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The cancer microbiome

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Abstract

Collectively known as the microbiota, the commensal bacteria and other microorganisms that colonize the epithelial surfaces of our body have been shown to produce small molecules and metabolites that have both local and systemic effects on cancer onset, progression and therapy response. To date, most studies focusing on the microbiome have used traditional preclinical mouse models and identified correlative relationships between m icrobial species and cancer phenotypes. Now, the profound influence of the microbiota on the efficacy of cancer treatments, such as im m unotherapies, has begun to be extensively characterized in humans. Paramount to the development of microbiota-based therapeutics, the next challenge in m icrobiom e research will be to identify individual microbial species that causally affect cancer phenotypes and unravel the underlying mechanisms. In this Viewpoint article, we asked four scientists working on the cancer

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microbiome for their opinions on the current state of the field, where the research is heading and how we can advance our understanding to rationally design microbial-based therapeutics to transform treatment strategies for patients with cancer.

Q *How should we best model the microbiota effects on cancer onset, progression and therapy response and improve upon current preclinical models?*

Eran Elinav. The effect of the gut microbiota on cancer depends on an interaction between three extremely complex, constantly evolving, biological entities — the microbiota, the tumour and the immune system. Unravelling this intricacy throughout the clinical course of malignancies is best modelled by an integrated approach combining microbiome and host multi-omic characterization (for example, by 16S ribosomal RNA gene amplicon sequencing, shotgun metagenomic sequencing and metabolomic characterization of the microbiome, and single cell RNA sequencing of the host) coupled with the use of preclinical models (that is, germ-free mice, patient-derived xenografts and organoids) and advanced computational tools. This multimodal approach was recently utilized by some researchers in cancer studies with notable success. For example, faecal microbiome transplantation (FMT) of stool samples from patients who responded to therapy into germ-free mice improved the effect of immune checkpoint inhibitor therapy in transplanted mice, whereas FMT from non-responding patients did not[1]. Similar findings were also observed by other groups[2], some integrating multi-omics with preclinical models to enable the identification of several commensal members suggested to contribute to the clinical effect.

Human-compatible preclinical models of cancer are becoming more sophisticated and will certainly contribute to the understanding of the role of the microbiome in cancer. For example, in vivo patient-derived xenografts, which involve the implantation of human tumours in living mice, are increasingly used for high-throughputdrug screening to discover patient-specific effective treatments[3]. In vitro models such as organ-chips are 3D organ-level structures that simulate in vivo cancer pathophysiology while conserving some aspects of its microenvironment[4]. This allows experimentalists to study tumour expansion, invasion and metastasis in a highly controlled environment that is otherwise unamenable for research with in vivo models. Implanting a patient’s individual microbiome and tumour cells in such an in vitro system may help to integrate microbial effects and contributions and to predict patient-specific responses to therapy while avoiding unwanted toxicities.

Predictive machine-learning and artificial intelligence algorithms may greatly contribute to the ability of clinicians to deliver personalized patient care. These algorithms can unbiasedly process an enormous amount of patient data as input, including complex variables such as the microbiota composition and function, and predict patient-specific physiological or clinical outcomes such as glycaemic response to different foods[5] and, in the context of cancer, can be used to predict positive responders to particular treatments. Algorithms of this sort are expected to play a growing role in the field of oncology.

Wendy S. Garrett. Ideally, we would model the effects of the microbiota on cancer onset, progression and therapy with virtual avatars. Virtual avatars or digital twins are widely used in the aerospace industry. If jet engines have digital twins, why not people[6]? The virtual

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avatar is core to the achievement of bona fide personalized precision medicine. However, data scientists have some exciting challenges to tackle before this becomes a reality. In the near term, there are some bridging approaches that warrant further development and effort. Organoids represent one approach for many solid tumour malignancies[4,7]. There is an increasing number of biobanks, both academic and commercial, that offer human-derived organoids with excellent genotyping, and organoid propagation is now within the financial reach of many labs. Genetic manipulation of many different types of organoids is now achievable owing to CRISPR-Cas technology[8]. An increasing number of cell types (for example, immune, stromal and microbial) are being integrated into organoid cultures, making these systems better models of the tumour microenvironment. Additionally, the ability to flip organoids inside-out offers opportunities to model exposures (to microbial products or microbial metabolites) that influence cancer onset and progression as well as more easily evaluate the effects of therapeutics that are delivered luminally.

Mice remain an outstanding but far from perfect in vivo model for cancer studies. Site-specific recombinase, CRISPR-Cas and inducible expression (small molecule and viral) technologies enable the more efficient capture of human genetics within mice in a time-tunable and tissue-specific fashion. The technologies that humanize mice are astounding, and some are rather simple. Gnotobiotics (specialized animal husbandry practices that allow for experimental animals that are microorganism-free or colonized with specific microbial consortia) is really rather ‘low tech’ yet enables mice to harbour human microbial communities via inoculation from tumours themselves or, more commonly, stool samples. This husbandry practice, while far from new, is enabling a tremendous amount of cancer microbiome studies that are shedding light on why immuno-oncology therapeutics work or fail in patients, and such knowledge is being translated to benefit patients[1,9,10]. Intrinsic to husbandry is, of course, diet and other environmental exposures (for example, light exposures and xenobiotics). Microbiome and population health studies are revealing how important the environmental factors of food and pharmaceuticals are for health, and increasingly, these factors are being considered in making mice a more effective model for human cancer. Beyond humanizing mice via genome editing, gnotobiotics, and diet and environmental perturbations, there are also means to humanize the mouse immune system via adoptive transfer of human $CD34^+$ stem cells with autologous human thymic grafts[11].

Giorgio Trinchieri. Most of the studies investigating the effect of the microbiota or specific bacterial species on cancer onset and progression have utilized genetically induced or chemical carcinogenesis mouse models, while the studies of cancer therapy have been largely based on mouse transplantable tumours. Indeed, the preclinical models that allowed the development of immune checkpoint inhibition therapy mostly used transplantable tumours. These models are often criticized as not being representative of human tumours. However, in studying the effect of the microbiota on cancer therapy, genetic cancer models present difficulties including limited numbers of mutations and reduced immunogenicity compared with many human tumours. Transfection efficiencies of the viral vectors used in those models and the tumour-promoting effect of inflammation may be affected by the presence and composition of the microbiota. This may be a source of confusion not only in cancer therapy studies but also in evaluating the role of the microbiota on cancer onset and

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progression. Important caveats when using transplantable tumours are that most cell lines originating from B6 mice have been infected by rearranged replicating endogenous murine leukaemia viruses (MLVs) and that MLV antigens are tumour-associated rejection antigens[12]. Also, the common use of transfected artificial antigens in transplantable tumours may generate mimicry with bacterial antigens, creating resistance mechanisms that may not be real in autologous human tumours.

Colonization of mice with human commensals is a common procedure to evaluate the effect of the human microbiota on carcinogenesis or therapy. However, human commensal bacteria do not always colonize the mouse in the same way as they do humans. Also, the response of the mouse mucosal and immune systems to introduced commensals is not always identical to that of humans. Studies using monocolonization are also hampered by the concern that, in the absence of an ecologically balanced microbiome, the results obtained may not be representative of the role of that individual bacterial species in human pathology. Robust approaches are those using colonization of mice with a complete human microbiome with or without the specific species or strain being investigated. A more accurate mechanistic analysis can be obtained by using an ecologically and metabolically balanced consortium of a small number of human commensal bacteria. Genetic modification of the strains being studied or the use of bacterial strains with partially different genomes may help in identifying mechanisms and molecules by which the microbiome and defined strains affect carcinogenesis or therapy.

In cancer therapy studies, the main objectives are to identify the microbiota composition that favours therapy response and to devise methods to target the microbiome to improve therapy effectiveness. To study the role of human commensal bacteria, gnotobiotic mice (that is, germ-free mice) reconstituted with microbiomes from healthy donors, patients or human commensal-defined consortia have been used. Clinical trials using faecal microbiota transplant aiming to improve the success of immunotherapy have been initiated[13]. Gnotobiotic mice associated with the transplant donor microbiota may ideally help to predict whether the transplant may be effective in improving the responseto therapy. Most studies used gnotobiotic mice that were associated with patients' faecal microbiota shortly before being tested for tumour growth and response to therapy[14,15]. However, the immune system of germ-free mice does not develop normally, and the sudden exposure to commensal bacteria induces inflammatory and immune stress that may alter the response to therapy. The use of mice treated with antibiotics instead of germ-free mice adds complications owing to the effects of antibiotics on host cells and possibly the competition of the remaining host commensals with the transferred human microbiome. An alternative approach is to associate breeding pairs of germ-free mice with the human microbiome and then test their progeny that have been exposed to the human microbiota from birth. More studies are needed to analyse whether the faecal transplant used for creation of gnotobiotic mice reproduces faithfully the microbiota of the human donors and how stable a human-type microbiota is in mice through time and generations.

Jennifer Wargo. Now that we have established that the microbiota can impact cancer onset, progression and therapy response, we need to better understand the mechanisms behind this via analysis of additional patient cohorts and through optimized in vitro and in vivo models.

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Integrating data from existing and prospective cohorts of patients with cancer is critical to this effort. However, this is difficult, as a wide range of techniques have been used in these studies (for example, with differences in how samples were collected, stored and processed and in the sequencing methods and the pipelines used for analysis). An important and necessary first step will be to harmonize and standardize such approaches so that data may be more easily compared between groups[16].

In such studies, it is important to remember that these gut microorganisms are not sole influencers of cancer and that numerous other factors are at play — including host genomics, host immunity, environmental exposures (such as ultraviolet radiation and smoking) and tumour-intrinsic factors (such as alterations in the genome, epigenome and tumour microenvironment)[17,18]. Thus, these factors must be taken into account as we assess data from new studies and as we optimize existing and novel preclinical models. The interaction of microorganisms with each of these other factors must also be carefully considered, as bidirectional influences have been noted[19].

With regard to preclinical models, germ-free mouse models are an important resource; however, critical efforts are needed to optimize novel models for microbiome research. This includes models such as the ‘gut-on-a-chip’[20].

Q *How do you envisage our understanding of the microbiome being used in cancer diagnostics and therapeutics, and what are key challenges for translation?*

E.E. Understanding the contribution of the microbiome to cancer development, progression and treatment responsiveness could revolutionize patient management strategies. It is now established that some distinct members of the microbiota are involved in carcinogenesis. A classic example is seen in *Helicobacter pylori*, a World Health Organization (WHO) class I carcinogen, which causes gastric cancer. Other bacteria such as *Fusobacterium* spp. are associated with colorectal adenocarcinoma, and patients with colon cancer have an increased abundance of *Escherichia* coli[21,22].

Further to being involved in cancer causation, the microbiota may also contribute to responsiveness or resistance to chemotherapy treatment regimens. Exciting new antibody-based immune checkpoint inhibitors show variable efficacies, with treatment success suggested to be influenced by host factors and gut microbiota composition[23]. Furthermore, microbiota taxa residing within tumours have been found to confer tumour chemo-resistance brought about by microbial drug metabolism[24].

Overall, I envision the future of cancer care as involving a holistic treatment approach personalized to patient genetic and microbiome characteristics. Involvement of gut microbiota species in carcinogenesis or in modulation of treatment efficacy may also pave the way towards new interventions altering microbiota composition and function. For example, prebiotic or personalized nutritional approaches may alter the microbiome configuration towards one that favours cancer treatment responsiveness. Patient-tailored probiotics may supplement commensals crucial for cancer treatment success. ‘Postbiotic’ interventions, consisting of molecules generated or modified by commensal bacteria, may enable the supplementation or inhibition of microbiome-derived small molecules, thereby

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impacting the human host while bypassing the variable microbial ecosystem itself. In cases in which bacterial elimination is a need, novel approaches such as phage cocktail treatment may help to eliminate cancer-promoting bacteria while avoiding disadvantageous alterations to the microbiota as a whole. Impacting the host side of the host-microbiome interface may enable gut barrier function to be relaxed, thereby allowing better influx of chemotherapeutic drugs, or alternatively the barrier to be tightened, thereby avoiding microbial influx inducing infectious and inflammatory adverse effects. Collectively, I envision these modalities to be used in combinations in various patient-specific, cancer-specific and symptom-specific contexts in optimizing cancer patient care.

W.S.G. There is tremendous opportunity for the microbiome as a prognostic biomarker, a guide for the selection of appropriate preventive and therapeutic strategies for individuals, a primary and second prevention measure and an adjuvant therapeutic — as both a target and a treatment. One key challenge is the execution of the appropriate population-health-scale studies for the microbiome in cancer. We desperately need studies of the microbiome both across the cancer continuum and across cancer types on a greater scale — thousands and tens of thousands of subjects rather than hundreds. Hand in hand with the need for population-health-scale studies is the continued commitment to mechanistic microbiome studies to move beyond correlation, pinpoint mechanism — to the extent to which one can in preclinical models — and validate host-microbiome targets using multiple complementary assays. Also, it is important to point out that the organisms within the human microbiome, or more precisely those within the microbiota, are not the only microbial taxa that live within and on the human body. The human microbiome also encompasses the proteins and metabolites produced by individual members of the community, by larger networks within the microbial community and by humans in concert with the microbiota (for example, cometabolites).

G.T. Assaying microbiome composition for cancer diagnosis has been proposed for a few types of human cancers; however, the most promising results are those based on the identification of certain strains of *Fusobacterium* spp. as an independent diagnostic assay for colon cancer[25]. The approach has generated interest because of the low invasiveness of the test but has not yet reached a high level of accuracy, and it would not detect colon cancer associated with bacteria other than *Fusobacterium* spp.[25].

Because certain bacteria, when administered systemically, tend to accumulate and proliferate selectively in the anaerobic microenvironment of tumours, genetically modified bacterial strains have been proposed to be used in cancer therapy in a therapeutic approach that is promising and worth pursuing[26].

Recent data in experimental animals and to some extent in patients showed that the composition of the gut microbiota modulates the efficacy of cancer chemotherapy and immunotherapy and that targeting the microbiota could lead to an increased immunotherapy success rate[14,15,27,28]. Several roadblocks, however, still exist. Colonization of mice with patients' microbiomes has been used to characterize the mechanisms by which certain microbiota compositions enhance the response to immunotherapy[14,15]. However, as discussed above, the human microbiome transferred into mice does not always perfectly

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reproduce the donor microbiome; it may be unstable, and the response of the mice to the human microbiome may not be identical to that of the patients. Studies in anti-programmed cell death 1 (PD1)-treated patients identified bacterial species that appear to correlate with successful response, but unfortunately, each study identified completely different and unrelated species[15]. Thus, a challenge remains to identify reliable microbiome-related biomarkers for prediction of response. These controversial results may be due to the heterogeneity of the human microbiome between individuals and in different geographical areas. Particularly instructive in this respect is a recent study in southern China[29]. In that study, the district in which the individuals lived was shown to be the major determinant of microbiome diversity, and the microbiome composition was predictive of susceptibility to metabolic disease within each district but not across districts[29]. Also, the individual bacterial species identified in different clinical studies correlating with response may just be the tip of an iceberg of more complex ecological changes.

Faecal transplant trials have been planned or initiated to treat patients undergoing immunotherapy[13]. Because we are still unable to define a favourable microbiome, these clinical protocols have been based on the transfer of faecal microbiomes from patients with cancer that have successfully responded to anti-PD1 therapy into patients who have failed therapy[13]. When we are able to define a favourable microbiome for cancer therapy, it would be preferable to utilize balanced faecal microbiomes from healthy donors rather than dysbiotic microbiomes from sick patients. It will also be important to characterize the effect of diet in improving therapy efficacy by modifying the composition of the microbiota.

Rather than faecal transplant that may also transfer pathogens or pathobionts, an important goal remains to identify the mechanisms shared by different bacteria that enhance therapy response and to design ecologically balanced consortia of commensal bacteria that could enhance therapy response in any clinical setting. A consortium of human commensals was shown in mice to induce interferon-γ (IFNγ)-secreting $CD8^+$ T cells and to enhance the therapeutic efficacy of immune checkpoint inhibitors[9]. On the basis of these results, a similar consortium is planned to be tested in patients with cancer treated with anti-PD1[30]. Trials have also been initiated using oral treatment with a monoclonal microbial product (a single strain of *Bifidobacterium* spp.) that was found to be associated with favourable response to anti-PD1 and anti-PD1 ligand 1 (PDL1) in mice and in patients[2,31].

J.W. The microbiome is emerging as a potential biomarker as well as a tractable therapeutic target in improving responses to cancer therapy. Perhaps the most compelling clinical data to date are in the setting of treatment with immune checkpoint blockade, where several studies have now demonstrated differential signatures in gut microorganisms of responders versus non-responders (with cohorts of patients with several different cancer types treated with monoclonal antibodies targeting cytotoxic T lymphocyte antigen 4 (CTLA4) and/or PD1)[1,2,10,32,33].

The development of the microbiome as a biomarker is appealing, as several studies have demonstrated strong associations of specific gut microbiome signatures with response to immune checkpoint blockade[1,2] — with signatures in the gut microbiota outperforming other known biomarkers in selected studies[2]. However, we are clearly in the early stages of

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the development of these signatures as biomarkers, and complexities with such an approach certainly exist. Specifically, regarding gut microorganisms, it is unclear which metrics are most important (diversity of the microbiota, relative abundance of specific bacterial taxa or functional status of the microorganisms). Furthermore, numerous methods exist to profile the microbiota (including PCR-based approaches, 16 S sequencing, metagenomic sequencing, metabolomic profiling, culturomics (culturing specific taxa from samples) and other strategies), and it is unclear at present which approach should be used (in the short term, as well as in the long term). Several factors should be taken into consideration when contemplating using such approaches in patients, such as the length of turnaround for a particular assay and the positive and negative predictive value. Ultimately, the gut microbiome should be used in conjunction with other known and novel biomarkers (optimally via an integrated approach) to improve diagnostic accuracy — though strategies using integrative biomarkers are somewhat under-developed at present.

In addition to its role as a potential biomarker, there is intense interest and ongoing efforts to target gut microorganisms within the microbiota therapeutically to impact a number of medical conditions— including cancer. Such efforts have demonstrated marked success in diseases such as *Clostridium difficile* colitis — a condition characterized by profound dysbiosis and overgrowth of a specific bacterial species in the gut. Refractory cases can be treated via modulation of the gut microbiota via FMT — demonstrating proof of principle for such an approach[34]. Strategies to modulate the gut microbiota are now being used in the treatment of cancer; however, there are extensive considerations in using such an approach with respect to the type of strategy to use, conditioning regimens and numerous other considerations[35]. Examples include use of FMT from healthy individuals versus from patients with cancer who experienced a complete response after being treated with immune checkpoint blockade therapies[13,36], as well as use of defined bacterial consortia (with or without pre-conditioning regimens[31,37]) on the basis of insights gained from preclinical and clinical studies. Certainly, we must work together as a global community as we move forward with such approaches to learn how best to use them.

Beyond the gut microbiome, the tumour microbiome and microbiota at other sites (such as the skin, aerodigestive tract and other sites) must also be taken into consideration given their potential influence and impact[38].

Q *How do we advance towards showing causal rather than correlative relationships between microbiota species and cancer phenotypes?*

E.E. Indeed, while the association between commensal microorganisms and various features of cancer has been shown in many studies and in different cancer types, a mechanistic proof of causality constitutes a major challenge of the field. Different strategies are being utilized in demonstrating causal roles of whole microbiome signatures or of distinct bacterial strains in cancer treatment. These include FMT (replacing one microbiome with another), administration of broad-spectrum antibiotics (depleting the microbiome) and transfer of whole microbiomes, microbial consortia or isolated strains into cancer-harbouring germ-free mice. For example, Sethi et al.[39], reported that gut microbiota depletion, using a broad-spectrum cocktail of oral antibiotics, induced a significantly reduced melanoma burden in

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mice. This phenotype was related to an altered immune cell balance in the tumour microenvironment, that is, increased numbers of antitumour IFNγ-secreting T cells (T helper 1 cells and cytotoxic T cells), and decreased numbers of immune populations secreting both the pro-tumour interleukin-17A (IL-17A) ($IL\text{-}17A^{+}CD3^{+}$) and IL-10 ($IL\text{-}10^{+}CD4^{+}CD3^{+}$)[39]. In another study, FMT from patients (responder or non-responder to immunotherapy) into germ-free mice transferred the phenotype to recipient mice1. Mice that received responder FMT showed a higher density of $CD8^{+}$ T cells than those that received non-responder FMT, consistent with human data showing a higher density of $CD8^{+}$ T cells in baseline samples of responders versus non-responders.

W.S.G. Multidisciplinary team science that involves ‘wet lab’ experiments in robust and reproducible preclinical models (more than one model should be used to validate observations whenever possible) is necessary and essential for moving from identifying hypothesis-generating correlations to laying the groundwork for mechanisms of causality.

G.T. There is still much that can be done to optimize the interpretation of the clinical data and to obtain solid results unambiguously supportive of correlation and possibly hinting at causative effects. Strict standardization for sample collection, bacterial lysis, DNA purification sequencing, bioinformatics and statistical analysis should be applied to all the clinical studies to improve the ability to compare the results in different trials and clinical centres. For the analysis and interpretation of clinical studies using in-depth multiomics investigation of both host cells and commensal microorganisms, the most advanced systems analysis and machine-learning approaches should be utilized. Evidence of causal relationships will also be inferred from the results of clinical trials using faecal microbiome transplant or defined bacterial consortia. Eventually, precise mechanistic studies will have to rely on the use of gnotobiotic mice, with the caveat of differences between human and mouse physiology.

J.W. Although much of the published data regarding microbiota species and cancer or therapy response phenotypes show only correlative relationships, causal relationships are beginning to be demonstrated in some cases — with insights into mechanisms being gained.

This includes in the setting of treatment with immune checkpoint blockade, where studies showing associations in patient cohorts were bolstered by data in mouse models demonstrating that responder and non-responder phenotypes could be recapitulated by FMT into germ-free mouse models[1,2,10]. However, despite these publications showing specific bacterial taxa associated with response versus non-response among the cohorts, there was little to no overlap between each of the cohorts. This has been associated with some angst in the field, and careful analysis from expert investigators suggests that functional approaches (such as analysis of microbial gene expression using RNA sequencing or metabolomics) will likely be necessary to help identify the fundamental mechanisms mediating different phenotypes[15]. Ongoing and future analyses should take these findings into consideration and include standardized approaches to sequencing as well as functional assays to gain better insight.

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Key to these efforts are ongoing and planned clinical trials, of which there are many[35] — including efforts to modulate the gut microbiota using FMT and administration of bacterial consortia or probiotics, as well as dietary modulation. These trials should include intense biomarker assessment, ideally with standardization and harmonization of profiling techniques and collection of metadata.

Efforts to modulate the microorganisms at tumour sites or other sites in the body are also underway, though these efforts are less advanced than those targeting the gut microbiota.

Q *Beyond bacteria, could there be a role for other microbial groups, such as the virome and mycobiome, in cancer onset, progression and therapy response?*

E.E. Indeed, the gut virome, parasitome and fungome (mycobiome) are potentially important microbiome components that are much less studied than the bacterial microbiome. Some infectious non-commensal DNA and RNA viruses (that is, human papilloma virus, human herpesvirus 8, Epstein-Barr virus, cytomegalovirus, hepatitis C virus and human T lymphotropic virus 1) are widely known to be oncogenic, and these may play a role in tumour pathogenesis in certain contexts. Mycobiome alterations were suggested to be associated with acute graft versus host disease[40], squamous cell carcinoma of the tongue[41] and colorectal cancer[42]. However, a clear causative connection is yet to be proved for these interesting associations. Likewise, bacteriophages may hold a predator-prey relationship with the bacterial microbiome, therefore potentially driving cancer-associated bacterial expansion, which may impact tumorigenesis or the response to therapy. Of note, microbiomes other than the gut microbiome, including the skin, mouth, genitourinary and respiratory microbiomes, may also contribute to local cancer formation, progression, metastasis and response to treatment but are currently much less studied than the dense gut microbiome. Collectively, these ecosystems will constitute exciting new frontiers in cancer microbiome research in the next decade.

W.S.G. Thinking of the microbiota as just bacteria is regretfully narrow regarding the multi-faceted roles of the microbiota in cancer. There are viral sequences (phage and non-phage) in tumoural and stool samples from patients with cancer, and they represent a fascinating signal that warrants focus and investigation. Beyond eukaryotic organisms such as fungi (mycobiota), there are long-standing associations between human cancer and non-human eukaryotic organisms, for example, the trematode *Schistosoma haematobium* and bladder cancer and the trematodes *Opisthorchis viverrini* and *Clonorchis sinensis* and gall bladder and bile duct cancers[43]. Beyond cancer onset, progression and therapy response, microorganisms can also contribute to the morbidity and mortality of patients with cancer through infections. Cancer treatment can put patients at risk of typical and atypical infections following treatment-related immune compromise, hospital-acquired infections and multidrug-resistant microbial infections and second to complex clinical exposures and history.

G.T. Except for the role of *H. pylori* in stomach cancer, all the microorganisms officially recognized as human carcinogens are viruses and parasites[44]. Evidence is now emerging for a role of fungi in upper gastrointestinal neoplasia[45]. The role of components of the

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microbiome other than bacteria, including bacteriophages, in modulating cancer therapy still needs to be fully investigated.

J.W. Although many studies have focused mainly on bacteria and their roles in cancer onset, progression and therapy response, other microorganisms (such as viruses, protozoa and fungi) may also play an important role. These entities are somewhat less well studied in the current literature, where many of the studies utilized 16S sequencing and focused solely on bacteria taxa without taking other microorganisms into consideration. An example of these other microorganisms is bacteriophages, which are numerically more abundant than any other class of microorganism in the gut microbiome and more diverse[46]. There is evidence that these viruses can play a major role in mediating the therapeutic efficacy of strategies to modulate the gut microbiota, with data from a study demonstrating that sterile faecal filtrate transfer (filtering out live bacteria) is effective in treating patients with *C. difficile* colitis[47]. Other microorganisms have not been well studied in the tumour, gut and other sites in patients with cancer — however, efforts are underway to do so. Such efforts will require a movement towards more comprehensive means of profiling the microbiota (using metagenomic sequencing and other strategies), as well as optimization of reference databases to fully characterize these microorganisms. Nonetheless, this will certainly add substantially to our understanding of the impact and diagnostic and/or therapeutic potential of the microbiota in cancer.

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References

1. Gopalakrishnan V et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 359, 97–103 (2018). [PubMed: 29097493]
2. Matson V et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 359, 104–108 (2018). [PubMed: 29302014]
3. Pauli C et al. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov 7, 462–477 (2017). [PubMed: 28331002]
4. Sontheimer-Phelps A, Hassell BA & Ingber DE Modelling cancer in microfluidic human organs-on-chips. Nat. Rev. Cancer 19, 65–81 (2019). [PubMed: 30647431]
5. Zeevi D et al. Personalized nutrition by prediction of glycemic responses. Cell 163, 1079–1094 (2015). [PubMed: 26590418]
6. Bruynseels K, Santoni de Sio F & van den Hoven J Digital twins in health care: ethical implications of an emerging engineering paradigm. Front. Genet 9, 31 (2018). [PubMed: 29487613]
7. Drost J & Clevers H Organoids in cancer research. Nat. Rev. Cancer 18, 407–418 (2018). [PubMed: 29692415]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

8. Fujii M, Clevers H & Sato T Modeling human digestive diseases with CRISPR-Cas9-modified organoids. Gastroenterology 156, 562–576 (2019). [PubMed: 30476497]
9. Tanoue T et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. Nature 565, 600–605 (2019). [PubMed: 30675064]
10. Routy B et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 359, 91–97 (2018). [PubMed: 29097494]
11. Wege AK, Melkus MW, Denton PW, Estes JD & Garcia JV Functional and phenotypic characterization of the humanized BLT mouse model. Curr. Top. Microbiol. Immunol 324, 149–165 (2008). [PubMed: 18481459]
12. Young GR et al. Resurrection of endogenous retroviruses in antibody-deficient mice. Nature 491,774–778 (2012). [PubMed: 23103862]
13. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT03341143 (2019).
14. Vetizou M et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 350, 1079–1084 (2015). [PubMed: 26541610]
15. Gharaibeh RZ & Jobin C Microbiota and cancer immunotherapy: in search of microbial signals. Gut 10.1136/gutjnl-2018-317220 (2018).
16. Knight R et al. Best practices for analysing microbiomes. Nat. Rev. Microbiol 16, 410–422 (2018). [PubMed: 29795328]
17. Blank CU, Haanen JB, Ribas A & Schumacher TN The “cancer immunogram”. Science 352, 658–660 (2016). [PubMed: 27151852]
18. Cogdill AP, Andrews MC & Wargo JA Hallmarks of response to immune checkpoint blockade. Br. J. Cancer 117, 1–7 (2017). [PubMed: 28524159]
19. Zmora N, Soffer E & Elinav E Transforming medicine with the microbiome. Sci. Transl Med 11, eaaw1815 (2019). [PubMed: 30700573]
20. Shin W & Kim HJ Intestinal barrier dysfunction orchestrates the onset of inflammatory host-microbiome cross-talk in a human gut inflammation-on-a-chip. Proc. Natl Acad. Sci. USA 115, E10539–E10547 (2018). [PubMed: 30348765]
21. Rubinstein MR et al. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe 14, 195–206 (2013). [PubMed: 23954158]
22. Tahara T et al. Fusobacterium in colonic flora and molecular features of colorectal carcinoma. Cancer Res 74, 1311–1318 (2014). [PubMed: 24385213]
23. Bashiardes S, Tuganbaev T, Federici S & Elinav E The microbiome in anti-cancer therapy. Semin. Immunol 32, 74–81 (2017). [PubMed: 28431920]
24. Geller LT et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 357, 1156–1160 (2017). [PubMed: 28912244]
25. Zhang X et al. Fecal fusobacterium nucleatum for the diagnosis of colorectal tumor: a systematic review and meta-analysis. Cancer Med 8, 480–491 (2019). [PubMed: 30636375]
26. Forbes NS et al. White paper on microbial anti-cancer therapy and prevention. J. Immunother. Cancer 6, 78 (2018). [PubMed: 30081947]
27. Iida N et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 342, 967–970 (2013). [PubMed: 24264989]
28. Viaud S et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 342, 971–976 (2013). [PubMed: 24264990]
29. He Y et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. Nat. Med 24, 1532–1535 (2018). [PubMed: 30150716]
30. Biosciences Vedanta. Bristol-Myers Squibb and Vedanta Biosciences announce a new clinical collaboration to evaluate OPDIVO® (nivolumab) and VE800 in patients with advanced or metastatic cancers. Vedanta Biosciences https://www.vedantabio.com/news-media/press-releases/detail/2492 (2018).
31. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT03595683 (2018).

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32. Frankel AE et al. Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. Neoplasia 19, 848–855 (2017). [PubMed: 28923537]
33. Chaput N et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. Ann. Oncol 28, 1368–1379 (2017). [PubMed: 28368458]
34. Wang Y et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. Nat. Med 24, 1804–1808 (2018). [PubMed: 30420754]
35. McQuade JL, Daniel CR, Helmink BA & Wargo JA Modulating the microbiome to improve therapeutic response in cancer. Lancet Oncol 20, e77–e91 (2019). [PubMed: 30712808]
36. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT03353402 (2019).
37. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT03817125 (2019).
38. Byrd AL, Belkaid Y & Segre JA The human skin microbiome. Nat. Rev. Microbiol 16, 143–155 (2018). [PubMed: 29332945]
39. Sethi V et al. Gut microbiota promotes tumor growth in mice by modulating immune response. Gastroenterology 155, 33–37 (2018). [PubMed: 29630898]
40. van der Velden WJ et al. Role of the mycobiome in human acute graft-versus-host disease. Biol. Blood Marrow Transplant 19, 329–332 (2013). [PubMed: 23160005]
41. Mukherjee PK et al. Bacteriome and mycobiome associations in oral tongue cancer. Oncotarget 8, 97273–97289 (2017). [PubMed: 29228609]
42. Coker OO et al. Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer. Gut 68, 654–662 (2018). [PubMed: 30472682]
43. Brindley PJ, Costa J & Sripa B Why does infection with some helminths cause cancer? Trends Cancer 1,174–182 (2015).
44. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents Volume 100 B. A review of human carcinogens. IARC Monogr. Eval. Carcinog. Risks Hum 100, 1–441 (2012).
45. Zhu F et al. Autoreactive T cells and chronic fungal infection drive esophageal carcinogenesis. Cell Host Microbe 21, 478–493 (2017). [PubMed: 28407484]
46. Draper LA et al. Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. Microbiome 6, 220 (2018). [PubMed: 30526683]
47. Ott SJ et al. Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. Gastroenterology 152, 799–811 (2017). [PubMed: 27866880]

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