Critical Reviews in Oncology / Hematology 143 (2019) 139–147

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Gut microbiota and cancer: How gut microbiota modulates activity, efficacy and toxicity of antitumoral therapy

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ARTICLE INFO

Keywords:
Microbiota
Gemcitabine
Cyclophosphamide
Irinotecan
Cisplatin
5-Fluorouracil
Immune checkpoint inhibitors
Tyrosine kinase inhibitors

ABSTRACT

Gut microbiota is involved in gastrointestinal carcinogenesis. Also, it modulates the activity, efficacy and toxicity of several chemotherapy agents, such as gemcitabine, cyclophosphamide, irinotecan, cisplatin and 5-Fluorouracil, and target therapy, such as tyrosine kinase inhibitors. More recently, accumulating data suggest that the composition of gut microbiota may also affect efficacy and toxicity of cancer immunotherapy. Therefore, the manipulation of gut microbiota through antibiotics, probiotics, prebiotics or fecal transplantation has been investigating with the aim to improve efficacy and mitigate toxicity of anticancer drugs.

1. Introduction

The human microbiota is composed of bacteria and other microorganisms (fungi, protozoa and viruses) that inhabit the epithelial barrier surfaces (Costello et al., 2012). The gut microbiota gives the major contribution to human microbiota and comprises about 3×10^{13} bacterial cells, mostly in a commensal relationship with the host (Sender et al., 2016). Nonetheless, when the intestinal ecology is altered, commensal bacteria (for example, *Clostridium difficile, vancomycin-resistant Enterococcus*) may expand and acquire pathogenic characteristics (Chow et al., 2011). The bacteria most represented in the colon are: *Firmicutes*, *Bacteroides*, *Actinobacteria* and *Proteobacteria* (Cho and Blaser, 2012) (Fig. 1).

The composition of gut microbiota shows wide interpersonal variation (Eckburg et al., 2005; Bogaert et al., 2011) but relatively temporal stability in a single individual (Costello et al., 2009). It is conditioned by hereditary factors (inheritance from the mother through direct surface contact: vaginal during the passage through birth canal, cutaneous, oral, mammary through breastfeeding) (Cho and Blaser, 2012), ambient factors (as antibiotic exposure or enteric infections) and lifestyle (mainly diet).

Accumulating evidence show that gut microbiota is involved in the carcinogenesis and also modulates the activity, efficacy and toxicity of antitumoral therapy (Picardo et al., 2019) (Table 1). Targeting the microbiota could improve efficacy and prevent toxicity of anticancer drugs.

2. Intestinal microbiota and carcinogenesis

Accumulating evidence suggest that gut microbiota may be involved in intestinal carcinogenesis (Helmink et al., 2019). Besides known oncogenic bacteria, such as *Helicobacter pylori* implicated in the

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https://doi.org/10.1016/j.critrevonc.2019.09.003
Received 30 July 2019; Received in revised form 5 September 2019; Accepted 8 September 2019

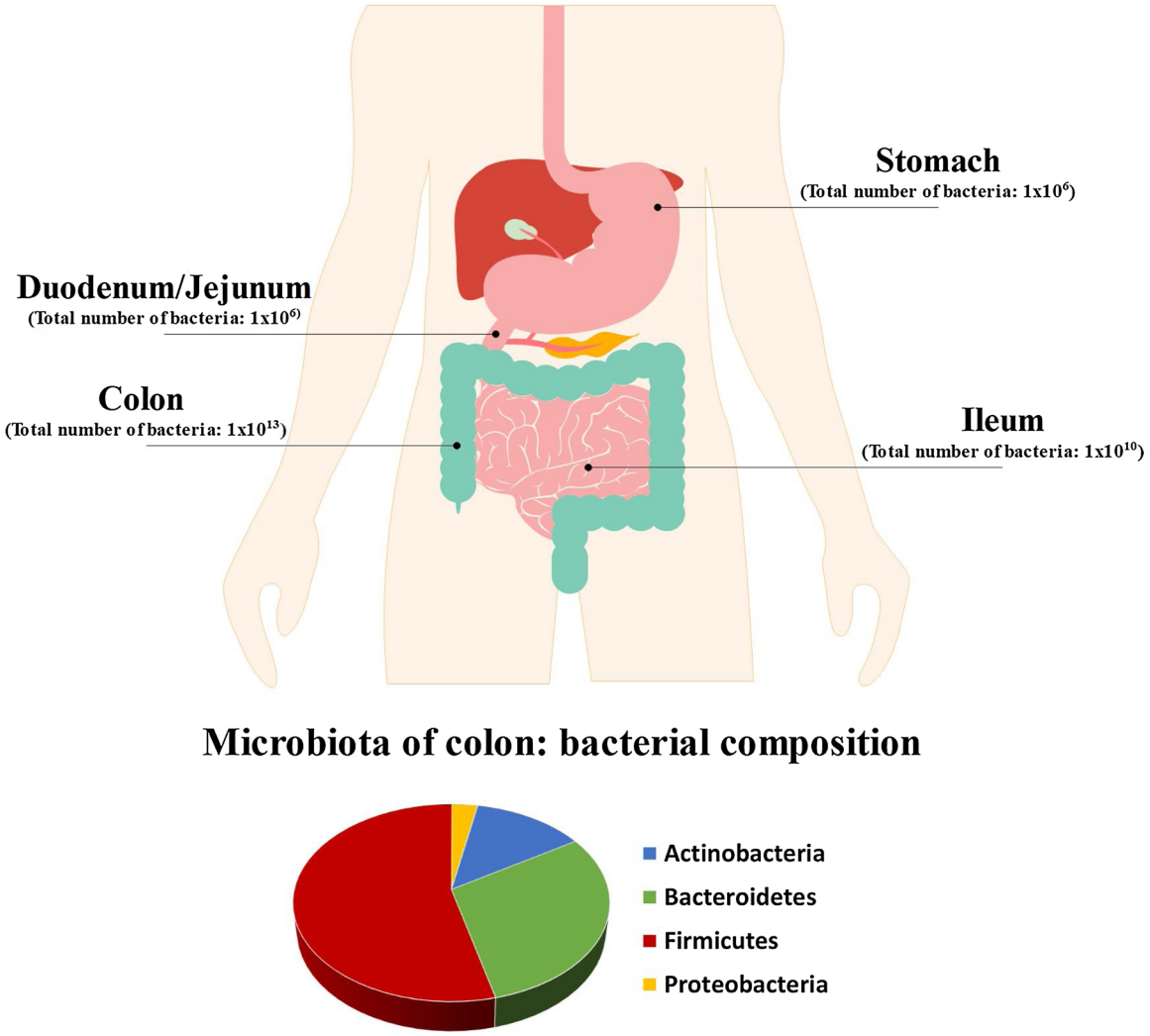

Fig. 1. Total number of bacteria in the gut microbiota according to anatomic site. Microbiota of colon: bacterial composition. The gut microbiota gives the major contribution to human microbiota and comprises about 3×10^{13} bacterial cells, mostly commensal with the host (Sender et al., 2016). The bacteria most represented in the colon are: *Firmicutes*, *Bacteroides*, *Actinobacteria* and *Proteobacteria* (Cho and Blaser, 2012).

Table 1
Impact of microbiota on activity, efficacy and toxicity of anticancer drugs.

DRUG	ACTIVITY/EFFICACY	TOXICITY	References
5-FU	Antibiotics diminished the antitumor efficacy of 5-FU in mice	According to preclinical studies, 5-FU causes an imbalance of gut microbiota (with a shift from commensal bacteria to *Escherichia*, *Clostridium* and *Enterococcus spp.*), potentially leading to intestinal mucositis, bacteremia or sepsis.	Hamouda et al. (2017), Yuan et al. (2018)
CISPLATIN	Anti-Gram-positive antibiotics were associated to reduced anticancer efficacy of platinum salts in animal models	D-methionine protects against cisplatin toxicity by promoting growth of beneficial bacteria (*Lachnospiraceae and Lactobacillus*)	Pflug et al. (2016), Perales-Puchalt et al. (2018)
CYCLOPHOSFAMIDE	Anti-Gram-positive antibiotics were associated with resistance to cyclophosphamide in murine models. The anticancer effect of cyclophosphamide was re-established by oral administration of *Enterobacter Hirae*	–	Viaud et al. (2013); Daillère et al. (2016)
GEMCITABINE	The co-administration of antibiotics could potentiate activity of gemcitabine	–	Geller et al. (2017)
IMMUNOTHERAPY	Dysbiosis from prior antibiotic therapy resulted in shorter survival in patients treated with immune checkpoint inhibitors	Intestinal reconstitution of antibiotic-treated mice with the combination of *Bacteroides fragilis* and *Burkholderia cepacia*, reduced histopathological signs of colitis induced by anti-CTLA-4 antibody	De Rosa et al. (2018); Routy et al. (2018), Vétizou et al. (2015)
IRINOTECAN	–	Inhibition of bacterial ß-glucuronidase produced by *Escherichia coli* reduces incidence of irinotecan-induced diarrhea	Wallace et al. (2010)
TKIs	Targeting stool *Bacteroides spp.* with antibiotics improves PFS in mRCC patients receiving VEGF-TKIs	VEGF-TKI-induced diarrhea is associated with higher levels of stool *Bacteroides spp*	Pal et al. (2015a, 2015b), Hahn et al. (2018)

5-FU: 5-fluorouracil; CTLA-4: cytotoxic lymphocyte antigen 4; VEGF: vascular endothelial growth factor; TKIs: tyrosine kinase inhibitors;

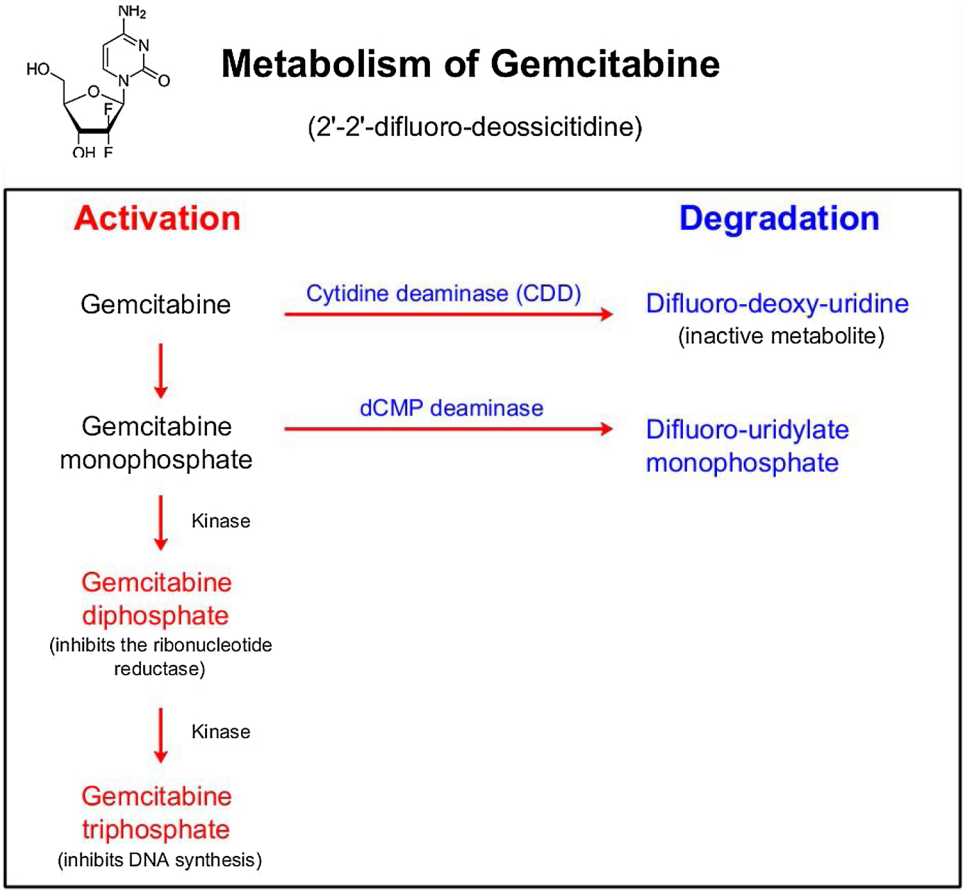

Fig. 2. Gemcitabine metabolism. The antitumor activity of gemcitabine is the result of intracellular activation and degradation. Gemcitabine diphosphate and monophosphate are active metabolites that inhibit ribonucleotide reductase and DNA synthesis, respectively, leading to cancer cell death. Cytidine deaminase (CDD) is the main enzyme involved in gemcitabine inactivation. A long isoform of CDD (CDD_L) is produced by *Gammaproteobacteria* in the intestinal lumen and may contribute to gemcitabine resistance (Geller et al., 2017).

development of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT lymphoma), gut microbiota may contribute to carcinogenesis through dysbiosis.

Dysbiosis is an alteration in microbiota composition that could lead to disruption of physiological homeostasis of intestinal epithelial cells (von Frieling et al., 2018). Causes of dysbiosis include diet changes, antibiotic therapies, and inflammatory intestinal diseases (Cho and Blaser, 2012). In presence of dysbiosis, a breach in the mucosal barriers may occur. Once mucosal barriers are permanently breached, microbiota may affect carcinogenesis through a number of several mechanisms including DNA damage, production of carcinogenic metabolites, alteration of β-catenin signaling, engagement of pro-inflammatory pathways, and inhibition of immune system (Garrett, 2015):

a) ***DNA damage.***

Bacterial toxins can directly or indirectly damage host DNA. Several bacterial toxins can directly damage host DNA, as the Cytolethal Distending Toxin (CDT), produced by some ε- and γ-proteobacteria, and the Colibactin, produced by polyketide synthases–positive (pks+) *Escherichia coli* (Nougayrède et al., 2006). Both CDT and Colibactin can cause damage of DNA in mammalian cells (Guerra et al., 2011b). Some bacterial toxins can indirectly damage host DNA, such as *Bacteroides fragilis* toxin (Bft) produced by enterotoxigenic *Bacteroides fragilis*. This toxin acts indirectly by eliciting high levels of reactive oxygen species (ROS) which in turn damage host DNA (Goodwin et al., 2011).

When DNA damage exceeds the capacity of host cell repair, cell death or oncogenic mutations occur.

a) ***Metabolism.***

Gut microbiota affect the metabolism of vitamins and nutrients, xenobiotic and host-derived compounds, and this could at least in part explain the observed association between dietary habits and cancer development (Nicholson et al., 2012). In fact, bacterial metabolism can lead to the formation of carcinogenic products, such as aromatic amines and sulphide from bile acids and proteins, or acetaldehyde from alcohol.

a) ***Alteration of β-catenin signaling pathway.***

An alteration of β-catenin pathway causes dysregulation of cellular growth, acquisition of stem cell-like characteristics, and cellular polarity loss (Clevers and Nusse, 2012).

Activation of this pathway can be due to direct bond of microbial proteins with E-cadherin of host epithelial cells, as reported for FadA adhesin, a cell surface adhesion component expressed by *Fusobacterium nucleatum,* that is a bacterium associated with human colorectal cancer (Rubinstein et al., 2013). Instead, other microbes active β-catenin injecting directly into the cytoplasm of host cells some effectors (for example, CagA expressed by *Helicobacter pylori*) (Abreu and Peek, 2014).

a) ***Engagement of pro-inflammatory pathways.***

Loss of integrity of mucosal barriers stimulates pro-inflammatory programs with activation of pathways (such as NF-κB and STAT3) that are known to be involved in carcinogenesis (Garrett, 2015).

a) ***Immune dysregulation.***

Although human microbiota seems to sustain anticancer immunosurveillance by broadening T-cell receptor repertoire and enhancing immune response, some bacteria may suppress host immunity. For example, *F. nucleatum* expresses Fap2 cell surface protein that inhibits

immune cytotoxicity through interaction with T and Natural Killer cells (Gur et al., 2015).

Despite growing evidence from animal and human studies, large longitudinal cohort studies are needed to confirm the role of human microbiota as a key driver in the pathogenesis of cancer (Scott et al., 2019).

3. Intestinal microbiota and chemotherapy

Gut microbiota can modulate the metabolism of chemotherapy drugs thus affecting the cancer response to chemotherapy and also the susceptibility of host to toxic effects (Table 1).

3.1. Gemcitabine

Gemcitabine (2′-2′-difluoro-deossicitidine) is an antimetabolite drug. It is an antagonist of pyrimidines, which competes with the physiological nucleotide deoxycytidine during DNA synthesis.

The antitumor activity of gemcitabine (used in the treatment of pancreatic cancer, non-small cell lung cancer, breast cancer, bladder cancer, ovary cancer, sarcoma) is the result of intracellular activation and degradation (Fig. 2). Degradation of gemcitabine occurs through transformation into the inactive metabolite difluoro-deoxy-uridine by cytidine deaminase (CDD).

Data from murine models of colon cancer showed that resistance to gemcitabine can be the result of increased metabolic degradation of the drug into difluoro-deoxy-uridine, due to the expression of a long isoform of the bacterial enzyme cytidine deaminase (CDD_L), seen primarily in *Gammaproteobacteria* (Geller et al., 2017; Choy et al., 2018). In fact, in mice with a subcutaneous model of colon cancer (MC-26 cells), the injection into the tail vein of CDD-wild-type *Escherichia coli* but not that of CDD-deficient *Escherichia coli* was associated with resistance to gemcitabine. This resistance was caused by increased degradation of gemcitabine by bacterial CDD. Instead, in presence of CDD-deficient *Escherichia coli,* gemcitabine was not degraded into the inactive metabolite and inhibited tumor growth. Moreover, the addition of ciprofloxacin to gemcitabine increased the gemcitabine antitumor activity through the inhibition of bacterial growth obtained by ciprofloxacin itself (Geller et al., 2017). These results also demonstrated that the modulation of intestinal microbiota can influence the activity of gemcitabine in murine models.

Several clinical trials showed only modest activity (objective response rate of 7–9.4%) and poor efficacy (median overall survival of about 6.8 months) for gemcitabine in advanced pancreatic adenocarcinoma (Burris et al., 1997; Cunningham et al., 2009; Conroy et al., 2011). To evaluate the hypothesis that the resistance to gemcitabine could be caused by intratumor bacteria, Geller et al (Geller et al., 2017) analyzed tissue samples obtained from normal human pancreas and from pancreatic cancer. Bacterial DNA was found in 86 out of 113 (76%) human pancreatic tumor samples obtained during cancer surgery and in only 3 out of 20 (15%) normal human pancreatic tissues obtained from organ donors ($p < 0.005$). The most common species identified in human pancreatic tumor samples were *Gammaproteobacteria*, mostly members of the *Enterobacteriaceae* and *Pseudomonaceae families* (Geller et al., 2017). *Proteobacteria* were abundant in duodenum, suggesting that retrograde bacterial migration from the duodenum through pancreatic duct could be a source of bacteria in pancreatic ductal adenocarcinoma.

Moreover, patients who underwent instrumentation of the pancreatic duct had significantly more bacteria in their tumors compared to patients that did not receive this procedure ($p < 0.05$) (Geller et al., 2017). Probably, pancreatic duct instrumentations could increase the risk of bacterial contamination of the tumor microenvironment and, consequently, may lead to gemcitabine resistance.

All these results show that human pancreatic ductal adenocarcinoma contained bacteria that can potentially modulate activity of gemcitabine (Geller et al., 2017; Choy et al., 2018).

3.2. Cyclophosphamide

Cyclophosphamide is an alkylating agent used in many solid and hematological cancers, but, as other chemotherapy drugs, also acts by stimulating the anticancer immune response (Kroemer et al., 2013). Studying murine models, it was demonstrated that cyclophosphamide, by inducing dysbiosis in small intestine with consequent destruction of the mucosa integrity, causes accumulation of mononuclear cells in the lamina propria and translocation of Gram-positive germs in the mesenteric lymph nodes and in the spleen (Viaud et al., 2013). In splenic cells, cyclophosphamide increases the production of interleukin-17 (IL-17), interferon gamma (IFNγ) and T-helper cells (Th17) producing IL17 (Viaud et al., 2013). Germ-free mouse models of cancer that have been treated with antibiotics to kill Gram-positive bacteria show a reduction of Th17 responses and resistance to cyclophosphamide.

These results obtained in murine models underline these concepts:

1 the administration of antibiotics during cancer treatment blocks the cyclophosphamide-mediated immune response;
2 the manipulation of intestinal microbiota could be of therapeutic utility; in fact, in mouse models treated with antibiotics, the anticancer effect of cyclophosphamide is re-established by oral administration of *Enterobacter Hirae* (Gram-positive germ) (Daillère et al., 2016).

3.3. Irinotecan (CPT-11)

Irinotecan (CPT-11), a topoisomerase I inhibitor, which inhibits DNA replication, is used in the treatment of advanced colorectal, gastric, pancreatic and small cell lung cancer.

Irinotecan is a prodrug, which is activated to SN-38 by cleavage of the side chain by the enzyme carboxylesterase (CE) in plasma, intestinal mucosa, liver and tumor cells (Wallace et al., 2010). The inactivation of the active form (SN-38) to glucuronide form (SN-38 G) is catalyzed by uridine 5′-diphospho-glucuronosyltransferase (UGT). The inactive form (SN-38 G) is excreted with the bile into intestinal lumen (Fig. 3).

Specifically, hepatic UGTA1 and UGT1A9, as well as extrahepatic UGT1A7, play a major role in the detoxification of SN-38. UGT1A1 gene polymorphisms have been linked to elevated and prolonged levels of SN-38 in the plasma with myelosuppressive effects of irinotecan.

Irinotecan can also cause early or delayed diarrhea. Early-onset diarrhea occurs during drug infusion and up to six hours after the infusion and is caused by a cholinergic-mediated event; it is not associated with metabolism and can be prevented or ameliorated with atropine. In contrast, delayed diarrhea generally occurs more than 24 h after irinotecan administration and is associated with metabolism. When in the intestinal lumen biliary-excreted SN-38 G (inactive form) is converted back to SN-38 by enteric bacterial ß-glucuronidase, produced by *Escherichia coli*, the re-generated active metabolite SN-38 can cause direct enteric injury and diarrhea. This type of diarrhea is generally treated with loperamide. Four potent inhibitors of ß-glucuronidase were identified (Wallace et al., 2010). These inhibitors are active against the bacterial ß-glucuronidase enzyme, both *in vitro* and *in vivo*, without affecting bacterial cell growth or survival under aerobic or anaerobic conditions and without killing mammalian epithelial cells. In murine models, the administration of CPT-11 together with an inhibitor of bacterial ß-glucuronidase protected mice from irinotecan-related diarrhea (Wallace et al., 2010). These data support the hypothesis that the inhibition of ß-glucuronidase enzyme present in bacterial symbiotes can prevent the gastrointestinal toxicity of CPT-11 metabolites.

3.4. Cisplatin

Cisplatin is effective and widely used alone or in combination for

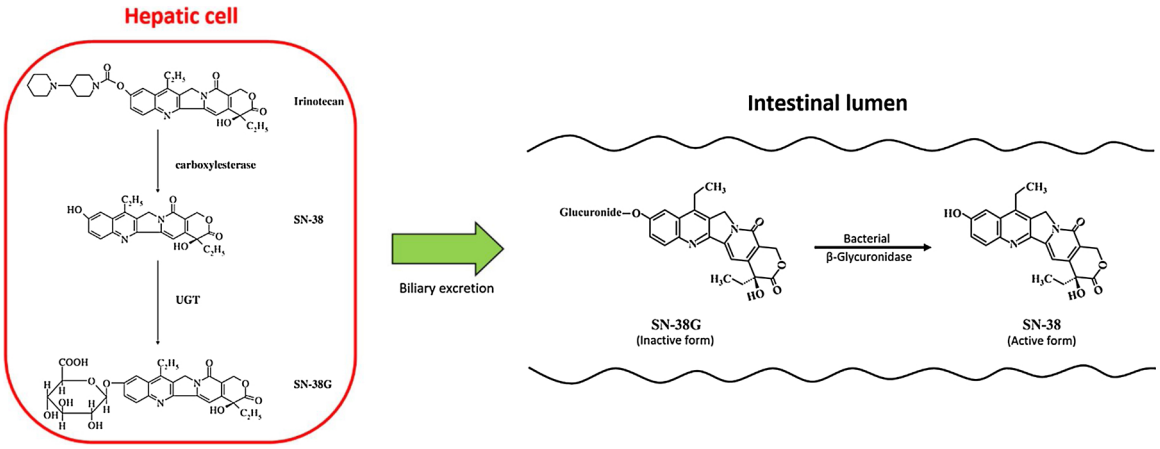

Fig. 3. Irinotecan (CPT-11) metabolism. In the liver, irinotecan is activated to SN-38 by carboxylesterase (CE) 1 and 2. The inactivation of SN-38 to the glucuronide form (SN-38 G) is catalyzed by enzyme uridine-diphosphate-glucuronosyltransferase (UGT) 1A1 and 1A9. The inactive form (SN-38 G) is excreted with the bile into intestinal lumen, where its conversion back to active form (SN-38) is catalyzed by enteric bacterial β-glucuronidase (Wallace et al., 2010).

the treatment of several advanced solid tumors, such as head and neck, ovarian, cervical, biliary tract, and lung cancer. Cisplatin is known to have antibiotic effects on both Gram-negative and Gram-positive bacterial strains, such as some *Bacillus* and *E. coli*, and may induce dysbiosis (Joyce et al., 2010). At the same time, cisplatin may also cause loss of integrity of the intestinal mucosa by binding to DNA thus impairing DNA replication of rapid proliferating epithelial cells. This damage results in a breach of mucosal barriers, thus leading to infections that can be also life-threatening. (Taur and Pamer, 2016). The reconstitution of the full repertoire of intestinal bacteria altered by cisplatin accelerates healing of the intestinal epithelium and ameliorates systemic inflammation. Therefore, fecal microbiota transplant may potentially prevent life-threatening sepsis in cancer patients treated with cisplatin (Perales-Puchalt et al., 2018).

Gut microbiota is involved in the modulation of other common side effects of cisplatin, such as ototoxicity, mucositis and weight loss.

Campbell *at al.* showed that D-methionine protects against cisplatin-induced ototoxicity in rats (Campbell et al., 1996). Double-blind placebo-controlled multicenter phase II trial revealed that oral D-methionine exhibited a protective effect against cisplatin induced mucositis (Hamstra et al., 2018) and did not influence tumor response to cisplatin (Vuyyuri et al., 2008; Hamstra et al., 2010). Cheng-Hsi Wu et al. demonstrated that D-methionine protects against cisplatin toxicity through its antioxidant and anti-inflammatory properties, but also by promoting growth of beneficial bacteria (*Lachnospiraceae* and *Lactobacillus*), thereby regulating gut microbiome imbalance induced by cisplatin (Wu et al., 2019). In addition, another study also suggests that altered gut microbiota specifically diminished *Firmicutes* and *Lactobacillus* would be the possible mechanism responsible for the observed body weight loss and cardiac dysfunction of cisplatin-associated side effects. Oral supplementation of Lactobacillus might prevent the body weight loss and restablish the cardiac function (Zhao et al., 2018).

Gut microbiota also seems to affect the anticancer activity of cisplatin. Reduced anticancer efficacy of platinum salts, in fact, has been reported in animals treated with anti-Gram-positive antibiotics. These effects were related to translocation of Gram-positive bacteria during mucositis with subsequent induction of cytotoxic ROS and tumor infiltration by pathogenic Th17 cells. Indeed, Natali Pfluga et al described a potential negative impact of antibiotics against Gram-positive bacteria on the anticancer activity of cisplatin (Pflug et al., 2016).

3.5. 5-Fluoruracil (5-FU)

5 Fluorouracil (5-FU), a thymidylate synthase inhibitor, is widely used for the treatment of gastrointestinal tumors. However, its clinical utility is limited by acquired resistance and gastrointestinal toxicities.

One of the most relevant side effects of 5-FU is intestinal mucositis. Mucositis usually appears along the entire gastrointestinal tract from mouth to anus, leaving the mucosal tissue open to ulceration and infection (Pereira et al., 2016).

Several factors or genes contributing to the 5-FU-induced mucositis have been previously investigated; the formation of ROS and the production of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) have been implicated in this process (Chang et al., 2012; Ferreira et al., 2012; Nijhuis et al., 2017).

However, results from preclinical studies failed to provide a complete understanding of 5-FU-induced mucositis pathogenesis. There is growing evidence that gut microbiota may play a role: 5-FU, in fact, leads to an imbalance of gut microbes and the ensuing inflammation leads to exacerbated intestinal mucositis and, potentially, to bacteremia and sepsis. Several preclinical studies have reported a drastic shift from commensal bacteria (i.e., *Bifidobacterium* and *Lactobacillus* spp.) to *Escherichia*, *Clostridium*, and *Enterococcus spp*. following even a single intraperitoneal dose of 5-FU (Hamouda et al., 2017). Mechanistic support for this relationship is provided by the reduced intestinal mucositis and decreased cytokine levels in 5-FU treated mice after antibiotic-induced depletion of microbes. Understanding the influence of gut microbes on 5-FU related toxicity may lead to the identification of targets (i.e. bacteria themselves or bacteria-mediated pathways) to reduce side effects of chemotherapy.

Recently, Lu Yuan et al. profiled the gut microbiota of mice treated with 5-FU, combined with probiotics or a cocktail of antibiotics (Vancomycin, Ampicillin, Neomycin and Metronidazole) by using the Colorectal Cancer mouse model and high-throughput sequencing. They demonstrated that this antibiotic cocktail administration diminished the antitumor efficacy of 5-FU in mice, however supplementation of probiotics upon 5-FU treatment did not significantly increase the efficacy of 5-FU treatment (Yuan et al., 2018).

4. Intestinal microbiota and tyrosine kinase inhibitors

Only few data are available about the relationship of gut microbiota and Tyrosine Kinase Inhibitors (TKIs), in particular Vascular Endothelial Growth Factor (VEGF) TKIs (Table 1).

VEGF TKIs, such as Sunitinib, Sorafenib, Pazopanib, Axitinib and Cabozantinib, are a mainstay of treatment for metastatic renal-cell carcinoma (mRCC). One of the most common toxicities of VEGF-TKIs is diarrhea. The etiology of VEGF-TKI-related diarrhea is poorly understood. It has been suggested that these drugs may cause a direct injury to intestinal mucosa. Another hypothesis is that diarrhea could

potentially be inflammatory in nature (Xin et al., 2009; Yang et al., 2010; Pal et al., 2015a), providing a potential relationship between microbiota and VEGF-TKI-induced diarrhea.

It has been demonstrated, by stool bacteriomic profiling, that VEGF-TKI-induced diarrhea was associated with higher levels of stool *Bacteroides spp*, showing for the first time that stool flora may be associated with this specific toxicity (Pal et al., 2015b). Hahn A. W. et al. found that targeting stool *Bacteroides spp*. with antibiotics improves PFS in patients receiving first-line VEGF-TKIs and, in contrast, targeting non-*Bacteroides spp*. does not affect PFS in patients receiving VEGF-TKIs. These findings suggest that antibiotics, in particular in patient with mRCC, may affect response to VEGF-TKI (Hahn et al., 2018). A recent open-label, randomized trial showed efficacy and safety of fecal microbiota transplantation (FMT) compared with probiotics in treating Pazopanib or Sunitinib related diarrhea among patients with mRCC (Rossi et al., 2019).

5. Intestinal microbiota and immunotherapy

Immune checkpoints inhibitors (ICIs) act by blocking pathways of negative regulation of the immune system, in order to enhance anti-tumor immune response. ICIs are monoclonal antibodies that target receptor molecules on the surface of T lymphocyte, such as cytotoxic lymphocyte antigen 4 (CTLA-4) and programmed death receptor 1 (PD-1), or PD-1 ligands (PD-L1 or PD-L2).

Mechanisms of action of anti-CTLA-4 antibodies (ipilimumab, tremelimumab), and anti-PD-1 (nivolumab, pembrolizumab) or anti-PD-L1 (atezolizumab, avelumab, durvalumab) antibodies are different.

CTLA-4 acts in the priming phase. The naïve T lymphocyte, after interaction with the antigen, to be activated requires a costimulatory signal represented by the interaction between B7 expressed on the surface of the antigen presenting cell, and CD28 expressed on the surface of T cell. CTLA-4, a negative receptor expressed by activated T-cell, by binding B7 blocks the second signal thus causing an inactivation of the response. Using anti-CTLA4 antibodies, CD28 signaling is re-activated and immune response restored.

PD-1 is mainly involved in the effector phase. Especially in case of long exposure to the antigen, such as cancer or chronic inflammation, T-cell expresses PD-1 which, by binding its ligands (PD-L1 or PD-L2) on the surface of the tumor cell, sends an inhibitory signal to the lymphocyte. Anti-PD-1 or anti-PD-L1 antibodies interrupt this inhibitory signaling unleashing the cytotoxic activity of activated T-cell.

Due to dysregulation of immune system, ICIs induce a broad spectrum of side effects potentially involving any organ, known as immune-related adverse events (irAEs) (Champiat et al., 2016). However, based on the different role of CTLA-4 and PD-1 pathways in the regulation of the immune response, the toxicity profile of anti-CTLA-4 and anti-PD-1/PD-L1 antibodies is different (Inno et al., 2017). In general, incidence and severity of irAEs is higher for CTLA-4 than for PD1/PD-L1 blockade, and a recent meta-analysis reported lower toxicity for anti-PD-L1 compared to anti-PD-1 antibodies (Bertrand et al., 2015; Wang et al., 2019). Particularly, diarrhea/colitis are more frequently observed with anti-CTLA-4 antibodies (Bertrand et al., 2015), whereas dysthyroidism or pulmonary toxicity is more frequent with anti-PD-1/PD-L1 (Wang et al., 2019).

ICIs have demonstrated efficacy in several solid tumors and hematologic malignancies, such as melanoma, NSCLC, SCLC, renal cell carcinoma (RCC), urothelial cancer, head and neck cancer, triple-negative breast cancer, Merkel cell carcinoma, microsatellite instability (MSI)-high tumors, and classical Hodgkin lymphoma (Hargadon et al., 2018; Robert et al., 2015; Larkin et al., 2015; Wolchok et al., 2017; Borghaei et al., 2015; Brahmer et al., 2015; Topalian et al., 2019; Carretero-González et al., 2018). Although ICIs achieve long lasting response and prolonged survival, a not-negligible percentage of patients do not derive any benefit (primary resistance) or eventually progress (secondary resistance), and there is accumulating evidence that in some patients the immune checkpoint blockade may even enhance tumor growth (hyperprogression) (Ferrara et al., 2019).

At this regard, several studies have been conducted to identify predictive factors for efficacy of immune checkpoint blockade (Zhang et al., 2019), as well as strategies to overcome resistance (Shergold et al., 2019).

Some data showed that the composition of intestinal microbiota modulates activity, efficacy and toxicity of ICIs (Table 1) (Picardo et al., 2019). Early data from cancer murine models were published 2015, reporting that the composition of intestinal microbiota can influence the response to anti-CTLA-4 (Vétizou et al., 2015) and anti-PD-1 antibodies (Sivan et al., 2015).

After a single injection of anti-CTLA-4, a relative increase in *Bacteroidales (Bacteroides fragilis* and/or *B. thetaiotaomicron)* and *Burkholderiales* was observed in mice gut microbioma (Vétizou et al., 2015). The cause-and-effect relationship between the dominance of distinct Bacteroides spp. in the small intestine and anticancer activity of anti-CTLA-4 antibody was established with recolonization (by oral feeding) of antibiotic-treated or germ-free mice with *Bacteroides fragilis* in combination with either *Bacteroides thetaiotaomicron or Burkholderia cepacia*. After intestinal murine recolonization, the anticancer response to CTLA-4 antibody was restored by inducing IL-12-dependent T-helper 1 (TH1) immune responses in the tumor-draining lymph nodes and by promoting the maturation of intra-tumoral dendritic cells (Vétizou et al., 2015). The clinical relevance of these observation was evaluated by the analysis of the composition of the gut microbiome before and after treatment with ipilimumab in 22 patients with metastatic melanoma. Such analysis revealed 3 clusters with different composition of the stools. Two weeks before tumor inoculation into germ-free mice that were subsequently treated with anti-CTLA-4 antibody, a fecal microbial transplantation (FMT) was performed with feces of different metastatic melanoma patients from each cluster. Improved tumor control was observed in mice that had been transplanted with feces from patients having dominant *Bacteroides* species, compared to mice transplanted with feces from patients having *Prevotella* or distinct *Bacteroides* species (Vétizou et al., 2015).

Furthermore, intestinal reconstitution of antibiotic-treated mice with the combination of *Bacteroides fragilis* and *Burkholderia cepacia,* reduced histopathological signs of colitis induced by anti-CTLA-4 (Vétizou et al., 2015). This observation was confirmed by analyses of intestinal microbioma of 34 metastatic melanoma patients treated with the anti-CTLA-4 antibody ipilimumab: increased representation of a *Bacteroidetes phylum* was correlated with resistance to the development of immune-related colitis (Dubin et al., 2016).

Similar observations were reported in preclinical melanoma mouse models also for anti-PD-1 antibodies (Sivan et al., 2015). Over-representation of *Bifidobacterium* species in gut microbiome of mice increased the response to anti-PD-L1 antibody and tumor-specific immunity; oral administration of commensal *Bifidobacterium* restored antitumor activity of anti-PDL-1 antibody in mice with unfavorable gut microbiota, facilitating maturation of dendritic cells with increased tumor-specific CD8 + T cell activity (Sivan et al., 2015).

Several studies evaluated the role of the gut microbiota in patients treated with ICs. Intestinal microbiome of metastatic melanoma patients undergoing anti-PD-1 immunotherapy was examined to evaluate the relation between the composition of gut microbiota and the efficacy of treatment (Gopalakrishnan et al., 2018a). Significant differences were observed in the composition of gut microbiome of 30 responder versus 13 non-responder patients: higher microbial fecal diversity and abundance of *Faecalibacterium* were reported in responders and lower fecal diversity and abundance of *Bacteroilades* in non-responders. Furthermore, microbial fecal diversity was related to progression-free survival (PFS), with longer PFS in patients with higher diversity compared to patients with lower diversity in gut microbiota (HR 3.57, 95% C.I. 1.02–12.52; $p < 0.01$). High abundance of *Faecalibacterium* in gut microbiota of patients was associated with prolonged PFS, while high

abundance of *Bacteroilades* was correlated with poor PFS. The enhanced systemic and antitumor immune responses in patients with favorable gut microbiota (higher diversity and abundance of *Faecalibacterium*) was mediated by increased antigen presentation and improved effector T-cell function in the periphery and in the tumor microenvironment (Gopalakrishnan et al., 2018a).

In order to assess the interaction between gut microbiota and clinical activity of ICIs, De Rosa et al retrospectively evaluated patients with advanced RCC or NSCLC treated with anti-PDL-1 antibody monotherapy or combination. Patients receiving antibiotics within 30 days of beginning immune checkpoint inhibitors were compared with those who did not, to evaluate the role of dysbiosis on objective response, PFS and overall survival (OS) (Derosa et al., 2018). Antibiotic therapy (most common β-lactam or quinolones for pneumonia or urinary tract infections) was administered to 16 out of 121 (13%) patients with RCC and 48 out of 239 (20%) patients with NSCLC. Among patients with RCC, those previously treated with antibiotics had shorter median PFS (1.9 vs 7.4 months; HR 3.1, 95% C.I. 1.4–6.9, $p < 0.01$) and median OS (17.3 vs 30.6 months, HR 3.5, 95% C.I. 1.1–10.8, $p = 0.03$) when compared to those not treated with antibiotics. Decreased PFS (median 1.9 vs 3.8 months, HR 1.5, 95% CI 1.0–2.2, $p = 0.03$) and OS (median 7.9 vs 24.6 months, HR 4.4, 95% CI 2.6–7.7, $p < 0.01$) were associated with antibiotic therapy also for patients with advanced NSCLC. In multivariate analyses, use of antibiotics remained significant for PFS in RCC and for OS in NSCLC. Although limitations due to retrospective collection of data and lack of information about other factors with a potential impact on the microbiota composition (such as diet or other medications), the authors supposed that dysbiosis related to antibiotic therapy may decrease the bacterial diversity of microbiota and eradicate the most immunogenic bacteria required to engage the immune system unleashed by PD-1/PD-L1 blockade (Derosa et al., 2018).

Similar results were obtained by Routy et *al.* (Routy et al., 2018), who addressed the impact of antibiotic therapy on 249 patients with advanced NSCLC (n = 140), RCC (n = 67) and urothelial carcinoma (n = 42) receiving anti-PD-1/PDL-1 antibodies after one or several line of treatment. They observed a significantly shorter PFS and OS with ICIs in 69 patients pretreated with antibiotic therapy, when all patients were included in the analysis. Similarly, worse PFS and /or OS was reported for patients treated with antibiotic therapy, when individual tumor types were considered. In univariate and multivariate analyses, prior antibiotic therapy was confirmed as an independent predictive factor of resistance to PD-1 blockade and it was hypothesized that dysbiosis might influence the efficacy of ICIs. To further evaluate this hypothesis and explore the composition of the gut microbiota, total DNA was extracted from 100 patients with NSCLC (n = 60) and renal cancer (n = 40) before starting immunotherapy and serially after PD-1 blockade. The higher richness of fecal samples (evaluated at gene count or metagenomic species levels) correlated with the clinical outcome (6-month PFS).

Furthermore, there was a correlation between clinical objective response to ICIs and the relative abundance of *Akkermansia muciniphila* in the feces of the patients: *A. muciniphila* was detectable in 69% (11/16) of patients with partial response and 58% (23/40) of patients with stable disease, but only in 34% (14/44) of patients who progressed or died ($p = 0.007$). These findings showed that *A. muciniphila* was over-represented at diagnosis in the feces of patients who later benefited from PD-1 inhibition.

To establish a cause-effect relationship between the anticancer efficacy of PD-1 blockade and the predominance of distinct commensal species, germ-free or antibiotic-treated mice were recolonized by fecal microbiota transplantation (FMT) using stool from responder and non-responder patients. FMT from responder patients (but not from non-responder patients) conferred sensitivity to treatment with anti-PD-1 antibody in the mice, with tumor growth delay, and accumulation of CXCR3+ CD4 + T cells in the tumor microenvironment.

These results suggested that fecal microbiota transplantation from patients influenced the outcome of mice treated with ICIs.

To validate the biological importance of the microbiota identified in patients with favorable clinical outcome, oral supplementation with *A. muciniphila* was used in mice after FMT from non-responder patients to colonized murine intestines. This restored the efficacy of PD-1 blockade, but the mechanisms of the immunomodulatory effects of *A.muciniphila* remain unclear (Collado et al., 2007).

However, Sen et al (Sen et al., 2018) analyzed 172 patients (105 treated with anti-CTLA-4-based and 67 with anti-PD-1-based therapies) enrolled in phase I trials. Fifty-seven patients used antibiotic therapy: 54 while on trial, 19 within 30 days before treatment and 14 patients 30–60 days before treatment. They did not report differences in the rate of primary progressive disease or in median PFS, but OS was significantly decreased in patients treated with antibiotic therapy in the 30 days before starting ICIs (Sen et al., 2018).

Taken together, all these data underline the complexity of the interaction between the gut microbiota and the immune system and confirm that dysbiosis associated with antibiotic therapy could reduce survival of patients treated with ICIs, suggesting that an intact gut microbiota is needed to mobilize the immune system regardless of the tumor site. It is advisable to avoid unnecessary antibiotic therapy in cancer patients, both to prevent the emergence of multidrug resistant organisms that can be dangerous for these patients, and to improve their outcome.

These observations should be confirmed in large prospective clinical trials.

6. Conclusions

Several studies in murine models and in patients with cancer reported that commensal bacteria of gut microbiota influence the activity and the efficacy of antitumoral drugs and the host susceptibility to toxic effects.

Some evidence also showed that targeting the gut microbiota could improve activity and efficacy of anticancer drugs:

a) the co-administration of antibiotics could potentiate activity of gemcitabine;
b) instead, dysbiosis from prior antibiotic therapy resulted in shorter survival in patients treated with checkpoint inhibitors (Derosa et al., 2018; Routy et al., 2018; Collado et al., 2007);
c) fecal microbiota transplantation (FMT) from cancer patients who had responded to immunotherapy into germ-free or antibiotic-treated mice increased the antitumor effects of immune checkpoint inhibitors (Routy et al., 2018; Collado et al., 2007).

Other data demonstrated that targeting intestinal microbiota can prevent toxicity of antitumoral therapy:

a) the incidence of diarrhea related to irinotecan therapy can be reduced by inhibition of bacterial ß-glucuronidase produced by *Escherichia coli;*
b) intestinal reconstitution of antibiotic-treated mice with the combination of *Bacteroides fragilis* and *Burkholderia cepacia,* reduced histopathological signs of colitis induced by anti-CTLA-4 antibody (Vétizou et al., 2015).

The intestinal microbiota could be modulated in many different ways:

a)
a) use of probiotics (living micro-organisms that, when administered in adequate quantities, can confer beneficial benefits to the guest) or symbiotics (formulations in which prebiotics selectively favor the growth / activity of probiotic organisms with

synergistic effect);
b) use of prebiotics in the diet (non-vital components of food, primarily fiber, that stimulate the growth / activity of beneficial bacteria);
c) use of antibiotics (administration of very selective antibiotics aimed at killing or stopping the growth of dangerous bacteria);
d) fecal microbiota transplantation (administration of a fecal matter solution from donor to the intestinal tract of a recipient to change the recipient's microbial intestinal composition and confer a benefit to its health) (Gopalakrishnan et al., 2018b).

Several trials are ongoing to evaluate these strategies (Gopalakrishnan et al., 2018b) and further studies are necessary to confirm the initial observations in large prospective trials.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

None.

Acknowledgements

The authors thank Matteo Valerio (Medical Oncology Unit, IRCSS Sacro Cuore – Don Calabria Hospital, Negrar, Verona) for his support in editing the artworks.

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