Resolving the Paradox of Colon Cancer Through the Integration of Genetics, Immunology, and the Microbiota

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While colorectal cancers (CRC) are paradigmatic tumors invaded by effector memory lymphocytes, the mechanisms accounting for the relative resistance of MSI negative CRC to immunogenic cell death mediated by oxaliplatin and immune checkpoint inhibitors has remained an open conundrum. Here, we propose the viewpoint where its microenvironmental contexture could be explained -at least in part- by macroenvironmental cues constituted by the complex interplay between the epithelial barrier, its microbial ecosystem, and the local immune system. Taken together this dynamic ménage-à-trois offers novel coordinated actors of the humoral and cellular immune responses actionable to restore sensitivity to immune checkpoint inhibition. Solving this paradox involves breaking tolerance to crypt stem cells by inducing the immunogenic apoptosis of ileal cells in the context of an ileal microbiome shifted towards immunogenic bacteria using cytotoxicants.

Abbreviations:
- 5-FU, 5-fluorouracil
- Ab, antibody
- ADCC, antibody-dependent cellular cytoxicity
- ATB, antibiotics
- Casp, caspase
- cDC, conventional dendritic cell
- CDD, enzyme cytidine deaminase
- CIMP, CpG island methylator phenotype
- CIN, chromosomal instability
- CMS, consensus molecular subtype
- CPT-11, irinotecan
- CRC, colorectal cancer
- CTL, cytotoxic T lymphocyte
- DAMP, danger associated molecular pattern
- d/pMMR, deficient/proficient DNA mismatch repair
- EMT, epithelial-mesenchymal transition
- ETBF, enterotoxigenic Bacteroides fragilis
- FMT, fecal microbial transplantation
- GALT, gut-associated lymphoid tissues
- GF, germ free
- GI, gastrointestinal
- HMGB1, high mobility group box 1 protein
- I.S., immune subtypes
- ICD, immunogenic cell death
- ICI, immune checkpoint inhibitors
- IEC, intestinal epithelial cells
- KEGG, Kyoto Encyclopedia of Genes and Genomes
- KO, knock-out
- LP, Lamina Propria
- MDSC, myeloid-derived suppressor cell
- MGS, metagenomic shotgun
- mLN, mesenteric lymph node
- MSI/MSS, microsatellite instability/stability
- NK, natural killer
- NSCLC, non-small lung cancer
- NTBF, non-toxigenic Bacteroides fragilis
- OS, overall survival
- OXA, oxaliplatin
- pCC, proximal colon adenocarcinoma
- pDC, plasmacytoid dendritic cell
- PFS, progression-free survival
- ROS, reactive oxygen species
- SCFA, short-chain fatty acid
- Tc1, T cytotoxic type 1
- TCGA, The Cancer Genome Atlas
- tdLN, tumor draining lymph node
- TFG, T follicular cell
- Tfh, T follicular helper cell
- Th, T helper
- TILs, tumor-infiltrating lymphocytes
- TLR, Toll-like receptor
- TME, tumor microenvironment
- TNM, T-primary tumor, N-regional lymph nodes, M-distant metastases
- Treg, T regulatory cell
- WT, wild type
productive Tfh and B cell dialogue in mesenteric lymph nodes culminating in tumor-specific memory CD8+ T cell responses sparing the normal epithelium.

Keywords: colon cancer, immunity, Bacteroides fragilis, Fusobacterium nucleatum, ileum, microbiome, immune checkpoint

THE PARADOX OF COLON CANCER

Introduction
Colorectal cancer (CRC) is the third leading malignancy worldwide and the second most common cause of cancer mortality, regardless of gender. CRC is expected to increase in incidence by 60% by 2030, posing an increasing burden on health care systems worldwide (1). The typical development of CRC takes place in aberrant crypts, evolving over time into a neoplastic precursor lesion called a “polyp.” The carcinogenic evolution is thought to occur over 10–15 years, following a traditional adenoma-carcinoma tumor progression. CRCs are often divided into two groups delineated by location: left-sided and right-sided (proximal) colon cancers. These anatomical sites help to capture the heterogeneous features of CRC in relation to key physiological landmarks (2).

CRC results from a progressive accumulation of epigenetic and genetic alterations resulting in the transformation of normal colonic mucosa to adenocarcinoma. 60%–65% of CRCs are classified as sporadic, occurring in people without a family history or genetic predisposition (3). Sporadic CRC development is often associated with numerous risk factors related to health determinants such as lifestyle, diet, smoking, and alcohol consumption (4). Key determinants such as smoking status is linked to proximal colorectal cancer (pCC) and rectal cancer development (5). While dietary habits such as overt consumption of animal fat, red and processed meat, low intake of dietary fibers, unrefined grains and vegetables promote key inflammatory pathways associated with CRC (6, 7). The current staging or classification of CRC malignancies, such as TNM (8, 9) only takes into account primary tumor size, regional lymph node involvement, and metastatic spread but fails in the ability to further delineate how best to approach therapeutic management of malignancies. Indeed, key aspects of classification, such as genetic specificities, immunological contexture, and gut dysbiosis, play a vital role in the physiopathology of CRC and should be considered in context (Figure 1) (10).

Genetic and Immunological Traits of Colon Cancers
CRC arises from mutational activation of oncogenes associated with the mutational inactivation of tumor suppressor genes (11). Three non-exclusive major types of genomic instability have been described in CRC. The first type, occurring in 85% of CRC, concerns gene mutations in APC or other tumor suppressor genes resulting in activation of the Wnt pathway characterized by a chromosomal instability (CIN) phenotype. The second type found in 20%–30% of CRC, accounts for global genome hypermethylation coinciding with the inactivation of tumor suppressor genes, known as CpG island methylator phenotype (CIMP) (12). The last type is found in ~15% of patients who encounter the loss of DNA mismatch repair (MMR), leading to a high level of microsatellite instability (MSI-High), a hypermutable phenotype (13). The MSI-H phenotype results from either a somatic inactivation of MLH1 MMR gene (sporadic cases, 12%) or from a germline mutation in MMR genes (MLH1, MSH2, MSH6, PMS2) leading to a deficient DNA mismatch repair (dMMR); such as in the case of the Lynch syndrome (3%) (14). The CIMP phenotype can lead to the MSI phenotype when hypermethylation of the MLH1 gene promoter occurs. This particular MSI phenotype generates neoantigens accounting for their intrinsic immunogenicity (15).

CRC outcomes are not only dictated by genetic features but also by the immune contexture (Figure 2). Several cell types associated with innate and adaptive immune responses cooperate and dictate the prognosis of patients diagnosed with CRC. γδT cells expressing a heterodimeric T-cell receptor (TCR) are often enriched in epithelial barriers of various mucosa to sense cellular stress at portal of entry (16). However, preclinical murine models of colitis and clinical CRC data have shown that the γδT17 cell subset, producing the IL-17A or IL-17F cytokines, promotes tumor progression through the accumulation of myeloid-derived suppressive cells (MDSC) (17, 18). MDSCs accumulate in the tumor microenvironment (TME), as compared to the adjacent healthy tissue, in patients with CRC and their circulation correlates with cancer stage and metastasis (19, 20). Moreover, Th17 cells through the secretion of IL-17A and the transduction of the STAT3 pathway lead to the downregulation of CXCR3 expression on CD8+ T cells. Consequently, these Th17 cells dampen the CXCL10-dependent recruitment of cytotoxic CD8+ T cells (CTLs) in advanced stages of CRC (21). In addition, the IL-17R signaling in tumor cells blunts CXCL10 release thereby limiting CTLs influx in tumor bed (22). Furthermore, Th17 cells secrete IL-22 which promotes colitis associated with CRC (23). Contrasting with γδT17 and Th17 cells, IFN-γ producing conventional CD4+ T cells, namely, Th1 lymphocytes, are associated with a favorable prognosis in CRC (24).

Another subset of auxiliary T cells, the T follicular helper (Tfh) CD4+ lymphocytes, defined by CXCR5 chemokine receptor expression and the Bcl6 transcription factor, are found within and around CRC tumor nests and tumor draining lymph nodes (tdLN). Their density is negatively correlated with CRC tumor progression (25, 26). A positive Th1/B cell signature associated with increased CD8+ T cell infiltrates has been reported in CRC cases with favorable outcomes (25, 27). The Th1/B cell dialogue is pivotal to orchestrate CD8+ T cell effector functions, which are believed
to keep in check CRC at early stages of development (28, 29) and may pave the way to the efficacy of immune checkpoint inhibitors (30). Indeed, B cells, FcγR and Ig as well as Th2 and IL-4 have been associated with chronic inflammatory processes, leading to tumor development in T cell-dependent (31–33) or -independent tumor models (34). Likewise, bladder tumors, amenable to therapeutic immune checkpoint blockade, have a dismal prognosis when their TILs contain CD8+ T cells producing IL-10 and expressing inhibitory receptors (TIGIT, Lag3, Tim3, and CTLA-4), associated with GATA3+ CD4+ Th2 cells and Treg (35, 36). Moreover, PD-1 expressing B cells are memory B cells exerting suppressive activity that can be alleviated by anti-PD-1 Abs (37). Another report described PD-1+ B cells that possessed regulatory capacity toward T cell responses, and although rare in peripheral blood, they were found enriched in thyroid tumors (38).

However, recent accumulating evidence shows that B cells can orchestrate favorable TCF7+ naive/memory CD8+ T cell-based immune contexture, specifically when tertiary lymphoid organs can be formed to prime naive CD8+ T cells in human tumors, paving the way to clinical benefit to anti-PD-1 Abs (39–41). In breast tumor models genetically modified to express neoantigens, CD8+ T cell effector memory cells could be elicited with anti-PD-1 Abs when Treg were depleted (using a mouse anti-CTLA-4 Ab with ADCC properties), therefore triggering a cascade whereby B cells became activated, capable of triggering Tfh activation and
IL-21 production. B cell depletion or inhibition prevented class switching, plasma cell generation, and the release of anti-tumor IgG indispensable for tumor rejection following therapy with ICBs (30). In these models of combined immune checkpoint inhibitors (ICIs), effector Tc1 CD8+ T cells cannot be generated in the absence of Tfh, IL-2, or B cells. Hence, the optimal B cell activity and therapeutic benefits derived from anti-PD-1/anti-CTLA-4 therapy correlated with concurrent Treg inhibition and

![Cellular components](image)

**Associated cytokines/chemokines**

- CXCL13
- CXCL9/CXCL10
- IFNγ
- CCL20
- TGF-β
- IL-17

**Immune checkpoint expression**

| High (PD-L1, PD-1, CTLA-4, IDO1, LAG3) | Low |

**Ileal microbiome**

- *B. fragilis, C. ramosum, A. ondordonkii*
- *Erysipelotrichaceae, Acidaminococcaceae*
- *P. clara, F. nucleatum, Fusobacteriaceae, Prevotellaceae*

**Clinical outcome**

| Good | Poor |
|------|------|
| Prolonged survival | High recurrence risk |
Tfh activation (30) or, as described in our study, by lamina propria IL-12 and IL-1β producing DC after stimulation with immunogenic ileal commensals (26).

Tumor-associated Tfh secrete CXCL13, attracting CXCR3+ CTLs (42) and via IL-21 secretion promotes their effector functions (43). Tfh are involved in the orchestration of the humoral responses through interactions with B cells. Nevertheless, the Tfh/B cells/CTLs collaboration is limited by an increased expression of PD-1 on Tfh during tumor development, dominated by a PD-L1-associated immunosuppression (43, 44). It has been shown that germinal center B cells and/or plasma cells play a protective role, while the T cell suppressive function of regulatory B cell subsets was associated with the dissemination of metastases (45–49). The CD8+ Tfh counterpart has also been described in the tdLN of CRC. CXCR5+ CD8+ T follicular cytotoxic cells (Tfc) express high levels of effector molecules and convey a better prognosis in CRC (50). In line with these contentions, Roberti et al. have recently described the beneficial role of antibody producing B cells in cancer immunosurveillance in murine transplantable colon tumors (26). Hence, as described for other tumor types, terminally differentiated memory B cells and/or plasma cells and Tfh could predict increased patients survival in CRC (49, 51, 52).

Pagès and Galon (Figure 2) were the first to report the major role of cytotoxic and memory T lymphocytes within primary tumors in predicting survival of CRC patients (28, 53, 54). These studies included early-stage cancer patients (55) and described distinct cellular and molecular cues modulating tumor-infiltrating lymphocyte (TIL) densities (56). A scoring system “Immunoscore” based on the quantification of CD3 and CD8 in the core (CT) and at the invasive margin (IM) of primary tumors has a prognostic value superior to the AJCC/UICC TNM-classification (28, 54, 57, 58). Immunoscore predicts survival of non-metastatic patients, as does the immune infiltrate evaluation in the metastasis for metastatic patients (59–61). These authors have also demonstrated the superiority of Immunoscore over microsatellite instability and PD-L1 expression in predicting survival (62, 63). Focusing on metastasis profiling in CRC, Galon’s group brought forward evidence that the cytotoxic T lymphocytes-based adaptive immune response plays a key role in preventing tumor recurrence and metastatic dissemination; despite evidence for clonal expansion of distinct T lymphocyte subsets culminating in immunoeediting (60, 64). Disease-free survival (DFS) and OS in stage IV patients are largely governed by the state of the local adaptive immune response within the metastases being the most likely site of tumor immune escape (63). However, further studies are ongoing to better understand how dynamics of genomic and immune patterns shape the CRC metastatic landscape.

Recent Reclassifications of Colon Cancers
Given the potential significance of the intertwined relationships between genetics and immunometrics, the Cancer Genome Atlas Consortium (TCGA) proposed, in 2012, a different stratification of CRC (65). Four consensus molecular subtypes (CMS1–4) have been discussed to classify CRC based on transcriptomic of the tumor microenvironment (TME) (Figure 1) (66, 67).

The CMS1 group (14% of early-stage CRC) showing hypermutation, hypermethylation, and high antigenicity (neoepitopes/neoantigens) is heavily immune infiltrated and predominately constituted by MSI-H tumors (76%). BRAF mutations are often found in the CMS1 subtype. The CMS1 group is characterized by a higher expression of immune checkpoints conveying a dismal prognosis (67–70) yet potentially favoring the relative efficacy of immune checkpoint inhibitors (ICIs) (69). CMS2 “canonical” and CMS3 “metabolic” groups (37% and 13% of early-stage tumors, respectively) correspond to “immune-neglected” CRC, in that CMS2 represents an “immune desert” and CMS3, an “immune excluded” tumor microenvironment. The CMS2 group shows WNT and MYC activation. The CMS2 “immune-desert” subtype is characterized by a poor intratumoral T cell infiltrate. After relapse, the CMS2 group has a superior survival rate than the CMS1 (67). Compared to CMS2, the CMS3 “immune-excluded” subtype often harbors KRAS-activating mutations associated with memory Th and naïve B cell-based immune components (71). The CMS4 “mesenchymal” subtype (23% of early-stage tumors) exhibits the prototypic fingerprint of the epithelial-mesenchymal transition (EMT) characterized by a stroma-related gene transcription centered by the transforming growth factor-β (TGF-β) metagene. Stromal cells secret immunosuppressive chemokines that interact with cancer cells and inhibit cytotoxic immune cells, thus contributing to the proliferation of MDSCs (66). CMS4 has the worst relapse-free and overall survival (67). A small fraction of CRC tumors have indeterminate features (13%), and possibly represent a transitional state, or result of a mixed phenotype, due to intratumoral heterogeneity (67).

Recently, based on TCGA transcriptomic analysis of more than 10,000 solid primary tumors, Thorsson et al. have identified six immune subtypes (I.S.) of the tumor microenvironment (C1-C6), namely: wound healing (C1), IFN-γ dominant (C2), inflammatory (C3), lymphocyte depleted (C4), immunologically quiet (C5), and TGF-β dominant (C6). Across all cancer types, the C3 “inflammatory” I.S. was associated with the best overall survival. C1 and C2 I.S. were characterized by a high proliferation rate and exhibited less favorable prognosis even if a higher lymphocyte signature ameliorated the clinical outcome (72). The “wound healing” (C1) and “IFN-γ dominant” (C2) subtypes are the two main I.S. represented in CRC (72, 73). The C1 “wound healing” I.S. showed a prominent expression of angiogenesis-related genes and a low Th1/Th2 ratio. The C2 “IFN-γ dominant” I.S. has the highest intratumoral heterogeneity; containing higher levels of TILs and Tfh cells (73). Nevertheless, the C1 “wound healing” I.S. harbors a better prognosis than the C2 “IFN-γ dominant” I.S. (5-year overall survival (OS) 65% and 49%, respectively), perhaps in line with the upregulation of PD-L1, PD-1, CTLA-4, IDO1, andLAG3 molecules related to immune exhaustion in C2.

Clinical Management of Colon Cancers
Depending on the TNM score, the main treatment for colon cancers remains the surgical resection of the tumor; especially for tumors with a low risk of recurrence (74). For tumors with stage
III or high-risk stage II, a (neo) adjuvant therapy based on chemotherapy is performed prior to surgery to reduce tumor burden and recurrence rates (74). For stage III or high-risk stage II CRC, adjuvant chemotherapies with FOLFOX (5-FU/leucovorin/oxaliplatin) is the standard of care (75–77). As for metastatic diseases, a form of immunotherapy called immune checkpoint inhibitors (ICIs) has transformed the standard-of-care for cancer patients (78–81).

Six months of adjuvant chemotherapy frequently benefit immunoscore-high-patients with stage III primary CRC (82). Moreover, patients treated with chemotherapy and anti-EGFR (Epidermal Growth Factor Receptor) had greater infiltration of T lymphocytes at the core of the metastatic lesion and a higher Immunoscore than patients who received chemotherapy and anti-VEGF (Vascular Endothelial Growth Factor) (63). Hence, Immunoscore® is improved by chemotherapy and predicts the beneficial effects. Oxaliplatin (OXA), a platinum salt used as a cornerstone chemotherapy for CRC (77) is one of the best cytotoxicants capable of inducing immunogenic cell death (ICD) (83). ICD triggers an endoplasmic reticulum (ER) stress response and the activation of the autophagic machinery culminating in tumor cell surface exposure and/or secretion of the mandatory damage-associated molecular patterns (DAMPs); stimulating anticancer immune responses (84). Moreover, OXA increased Tfh density in tumor nests and eventually TILs accumulation as a function of its capacity to induce ileal crypt apoptosis (26).

In CRC, MSI status constitutes a predictive biomarker for response to ICIs. Metastatic CRC with MSI-H phenotype show a high disease control rate and a favorable progression-free survival (PFS) after anti-PD-1 antibody while the 85% patients presenting with microsatellite stable (MSS) or proficient MMR (pMMR) CRC have failed to benefit from various immunotherapy approaches (85, 86). POLE proofreading domain mutations are also associated with a favorable response to ICIs (87). Hence, anti-PD-1 therapies, such as nivolumab and pembrolizumab, have been approved by the U.S. Food and Drug Administration (FDA) in 2017 for patients with MSI-H/dMMR metastatic CRC that have progressed following treatment with a fluoropyrimidine, oxaliplatin, or irinotecan-based chemotherapy (88, 89). Though, the response rate after anti-PD-1 antibodies often remains less than 50% (85, 90), in relation to tumor heterogeneity within MSI-H CRC (91), or immune evasion based on alterations in the antigen processing and presentation machinery (92). However, the immunoscore turned out to exhibit a superior prognostic value for overall survival regardless of the MSI/MMR status. MSI-H patients with a low immunoscore do not have any survival benefit compared with MSS patients. Additionally, in MSS CRC cases, the immunoscore correlates with higher disease-free survival and overall survival (54). Actually, there is no prospective study confirming the clinical significance of the immunoscore for CI response yet. An ancillary study coinciding with a clinical trial in metastatic MSS CRC (the POCHI trial: NCT04262687) just started to analyze the predictive value of the immunoscore for the clinical benefit to expect from chemotherapy and immunotherapy (93).

The degree of microsatellite instability (MSI) and resultant tumor mutational burden (TMB) have also been shown to underlie the variable response to PD-1 blockade in dMMR, with indel mutations strongly associated with objective response (85, 94). In one of the largest number of patients treated with ICI analyzed for TILs and TMB, Loupakis et al. found that TILs correlate to TMB and the higher the number of TILs, the better the outcome (95). Thus, pembrolizumab is FDA approved for TMB-high tumors (96). In other cases, such as in dMMR/MSI-H metastatic CRC, the combination of ICIs (such as PD-1 and CTLA-4 co-blockade) are likely to be more effective than monotherapy (97). The vast majority of CRC cases, MSS or pMMR CRC, are naturally resistant to immunotherapy; at least in part due to the low antigenicity (TMB) of the malignancy. However, based on TCGA dataset analysis, a small subset of patients among MSI-Low/MSS-CRC cases exhibiting a high CD8+ T cell infiltrate and an upregulation of IFN-γ could benefit from immunotherapies (98).

To overcome the lack of efficacy of ICIs in MSS and pMMR CRC, investigators have combined ICIs with chemotherapy and targeted therapeutics (99). Despite these efforts, the combination of oxaliplatin and pembrolizumab has failed to prove superior over OXA-based chemotherapy alone in advanced CRC (100).

Taken together, CRC is the first neoplasia found to be under immunological control, and the first to be treated with OXA, a chemotherapy endowed with immunogenic cell death properties. However, we can see that most attempts to overcome this malignancy have failed, with the exception of a minority of lesions characterized by MSI. This paradox may be solved by a more complete understanding of the role played by the macroenvironment in which this cancer develops (Figure 1).

THE GUT MICROBIOME AND THE INTESTINAL IMMUNE SYSTEM

The gastrointestinal (GI) tract is the major mucosal surface of the human body and the most densely colonized organ. The overall bacterial load of the GI tract has been described to contain between 10^{13}–10^{14}; nearly equaling the number of the mammalian cells in the body (101, 102).

In mammals, and more specifically in humans, colonization by the intestinal microbiome occurs rapidly during and after birth (103, 104). Successful colonization is determined by microbial selection and competition that begins in the first hours of life (105). Throughout our lifetimes, the microbial population in the human GI tract is affected by a multitude of environmental factors such as age (103, 106), geography (106), dietary habits (107, 108), use of antibiotics (109, 110), host genetics (111, 112), and the pressure of the immune system (113).

The intestinal microbial ecosystem has a significant role in host physiology in multiple ways. The processing of food and xenobiotics enables a host to obtain essential nutrients (101). The intestinal microbiota promotes post-natal maturation of physiological gut functions such as the integrity of the
epithelial barrier, the expression of essential enzymes (114), the development of the intestinal vascularization (114, 115), and of the enteric nervous system; all essential for motility (116). Most importantly, the gut microbiota participates in the maturation of the local and systemic immune system to ensure tolerance vis-à-vis of food antigens and the elimination of pathogens, maintaining a mutual symbiosis between commensals and self-tissues for the homeostasis of the meta-organism (117, 118).

Various bacterial communities are disseminated along the GI tract with diversity affected by anatomical and environmental differences from the esophagus to the rectum. There is a limited diversity of the microbiome in the esophagus where Streplococci remain the dominant species (119). Similarly, the stomach has limited microbial diversity due to low pH of the gastric lumen (120). While the small intestine and colon harbor a wider commensalism compared to that of the upper GI tract. Different physiologies and environments such as chemical, oxygen and nutrient gradients, and the compartmentalized immune system along the GI tract result in distinct distribution of the intestinal microbiota (121). The small intestine contains a smaller load of bacteria and with less diversity, than the colon. This is due to a number of factors such as harsh environment for bacterial communities, a shorter transit time, an increased influx of digestive enzymes of antimicrobial peptides and bile acids, and an intermittent food substrate delivery (121, 122). Although the taxonomic classification has been inconsistent across studies, several reports show the predominance of two major phyla, i.e., Firmicutes and Proteobacteria (121, 123, 124), followed by others (Bacteroidetes, Fusobacteria, Verrucomicrobia, Actinobacteria) residing in the human small intestine (124). At the genus level, several genera are commonly found in the small intestine, such as Lactobacillus, Clostridium, Staphylococcus, Streptococcus, and Bacteroides (124–127). Sample collection from the distal ileum has shown that Streptococcus, Granulicatella, Actinomyces, Solobacterium, Rothia, Gemella, and TM7(G-1) are the most frequently detected bacterial genera by 16S gene sequencing (127) with Streptococcus, Actinomyces, Rothia and Lactobacillus species most frequently identified by culturomics and mass spectrometry (128). In contrast, the colon contains roughly 70% of all the bacteria of the human body (129). The microbial population in the colon is a result of environmental factors such as lower concentrations of antimicrobials, slower transit time, and fermentation of polysaccharides (121, 129). Interestingly, the two major phyla in the colon are Bacteroidetes and Firmicutes (121, 129). At the genus level, Bacteroides, Prevotella, and Ruminococcus are predominant (129) (Figure 3).

Fecal profiling of the microbial populations is investigated most frequently because the sampling of feces is non-invasive and convenient for patients compared to performing an invasive biopsy or collecting luminal content of ilea or colons (130). However, there is increasing evidence that the observed microbial composition in feces is different from the content of mucusosal samples of the GI tract (123, 131–135). Bacteroidetes and Firmicutes are the main phyla in the healthy human stool samples followed by other phyla such as Actinobacteria, Proteobacteria, Synergistetes, and Verrucomicrobia (123, 134, 136–142). At the genus level, Bacteroides is the most abundant genus (136, 140, 141) followed by Faecalibacterium, Bifidobacterium, Lachnospira, Roseburia, Subdoligranulum, Collinsella, Ruminococcus, Prevotella, Alistipes, and Akkermansia (123, 132, 136–142). Of note, mucosal microbiome of the colon differs from that of fecal composition. James et al. reported that Enterococcus was found to be more prevalent in the proximal colon while Coprobacillus and Escherichia/Shigella were more abundant in the distal colonic mucosa (143).

The symbiotic relationship between the gut microbiome and the associated local immune system is central for the maintenance of the systemic immune tonus. The lamina propria and gut-associated lymphoid tissues (GALT) contain the largest pool of cells mediating innate and cognate immune responses (Figure 3). There is marked regional variation in immune cells along the GI tract, with Th17 decreasing in number from the duodenum to the colon, and regulatory CD4+ T cell (Treg) being most abundant in the colon (144). Pioneering mouse studies demonstrated that distinct bacterial species can fine-tune intestinal immune responses, such as Th17 (145, 146), Treg (118, 147), or Th1 (148, 149), Th17, and B cell activation (150). James et al. cataloged the mucosal microbiome in different regions of the human colon and reported the annotated colon microbiome single-cell dataset at the Gut Cell Atlas (https://www.gutcellatlas.org/) (143). There are 25 cell types in the lamina propria (LP) and mesenteric lymph nodes (mLNs), draining the healthy colons. Among these, are follicular and memory B cells, IgA+, and IgG+ plasma cells, effector and memory CD4+ T cells, Treg cells, CD8+ T cells, innate lymphoid cells, natural killer cells (NK), mast cells, and myeloid cells (cDC1, cDC2, pDC, LYVE1+, or CD16+ macrophages, monocytes). B cells, dendritic cells and γδ T cells are Ki67+ cycling populations compared to other colonic immune cell populations. The cecum and the sigmoid were reciprocally enriched in CCL20+ Th17 and Th1 respectively. While mLN contained follicular B cells and memory CD4+ T cells, colonic mucosa, and most specifically the sigmoid LP is rich in effector CD4+ T cells and plasma cells. Region-specific transcriptional differences linked activation and tissue migration of Th1 and Th17 cells of the proximal and sigmoid colon as well as the identification of clonal sharing between these colonic regions plead for cell-extrinsic rather than cell-intrinsic factors regulating T cell functions. However, the relative proportion of Treg cells do not change significantly from proximal to distal colon. There is heterogeneity in Treg cell states in the mLNs and colon with a transient loss of the FoxP3-expressing cell population. Data infer a continuous activation trajectory of these Treg cell states between draining lymph nodes and colon, with genes regulating Treg cell migration and adoption of Th-like profiles in tissues (143). Treg transiently losing FoxP3 expression could re-express it transcription under activation to achieve their bona fide immunosuppressive functions (151). There is a highly activated state of plasma cells found in the...
distal colon compared with proximal colon plasma cells, which are characterized by greater accumulation, somatic hypermutation, clonal expansion, and stronger homing to the colonic mucosa (143). Compared with the cecum, IgA+ plasma cells of the sigmoid colon respond to a rich and unevenly represented community of bacterial species; most likely accounting for the increased activation, migratory and greater clonality status of plasma cells (143). Indeed, by means of a novel method for identifying and isolating lymphoid follicles along the length of the human intestine and IgA sequencing analysis, Agace’s group showed that IgA adaptive immune responses are initiated in an anatomically restricted manner in the human ileal and colonic plasma cell repertoires, respectively (152).

**FIGURE 3** | Geographical distributions of immune and microbial components highlight specificities between the ileal and colonic segments. The most dominant microbial and immune cells accounting for the diversity and specificity of each organ are depicted. The main specialized functions (metabolic, immune, motility, etc.) for each segment are indicated. A graphical abstract is lining below the boxes where these cells and functional specificities are listed. Immune cells are found either clustered within organized lymphoid structures or scattered within intestinal epithelium (IE) and lamina propria (LP). AMP, anti-microbial peptides; B cell, B cell lymphocyte; DC, dendritic cell; ILC3, innate lymphoid cell type 3; IL-22, interleukin-22; Th1, T helper 1 cell; Th17, T helper 17 cell; Treg, regulatory T cell.

**DYSBIOSIS ASSOCIATED OR CAUSALLY LINKED WITH COLON CARCINOGENESIS**

Western style diets (high fat, high sugar) and meat consumption may in fact be major risk factors in the development of CRC (153). Diet displays a dominating role in shaping the structure of
gut microbiota, occasionally leading to a detrimental alteration of microbiota ecology and functionality. Indeed, increasing dietary fiber can lead to the establishment of a favorable microbiota regulating the production of the anti-inflammatory short chain fatty acids (SCFA) (154). An unbalanced microbiota, known as dysbiosis, has been associated with many maladaptive states, including CRC (155–159). (Figure 4A) However, the contribution of the microbiota in CRC initiation and development remains a subject of debate. Preclinical and human clinical studies have linked the intestinal microbiota to CRC. Preclinical studies performed with sporadic CRC rodent models showed that germ-free mice and rats display less intestinal tumorigenesis than animals conventionally reared, highlighting the role of the microbiota in CRC emergence (160). Confirming this functionality, the first mouse model of spontaneous invasive CRC has been described, in which resident microbiota play a key role in disease outcome (161). Indeed, intestinal epithelial cell (IEC)-specific transgenic expression of the epithelial-mesenchymal transition regulator Zeb2 in mice (Zeb2IEC-Tg/+ mice) lead to increased intestinal permeability and spontaneous invasive colon carcinoma development in a microbiota-dependent manner (161). However, this work did not yet identify CRC-associated microbial entities. Several independent studies comparing tumor versus paired adjacent healthy tissues showed marked differences in the gut microbiota composition (162–165), with either depletion or enrichment of selected bacterial species. Meta-analysis of five publicly available datasets and two new cohorts with validation using two additional cohorts, considering n=969 and n=768 fecal metagenomes revealed an enrichment of bacterial species within CRC and feces of patients, not found in homeostatic controls. They identified 29 species, mostly from the oral cavity, associated with CRC development (166, 167). At the functional level, the choline trimethylamine-lyase gene (166) as well as protein and mucin catabolism genes were overabundant while carbohydrate degradation genes were depleted (167) in CRC.

Of note, this particular microbial shift distinguishes benign from malignant colon tumors (166). Alterations of microbiota composition are distinct across major stages of colorectal carcinogenesis (168, 169), suggesting a role of specific bacterial communities in this process. Moreover, besides the important shifts observed in the colonic microbiome of CRC, significant variations have been reported in the ileal microbiome. Using healthy ileal mucosa-associated microbiome, lining upstream from Bauhin’s valve and collected during hemicolectomy in patients suffering from different stages of proximal CC, Roberti et al. (26) identified distinct species and family members conveying immunogenicity to CRC by regulating the accumulation of Tfh instead of Th17 cells in the tumor microenvironment.

Furthermore microbiome diversity and richness changes during carcinogenesis, bacteria can be found with a specific organization in normal colon mucosa, mirroring the bacterial composition within the tumor. Bacterial organization in biofilms protects commensals against external agents and induces pro-oncogenic properties by inducing major deregulation of epithelial cell biology culminating in sustained inflammation as detailed below (Figure 4). Biofilms are associated with increased risk of developing sporadic CRC (170, 171). Formation of biofilms is mostly a characteristic of right-sided colon cancer (170).

Altogether, these studies showing significant variations of taxonomic footprints suggest a role of the gut microbiota in the emergence of CRC. However, whether one or more species are necessary for CRC development remains unclear. Notable steps forward have been made in the identification of single bacterial species or bacterial communities associated with CRC. Several studies have nailed down mechanistic cues that link distinct bacteria strains and species to CRC carcinogenesis. Bacteria can exert their pro-oncogenic effects through multiple mechanisms. The inflammatory contexture can modulate microbial gene functions and increase the cancer-promoting activity of some bacteria strains, as exemplified for E.coli NC101 (172). Bacteria can produce toxins and metabolites from the fermentation of by-products or induce the formation of superoxide radicals that lead to subsequent genetic mutations in the colonic epithelium (173, 174). Several theories have also been proposed to delineate the involvement of specific microbiota in CRC initiation or progression (Figure 4). The “driver-passenger” model supports the idea that a microbial leader recruits a consortium of disease-facilitating microbes to initiate the biological events causing CRC (175–177). This model suggests a chronological recruitment of specific bacteria concurring to CRC. First, “driver” bacteria create a pro-oncogenic environment through DNA-damage and malignant transformation of epithelial stem cells. After initiation of tumorinitiation processes, there is an emergence of “passenger” bacteria, more adapted to the tumor environment such as Fusobacterium nucleatum and Streptococcus bovis/gallolyticus (177) (Figure 4B). Secondly, the “keystone hypothesis,” or “alpha-bug hypothesis,” suggests that certain low-abundance bacteria possessing unique virulence traits can reshuffle a benign environment into a carcinogenic one. For instance, enrichment of Fusobacterium species is associated with a depletion of the Bacteroidetes and Firmicutes, in malignant colon relative to normal colon tissue (175, 178–180). Thus far, several “alpha-bugs” that promote intestinal carcinogenesis in animal models have been described such as Fusobacterium nucleatum (181, 182), colibactin-producing Escherichia coli (183–185) and enterotoxigenic Bacteroides fragilis (ETBF) (186) (Figure 4C).

Fusobacterium nucleatum is frequently associated with advanced proximal CC tumors, metastasis, chemoresistance, and poor prognosis (26, 187–189). These pathogenic properties occur through the expression of two different virulence factors, FadA, and Fap2 on the intestinal CRC and non-CRC epithelium, which induce the expression of a large spectrum of transcription factors, oncogenes, and genes coding for inflammatory functions or tumor progression (182, 190). F. nucleatum shapes the immune environment to promote tumor growth through the elicitation of a pro-inflammatory cytokine cascade (IL-8, CXCL1) and the direct inhibition of T and NK cell functions (191, 192). Furthermore, F. nucleatum has been shown to
modulate autophagy in intestinal epithelial cells (IECs) by activating regulatory microRNAs that consequently alter colorectal cancer chemotherapeutic responses (189) (Figure 4).

Regulatory T cells (Treg) can be anti-tumorigenic or pro-tumorigenic in colorectal cancer (CRC) depending on the presence of different Treg subsets with various immunosuppressive molecules. FoxP3 expression intensity dictates the prognosis of CRC; FoxP3(hi) Treg being associated with poor clinical outcome in contrast to producing-FoxP3(lo) Treg producing inflammatory cytokines (193). Earlier work described non-effector T cells comprising regulatory and anergic T lymphocytes that specifically accumulate in tumor tissues and eventually recirculate (194). Some reports suggested synergistic associations between PD-1/CTLA-4 and PD-1/CD39 within Helios+ or Heliosneg FoxP3+ CD4+ T cells to dampen T-cell activation and functions in CRC (195, 196).

Escherichia coli, although commonly found in homeostatic gut microbiota, is another bacterium frequently correlated with tumor staging and prognosis (184). The association between E. coli and CRC specifically implies E. coli strains that produce the genotoxin colibactin. This toxin accelerates tumor progression (185, 197) by damaging host DNA (172, 183, 198) and by

FIGURE 4 | Instrumental links between intestinal dysbiosis and colon carcinogenesis. Risk factors contributing to colorectal cancer (CRC) initiation and development encompass the lifestyle, diet, the exposure to environment (xenobiotics), host genetics and the gut microbiome (A). Several hypotheses have been formulated to account for the toxicity of the microbiome for the epithelium. A driver bacterium could recruit a consortium of disease-facilitating microbes (B). The “keystone hypothesis” suggests that specific bacteria lead to dysbiosis and pro-carcinogenic microenvironment (C). Mucus digestion by some bacteria could expose the epithelium to toxins produced by virulent bacteria organized in biofilms (D).
inducing cellular senescence (Figure 4). This process may involve the production of several growth factors in human CRC (197).

ETBF is an enterotoxin-producing bacterium that is thought to play a role in the occurrence and progression of CRC (186). The toxicity of this bacterial compound is linked to its capacity to damage DNA strands via the generation of reactive oxygen species (ROS) and through the induction of Th17-dependent inflammatory responses (186). The ability of Bacteroides fragilis to promote tumor growth presumably relies on the enterotoxin production, as nontoxigenic B. fragilis (NTBF) does not induce Th17 responses and fails to facilitate cancer outgrowth (186), offering prophylactic effects against colitogenic B. fragilis (199). While IL-17 initiates a NFκB-mediated recruitment of protumoral CXCR2+ myeloid cells (200), other mechanistic events mediated by toxigenic B. fragilis contribute to colon tumor development. In particular, digestion of the mucus layer by ETBF enabled enterotoxigenic species, namely pks+ E. coli, to adhere to colonic IEC, thus facilitating access to the toxin (201).

Finally, mucus-invasive bacterial biofilms were identified on the colon mucosa of 50% of CRC patients and approximately 13% of healthy individuals. Remarkably, biofilm-positive communities from healthy colonoscopy induced colon inflammation and tumors similarly to biofilm-positive tumor tissues in three independent mouse models of CRC (202). Bacterial genera shown to be amplified in CRC patients such as Clostridium XI, Clostridium XVIII, Erysipelotrichaceae incertae sedis, Escherichia/Shigella, Eubacterium, and Parabacteroides were increased in biofilm-positive-associated mice. These bacteria may interact with one another. Collaboration between multiple types of bacteria is likely to be a contributing factor, as suggested by the identification of ETBF and pks+ E. coli within familial adenomatous polyposis mucosal biofilms. In contrast, Bifidobacterium was depleted in mice inoculated with biofilm-positive tissues, confirming the metagenomics data of stool composition in CRC patients (162, 203). Mechanistically, biofilm-positive colon cancers have been associated with decreased E-cadherin expression, an increased release of IL-6, and the polyamine metabolites N(1),N(12)-diacetylspermine, activation of STAT3, all culminating in IEC proliferation and cell transformation (170) (Figure 4D). This work converged into the demonstration that biofilm formation plays a key carcinogenic role (202).

The composition of the ileal microbiome influenced the accumulation of T follicular helper (Tfh) cells and CD8+ T cells in proximal colon cancers (26). Cremonesi et al. showed that distinct bacteria species can directly stimulate colon cancer cells producing a chemokine array closely correlating with T cell subsets expressing the appropriate chemokine receptors, which in turn, may modulate tumor immunosurveillance and dictate the prognosis of colon malignancies. Hence, Fusobacteria and Prevotella can trigger CCL20 release through tumor cells in vitro, while Th17 infiltration in vivo was associated with “cold” (poor in tumor infiltrating lymphocytes) CRC (204, 205).

Aside from Th1 and Th17 responses, the tumor contexture can also reveal the presence of beneficial Th9 that could be associated with specific local microbes. In recent reports, IL-9 producing-Th9 cells resulted from the conversion of Th2 cells into Th9 cells endowed with pro-apoptotic tumoricidal activity and immunizing capacities (206). Moreover, positive and negative correlations between intratumoral IL-9 and abundance of Prevotella or Bacteroides spp. were reported in CRC respectively (207).

Wong et al. also demonstrated a causal relationship between the composition of CRC patients’ stools and carcinogenesis. Wong et al. fed fecal samples from patients diagnosed with CRC (versus healthy volunteers) to germ-free mice and conventional mice treated with the procarcinogenic azoxymethane. CRC-derived feces promoted polyp formation, intestinal dysplasia, epithelial proliferation and stemcellness, as well as inflammation (IL-17A, IL-22, IL-23A) associated with the colonic recruitment of Th1 and Th17 cells (208).

**COLON CANCER THERAPIES AND GUT MICROBIOTA**

Most pharmacological compounds in the oncological armamentarium have the capacity to perturb the delicate triangle of fitness and integrity of the intestinal barrier, the microbiota ecosystem, and the gut immune system. Such “off target” side effects of targeted therapeutics drugs may eventually affect their anticancer efficacy and/or the safety profile. Multiple chemotherapies and immune checkpoint inhibitors have been studied in preclinical models of colon carcinoma (26, 209, 210) and pancreatic cancer (211) expanding our current understanding of mechanisms underlying response.

Oxaliplatin (OXA) is a clinically effective tumoricidal platinum salt commonly used against colon, breast, ovarian and lung carcinomas (212, 213). The initial mode of action described for OXA was that it creates DNA damage, inducing formation of intra- and interstrand DNA adducts, generated by crosslinking between activated platinum species and specific base sequences (214). However, in the absence of a functional immune system, in particular a TLR4 signaling pathway and T lymphocytes, OXA fails to mediate full blown anticancer activity in tumor bearing mice and in patients diagnosed with CRC (215, 216). Indeed, following OXA-based chemotherapy, antitumor adaptive immune responses are activated (217). This activation occurs through the induction of immunogenic cell death (ICD) of colon cancer cells due to the initiation of executioner caspases (216, 218–220) and through the subsequent release of several damage associated molecular patterns (DAMPs) including surface exposed-calreticulin (220), high mobility group box 1 protein (HMGB1) (221), CXCL10 (223), and Annexin A1 (224). Moreover, the antitumor efficacy of OXA largely depends on the presence of an intact microbiota. Disruption of murine microbiota with broad-spectrum antibiotics was shown to reduce the cytotoxicity and production of reactive oxygen species (ROS) via the NADPH oxidase NOX2 by tumor-infiltrating myeloid cells after OXA treatment (209). ROS are needed for the OXA antitumor effect. Although the genotoxic effect of platinum...
compounds was known to require ROS and particularly H$_2$O$_2$ production by tumor cells in vitro, ROS is produced by tumor-associated myeloid cells in vivo. Thus, the microbiota affects OXA early tumor genotoxicity by systemically priming tumor-associated myeloid cells for ROS production (209) (Figure 5A).

Finally, OXA does not just induce apoptosis of tumor cells. Chemotherapy can promote apoptotic cell death in the intestinal crypts of healthy tissues, known as chemotherapy-induced mucositis (224). As many other anticancer agents, OXA compromises the integrity of the intestinal barrier which may create a dysbiosis and potentiate systemic inflammation and immunity (225–228). It has been shown that OXA is associated with cell apoptosis in ileal, but not colonic, crypts sparing the villi and the lamina propria, in tumor bearing mice and in patients diagnosed with proximal colon cancer (pCC) (26). Ileal crypt apoptosis was correlated with progression-free survival and the accumulation of TILs and Th1 in the tumor bed (26). (Figure 5A) Ileal cell apoptosis requires caspase 3 and caspase 7 executioners as well as ileal commensals. First, tumor bearing mice in which caspase 3/7 expression was ablated specifically in intestinal epithelial cells (IEC) (Casp3/7$^{IEC}$) failed to respond to OXA while RIPK3-deficient animals disabled for necroptosis of the IEC controlled tumor outgrowth during OXA therapy (26). Secondly, when broad spectrum antibiotics were administered along with OXA, the apoptosis of the crypts was blunted and the anticancer effects were abrogated. To consolidate the impact of patient microbiota on the efficacy of OXA, Roberti et al. utilized an avatar model (229), in which the intestines of germ free mice were colonized with human colonic content collected from pCC patients. Two weeks later, these mice were inoculated with subcutaneous MC38 and 7 days later, treated with OXA. While 66% of patient microbiota resulted in antitumor efficacy of OXA comparable to that observed in mice reared in specific pathogen-free conditions, 33% patients’ microbiota induced complete resistance to this immunogenic chemotherapy. Ileal crypt apoptosis was highly correlated with major changes in the composition of the ileal microbiome, with a dominance of Erysipelotrichaceae at the expense of Fusobacteriaceae.

**FIGURE 5** | Pharmacological-microbiota interactions. Drugs and microbes can mutually influence each other. A synergy - exemplified by oxaliplatinum - can occur culminating in triggering the local immune system eventually controlling the tumor microenvironment. This synergy is severely compromised by antibiotics. (A) Drugs can shape gut microbiota and activate bacterial functions beneficial for the anticancer therapy. For instance, CTLA-4 blockade could enrich the ileal microbiome with distinct Bacteroides spp. that in turn fosters Th1 responses beneficial against cancer. (B) On the other hand, commensals can metabolize anticancer prodrugs, thus activating or inactivating their bioactive metabolite, sustaining or compromising the therapeutic effect. Conversely, increased recycling of bioactive compounds may be accelerated by enzymatic machineries associated with distinct microbes (such as camptothecin-11), generating severe side effects. (C).
Capecitabine, an oral prodrug of fluorouracil, inhibits the enzyme activity of thymidylate synthase during DNA replication (230). It has been proven effective in conjunction with OXA in metastatic colon cancers and other cytotoxicants in breast cancer and is widely used in adjuvant setting. A pioneering study explored the role of microbes in modulating the effect of 5-FU and other fluoropyrimidines on Caenorhabditis elegans and found that bacteria are key determinants of fluoropyrimidine efficacy on host metabolism (231). Microbes can boost or suppress the effects of fluoropyrimidines through metabolic drug interconversion involving bacterial vitamin B_6, B_9, and ribonucleotide metabolism. (Figure 5C) Also, modulations in bacterial deoxynucleotide storage amplify 5-FU-induced autophagy and cell death in host cells. A Chinese prospective study of the fecal composition of 31 females treated for HER2 negative breast cancer used 16S RNA sequencing to analyze the variations of the gut microbiota during a maintenance chemotherapy comparing metronomic versus conventional dose of capecitabine (232). While alpha diversity was not different between the two treatment modalities, beta diversity varied significantly between the two groups, with a relative depletion of Cyanobacteria, Chloroplast, Blautia, and Streptophyta in stools of the metronomic capecitabine -treated females. Multivariate analyses of progression-free survival of all 31 patients regardless of capecitabine dosing revealed that Blautia obeum was significantly associated with clinical benefit (HR 3.4) in contrast with Slakia (HR 0.2). For the study of relative bacteria functions, the Kyoto Encyclopedia of Genes and Genomes (KEGG) modules were quantified, indicating that metronomic capecitabine tended to enrich for bacteria involved in nitrification, and putrescine, lysine/arginine/ornithine transport system. These metabolic traits are reminiscent of polyclamine synthesis closely linked with the activation of the autophagic machinery mandatory for the immunogenicity of cancer cell death (233) (Figure 5B). Although concerning for breast cancer females, and underpowered, this pioneering study suggests that distinct chemotherapeutic regimen influence the composition of the gut microbiota that in turn, could impact clinical outcome (232).

**Gemcitabine and Camptothecine**

Bacteria can metabolize chemotherapeutic drugs, to increase or decrease their pharmacological effect. The presence of bacteria inside human tumors may paradoxically result in drug concentrations that are lower in the tumor than in other organs. Gemcitabine is a nucleoside analog (2',2'-difluorodeoxycytidine) used to treat patients with pancreatic, lung, breast, or bladder cancers. It has been occasionally combined with FOLFOX against colon cancer (234). Bacteria can metabolize the chemotherapeutic drug gemcitabine (2',2'-difluorodeoxycytidine) into its inactive form, 2',2'-difluorodeoxyuridine (211). Metabolism is dependent on the expression of a long isoform of the bacterial enzyme cytidine deaminase (CDD)_{2}, seen primarily in Gammaproteobacteria found in pancreatic ductal adenocarcinoma or colon cancers (211). In a colon cancer mouse model, the authors demonstrated that gemcitabine resistance was induced by intratumor Gammaproteobacteria, dependent on bacterial CDD_{2} expression, and abrogated by the ciprofloxacin fluoroquinolone. Antibiotic-treated mice displayed a better antitumor response to gemcitabine than control mice (211) (Figure 5C).

Bacteria could confer drug resistance through the induction of autophagy in colorectal cancer cells, therefore interfering in the tumoricidal activity of chemotherapy. *F. nucleatum* is gradually increased from normal tissues to adenoma tissues and to adenocarcinoma tissues in colorectal carcinogenesis (178, 179). Moreover, the amount of *F. nucleatum* in CRC tissues was associated with shorter survival (187). Another independent group found that the five-year recurrence survival was substantially shorter in patients with CRC rich in *F. nucleatum* than in those poor in *F. nucleatum* (189). Multivariate regression analyses demonstrated that the amount of intratumor *F. nucleatum* was an independent predictor of CRC aggressiveness and recurrence post-chemotherapy with significant hazard ratios for predicting clinical outcome (189). ULK1/ATG7 -dependent autophagy contributed to *F. nucleatum*-mediated CRC resistance to OXA and 5-FU regimens (189). *F. nucleatum* induced- genomic loss of miR-18a* and miR-4802 depended upon the TLR4/MYD88 signaling pathway (189) (Figure 5C).

**Immune Checkpoint Inhibitors (ICIs)**

DNA mismatch repair-deficient or microsatellite-instable tumors particularly benefit from the immune checkpoint blockade (85). Accumulating evidence points to the critical role of the gut microbiota in the efficacy of ICIs. First, antibiotics taken within the month preceding start of anti-CTLA-4 or anti-PD-1 antibodies, markedly attenuated the clinical benefit, reducing both progression-free and overall survival across many metastatic and stage III malignancies amenable to therapies based on immune checkpoint inhibition (239, 240). Second, fecal microbial transplantation of stools from patients prone to respond to ICI or doomed to fail first or second line ICI-based therapy confer sensitivity or resistance to tumor bearing mice treated with anti-PD-1 antibodies respectively (241). Third, shot gun metagenomics sequencing of patients’ stools at diagnosis may help predicting primary resistance to PD-1
blockade in metastatic melanoma (242), kidney (241) and lung cancers (243). Fourth, oral compensation of antibiotics-treated tumor bearers or dysbiotic hosts with monoclonal anticancer probiotics (such as Akkermansia muciniphila (243), or Bifidobacterium pseudolongum (244) restored full blown immunostimulatory activity of anti-PD-1 antibodies. (Figure 6). Conversely, ICIs also have an impact on the gut composition. Vetizou et al. reported that anti-CTLA-4 antibody modulated the ileal bacteria composition, increasing the relative abundance in Bacteroides spp. (namely B. fragilis) and Burkholderiaceae family members, involved in the IL-12-dependent priming of Th1 cells as well as the immunostimulatory and anti-cancer effects of CTLA-4 blockade (210). In line with these preclinical data, six months of therapy with nivolumab ameliorated the alpha diversity of metastatic kidney cancer bearing patients in those individuals benefiting from the antibodies (241). Such studies are awaited in MSIhigh CRC amenable to PD-1 blockade. Finally, memory Th1 and Tc1 immune responses directed against immunogenic bacteria E. hirae, A. muciniphila, and B. fragilis dictated progression free survival in cancer patients treated with anti-PD-1 or anti-CTLA-4 Abs (210, 243).

TOWARDS A SOLUTION TO THE PARADOX OF COLON CANCERS

As stated above, the reasons why DNA mismatch repair-proficient or microsatellite-stable CRC, endowed with tumor infiltrating lymphocytes at the primary or metastatic stage and treated with a cytotoxic compound mediating ICD, fail to benefit from immune checkpoint blockade are enigmatic (85, 100). Indeed, FOLFOX could induce T-bet-dependent PD-1 expressing CD8+ T cell infiltration and IFN-γ-mediated PD-L1 upregulation in mouse models of colon cancers, and in CRC patients (245, 246).

The molecular and cellular cues underlying this delicate equilibrium between tolerance (vis-à-vis of self and food antigens) and immunity (against pathogens and tumors) in the intestinal barrier remain an immunological challenge. Recent work has highlighted how ileal bacteria may contribute to break tolerance to self-antigens shared between ileal crypts and cancer stem cells. Roberti et al. reported that the immunogenicity of ileal epithelial cell death mediated by OXA to treat a proximal CRC relied on two biological features: (1) antigenicity provided by the caspase 3/7-dependent apoptosis of crypt-derived IEC and (2) the adjuvanticity of selected ileal bacterial families or species (Erysipelotrichaceae, B. fragilis). These features cooperated to elicit Tfh immune responses and antibody producing cells protective against tumor progression. The serum IgG levels were increased by immunogenic bacteria but not by tolerogenic bacteria and could be associated with IgG responses directed toward bacteria or tumor cells (Figure 7A). A vaccine composed of OXA-exposed dying ileal IEC was more effective to immunize naive hosts against a lethal dose of colon cancer cells (CT26 and MC38) when harvested from SPF wild type mice than TLR2/4 or MYD88 KO mice or germ-free animals. These data suggest that self-antigens derived from ileal crypts could elicit an immune response in the presence of microbial adjuvants. Using 16S rRNA gene

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**FIGURE 6** | Evidence pointing to a key role of the gut microbiota in immune checkpoint inhibitors (ICI)-mediated anticancer effects. Recently, pre- and clinical studies have shown the clinical significance of the composition of the gut microbiota in ICI efficacy. 1) Retrospective and prospective studies have highlighted the detrimental effect of antibiotics administration within the month preceding the start of immunotherapy in multiple stage III and IV cancers. 2) Metagenomic analysis (MGS) of patients’ stool composition predicts primary resistance to ICI in 1L and 2L melanoma, kidney and lung cancers. 3) Avatar mouse models where the intestines of tumor bearing rodents are colonized by human stools from cancer patients at diagnosis allowed to predict the response to immunotherapy in patients. 4) The identification of beneficial bacteria, such as Akkermansia muciniphila or Bifidobacterium pseudolongum for immunotherapy efficacy opens up prospects to restore cancer-associated dysbiosis in patients.
sequencing of patients’ ilea and culturomics, the authors concluded that distinct bacteria residing in ilea may convey the immunogenicity of apoptotic cell death of the crypt, shifting the antitumor immune response from Th17 (observed in progressive tumors) to Tfh cells accumulating in tumor draining lymph nodes (tdLN) of mice responding to OXA. Indeed, Bacteroides fragilis, or Erysipelatoclostridium ramosum could potentially restore responsiveness to chemotherapy in germ free mice or ATB-treated SPF animals or could confer immunogenicity to sterile ileal apoptosis. In contrast, Fusobacterium nucleatum or Prevotella clara failed to do so. In fact, OXA could mobilize migratory conventional type 1 DCs (cDC1) (CD103⁺CD11b⁺ DC) from the ileal lamina propria to the mLN, and contribute to IL-1β and IL-12-dependent priming of CXCR5⁺Bcl6⁺PD-1⁺ CD4⁺ Tfh cells and systemic IgG responses. In the absence of Tfh or B cells, the vaccine composed of OXA-exposed crypt ileal enterocytes failed to induce long term memory autoreactive CD4⁺ and CD8⁺ T cell responses protective against colon
carcinoma. In Batf3 gene deficient animals defective in cDC1, Tfh were not primed during OXA administration. Moreover, cDC1 exposed to immunogenic ileal-residing bacteria (B. fragilis or C. ramosum) could produce IL-1β and IL-12p70 while they could not produce IL-12p70 when stimulated with F. nucleatum or P. clara. The authors postulated that PD-1 expressing Tfh induced by OXA-mediated ileal apoptosis could be bolstered by anti-PD-1 Abs and carried out to modulate the efficacy of a combination of OXA with this immune checkpoint inhibitor with a concomitant oral gavage with immunogenic (B. fragilis, C. ramosum) versus tolerogenic (F. nucleatum, P. clara) bacteria in MSS and MSI mouse colon cancers. They could bring evidence that the ileal residence and/or colonization of bacteria fostered (as for B. fragilis, C. ramosum) or blunted (as for F. nucleatum, P. clara) the immunostimulatory anticancer effects of the combinatorial regimen. These additive effects of both therapeutic modalities were accompanied by a rise in IgG2b serum levels.

Initially, ileal bacteria participate in ileal crypt apoptosis but not in the release of DAMPs. Indeed, in the presence of antibiotics, OXA-induced ileal cell demise was significantly reduced. Interestingly, OXA alone could promote the release of ATP and HMGB1, two hallmarks of ICD, that were not mandatory for the immunizing capacities of dying IEC, in contrast to live bacteria or pathogen associated molecular patterns (26). This is in contrast with a report utilizing gram negative bacteria ghosts injected intraperitoneally with OXA that could turn on ICD in the peritoneal cavity and elicit potent NKT and CD8+ T cell responses associated with regression of peritoneal carcinogenesis (247). These observations emphasize that the tumor macroenvironment and organ may critically influence the requirements for efficient priming of a T cell response.

Secondly, it is intriguing that ileum may be more appropriate than the colon mucosae to elicit Tfh immunity in the MLN. As outlined above (248, 249), the immune cell types and microbiota composition are highly compartmentalized (250, 251), lymphatics draining the small and large intestines differ and ileal crypts can undergo apoptotic cell death after chemotherapy while colonic crypts failed to do so (26).

Third, breaking tolerance to self-tissues in colon cancer has been previously achieved (252). The authors showed the capacity of Stat3-deficient IEC to promote adaptive immunity in spontaneous models of carcinogenesis (252). Mitophagy, the specific degradation process of damaged or aged mitochondria, triggered lysosomal membrane permeabilization which enhanced MHC-I expression in IEC that in turn allowed cross-dressing of DC with IEC derived-MHC-I/peptide complexes. This pathway could also come into action upon OXA treatment, knowing that STAT3 deficiency in epithelial cancer cells amplified an OXA-elicited type 1 IFN response and triggered an immunogenic cell death pattern, ameliorating antitumor T cell responses (253).

Fourth, our findings raise the theoretical possibility of a self-reactive T cell-dependent immunity that spares healthy tissues to specifically combat cancer (26). Uncoupling antitumor effects from ileitis or colitis remains an open conundrum in cancer chemotherapy and immunotherapy. We postulate that Lgr5 negative crypt-derived cells devoid of stemcellness share common antigens with pCC that elicit therapeutically relevant immune responses, at least in the context of chemotherapy with OXA. As a possibility, the fragments of apoptotic ileal IEC that contain colon cancer-crossreactive antigens and immunogenic commensals (with their TLR ligands) could end up in the same phagosomes of antigen-presenting cells in the GALT, thus providing an opportunity for both self-and non-self-peptides to be concomitantly loaded into MHC class II molecules (254). Actually, interactions between cytokine-producing Th cells and MHC class II+ Lgr5+ intestinal stem cells were shown to be critical for the self-renewal and/or differentiation of this latter cell type (255).

Finally, although Tfh and Tfh-like populations have been associated with poor prognosis in lymphoma (256, 257) and ICI-treated melanoma (258), numerous pre-clinical and clinical studies revealed their positive impact on the outcome of many solid tumors (259), including breast cancer (260, 261), non-small-cell lung cancer (NSCLC) (262), ovarian cancer (263), and colorectal cancer (25, 26). Mechanisms underlying the protective role of Tfh cells are not fully understood, although some evidence point to cooperation with CD8+ T cells and IL-21 (25, 43). CXCL13-producing Th act as “organizers” of germinal centers in secondary and tertiary lymphoid organs, sustaining local humoral immunity by attracting antibody-secreting B cells (264), which control tumor progression (260, 265–268). ICIs are used to reinvigorate cellular immunity and accumulating evidence show a direct impact of ICIs on humoral response. Indeed, anti-PD-1 antibodies increase germinal center formation, CD4+ T infiltrates and terminal B cell differentiation (269). The cooperation between Tfh and B cells appears to be associated with a favorable clinical outcome in colorectal cancer (25) and in breast cancer, in both human (260, 270) and murine models of tumors harboring high tumor mutational burden (30). The humoral response in CRC may be directed towards several tumor-associated antigens, such as Carcinoembryonic antigen (CEA) (271, 272), Epidermal Growth Factor Receptor (EGFR) (273), Human Epidermal Growth Factor Receptor 2 (HER2 or ErbB2) (274), MUC5AC (275), and ribosomal P proteins (276) and facilitate antibody-dependent cellular cytotoxicity (30) (Figure 7B).

Intratumoral terminally differentiated memory B cells and/or plasma cells can predict prolonged survival (49, 51, 52). Tumor-infiltrating B cells have been associated with positive clinical outcome following therapy with ICIs in melanoma (40, 277), with (41) or without metastasis (278), in sarcoma (39) and in renal cell carcinoma (40). Of note, B cells can exert direct tumoricidal activity against cancer cells, in an antigen-specific and Fas ligand-dependent manner (279).

Intratumoral bacterial colonization and residency, whether localized within myeloid cells or in tumor cells have recently been described to modulate local metabolism, change tumor cell biology or interfere in the drug catabolism (211, 280). IgG responses directed against intratumoral bacteria may contribute to preventing tumor colonization and/or tumor killing, in both mouse models and patients (30, 281, 282).
Collectively, these studies highlight the potential clinical significance of B cells, antibody secreting cells, and antibodies as well as Tfh in dictating the efficacy of the combination of FOLFOX+ICIs.

Moreover, Mager et al. have recently shown that metabolites of small intestine-derived immunogenic species, such as *Bifidobacterium pseudolongum*, can act as co-stimulatory molecules on the TCR signaling of Th1 and Tc1 cells. Indeed, *B. pseudolongum* produces the inosine, a metabolite that acts through the adenosine A2A receptor expressed on TILs and potentiates the effect of PD-1 or CTLA-4 antibodies in mice bearing transplantable MC38 colon cancer in conjunction with co-stimulatory signal. This effect was lost in mice harboring A2A receptor-deficient T cells. The anti-tumor effect of the inosine to reduce tumor growth is still dependent on the presence of a microbiota since in germ-free mice the administration of inosine alone did not ameliorated ICIs efficacy while it did in the presence of a complex microbiota. Additionally, *B. pseudolongum* modulated the intrinsic local immunity as it increased the T-bet expression among CD4⁺ T cells in the GALT and the nLN but not in the periphery. On the other hand, *B. pseudolongum* induced systemic immune modulation when associated with ICIs leading to the activation of effector cells and the secretion of IFN-γ by CD4⁺ and CD8⁺ T cells. The systemic effect can be explained by the fact that anti-CTLA-4 alters the gut barrier integrity increasing the systemic translocation of metabolites from the gut. The activation of T cells requires the presence of an IL-12 co-stimulatory signal provided by cDCs. Thus, in the absence of cDCs (cDC-DRT mouse model), *B. pseudolongum* associated with anti-CTLA-4 failed to induce IFN-γ-T cell producers and anti-tumor effect. Furthermore, anti-IL-12p75 neutralization dramatically dampened the effect of anti-CTLA-4 combined with immunogenic species in a genetically modified mouse model presenting dMMR intestinal tumors mismatch repair (*Msh2LoxP/LoxP* Villin-Cre) tumors (Figure 7C) (244).

Altogether, these studies point to the central role of the ileal, but not the colonic, epithelial cells and its natural microbial ecosystem, in mobilizing an efficient immune response against self-antigens expressed by colon cancers, presumably tumor stem cells. The solution to the paradox of colon cancers—which has the propensity to elicit and attract CD3⁺/CD8⁺ T cells (so called “high immunoscope”) but remains resistant to immunotherapy when devoid of mutated neoantigen-relies on two pillars, i) ileal apoptosis through activation of the executioner caspases, and ii) a proper ileal microbiome composition balancing immunogenic and tolerogenic commensals. These two conditions can be achieved by cytotoxic regimen inducing immunogenic cell death of tumor and/or crypt stem cells and by doing so, favor the dominance of immunogenic bacteria. Such suitable therapeutic regimens include FOLFOX (5-FU/leucovorin/OXA), FOLFIRI, or any type of antibody-dependent cytoxicity through various payloads releasing compounds endowed with ICD properties (283). The ensuing immune response is composed of humoral (Ab secreting cell and B cell-based) and cellular (Tfh and memory CD8⁺ T cell-based) based-immune responses towards crypt -derived- self antigens shared between the small intestine and tumor cells. These premises allowed the demonstration of synergistic effects between ICD-mediating chemotherapy and PD-1 blockade in pMMR or MSS mouse colon tumors.

**CONCLUSION**

Despite tremendous advances in the classification of CRC based on genetics, transcriptomics and immunometrics, few patients benefit from combinatorial regimens that combine ICD-mediating cytotoxicants and ICIs or targeted therapies. Increasing knowledge on the macroenvironment of colon carcinoma, mainly on the connections between the local microbial ecosystem and the systemic immune tonus, has shed new light on the role of intratumoral and ileal bacteria in modulating the immune contexture as well as tumor signaling pathways. This review gives novel insights into the development of potential biomarkers of response (ileobiome, ileal immune patterns) or novel therapies based on live biotherapeutics consisting of appropriate mixtures of immunogenic commensals or phages (284) to complement current combinatorial regimen. Moreover, attention should be paid to new actors playing a role in colon cancer immunosurveillance, including antibodies to self-antigens, B cells, and Tfh. Moreover, technological advanced such as single cell sorting and sequencing will also enable a comprehensive characterization of these actors’ functions and specificities.

**AUTHOR’S NOTE**

The figures were conceived using https://smart.servier.com/.

**AUTHOR CONTRIBUTIONS**

MF, SY, MP, AH, AC, MR, and LZ contributed to the writing of this manuscript. MF and MR conceived the figures using https://smart.servier.com/. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut (2017) 66:683–91. doi: 10.1136/gutjnl-2015-310912
2. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet (2019) 394:1467–80. doi: 10.1016/S0140-6736(19)32319-0
3. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev (2007) 21:2525–38. doi: 10.1101/gad.1593107
4. Dashiti SG, Buchanan DD, Jayasekara H, Ouakrim DA, Clendenning M, Rosty C, et al. Alcohol Consumption and the Risk of Colorectal Cancer for Unstable Cancer. Fam Cancer (2016) 15:405. doi: 10.1007/s10434-015-0757-z
5. Murphy N, Ward HA, Jenab M, Rothwell JA, Boutron-Ruault M-C, Key TJ, Fentiman IS, et al. Dietary fiber and colorectal cancer risk: a nested case-control study using food diaries. J Natl Cancer Inst (2010) 102:614–26. doi: 10.1093/jnci/djq092
6. Chan AT, Giovannucci EL. Primary Prevention of Colorectal Cancer. Gastroenterology (2010) 138:2029–2043.e10. doi: 10.1053/j.gastro.2010.01.057
7. Dahm CC, Keogh RH, Spencer EA, Greenwood DC, Key TJ, Fentiman IS, et al. Deficiency of epithelial barrier tissues. Gut (2017) 66:683–91. doi: 10.1136/gutjnl-2015-310912
8. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to cancer staging. J Natl Cancer Inst (2017) 109:2073–2087. doi: 10.1093/jnci/djx310
9. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell (1990) 61:259–67. doi: 10.1016/0092-8674(90)90186-4
10. Advani SM, Advani PS, Brown DW, DeSantis SM, Korphaisarn K, vanVille HM, et al. Global differences in the prevalence of the CpG island methylator phenotype of colorectal cancer. BMC Cancer (2019) 19:964. doi: 10.1186/s12885-019-1644-9
11. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology (2010) 138:2073–2087.e3. doi: 10.1053/j.gastro.2009.12.004
12. Gatalica Z, Vranic S, Xu J, Swensen J, Reddy S. High microsatellite instability (MSI-H) colorectal carcinoma: a brief review of predictive biomarkers in the era of personalized medicine. Fam Cancer (2016) 15:405–12. doi: 10.1007/s10689-016-9884-6
13. Wu P, Wu D, Ni C, Ye J, Chen W, Hu G, et al. γT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. Immunity (2014) 40:785–800. doi: 10.1016/j.immuni.2014.03.013
14. Toor SM, Syed Khaja AS, El Saltah H, Bekdache O, Kanbar J, Jaloudi M, et al. Increased levels of circulating and tumor-infiltrating granulocytic myeloid cells in colorectal cancer patients. Front Immunol (2016) 7:560. doi: 10.3389/fimmu.2016.00560
15. Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, et al. Circulating and Tumor-Infiltrating Myeloid-Derived Suppressor Cells in Patients with Colorectal Carcinoma. PLoS One (2013) 8:e57114. doi: 10.1371/journal.pone.0057114
16. Wang D, Yu W, Lian J, Wu Q, Liu S, Yang L, et al. Th17 cells inhibit CD8+ T cell migration by systematically downregulating CXCR3 expression via IL-17A/STAT3 in advanced-stage colorectal cancer patients. J Hematol Oncol (2020) 13:68. doi: 10.1186/s13045-020-00897-z
17. Chen J, Ye X, Pitmon E, Lu M, Wan J, Jellison ER, et al. IL-17 inhibits CXCL9/10-mediated recruitment of CD8+ cytotoxic T cells and regulatory T cells to colorectal tumors. J Immunother (2019) 7:524. doi: 10.1186/s41467-019-01636-w
18. Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. Cancer Res (2011) 71:1263–71. doi: 10.1158/0008-5472.CAN-10-2907
19. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity (2013) 39:782–95. doi: 10.1016/j.immuni.2013.10.003
20. Roberti MP, Yonekura S, Duong CPM, Picard M, Ferrere G, Tidjani Alou M, et al. Chemotherapy-induced ideal crypt apoptosis and the ideal microbiome shape immunosurveillance and prognosis of proximal colon cancer. Nat Med (2020) 26:919–31. doi: 10.1038/s41591-020-0882-8
21. Edin S, Kaprio T, Hagström J, Larsson P, Mustonen H, Böckelman C, et al. The Prognostic Importance of CD20+ B lymphocytes in Colorectal Cancer and the Relation to Other Immune Cell subsets. Sci Rep (2019) 9:19997. doi: 10.1038/s41598-019-5644-8
22. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. Science (2006) 313:1960–4. doi: 10.1126/science.1129139
23. Galon J, Fridman W-H, Pagès F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res (2009) 69:886–904. doi: 10.1158/0008-5472.CAN-08-4356
24. Hollern DP, Xu N, Thennavan A, Glodowski C, Garcia-Recio S, Mott KR, et al. B Cells and T Follicular Helper Cells Mediate Response to Checkpoint Inhibitors in High Mutation Burden Mouse Models of Breast Cancer. Cell (2019) 179:1206.e21. doi: 10.1016/j.cell.2019.10.028
25. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawil M, et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell (2009) 16:91–102. doi: 10.1016/j.ccr.2009.06.018
26. Andreu P, Johansson M, Afara NL, Pucci F, Tan T, Junankar S, et al. FcRgamma activation regulates inflammation-associated squamous carcinogenesis. Cancer Cell (2010) 17:121–34. doi: 10.1016/j.ccr.2009.12.019
27. De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer (2010) 10:747–74. doi: 10.1038/nrc2874
28. Kammerloens T, Qin Z, Briesemeister D, Bendelac A, Blankenstein T. B-cells and IL-4 promote methylcholanthrene-induced carcinogenesis but there is no evidence for a role of T/NKT-cells and their effector molecules (Fas-ligand, TNF-α, perforin). Int J Cancer (2012) 131:1499–508. doi: 10.1002/ijc.27411
29. Liu Z, Zhou Q, Wang Z, Zhang H, Zeng H, Huang Q, et al. Intratumoral TIGIT+ CD8+ T-cell infiltration determines poor prognosis and immune response. Ann Surg Oncol (2019) doi: 10.1038/s41591-020-01636-w
evasion in patients with muscle-invasive bladder cancer. *J Immunother* (2020) 8:e000978. doi: 10.1136/jitc-2020-000978

36. Miller BC, Sen DR, Al Aboos K, Bi K, Virkud YV, LaFleur MW, et al. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* (2019) 20:326–36. doi: 10.1038/s41590-019-0312-6

37. Thibault M-L, Mammessier E, Gartner-Dardenne J, Pastor S, Just-Landi S, Xerri L, et al. PD-1 is a novel regulator of human B-cell activation. *Int Immunol* (2013) 25:129–37. doi: 10.1093/intimm/dxt098

38. Wang X, Wang G, Wang Z, Liu B, Han N, Li J, et al. PD-1-expressing B cells suppress CD4+ and CD8+ T cells via PD-1/PD-L1-dependent pathway. *Mol Immunol* (2019) 109:20–26. doi: 10.1016/j.molimm.2019.02.009

39. Petippets F, Reyniès A, Keung E, Chen W-W, Sun C-M, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature* (2020) 577:1–5. doi: 10.1038/s41586-019-1906-8

40. Helmink BA, Reddy SM, Gao J, Zhang S, Liu B, Han N, Li J, et al. Anti-CD20 antibody promotes cancer escape via enrichment of tumor-infiltrating plasma cells impeding T-cell-dependent immunogenic edema and overall survival time in patients with brain metastases. *Oncoimmunology* (2016) 5:e1057388. doi: 10.21429/1262402X.2015.1057388

41. Cabrita R, Lauss M, Sanna A, Donia M, Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Oncoimmunology* (2016) 5:205. doi: 10.1080/2162402X.2015.1057388

42. Jenh C-H, Cox MA, Hipkin W, Lu T, Pugliese-Sivo C, Gonsiorek W, et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic properties of tumors associated with local immune cytolytic activity. *Immunol J* (2017) 47:95–101. doi: 10.1016/j.jimmunol.2017.05.018

43. Shahpour S, Font-Burgada J, Di Caro G, Zhong S, Sanchez-Lopez E, Dhar D, et al. Immunosuppressive plasma cells and Tim-3 expression in Tumor tissue in Stage I-III Colorectal Cancer. *Cancer Res* (2013) 73:2127–38. doi: 10.1158/0008-5472.CAN-12-4184

44. Mao H, Han F, Wu Z, Wang Z, Zhou Y, Zhang P, et al. Colorectal tumors are enriched with regulatory plasmablasts with capacity in suppressing T cell function. *DNA Cell Biol* (2017) 36:794–800. doi: 10.1089/dna.2017.3669

45. Bodoagi M, Lee Chang C, Weyksza K, Lai J, Merino M, Wersto RP, et al. Anti-CD20 antibody promotes cancer escape via enrichment of tumour-evoked regulatory B cells expressing low levels of CD20 and CD137L. *Cancer Res* (2013) 73:2127–38. doi: 10.1158/0008-5472.CAN-12-4184

46. Mlecnik B, Gryschok L, Malcher J, et al. PD-1 is a novel regulator of human B-cell activation. *Int Immunol* (2013) 25:129–37. doi: 10.1093/intimm/dxt098

47. Jeng C-H, Cox MA, Hipkin W, Lu T, Pugliese-Sivo C, Gonsiorek W, et al. HUMAN B CELL-ATTRACTING CHEMOKINE 1 (BCA-1; CXCL13) IS AN ANAGONIST FOR THE HUMAN CXCR3 RECEPTOR. *Cytochemistry* (2007) 15:113–21. doi: 10.1016/j.cytobio.2001.09.001

48. Shi W, Dong L, Sun Q, Ding H, Meng J, Dai G. Follicular helper T cells promote the effector functions of CD8+ T cells via the provision of IL-21, which is downregulated due to PD-1/PD-L1-mediated suppression in colorectal cancer. *Exp Cell Res* (2018) 372:35–42. doi: 10.1016/j.yexcr.2018.09.006

49. Gao W, Zhou J, Ji B. Evidence of Interleukin 21 Reduction in Osteosarcoma Patients Due to PD-1/PD-L1-Mediated Suppression of Follicular Helper T Cell Functionality. *DNA Cell Biol* (2017) 36:794–800. doi: 10.1089/dna.2017.3669

50. Bodoagi M, Lee Chang C, Weyksza K, Lai J, Merino M, Wersto RP, et al. Anti-CD20 antibody promotes cancer escape via enrichment of tumour-evoked regulatory B cells expressing low levels of CD20 and CD137L. *Cancer Res* (2013) 73:2127–38. doi: 10.1158/0008-5472.CAN-12-4184

51. Mao H, Han F, Wu Z, Wang Z, Zhou Y, Zhang P, et al. Colorectal tumors are enriched with regulatory plasmablasts with capacity in suppressing T cell function. *Int Immunopharmacol* (2017) 49:95–101. doi: 10.1016/j.intimp.2017.05.018

52. Shahpour S, Font-Burgada J, Di Caro G, Zhong S, Sanchez-Lopez E, Dhar D, et al. Immunosuppressive plasma cells and Tim-3 expression in Tumor tissue in Stage I-III Colorectal Cancer. *Cancer Res* (2013) 73:2127–38. doi: 10.1158/0008-5472.CAN-12-4184

53. Shahpour S, Lin X-J, Bastian IN, Brain J, Burt AD, Akesson AA, et al. Inflammation-induced IgA+ cells dismantle anti-tumor immunity. *Nature* (2017) 551:340–5. doi: 10.1038/nature24302

54. Shimabukuro-Vornhagen A, Schlößer HA, Gryschok L, Malcher J, Bennhold K, Garcia-Marquez M, et al. Characterization of tumor-associated B-cell subsets in patients with colorectal cancer. *Onco-target* (2014) 5:6451–64. doi: 10.18632/oncotarget.1701

55. Jifu E, Yan F, Kang Z, Zhuo L, Xing J, Yu E. CD8+CXCR5+ T cells in tumor-draining lymph nodes are highly activated and predict better prognosis in colorectal cancer. *Hum Immunol* (2018) 79:446–52. doi: 10.1016/j.jhimmunol.2018.03.003

56. Lohr M, Edlund K, Botling J, Hammad S, Hellwig B, Othman A, et al. The prognostic relevance of tumour-infiltrating plasma cells and immunoglobulin kappa C indicates an important role of the humoral immune response in non-small cell lung cancer. *Cancer Lett* (2013) 333:222–8. doi: 10.1016/j.canlet.2013.01.036

57. Wouters MCA, Nelson BH. Prognostic Significance of Tumor-Infiltrating B Cells and Plasma Cells in Human Cancer. *Clin Cancer Res* (2018) 24:125–35. doi: 10.1158/1078-0432.CCR-17-1481

58. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindeja G, Meatchi T, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol* (2011) 29:610–8. doi: 10.1200/JCO.2010.30.5425
consensus molecular subtypes in colorectal cancer identifies novel tumour microenvironment profiles, with prognostic and therapeutic implications. Euro J Cancer Oxf Engl (1990) (1990) 123:118–29. doi: 10.1016/j.ejca.2019.09.008

74. Horvat N, Tavares C, Rocha C, Clemente Oliveira B, Petkovska I, Golub MJ. MRI of Rectal Cancer: Tumor Staging, Imaging Techniques, and Management. RadioGraphics (2019) 39:367–87. doi: 10.1148/rg.2019180114

75. deBraud F, Munzone E, Nolè F, De Pas T, Bifulco C, et al. CRC Paradox and Microbiome

90. Schrock AB, Ouyang C, Sandhu J, Sokol E, Jin D, Ross JS, et al. Tumor activity of oxaliplatin and 5-fluorouracil in patients with metastatic colorectal cancer with progressive disease while on or after 5-fluorouracil. Am J Clin Oncol (1998) 21:279–83. doi: 10.1097/00000421-199806000-00015

96. Marabelle A, Fakh K, Lopez J, Shah M, Shapiro-Frommer R, Nakagawa K, et al. Association of tumour mutational burden with outcomes in patients with selected advanced solid tumours treated with pembrolizumab in KEYNOTE-158. Ann Oncol (2019) 30:v477–8. doi: 10.1093/annonc/mdz253.018

97. Overman MJ, Lonardi S, Wong KYM, Lens H-J, Gelsomino F, Aglietta M, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol (2018) 36:773–9. doi: 10.1200/JCO.2017.76.9901

99. Antoniotti C, Borelli B, Rossini D, Pietrantonio F, Morano F, Salvatore L, et al. Annu Rev Immunol (2013) 31:51. doi: 10.1146/annurev-immunol-032712-100068

102. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Capsule 1. Murray KM, Sehdev A, Shaib WL, et al. A phase II study of pembrolizumab in combination with mFOLFOX6 for patients with advanced colorectal cancer. J Clin Oncol (2017) 35:3541–1. doi: 10.1200/JCO.2017.35.15_suppl.3541

103. Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic Analysis of the Human Distal Gut Microbiome. Science (2006) 312:1355–9. doi: 10.1126/science.1124234

104. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PloS Biol (2016) 14.e1002533. doi: 10.1371/journal.pbio.1002533

105. Koenig JE, Spor A, Salfone N, Frickier AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci (2011) 108:4578–85. doi: 10.1073/pnas.1008081107

106. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. Complete and Prolonged Response to Immune Checkpoint Blockade in Microsatellite Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. JCO Precis Oncol (2017) 3:1–5. doi: 10.1200/JCO.2016.08.00214

107. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature (2012) 486:222–7. doi: 10.1038/nature10153

108. Diet rapidly and reproducibly alters the human gut microbiome. Science (2011) 332:106–10. doi: 10.1126/science.1196738

109. Marrow JJ, Nevins RM, Dearlock KM, et al. Synergistic effects of antibiotics on the gut microbiota in patients with ulcerative colitis by inhibition in MSI-high metastatic colorectal cancer. Ann Oncol (2019) 30:1096–103. doi: 10.1093/annonc/mdz134

110. Hu W, Yang Y, Qi L, Chen J, Ge W, Zhong S. Subtyping of microsatellite instability-high colorectal cancer. Cell Commun Signal (2019) 17:79. doi: 10.1186/s12964-019-0397-4

111. Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, Quist M, et al. Genetic Mechanisms of Immune Evasion in Colorectal Cancer. Cancer Discov (2018) 8:730–49. doi: 10.1158/2159-8290.CD-17-1327

112. Federation Françophone de Cancerologie Digestive. Pembrolizumab in Combination With Xelox Bevacizumab in Patients With Microsatellite Stable Metastatic Colorectal Cancer and a High Immune Infiltrate: a Proof of Concept Study. clinicaltrials.gov (2020). Available at: https://clinicaltrials.gov/ct2/show/NCT04262667 (Accessed October 5, 2020).

113. Mandal R, Samstein RM, Lee K-W, Havel JJ, Wang H, Krishna C, et al. Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response. Science (2019) 364:485–91. doi: 10.1126/science.aau0447

114. Loupakis F, Depestit I, Baisan P, Intrini R, Prete AA, Leone F, et al. Prediction of Benefit from Checkpoint Inhibitors in Mismatch Repair Deficient Metastatic Colorectal Cancer: Role of Tumor Infiltrating Lymphocytes. Oncologist (2020) 25:481–7. doi: 10.1634/theoncologist.2019-0611

115. Marabelle A, Fakh MG, Lopez J, Shah M, Shapiro-Frommer R, Nakagawa K, et al. Association of tumour mutational burden with outcomes in patients with selected advanced solid tumours treated with pembrolizumab in KEYNOTE-158. Ann Oncol (2019) 30:v477–8. doi: 10.1093/annonc/mdz253.018

116. Overman MJ, Lonardi S, Wong KYM, Lens H-J, Gelsomino F, Aglietta M, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol (2018) 36:773–9. doi: 10.1200/JCO.2017.76.9901

117. Kikuchi T, Mimura K, Okamaya H, Nakayama Y, Saito K, Yamada L, et al. A phase II study of pembrolizumab in combination with mFOLFOX6 for patients with advanced colorectal cancer. J Clin Oncol (2017) 35:3541–1. doi: 10.1200/JCO.2017.35.15_suppl.3541

118. Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic Analysis of the Human Distal Gut Microbiome. Science (2006) 312:1355–9. doi: 10.1126/science.1124234

119. Koenig JE, Spor A, Salfone N, Frickier AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci (2011) 108:4578–85. doi: 10.1073/pnas.1008081107

120. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The control of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis (2015) 26:26050. doi: 10.3402/mehd.v26.26050

121. Browne HP, Neville BA, Forster SC, Lawley TD. Transmission of the gut microbiome viewed across age and geography. Nature (2012) 486:222–7. doi: 10.1038/nature10153

122. Diet rapidly and reproducibly alters the human gut microbiome. Science (2011) 332:106–10. doi: 10.1126/science.1196738

123. Marrow JJ, Nevins RM, Dearlock KM, et al. Synergistic effects of antibiotics on the gut microbiota in patients with ulcerative colitis by
antibiotic combination therapy. *PloS One* (2014) 9:e86702. doi: 10.1371/journal.pone.0086702

110. Reese AT, Cho EH, Katzman B, Nichols SP, Wniesi A, Villa MM, et al. Antibiotic-induced changes in the microbiota disrupt redox dynamics in the gut. *eLife* (2018) 7:e35987. doi: 10.7554/eLife.35987

111. Goodrich JK, Waters JL, Porcel AC, Sutter JL, Koren O, Blevkin R, et al. Human genetics shape the gut microbiome. *Cell* (2014) 159:789–99. doi: 10.1016/j.cell.2014.09.053

112. Turpin W, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, et al. Association of host genome with intestinal microbial composition in a large healthy cohort. *Nat Genet* (2016) 48:1413–7. doi: 10.1038/ng.3693

113. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microbiota by toll-like receptors is required for intestinal homeostasis. *Cell* (2004) 118:229–41. doi: 10.1016/j.cell.2004.07.002

114. Hooper LV, Wong MH, Thelen A, Hansson L, Falk PG, Gordon JL. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* (2001) 291:881–4. doi: 10.1126/science.291.5505.881

115. Stappenbeck TS, Hooper LV, Gordon JL. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci U S A* (2002) 99:15451–5. doi: 10.1073/pnas.20260429

116. Sharan R, Deshpande A, Gitter Y, Koyuturk M, Karp RM. Let’s move beyond modules: toward functional networks. *Am J Transl Res* (2010) 2:250–6. doi: 10.3892/tcrm.2010.101

117. Barba G, Stanghellini V, Brandi G, Cremon C, Di Nardo G, Di Giorgio R, et al. Interactions between commensal bacteria and gut sensorimotor function in health and disease. *Am J Gastroenterol* (2003) 100:2560–8. doi: 10.1111/j.1572-0241.2003.00230.x

118. Bouska D, Bezzilion C, Berard M, Werts C, Varona R, Boneca IG, et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* (2008) 456:507–10. doi: 10.1038/nature07450

119. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System. *Cell* (2005) 122:107–18. doi: 10.1016/j.cell.2005.05.007

120. Pei Z, Bini EJ, Yang L, Zhou M, Francois F, Blaser MJ. Bacterial biota in the human gut microbiomes. *DNA Res* (2008) 14:1449–58. doi: 10.1093/dnares/dgn052

121. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* (2016) 14:240–2. doi: 10.1038/nrmicro3552

122. Kastl AJ, Terry NA, Wu GD, Albenberg LG. The Structure and Function of the Human Small Intestinal Microbiota: Current Understanding and Future Directions. *Cell Mol Gastroenterol Hepatol* (2020) 9:935–45. doi: 10.1016/j.jcmgh.2019.07.006

123. Leit GS, Weitman S, Parodi G, Celio S, Sedghi H, Sanchez M, et al. Mapping the Segments of the Microbiome in the Human Small Bowel Using in Comparison with Stool: A REIMAGINE Study. *Dig Dis Sci* (2020) 65:2595–2604. doi: 10.1007/s10620-020-02617-x

124. Wang X, Heazlewood SP, Krause DO, Florin THJ. Molecular characterization of the microbiota of the murine ileum and colon. *Cell* (2015) 163:367–72. doi: 10.1016/j.cell.2015.08.058

125. James KR, Gomes T, Elmentaite R, Kumar N, Gulliver EL, King HW, et al. Distinct microbial and immune niches of the human colon. *Nat Commun* (2020) 11:3453–50. doi: 10.1038/s41467-020-16062-z

126. Denning DL, Norris BA, Medina-Contrasor O, Manicasasay Y, Geem D, Madan R, et al. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the cell/APC ratio, source of mouse strain, and regional localization. *J Immunol* (2019) 203:18733–47. doi: 10.4049/jimmunol.2000720

127. Atarashi K, Tanoue T, Ando M, Kamada N, Narushima S, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* (2015) 163:367–80. doi: 10.1016/j.cell.2015.08.058

128. Ansaldo E, Slayden LC, Ching KL, Koch MA, Wolf NK, Plichta DR, et al. Akkermansia muciniphila induces intestinal adaptive immune responses during homeostasis. *Science* (2019) 364:1179–84. doi: 10.1126/science.aaw7479
Fidelle et al.  CRC Paradox and Microbiome

---

149. Atarashi K, Suda W, Luo C, Kawaguchi T, Motto I, Narushima S, et al. Ectopic colonization of oral bacteria in the intestine drives TH1 cell induction and inflammatory bowel disease.  Cell (2017) 170:548–563.e16. doi: 10.1016/j.cell.2017.07.008

150. Fenton TM, Jørgensen PB, Niss K, Rubin SJS, Mörbe UM, Riis LB, et al. Role for Isolated Lymphoid Follicles in Priming of Region-Specific Immunity.  Immunity (2020) 52:557–70. doi: 10.1016/j.immuni.2020.02.001

151. Mann S, Sidhu M, Gowin K. Understanding the Mechanisms of Diet and Metabolic Syndromes in Mice.  Metabolism links bacterial biofilms and colon carcinogenesis.  Cell Metab (2015) 21:891–7. doi: 10.1016/j.cmet.2015.04.011

152. Fenton TM, Jørgensen PB, Niss K, Rubin SJS, Mörbe UM, Riis LB, et al. Metagenomic analysis of colorectal cancer datasets identifies a role for isolated lymphoid follicles in the early stages of colorectal carcinogenesis.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

153. Fidelle et al. CRC Paradox and Microbiome

---

154. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

155. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

156. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

157. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

158. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

159. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

160. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

161. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

162. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

163. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

164. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050

165. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Dulal S, Keku TO. Gut microbiome and colorectal adenomas.  Cancer J (2014) 20:225–31. doi: 10.1097/PPO.0000000000000050
191. Casasanta MA, Yoo CC, Udayasuryan B, Sanders BE, Umaña A, Zhang Y, et al. Fusobacterium nucleatum host-cell binding and invasion induces IL-8 and CXCL1 secretion that drives colorectal cancer cell migration. Sci Signal (2020) 13:eaa89157. doi: 10.1126/scisignal.aaa9157

192. Gur C, Ibrahim Y, Isacson B, Yamin R, Abed J, Gamliel M, et al. Binding of the Fap2 Protein of Fusobacterium nucleatum to Human Inhibitory Receptor TIGIT Protects Tumors from Immune Cell Attack. Immunity (2015) 42:344–55. doi: 10.1016/j.immuni.2015.01.010

193. Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, et al. Patients with colorectal cancer show elevation of the paracrine cytokine IL-10 in the colonic mucosa. Gastroenterology (2017) 153:1621–1633.e6. doi: 10.1053/j.gastro.2017.08.022

194. Casasanta MA, Yoo CC, Udayasuryan B, Sanders BE, Umaña A, Zhang Y, et al. CRC Paradox and Microbiome

195. Cremonesi E, Governa V, Garzon JFG, Mele V, Amicarella F, Muraro MG, et al. Gut microbiota modulate T cell trafficking into human colorectal cancer. Gut Microbes (2018) 9:675–80. doi: 10.1089/gm.2017.00619

196. Toor SM, Murshed K, Al-Dhaheri M, Khawar M, Abu Nada M, Ellkore D, Immune Checkpoints in Circulating and Tumor-Infiltrating CD4+ T Cell Subsets in Colorectal Cancer Patients. Front Immunol (2019) 10:293626. doi: 10.3389/fimmu.2019.02936

197. Dalmaso G, Cognoux A, Delmas J, Darfeulle-Michaud A, Bonnet R. The bacterial genotoxin colibactin promotes colon tumour growth by modifying the tumour microenvironment. Gut Microbes (2014) 5:675–80. doi: 10.4161/19490976.2014.969989

198. Denizot J, Desrichard A, Agus A, Uhrhammer N, Dreux V, Vouret-Craviari V, et al. Diet-induced hypoxia responsive element demethylation increases CEACAM6 expression, favouring Crohn’s disease-associated Escherichia coli colonisation. Gut (2015) 64:238–47. doi: 10.1136/gutjnl-2014-309649

199. Chan JL, Wu S, Geis AL, Chan GV, Gomes TAM, Beck SE, et al. Non-toxigenic Bacteroides fragilis (NTBF) administration reduces bacteria-driven chronic colitis and tumor development independent of polysaccharide A. Mucosal Immunol (2019) 12:164–77. doi: 10.1038/s41385-018-0085-5

200. Chung LH, Thiele Orberg E, Geis AL, Chan JL, Fu K, DeStefano Shields CE, et al. Bacteroides fragilis toxin coordinates a pro-carcinogenic tumor microenvironment. Cell (2016) 164:104–19. doi: 10.1016/j.cell.2016.02.038

201. Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, et al. Effective immunotherapy with an ERM-T target identifies new cancers. Science (2017) 355:1156–60. doi: 10.1126/science.aha5043

202. Pelley RJ. Oxaliplatin: a new agent for colorectal cancer. Curr Oncol Rep (2001) 3:147–55. doi: 10.1007/s11912-001-0015-6

203. Van Cutsem E, Cervantes A, Andrade R, Sobrero A, Van Vancksen JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol (2016) 27:1386–42. doi: 10.1016/j.annonc.2016.06.022

204. Gur C, Ibrahim Y, Isacson B, Yamin R, Abed J, Gamliel M, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med (2007) 13:1050–9. doi: 10.1038/nm1622

205. Teixeira A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, et al. Immune dysfunction of colon cancer cells treated with oxaliplatin. Oncogene (2010) 29:482–91. doi: 10.1038/onsc.2009.356

206. Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, et al. An immunosurveillance mechanism controls cancer cell ploidy. Science (2012) 337:1678–84. doi: 10.1126/science.1224922

207. Casares N, Pequignot MO, Teixeira A, Ghiringhelli F, Roux S, Chaput N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med (2005) 202:1691–701. doi: 10.1084/jem.20050915

208. Galluzzi L, Kepp O, Kroemer G. Mitochondrial regulation of cell death: a phylogenetically conserved control. Microb Cell (2016) 3:101–8. doi: 10.1598/mic.2016.03.483

209. Amicarella F, Muraro MG, Hirt C, Cremonesi E, Padovan E, Mele V, et al. Chemotherapy-induced antitumor immunity requires for myeloid peptide receptor 1. Science (2018) 350:972–8. doi: 10.1126/science.aad0779

210. Apetoh L, Ghiringhelli F, Teixeira A, Ciollo A, Ortiz C, Lidereau R, et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. Immuno Rev (2007) 220:47–59. doi: 10.1111/j.1600-065X.2007.00573.x

211. Sistigo A, Yamazaki T, Vaccelli E, Chaba K, Enot DP, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med (2014) 20:1301–9. doi: 10.1038/nm.3708

212. Galvani E, Yu Y, Baracco EE, Sistigo A, Enot DP, Pietrocola F, et al. Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. Science (2015) 350:972–8. doi: 10.1126/science.aad0779

213. Yuan CJ. Roles of growth factors in chemotherapy-induced mucosal damage repair. Curr Pharm Biotechnol (2003) 4:260–9. doi: 10.1717/1389201033489793

214. Goldszmid RS, Dzutsev A, Viala S, Zitvogel L, Restifo NP, Trinchieri G. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Cancer Immunol Immunother (2015) 64:1037–49. doi: 10.1007/s00262-015-1845-7

215. Walko CM, Lindley C. Capecitabine: a review. Clin Cancer Res (2005) 11:3824–34. doi: 10.1158/1078-0432.CCR-04-2414
antitumor effects from experimental models to clinical trials. Recent Pat Anticancer Drug Discov (2012) 7:265–96. doi: 10.2174/157489212801820020

272. Zanetti BF, Ferreira CP, de Vasconcelos JRC, Han SW. scFv6.C4 DNA vaccine with fragment C of Tetanus toxin increases protective immunity against CEA-expressing tumor. Gene Ther (2019) 26:441–54. doi: 10.1038/s41434-019-0062-y

273. Foy KC, Wygle RM, Miller MJ, Overholser JP, Bekaii-Saab T, Kaumaya PTP. Peptide vaccines and peptidomimetics of EGFR (HER-1) ligand binding domain inhibit cancer cell growth in vitro and in vivo. J Immunol Baltim Md 1950 (2013) 191:217–27. doi: 10.4049/jimmunol.1300231

274. Wang J, Hollingshead J, El-Masy N, Horncastle D, Talbot I, Tomlinson I, et al. Expression of EGFR, HER2, phosphorylated ERK and phosphorylated MEK in colonic neoplasms of familial adenomatous polyposis patients. J Gastrointest Cancer (2012) 43:444–55. doi: 10.1007/s12029-011-9330-9

275. Kocer B, McKolanis J, Soran A. Humoral immune response to MUC5AC in patients with colorectal polyps and colorectal carcinoma. BMC Gastroenterol (2006) 6:4. doi: 10.1186/1471-230X-6-4

276. Benvenuto M, Sileri P, Rossi P, Masuelli L, Fantini M, Nanni M, et al. Natural humoral immune response to ribosomal P0 protein in colorectal cancer patients. J Transl Med (2015) 13:101. doi: 10.1186/s12967-015-0455-7

277. Griss J, Bauer W, Wagner C, Simon M, Chen M, Grabmeier-Pfistershammer K, et al. B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. Nat Commun (2019) 10:4186. doi: 10.1038/s41467-019-12160-2

278. Amini RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med (2018) 24:1649–54. doi: 10.1038/s41591-018-0197-1

279. Tao H, Lu L, Xia Y, Dai F, Wang Y, Bao Y, et al. Antitumor effector B cells directly kill tumor cells via the Fas/FasL pathway and are regulated by IL-10. Eur J Immunol (2015) 45:999–1009. doi: 10.1002/eji.201444625

280. Riquelme E, Zhang Y, Zhang L, Montiel M, Zoltan D, et al. Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes. Cell (2019) 178:795–806.e12. doi: 10.1016/j.cell.2019.07.008

281. Luna Coronell JA, Sergelen K, Hofer P, Gysurarjan I, Brezina S, Hettegger P, et al. The Immunome of Colon Cancer: Functional In Silico Analysis of Antigenic Proteins Deduced from IgG Microarray Profiling. Genomics Proteomics Bioinformatics (2018) 16:73–84. doi: 10.1016/j.gpb.2017.10.002

282. Wang H-F, Li L-F, Guo S-H, Zeng Q-Y, Ning F, Liu W-L, et al. Evaluation of antibody level against Fusobacterium nucleatum in the serological diagnosis of colorectal cancer. Sci Rep (2016) 6:33440. doi: 10.1038/srep33440

283. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol (2017) 17:97–111. doi: 10.1038/nri.2016.107

284. Zheng DW, Dong X, Pan P, Chen KW, Fan JX, Cheng SX, et al. Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. Nat Biomed Eng (2019) 3:717–28. doi: 10.1038/s41551-019-0423-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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