Polymorphic microbes: a new emerging hallmark of cancer
Mark P. Lythgoe 1,*, Benjamin H. Mullish 2, Adam E. Frampton 1,3 and Jonathan Krell 1

Recognition of the microbiome (and ‘polymorphic microbes’ within them) as a new emerging hallmark of cancer reflects a wide body of rapidly evolving research. Microbes may be directly carcinogenic, impact host immune responses to promote malignancy, and may be key effectors in determining the efficacy of anticancer therapy. Manipulation of the microbiome is showing promise as an opportunity to influence cancer outcomes.

Introduction
In 2000, Douglas Hanahan and Robert Weinberg published the seminal paper ‘The hallmarks of cancer’, conceptualising six core rules which orchestrate the multistep transformation of normal cells into malignant cells [1]. Over 20 years later, in the third update ‘Hallmarks of cancer: new dimensions’, these six original hallmarks have expanded to 14 (Figure 1) with the latest addition of four new emerging hallmarks and enabling characteristics, unlocking phenotypic plasticity, nonmutational epigenetic reprogramming, senescent cells, and polymorphic microbes.

The inclusion of polymorphic microbes reflects increasing appreciation that the complex microbial ecosystems (or ‘microbiome’) – including bacteria, fungi, and viruses, which symbiotically associate with the human body – have a profound impact on cancer pathogenesis [2]. Evidence now demonstrates that the microbiome plays a substantial role in tumorigenesis, cancer differentiation, and malignant progression [1,3]. Furthermore, the microbiome directly interacts, both positively and negatively, with other established cancer hallmarks, such as tumour inflammation, avoiding immune destruction, genome instability, and resistance to anticancer therapies [1]. The most significant evidence for this integrated role comes from the study of microbes within the gastrointestinal tract (gut microbiome), with this being the most extensively characterised area. However, more recently there has been growing appreciation of the role of polymorphic microbes in other tissues/organs, including those of other mucosal surfaces and/or in contact with the external environment (e.g., skin, genitourinary tract, and lung), as well as those living within tumours (intratumoural microbiome).

Gut microbiome
The human body is estimated to contain $\sim 4 \times 10^{13}$ microorganisms, with the cumulative microbial genome – or ‘metagenome’ – exceeding the human genome by a factor of $>100$ [4]. Over 97% of the microbiome consists of bacteria in the colon, and the high density is believed to drive most of the known microbial immunomodulatory effects. Mounting evidence has now demonstrated that polymorphic microbes within the gut play a key role in the pathogenesis of cancer, from tumorigenesis to determining the efficacy of anticancer therapy [3].

Functional studies utilising faecal microbiota transplants (FMTs) from patients with colorectal cancer into recipient mice predisposed to develop colon cancer have established a principle that microbes have both tumour-promoting and cancer-protective qualities [5]. Furthermore, perturbations of the intestinal microbial population, or dysbiosis, caused by infection or antibiotic administration, have been consistently linked to carcinogenesis in the colon and beyond [3,6]. Despite the clear delineation of this relationship, out of the estimated $\sim 10^{15}$ distinct microbial species on Earth, only 11 are identified as direct human carcinogens (oncomicrobes); for example, some strains of Escherichia coli produce a potent DNA alkylator, colibactin, linked to colorectal cancer [7]. More typically is that gut microbes are ‘complicit’, unable to cause carcinogenesis independently but promote other cancer hallmarks. Bacteroides fragilis produces toxins which bind to the surface of colonic epithelial cells, stimulating proliferation signalling (cancer hallmark; Figure 1) driving the development of colon cancer [6]. Furthermore, bacteria may work synergistically in a ‘driver–passenger’ relationship to trigger and sustain carcinogenesis; in this example, B. fragilis (‘driver’) promotes tumorigenesis causing a remodeling of the colonic microenvironment, allowing opportunistic commensal bacteria, such as Streptococcus galalyticus (‘passenger’), to dominate and further attenuate carcinogenic evolution [6].

Preclinical and clinical evidence for the influence of gut microbes on systemic immunity is highly pervasive. The gut microbiome has been shown to have a broad effect on both adaptive and innate immune systems, resulting in the expression of a diverse repertoire of chemokines and cytokines linked to creating both tumour-promoting and tumour-antagonising immune microenvironments [1]. Concordantly, the distinctive microbiomes of individual patients modulate other cancer hallmarks, such as eliciting tumour inflammation, escaping adaptive immune destruction, and responsiveness to anticancer therapies [e.g., immune checkpoint inhibitors (ICIs)], providing insight into modulation as a potential anticancer therapeutic strategy [3].

Modulation of the gut microbiome
Many different approaches – including prebiotics, probiotics, live biotherapeutic
products (LBPs), FMT, and dietary interventions – as strategies for microbiota modulation are under investigation in cancer (Table 1). Despite extensive preclinical evidence of benefit in cancer, translation of microbiota modulation approaches has not yet materialised into commercial therapies. However, there are encouraging signs this may be changing. Several proof-of-concept clinical studies have now emerged, demonstrating the potential of FMT, LBPs, and dietary modification to enhance the efficacy of cancer treatments, primarily focused on ICIs.

Studies have shown the ability to restore efficacy to ICIs in melanoma patients following FMT donated from therapy-responsive patients who had progressed during prior therapy with ICIs [8]. Significant variability in response rate (30–40%) was observed in subjects, and further mechanistic understanding is required. LBPs, typically composed of a single purified strain of bacteria or consortia of synergistic bacteria, have also demonstrated anticancer potential – for instance, administration of CBM588, consisting of Clostridium butyricum, a butyrate-producing anaerobe spore-forming bacterium, significantly enhanced clinical outcomes in patients with metastatic renal cell cancer treated with dual ICI
therapy [9]. Significant metabolic and chemokine changes were noted in patients in the active treatment arm compared to placebo, giving clues to the potential mechanism. Another LBP, consisting of Enterococcus gallinarum (MRx0518), under clinical investigation in a variety of cancers, has also demonstrated significant gene and metagenic changes in treatment-naïve cancer patients [10]. Lastly, dietary changes, such as increased intake of a high-fibre diet, have also been shown to improve ICI therapy response in melanoma, both preclinically and clinically [11]. These studies collectively provide intriguing insight into the potential viability of microbiota modulation for cancer patients receiving ICIs.

**Beyond the gut**

All tissues and organs exposed to external environment are colonised by different microorganisms. Considerable variation occurs in the microbiota present in these locations, based on a multitude of factors such as sex, age, and environment [1]. Unlike in the gut, where the symbiotic relationship between microbiota and host is more clearly delineated, the normal and pathogenic roles of the microbiota in cancer are still emerging. However, there is increasing evidence that tissue-specific microbiomes (e.g., in the vagina) have microbes present which may either contribute towards the development of cancer or protect against it [1,3].

Characterisation of the tumour microenvironment (TME) has consistently detected the presence of microbes residing within the stroma, immune cells, and cancer cells [12]. Until very recently the low microbial biomass in this environment presented a significant challenge for analysis. Estimates vary, but pooled evidence shows the presence of ~one bacterial cell for every ~147 cancer cells within the TME [4]. Over 500 distinct bacterial species have now been identified, and each tumour type analysed shows a unique and distinct pattern, which could potentially help in the elucidation of some of the more-difficult-to-characterise cancer subtypes (e.g., cancer of unknown primary) [12]. Studies have demonstrated that the intratumoural microbiota can modulate the immune phenotype, promote metastasis, and determine the response to anti-cancer therapy [3,12,13]. However, further work is required to corroborate these findings more ubiquitously.

**Impact on cancer care**

With the emergence of pleomorphic microbes as a new cancer hallmark, there is an opportunity to integrate profiling and targeting of the microbiome into precision

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**Table 1. Examples of different approaches under clinical investigation for microbiota modulation in cancer**

| Intervention | Example trial | Description | ClinicalTrials.gov identifier (sponsor ID) |
|--------------|---------------|-------------|------------------------------------------|
| Prebiotics (compounds inducing growth/activity of beneficial microorganism) | Prebiotic effect of eicosapentaenoic acid treatment for colorectal cancer metastasis | A randomised placebo-controlled Phase 3 trial of the effect of the omega-3 fatty acid eicosapentaenoic acid (EPA) on colorectal cancer recurrence and survival after surgery for resectable liver metastases | NCT04682665 and NCT03428477 (University of Leeds) |
| Probiotics (live microorganisms which, when administered in adequate amounts, confer a health benefit) | Effects of probiotics on the gut microbiome and immune system in operable stage I-III breast or lung cancer | A single-arm Phase 1 clinical trial to evaluate whether engineering the gut microbiome using probiotics will alter the body’s immune system to react to stage I-III breast or lung cancers | NCT04857697 ( Mayo Clinic and National Institute of Cancer) |
| Live biotherapeutic products (live organisms, such as bacteria applicable to the prevention, treatment, or cure of a disease) | CBM588 in combination with nivolumab and cabozantinib for the treatment of advanced or metastatic kidney cancer | A randomised open-label Phase 1 trial to investigate to determine the effect of CBM 588 (Clostridium butyricum) probiotic strain in combination with cabozantinib/nivolumab in modulation of the gut microbiome in patients with metastatic renal cell carcinoma | NCT05122546 (City of Hope Medical Centre and National Cancer Institute) |
| Faecal microbiota transplantation (transfer of biological materials, incorporating faecal microorganisms, from human donors) | Faecal microbiota transplantation and re-introduction of anti-PD-1 therapy (pembrolizumab or nivolumab) for the treatment of metastatic colorectal cancer in anti-PD-1 non-responders | A nonrandomised open-label Phase 2 trial studies the effect of faecal microbiota transplantation and reintroduction of anti-PD-1 therapy (pembrolizumab or nivolumab) in treating anti-PD-1 nonresponders with metastatic colorectal cancer | NCT04729322 ( MD Anderson Cancer Centre and National Cancer Institute) |
| Dietary intervention (modification of diet to promote health benefits) | Neoadjuvant dietary intervention in intermediate-risk prostate cancer | A single-arm clinical trial to investigate the effects of a dietary intervention prior to surgery in patients with intermediate-risk prostate cancer | NCT04985566 ( MD Anderson Cancer Centre and National Cancer Institute) |
cancer care. Profiling of the microbiota in the gut, mucosal surfaces, and the tumour is unlocking new discoveries about the complex relationship between microbes, humans, and cancer. Systemic characterisation of the cancer microbiome is providing opportunities to improve cancer detection and enhance the potential efficacy of cancer treatments, such as ICI [3]. Several clinical studies have shown microbiota modulation, by a variety of mechanisms, as a viable and feasible treatment for patients with advanced cancer [8–10]. However, further investigation is required to demonstrate clinical utility in a broader range of cancer subtypes and provide further greater insight into the effector action.

Concluding remarks
Polymorphic microbes are potentially instrumental and a quasi-independent variable in how cancers develop, progress, and respond to therapy. Inclusion as a new emerging and enabling cancer hallmark reflects increasing understanding and appreciation of the substantial role that microbes play in cancer. Greater comprehension may permit an opportunity to improve each stage of the cancer care cycle from prevention to treatment for advanced disease.

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Declaration of interests
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