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Redrawing Therapeutic Boundaries: Microbiota and Cancer

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Abstract

The unexpected roles of the microbiota in cancer challenge explanations of carcinogenesis that focus on tumor-intrinsic properties. Most tumors contain bacteria and viruses, and the host's proximal and distal microbiota influence both cancer incidence and therapeutic responsiveness. Continuing the history of cancer-microbe research, these findings raise a key question: to what extent is the microbiota relevant for clinical oncology? We approach this by critically evaluating three issues: how the microbiota provides a predictive biomarker of cancer growth and therapeutic responsiveness, the microbiota's causal role(s) in cancer development, and how therapeutic manipulations of the microbiota improve patient outcomes in cancer. Clarifying the conceptual and empirical aspects of the cancer-associated microbiota can orient future research and guide its implementation in clinical oncology.

Keywords

Cancer; microbiota; biomarkers; causality; therapeutic modulation; network medicine

1 **Introducing the microbiota to oncology**

2 Recent work elucidating the microbiota's various roles in cancer initiation, progression and
3 treatment challenges the predominant view that cancer can be explained primarily in terms
4 of tumor-intrinsic properties [1]. Not only do tumors contain bacteria [2,3], viruses [4] and
5 sometimes fungi [5], but both cancer incidence and response to various cancer therapies are
6 influenced by the host's microbiota [6]. Despite the overall enthusiasm around the
7 microbiota in most areas of current biology and medicine [7–10], these discoveries came as a
8 surprise because they expanded the scope of what was considered relevant for oncology.
9 This research has also been met with skepticism; amidst the controversial history of using
10 microbes to explain or treat cancer, it has often been difficult to establish the reproducibility,
11 efficacy and safety of these approaches [11]. Moreover, in certain cancer types, the relative
12 importance of microbial versus tumor cell mechanisms and their interactions in
13 carcinogenesis remains debated [12]. It is thus still an open question to what extent the
14 microbiota is relevant for clinical oncology [13–16], and whether the role of the microbiota in
15 cancer requires an explanatory shift beyond tumor-intrinsic features.

16 After providing some brief historical background on the use of microbes/infections
17 in cancer research and therapy, we illustrate how data on cancer-associated microbiota
18 question several tightly held assumptions in oncology [17–19]. We then evaluate the
19 relevance of the microbiota for oncology by clarifying three complementary perspectives.
20 First, can the microbiota be used as a *biomarker* of cancer growth and therapeutic
21 responsiveness? Second, can the microbiota be *causally* linked to cancer development?
22 Finally, can the microbiota be *therapeutically manipulated* to improve the treatment and
23 course of cancer, and if so, how? By clarifying several conceptual and empirical challenges
24 at the intersection of oncology and microbiology, this conceptual review proposes the
25 oncological utility of cancer-associated microbiota in patient diagnosis, prognosis, and
26 treatment.

27

28 **Historical background: from microbes to mutations**

29 There is a long tradition of using microbes—particularly bacteria and viruses—in cancer
30 *therapy*. In a broad sense, this may date back to observations in ancient Egypt and Greece
31 that tumor regression followed infections and/or fevers [11,20,21]. The first scientific attempts
32 at modulating the immune system to treat cancer appear to emerge in the late 19th Century
33 with the German physicians Wilhelm Busch and Friedrich Fehleisen who independently
34 noticed tumor regression in several patients following erysipelas infections caused by

1 *Streptococcus pyogenes*. In the early 20th Century, this tradition was advanced by one of the
2 ‘fathers’ of immunotherapy, William Coley, who, after tracking down a patient in New York
3 who experienced spontaneous regression of an egg-sized sarcoma following erysipelas,
4 started controversially injecting live and later heat-inactivated bacteria (“Coley’s toxins”)
5 into his patients with inoperable cancer [21]. Despite the apparent success of Coley’s method
6 to achieve ~30% long-term remission in 11 types of malignancies across ~210 patients before
7 1940 [22], his findings and methods were not well received by the oncology community due
8 to poorly understood mechanisms, painful fevers (often associated with therapeutic
9 responses), difficult reproducibility, and non-trivial risks of death in immunocompromised
10 patients. Some suggest that as surgery and radiotherapy advanced, and antibiotics and
11 antipyretics were commonly used to enhance hygiene or suppress undesirable immune
12 responses, this sterilizing environment may have obscured the potential anti-tumoral roles
13 for microbes and immune or febrile reactions [21].

14 Throughout the 20th Century, there was a parallel tradition of considering whether
15 microbes play a *causal role* in carcinogenesis and progression [23]. Amidst interest for cancer
16 vaccines, first Thomas Glover and later Virginia Livingston-Wheeler argued that bacteria
17 could be cultivated from tumors and that cancer had a bacterial origin [24,25]. However,
18 Glover’s findings were not reproducible by researchers at the National Institutes of Health
19 and Livingston-Wheeler’s research was criticized for not controlling for contamination [24].
20 As this bacterial research was being abandoned (though not entirely [26]), Peyton Rous made
21 the tentative observation in 1911 that specific ‘agents’ from a chicken tumor could be
22 transmitted to healthy chickens, thereby reproducing an avian tumor resembling human
23 neoplasms [27]. Initially met with outright rejection or considerable skepticism [28], Rous’s
24 findings were eventually vindicated, opening the field of tumor virology [23]—canonized by
25 his receipt of the Nobel Prize in 1966. While a variety of viruses have been linked to several
26 cancers, such as Epstein-Barr, hepatitis viruses, and human papilloma—the most recent
27 being the Merkel cell polyomavirus described in 2008—the viral origins of cancer proved
28 limited and ultimately gave way to a focus on *internal* etiologies such as cellular mechanisms
29 and mutations. Notably, the 1989 Nobel Prize to Michael Bishop and Harold Varmus signaled
30 this major shift in thinking, for they found that many retroviral oncogenes, including from
31 Rous’s sarcoma virus, had a cellular (non-viral) origin and were found in many animal
32 species [29]. As the presumed origin of cancer moved from external to internal factors, it
33 engendered research into cancer genomics.

1 Together, these historical traditions exhibit the persistent challenges, and even
2 reluctance, for evaluating the importance of microbes in oncology. One interesting tension
3 running through this history concerns the relative importance of microbes for
4 conceptualizing tumorigenesis: is cancer formation and progression primarily intrinsic or
5 extrinsic [28]? We have seen that throughout the 20th Century microbes were viewed as an
6 *extrinsic* factor that might have a direct causal or therapeutic role, but paradoxically, this
7 perspective eventually catalyzed the study of cancer genetics [30] and the focus on cell-
8 *intrinsic* mechanisms (while clearly acknowledging extrinsic triggers).

9 Recent research is not only examining specific microbes but also the *microbiota*, or
10 communities of microbes that inhabit and influence the human body and their potential role
11 in cancer progression and treatment [18]. The microbiota colonizes tumors and even
12 individual tumor cells [18], prompting us to once again ask, for instance, in what sense the
13 microbiota is an extrinsic or intrinsic causal and therapeutic factor. The additional findings
14 that patient responsiveness to cancer therapies (e.g., immune checkpoint inhibitors (ICI) or
15 chemotherapies) *depends on* the microbiota in the host appear to blur the intrinsic-extrinsic
16 distinction [14,31,32]. As advances continue to uncover complex interactions among the
17 microbiota, cancer cells, and the host's immune responses [33], the microbiota have become
18 a manipulable tool at once external to and part of the host and its tumor(s). As such, the
19 microbiota is no longer an accidental environmental factor to be suppressed but appears
20 necessary for understanding and effectively treating cancer.

21 22 **The microbiota provides distinct biomarkers in oncology**

23 A key area in oncology comprises the search for cancer-specific *biomarkers* that enable
24 accurate predictions about patient diagnosis, prognosis, and treatment. Going beyond
25 tumor-intrinsic factors, many have begun investigating whether the microbiota, and its
26 functions or metabolites, can alone serve as non-human biomarkers for cancer [34–36]. Here,
27 we discuss how the microbiota constitute distinct types of biomarkers [37].

28 First, blood-derived microbial DNA and specific alterations in localized microbial
29 communities have recently been put forth as possible *diagnostic* biomarkers for various
30 cancers [2,3,38], though their real-world performance remains to be seen [18]. As *prognostic*
31 and *risk* biomarkers for evaluating cancer progression, patterns in microbial communities in
32 patients may help explain inter-individual variation in which mutations become cancers
33 [39], personal risk of acquiring cancer [40], and the anti- or pro-tumor function of specific
34 mutations [12]. These various biomarkers can be derived by evaluating the microbiota

1 specific to a given cancer type (the ‘intratumoral microbiome’), but also the host microbiota
2 at distant sites. For instance, alpha diversity in the intratumoral microbiota is predictive of
3 short versus long-term survival in pancreatic cancer [15], patterns of microbial community
4 changes in lung tissues may be markers of lung cancer progression [41], and gut microbiota
5 composition can inform the risk of developing colorectal cancer [42]. Distally, gut microbiota
6 can help assess the risk of progression from liver disease to cancer [43] and determine
7 patients at risk of tumor metastasis in breast cancer [35], and periodontal microbes appear to
8 stratify risk for developing pancreatic cancer [44].

9 Next, as *therapeutic response* biomarkers, the microbiota can surprisingly determine a
10 patient’s likelihood of responding to treatment [45]. Bourgeoning evidence suggests how
11 clinical antibiotics is a predictor of poor survival in various cancers (with some exceptions
12 [14,46]) and might undermine cancer therapies [47]. Conversely, increasing attention is given
13 to the ability of the microbiota to support the efficacy of immune checkpoint inhibitors
14 [48,49], as well as traditional chemotherapies [14,50]. Researchers have extracted specific
15 microbial species from the feces of responders and non-responders and have shown how
16 these phenotypes are replicated in mice or humans upon receipt of concomitant microbes
17 [6,51,52]. These data provide persuasive examples of how the microbiota is linked to
18 therapeutic efficacy and in some cases may outperform traditional biomarkers such as tumor
19 mutational load [31]. Some researchers also suggest that the functional traits they exhibit
20 could have more explanatory value than the taxa compositions alone, although this remains
21 to be rigorously tested [53].

22 Ultimately, predictions about cancer risks and progression are not solely tied to
23 tumor-intrinsic properties but are coupled with, and in some cases superseded by, local and
24 distant microbial signatures [41,43]. As such, the microbiota may eventually allow for better
25 diagnoses and appears crucial for predicting the health and survival of the cancer-bearing
26 host, highlighting its relevance for oncology. However, the transition from correlative
27 signatures and biomarkers to causative factors raises challenges since many of these
28 microbial biomarkers comprise entire communities of organisms in comparison to
29 individually assayed host biomarkers [54].

30

31 **Establishing causality in cancer-microbiota interactions**

32 Although experts agree that a handful of microbes have causal roles in carcinogenesis [55],
33 many complexities exist, including how particular microbes and communities can aid in
34 tumorigenesis without being direct causal agents, or can even protect against tumorigenesis

1 [18]. Determining causality requires a careful analysis of the relevant context and the distinct
2 roles that microbes play under various physiological conditions. *Box 1* addresses some of the
3 mechanisms identified for the roles discussed below and Figure 1 offers a visual
4 representation.

6 **Pathogenic microbes in cancer**

7 From the perspective of microbes as *pathogens*—generally considered extrinsic causes—it is
8 well accepted that key bacteria and viruses have oncogenic effects in humans. There are
9 currently 11 agents recognized as bona fide ‘oncomicrobes’ in humans (IARC Working Group
10 2012), including *Helicobacter pylori*, human papillomavirus (HPV), hepatitis B virus (HBV),
11 and hepatitis C virus (HCV), Epstein-Barr, herpesviruses, and various polyomaviruses [57].
12 Moreover, several seemingly pro-carcinogenic bacteria, such as *Bacteroides fragilis*,
13 *Enterococcus faecalis*, *Fusobacterium* and *pks+* strains of *Escherichia coli*, all appear to have
14 carcinogenic capacities through their effects on the host immune system, mutagenesis, and
15 inflammation [48,58,59].

16 However, even where specific microbes are shown to have cell-transforming abilities,
17 they often occur alongside inflammation or specific changes to the microbial milieu, such
18 that “causal” microbes may be necessary yet insufficient for tumorigenesis and progression
19 [58,60]. Thus, while causal links exist, the influence of the broader microbial community and
20 the physiological responses of the host should also be considered.

22 **Microbes living in and traveling with the tumor**

23 Recent data supports the perspective that microbes pervasively *colonize* tumors—evidence
24 that falsifies the assumption of cancer’s sterility [2–5,61], a theory possibly kept alive by
25 sterility assumptions of various organs, such as the lungs or bladder [62]. In fact, distinct
26 cancers have cancer type-specific microbial signatures [2,3]: a microbiome unique to each
27 cancer with varying ratios of, e.g., *Proteobacteria* and *Firmicutes*, appearing in higher loads in
28 tumors than in adjacent normal tissue. It is perhaps not surprising to find microbes in
29 gastrointestinal cancers, which are anatomically proximal to the gut microbiota, but
30 intratumoral microbiomes have also been found in tissues distal from the gut, such as in
31 breast, lung, ovary, melanoma, bone, and brain tumors. In these studies, many bacteria
32 detected in tumors appear to be live, cell-wall deficient bacteria, which are exclusively
33 *intracellular* bacteria and are mainly found in cancer cells and immune cells [2]. It remains
34 unknown how many of these bacteria are merely *passengers* rather than active participants
35 in a nutrient-rich and immunosuppressed environment.

1 Additionally, the microbiota can be seen as *migrators*. For instance, microbes found
2 in primary colorectal tumors have also been found in matched metastatic liver tumors [61].
3 Although originally thought to hitchhike within metastatic cancer cells [61], new evidence
4 suggests that colorectal cancer bacteria may actually travel to the liver ahead of metastatic
5 cells and prepare a pro-tumoral, pro-inflammatory environment for them to later seed [63].
6 These metastatic processes remain uncharacterized in most cancer types.

7 While specific taxa were identified in these studies, their functional repertoire and
8 spatial distribution within tumors remain poorly characterized, obscuring causal roles they
9 may play in cancer progression [53]. Determining the pathogenicity of these tumoral
10 colonizers is complicated by the observation that, in some cases, the composition of specific
11 microbes in tumors may improve patient outcomes [15] or enhance immunotherapy
12 response [2].

13 14 **The role of the resident microbiota in promoting or inhibiting cancer**

15 Another perspective studies the microbiota as an oncological *regulator*. This idea is
16 supported by research showing how the resident microbiota can play a dual role in *promoting*
17 or *inhibiting* cancers.

18 There are multiple ways in which the microbiota promotes cancer growth and
19 progression. Bacterial infections trigger inflammation and innate immunity pathways,
20 which in turn create a tumor-promoting microenvironment [63–65]. A second pathway is
21 through dysbiosis, a widely discussed and sometimes contested concept, generally referring
22 either to a loss of beneficial microbes, an expansion of pathobionts, or reduced diversity [66–
23 68]. While in some cases it is unclear whether dysbiosis is driving inflammation and thus
24 tumorigenesis, or whether inflammation drives the dysbiosis, there are nevertheless strong
25 links between the microbial community changes, inflammation, and tumor promotion [58].
26 Furthermore, when barrier epithelial cells are damaged (an innate immune defense), the
27 “normal” resident microbiota can further damage these cells or underlying tissues, induce
28 genetic instability via DNA-damaging reactive oxygen and nitrogen species [48], or
29 translocate into circulation causing systemic inflammatory responses [69].

30 While certain microbes are pathogenic, others support the body’s antitumoral
31 responses. The microbiota can epigenetically prime myeloid cells, such as dendritic cells and
32 macrophages, for optimal responsiveness to tumors [65], an effect that is significantly
33 reduced in germ-free mice models. Several bacterial species have also been associated with
34 anticancer immunosurveillance [70], with specific intratumoral microbial compositions
35 linked to *better* chances of survival [15] or response to immunotherapy [2]. It remains

1 undetermined whether the diversity of gut microbes facilitating positive outcomes can be
2 explained by shared functional output of specific metabolites (e.g., short-chain fatty acids)
3 that contribute to reducing inflammation and/or ensuring intestinal barrier integrity [71,72].
4

5 6 7 8 **Box 1: Microbiota-Cancer Causal Mechanisms** 9

10 When viewed as cancer *pathogens*, we can distinguish direct and indirect mechanisms:

- 11 • Direct pathogenic mechanisms [18,73–75]: genotoxic or cytotoxic mutagenesis
12 (colibactin, cytolethal distending toxins) via DNA alkylation, DNase activity, and
13 ROS/NOS production; activating β -catenin or PI₃K/AKT pathways.
- 14 • Indirect pathogenic mechanisms [48,65,72,76,77]: following translocation, microbiota
15 promote inflammatory $\gamma\delta$ T cells, DNA damaging ROS-producing neutrophils; their
16 metabolites can hinder immunosurveillance of human NK and T cell activity;
17 following mucosal damage in gut, microbes regulate cytokines (e.g., IL-6, 11, 18, 22).
18

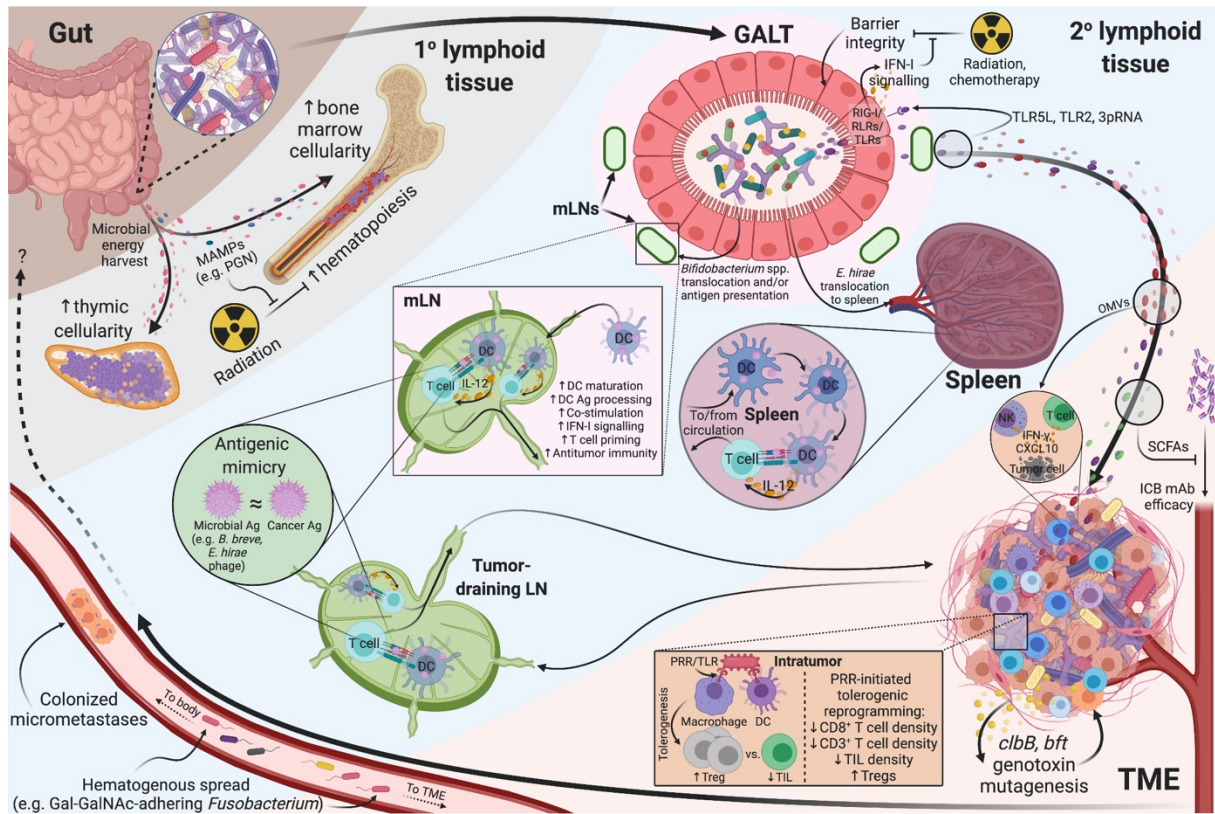
19 As tumoral *colonizers* and *migrators*, there are various potential mechanisms:

- 20 • Intratumoral TME-colonizing mechanisms [15,18,46,65,78]: producing genotoxins, T-
21 cell mediated inflammation, suppressing local antitumor immunity (short-chain
22 fatty acids (SCFAs) inducing Treg production), enzymatically aiding
23 chemoresistance (bacterial cytidine deaminase degrading gemcitabine), activating
24 the host's MBL-C₃ axis.
- 25 • Mechanisms of migrators [61,63]: potentially intracellular migration with metastatic
26 cancer cells; bacteria such as *E. coli* can open the gut vascular barrier and thereby
27 translocate to the liver where they recruit immune cells (macrophages and
28 inflammatory monocytes) and aid in the maturation of a premetastatic niche.
29

30 When viewed as cancer *regulators*, we find mechanisms for *promoting* and *inhibiting*
31 tumorigenesis:

- 32 • Promoter mechanisms [18,33,48,65,77,78]: contributing to a pro-inflammatory
33 microenvironment, stimulating IL-1 and IL23 from myeloid cells or IL-17 from Th17
34 cells; tumor-promoting pathogens escaping immune control (dysbiosis); activating
35 inflammasomes (NLRP3,6) via SCFAs, in turn increasing tumor promoting IL-22
36 (positive feedback loop); promoting metastasis by upregulating tumor matrix
37 metalloproteinases.
- 38 • Inhibiting mechanisms [79–83]: The microbes growing in and around tumors can
39 inhibit growth through the production of anti-inflammatory metabolites,
40 particularly SCFAs such as butyrate and propionate, which have been shown to
41 affect gene expression, cell proliferation and cell death; mimicry between microbial
42 or phage antigens and cancer antigens thereby causing antitumor immune response.
43

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4 Reevaluating causality

5 Several challenges remain for evaluating causal claims of the cancer-associated microbiota.
6 These include the primary way researchers conclude causality in microbiome studies:
7 rodent models. There are limitations of uneven colonization in recipient animals, the
8 prevention of novel communities forming upon colonization, the lack of ecological factors
9 in these models that were important for producing host disease states, and the difficulty of
10 getting recipient animals to adapt to microbes with which they did not co-evolve [84].
11 Although some cancer studies have performed human fecal microbiota transplants [51,52],
12 these are rare. Causal claims are further complicated due to some infections having long
13 latency periods (e.g., human T-cell leukaemia virus type 1), the fact that many microbes are
14 widespread and yet their associated cancer is rare, and that causal mechanisms may vary
15 during the time course of carcinogenesis [23].

16 Moreover, this research faces the immense challenge of specifying causality amidst
17 the complex variations of host-microbe and microbe-microbe dynamics [60]. Microbes exist
18 within ecosystems, and even if single microbes can be linked to or associated with various
19 cancers, they also tend to be accompanied by shifts in other microbial taxa [72]. This is
20 further complicated when considering that phages preying on bacteria may influence these

1 dynamics [83]. Finally, there is the issue of context-dependency [53,70,85], such as whether
2 specific microbes contributing to cancer depends on host physiology (e.g., inflammation).

3 One proposed way forward is to incorporate ideas from systems medicine or ecology
4 [72,86–92]. For instance, we can track how perturbations in one part of the microbe-host
5 ecology will result in adjustments, compensations, or disruptions to other parts of this
6 system [93]. The challenge is to determine how to accurately define these systems and their
7 relevant causal factors without sacrificing explanatory precision or clinical utility.

8

9 **Manipulating the microbiota for cancer therapy**

10 While establishing etiology is central to this research, there is a closely related issue of
11 investigating the effects of host-microbiota interactions on cancer therapies. Evidence that
12 the microbiota or its metabolites modulate, or in some cases enable, the outcomes of cancer
13 therapies is increasingly well-supported [6]. As microbes and the microbiota play the role of
14 *therapeutic mediators*, this suggests that host physiology is not solely responsible for whether
15 a cancer treatment is effective. Microbes are not just accidental aspects of host physiology
16 that can be ignored or even eradicated, as is commonly done to treat or prevent opportunistic
17 infections [94], possibly obscuring their beneficial roles in cancer [21]. In fact, they may
18 sometimes be necessary for treating cancer. Set against the above history, the microbiota is
19 perhaps less of a medical ‘breakthrough’ [95] than an increasingly promising and better
20 understood therapeutic target.

21

22 **What the microbiota can do to cancer therapies**

23 While modulating host immune responses to tumors has long been a target of
24 immunotherapies, it was only recently possible to ask whether a patient’s microbiota might
25 undergird treatment efficacy. This intriguing role of the microbiota as a mediator has been
26 confirmed, in part, by showing that antibiotic treatments reduce the efficacy of various
27 cancer therapies: in the absence of commensal microbiota, the immune system will either
28 not, or to a lesser degree, be activated by immunotherapies [70,71,96]. Returning to the
29 research on immune checkpoint inhibitors (ICIs), antibiotic-induced gut dysbiosis can
30 inactivate the antitumoral T cell responses, and re-introduction of several bacterial species,
31 their proteins, and/or their metabolites appears to restore the activity of these therapies
32 [71,97–99]. Moreover, while gut commensals seem to enhance some ICIs (anti-PD-1 or anti-
33 PD-L1), they appear to render others possible (anti-CTLA-4) [100]. The exact mechanisms by

1 which microbiota and/or their metabolites support immunotherapy efficacy remains under
2 investigation [101].

3 The microbiota also appears to mediate conventional cancer therapies. For instance,
4 alterations in gut commensals support the efficacy of total body irradiation (TBI) as a
5 conditioning regime for adoptive T-cell transfer therapy [58], and the beneficial effects of
6 TBI are reduced by antibiotics. Conversely, certain commensals may be important for
7 patient survivability of whole body radiotherapy [102], and intestinal fungi appear to
8 modulate antitumoral responses to radiation therapy in mice [103]. Synergistic antitumor
9 effects seem to be driven by translocating bacteria from the gut into neighboring tissues and
10 subsequently inducing immunostimulation [58], or by priming tumor-infiltrating myeloid
11 cells [65]. Similar antitumor effects have been found with chemotherapies using oxaliplatin
12 or cyclophosphamide: the antitumor immune responses are primed and/or enhanced by
13 commensal bacteria [50,97,104]. For instance, cyclophosphamide disrupts gut mucosal
14 integrity, thereby inducing the translocation of specific gram-positive bacteria into
15 secondary lymphoid organs, which then stimulate the production of antitumoral Th17 cells
16 and Th1 immune responses.

17 One key question is whether these responses are due less to the individual species
18 found in the so-called ‘responders’ than to specific communities, ‘consortia’, or even the
19 entire *ecosystem* with which these species are associated [70]. Deconvolving microbe-
20 microbe and host-microbe interactions are necessary to address this in detail.

21

22 **Redrawing the boundaries of therapeutic intervention**

23 Using microbes to treat and better understand cancer fits within the longer historical
24 traditions while expanding the current one [17,105–107]. A nuanced development, though,
25 concerns the shift from using *exogeneous* microbes (e.g., Coley’s treatments) to manipulating
26 *endogenous* ones to stimulate an antitumor immune response. In this sense, the extrinsic-
27 intrinsic distinction concerning the microbiota’s relation to cancer is dissolving: the
28 mechanism is at once external and internal, depending on one’s perspective.

29 However, a large problem for redrawing the therapeutic boundary becomes apparent
30 when considering the context-dependency of microbes in carcinogenesis. For instance,
31 while bacteria such as *H. pylori* and viruses such as herpesvirus and Epstein-Barr can all be
32 carcinogenic, they can also cooperate with commensals and thereby offer some protection
33 against other diseases and infections [48,108]. Evolutionary trade-offs will thus have to be
34 carefully considered.

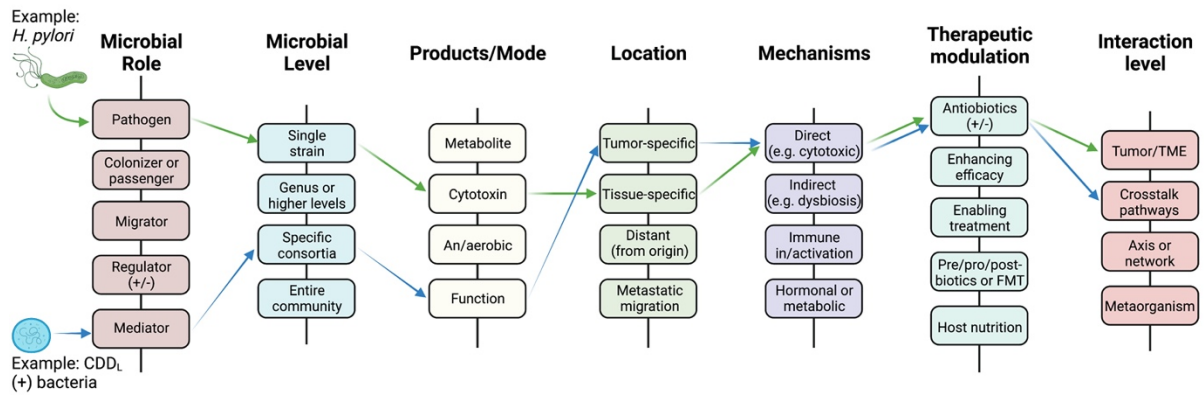
1 This ecological perspective motivates a systems-based approach for interrogating
2 and mapping the interactions between microbes and cancer or immune cells, similar to how
3 systems biologists have sought to systematically map protein-protein interactions and gene
4 knockout effects on cell phenotypes. Understanding the manipulable nodes and edges in
5 this multi-species network (including the host) can then guide rational interventions
6 directed towards a specific goal (e.g., cancer eradication). For example, researchers are
7 investigating how modulation of the estrobolome, or the aggregate of enteric bacteria that
8 metabolize estrogen, can influence hormonal or metabolic pathways that promote
9 tumorigenesis [109]. Microbial species with β -glucuronidase and β -glucuronide activities are
10 manipulable ‘nodes’ in this network that facilitate estrogen metabolism and secretion.
11 Targeting these microbial ‘nodes’ can modify the network so as to reduce the emergence of
12 hormone-dependent cancers (Type I (endometrioid) endometrial cancer, estrogen receptor-
13 positive breast cancer, and some ovarian cancers) [60]. However, what is needed to enable
14 this form of systems medicine are precision tools that alter individual or groups of microbes
15 rather than whole communities, which is currently limited by most antibiotics, prebiotics,
16 postbiotics, dietary interventions, and fecal microbiota transplants [34,51,110]. Nonetheless,
17 as the microbiota expands the cancer therapeutic armamentarium, oncologists are no longer
18 simply manipulating or targeting host-centric, tumor-intrinsic properties.

19

20 **Conclusions and future perspectives**

21 This review aimed to elucidate the historical, conceptual, and scientific implications of
22 recent data on the cancer-associated microbiota for oncology. We started with the notion
23 that the microbiota comprises not simply another environmental factor among others that
24 influences cancer growth and treatment but is intimately bound up with it. We then
25 evaluated the relevance of this intimacy for oncology and explored its implications for
26 establishing novel biomarkers, determining causality, and manipulating the microbiota to
27 enhance or hinder treatments (see Outstanding Questions). We strove to capture the distinct
28 conceptual aspects and therapeutic implications of this on-going research (Figure 2) while
29 acknowledging its limitations.

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Going forward, we encourage further refinement of the proposals that articulate microbiota-cancer-host ‘axes’ [18,92,111], ‘networks’ [72,86], and ‘systems’ [88–91]. Clarifying the precise components of these networks and how they interact holds promise to help contextualize novel biomarkers, to specify other causal mechanisms, and to rationally guide therapeutic interventions that focus on the most effective targets.

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9
10

11 **Disclosures**

12 G.D.S.-P. and R.K. are inventors on a US patent application (PCT/US2019/059647) submitted
13 by The Regents of the University of California and licensed by Micronoma; that application
14 covers methods of diagnosing and treating cancer using microbial biomarkers in blood and
15 cancer tissues. G.D.S.-P. and R.K. are founders of and report stock interest in Micronoma.
16 G.D.S.-P. has filed several additional US patent applications on cancer microbiome
17 diagnostics that are owned by The Regents of the University of California. R.K. additionally
18 is a member of the scientific advisory board for GenCirq, holds an equity interest in GenCirq,
19 and can receive reimbursements for expenses up to US \$5,000 per year.

20

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1 (Figure descriptions)

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3 **Figure 1. Microbiota-tumor-host interactions.** Visual depiction of some key mechanisms
4 and causal pathways being uncovered within the host-tumor-microbiota network.

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7 **Figure 2. Microbiota-Cancer Conceptual Matrix.** This matrix, depicted as a parallel
8 coordinates plot, reflects various conceptual and empirical issues in current microbiota-
9 cancer research while engendering novel questions. For instance, research has shown how
10 the microbiota can play different roles in tumorigenesis and treatment, but it remains an
11 open question whether these roles are based on a particular microbial species, function, or
12 anatomical location, and the precise mechanisms involved are often tentative due to the
13 complexity of the cancer-host interactions. It further remains important to elucidate how
14 these roles and levels intersect with immune-microbiota pathways and whether they are
15 conserved amongst multiple microbes in a particular environment. Next, we can modulate
16 treatments through the targeted use of antibiotics or host nutritional interventions and
17 explore their efficacy in altering not only tumor- or tissue-specific microbiota, but also
18 preventing microbial translocation and the formation of premetastatic niches. Finally, the
19 complexity of causal mechanisms and therapeutic interventions increases as we shift
20 analysis from the TME level to interacting networks or even the entire metaorganism.

21