. Altobelli A, Bauer M, Velez K, Cover TL, Müller A. *Helicobacter pylori* VacA targets myeloid cells in the gastric lamina propria to promote peripherally induced regulatory T-cell differentiation and persistent infection. *mBio*. 2019;10(2):e00261-19.
98. Zhuang Y, Cheng P, Liu XF, et al. A pro-inflammatory role for Th22 cells in Helicobacter pylori-associated gastritis. *Gut*. 2015;64(9):1368-1378.
99. Oster P, Vaillant L, Riva E, et al. *Helicobacter pylori* infection has a detrimental impact on the efficacy of cancer immunotherapies. *Gut*. 2022;71(3):457-466.
100. Clasen SJ, Bell MEW, Borbón A, et al. Silent recognition of flagellins from human gut commensal bacteria by Toll-like receptor 5. *Sci Immunol*. 2023;8(79):eabq7001.
101. Peng R, Liu S, You W, et al. Gastric microbiome alterations are associated with decreased CD8+ tissue-resident memory T cells in the tumor microenvironment of gastric cancer. *Cancer Immunol Res*. 2022;10(10):1224-1240.
102. Kosumi K, Baba Y, Yamamura K, et al. Intratumour Fusobacterium nucleatum and immune response to oesophageal cancer. *Br J Cancer*. 2023;128(6):1155-1165.
103. Sunakawa Y, Matoba R, Inoue E, et al. Genomic pathway of gut microbiome to predict efficacy of nivolumab in advanced gastric cancer: DELIVER trial (JACCRO GC-08). *JCO*. 2021;39(suppl 3):161.