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The microbiome and gynaecological cancer development, prevention and therapy

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Abstract

The female reproductive tract (FRT), similar to other mucosal sites, harbours a site-specific microbiome, which has an essential role in maintaining health and homeostasis. In the majority of women of reproductive age, the microbiota of the lower FRT (vagina and cervix) microenvironment is dominated by Lactobacillus species, which benefit the host through symbiotic relationships. By contrast, the upper FRT (uterus, Fallopian tubes and ovaries) might be sterile in healthy individuals or contain a low-biomass microbiome with a diverse mixture of microorganisms. When dysbiosis occurs, altered immune and metabolic signalling can affect hallmarks of cancer, including chronic inflammation, epithelial barrier breach, changes in cellular proliferation and apoptosis, genome instability, angiogenesis and metabolic dysregulation. These pathophysiological changes might lead to gynaecological cancer. Emerging evidence shows that genital dysbiosis and/or specific bacteria might have an active role in the development and/or progression and metastasis of gynaecological malignancies, such as cervical, endometrial and ovarian cancers, through direct and indirect mechanisms, including modulation of oestrogen metabolism. Cancer therapies might also alter microbiota at sites throughout the body. Reciprocally, microbiota composition can influence the efficacy and toxic effects of cancer therapies, as well as quality of life following cancer treatment. Modulation of the microbiome via probiotics or microbiota transplant might prove useful in improving responsiveness to cancer treatment and quality of life. Elucidating these complex host– microbiome interactions, including the crosstalk between distal and local sites, will translate into interventions for prevention, therapeutic efficacy and toxic effects to enhance health outcomes for women with gynaecological cancers.

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Author contributions

All authors contributed to researching data for the article, made substantial contributions to discussions of the content, and wrote and edited the manuscript before submission.

Competing interests

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The human body is colonized by a diverse community of commensal, symbiotic and pathogenic microorganisms, which include bacteria, archaea, fungi, protists and viruses[1]. When present in a particular environment, this community is referred to as microbiota, whereas the entire habitat — including microorganisms, their genomes and the surrounding environment — is referred to as the microbiome[2]. Technical limitations mean that human microbiome studies have previously been restricted to bacterial communities; however, over the past 5 years, studies characterizing viruses and fungi (the virome and mycobiome) residing on or in the human body have been emerging[3–7]. For the purpose of this Review, we focus on the bacterial communities. Notably, the number of bacteria in and on the human body is estimated to be of the same order as that of human cells[8]. Furthermore, the metagenome of bacterial communities in our body comprises at least 100 times more genes than the human genome[9]. Thus, the human microbiota and metagenome are broadly accepted to have a critical role in the maintenance of homeostasis and/or development of certain diseases, including cancer[10,11].

The majority of the bacteria in our body reside in the gastrointestinal tract, particularly the colon; however, other body sites — including the urogenital tract — also harbour unique microbiota, which are distinct from the population of the gut1. In the past, the healthy urogenital tract was thought to contain bacteria only in the distal urethra and the vagina[12]. However, with advances in molecular and culture techniques, many tissues that have been traditionally considered sterile (including the bladder[13–17], prostate[18], uterus[12,19–22], Fallopian tubes and ovaries[23]) have been shown to harbour low-abundant microbial communities, although these are present mostly in diseased states[12]. Studies also suggest the interconnection of the urogenital microbiota, as the same bacterial taxa are found in different organs (for example, the bladder and vagina in women or the urethra and prostate in men) and are shared between sexual partners (such as the penile skin, urethra and semen of male partners and the vagina of female partners)[14,24,25]. Furthermore, biological variables, including sex and age, influence changes in the urogenital microbiota that could be related to anatomy, hormones and hygiene practices[13,17]. The microbiota might have a role in the pathogenesis of urinary and male reproductive cancers[13,17,18]; this Review focuses specifically on the microbiota of the female reproductive tract (FRT) and gynaecological cancers. We highlight interactions between the host and the bacterial communities throughout the FRT and discuss how the genital microbiota might function as an important factor in the development and progression of gynaecological malignancies. We also consider the complex crosstalk between genital microbiota and distal mucosal sites, including the gut and bladder, and investigate the possible roles of microbiota in the aetiology of gynaecological cancer, prevention, therapeutic efficacy and toxic effects.

Microbiota of the FRT

Anatomically, the FRT can be divided into the lower (vagina and cervix) and upper (uterus, Fallopian tubes and ovaries) FRT and exhibits site-specific microenvironments. The majority of bacteria in the FRT reside in the vagina; in healthy women of reproductive age, the vaginal microbiota mostly exhibits low microbial diversity (defined as species richness and evenness) and consists of one or few *Lactobacillus* spp.[26] (Fig. 1). This characteristic is in contrast to other mucosal sites (for instance, the colon), where high microbial diversity

is considered to be a sign of health[27]. In contrast to the lower FRT, our knowledge about the normal microbiota that reside in the uterus, Fallopian tubes or ovaries (Fig. 1) is still rudimentary and challenging to assess[12], and whether bacteria in the upper FRT are residents that maintain homeostasis, tourists that are readily eliminated or invaders that contribute to disease remains uncertain[12].

Microbiota of the lower FRT

In the lower FRT, *Lactobacillus* dominance is associated with vaginal health and depletion of these microorganisms can lead to numerous adverse conditions, such as increased risk of acquiring sexually transmitted infections (STIs), preterm birth, spontaneous miscarriage or pelvic inflammatory disease[28]. Interestingly, only one or few *Lactobacillus* species seem to be predominant in the vagina, such as *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, *Lactobacillus jen*senii or *Lactobacillus vaginalis* (found mostly in women from sub-Saharan Africa), as demonstrated using both culture-dependent and molecular techniques[26,28–33]. These vaginal *Lactobacillus* species seem to be adapted for optimal colonization of the vaginal niche, as other *Lactobacillus* species, such as *Lactobacillus acidophilus*, do not colonize and dominate vaginal tissue[33,34]. Genetic and molecular mechanisms explaining this phenomenon still remain to be explored. Strikingly, *Lactobacillus* dominance seems to be unique to humans; distinct reproductive physiology, high risk of STI or a shift to diets rich in starch in agrarian societies (as opposed to hunter-gatherers) have been hypothesized to explain the evolutionary origin of the human vaginal microbiome[35]. Predominant vaginal *Lactobacillus* spp. exhibit a mutually beneficial relationship with their host, protecting the inhabited microenvironment against invading pathogens through various mechanisms, including production of anti-microbial products and by blocking adhesion of pathogens[26,28,29,36]. Production of lactic acid, which acidifies the vaginal microenvironment to pH <4.5, is a hallmark protective mechanism of vaginal *Lactobacillus* spp. in the lower FRT[28,37]. Numerous in vitro studies have demonstrated that protonated lactic acid kills or inactivates a broad range of sexually transmitted pathogens, including *Neisseria gonorrhoeae*[38], *Chlamydia trachomatis*[39], herpes simplex virus[40] and human immunodeficiency virus[41], as well as urinary tract pathogens, such as uropathogenic *Escherichia coli*[42]. The beneficial effect of *Lactobacillus* spp. has been also attributed to hydrogen peroxide; however, production of this antimicrobial compound seems unlikely in vivo owing to hypoxic conditions in the local microenvironment[43,44]. Furthermore, *Lactobacillus* spp. are able to block adhesion of invading pathogens to the vaginal epithelium through competitive exclusion and inhibit the growth of pathogens through the production of bacteriocins[37,45]. In return, *Lactobacillus* spp. thrive in the vaginal niche owing to the availability of glycogen by-products, which are used by these microorganisms as an energy source[46].

Intriguingly, in some women, the vaginal microbiome seems to be devoid of a high proportion of *Lactobacillus* spp. and to consist of a diverse polymicrobial mixture of obligate and strict anaerobes (including *Gardnerella vaginalis*, *Prevotella* spp., *Atopobium vaginae*, *Sneathia* spp., *Megasphaera* spp. and others)[26,47,48], many of which are commonly associated with bacterial vaginosis (BV). BV is the most common vaginal disorder among women of reproductive age, present in ~23–29% depending on the region[49]. However, the

vaginal microbiome can also be dominated by *G. vaginalis* and exhibits a low diversity[50]. Whether the non-*Lactobacillus*-dominant vaginal microbiome exists as another vaginal microbial community type or actually reflects asymptomatic BV is still debatable[30]. Notably, these non-*Lactobacillus*-dominant microbial communities have been reported to be more prevalent in Hispanic and black women (30–40%) than in white and Asian women (10–20%)[26,48,50,51]. Furthermore, the data from the USA, Canada, Europe and Asia consistently demonstrate that groups representing minority populations within a country studied had higher rates of BV and lower prevalence of *Lactobacillus* dominance after adjustment for sexual practices and other confounders[48,52–55]. These racial and ethnic differences could relate to socioeconomic, behavioural and environmental factors, which can shape the vaginal microbiome, and require further investigation (Fig. 2). Finally, in some women, vaginal microbiome communities are characterized by the overgrowth of human pathobionts, such as *Streptococcus* spp., *Staphylococcus* spp. or *Enterobacteriaceae*[50].

Lactobacillus depletion and dominance of diverse anaerobes, which is characteristic of dysbiosis, has been associated with numerous gynaecological and obstetric sequelae, including increased risk of acquiring STIs, preterm birth, spontaneous miscarriages, pelvic inflammatory disease, endometritis and gynaecological cancer[28] (Fig. 1). However, not all *Lactobacillus*-dominant communities benefit the host in the same manner. For example, one of the vaginal *Lactobacillus* species, *L. iners*, commonly occurs as a component of the diverse non-*Lactobacillus*-dominant communities[56]. Furthermore, *L. iners*-dominant vaginal microbiomes also frequently transition to the non-*Lactobacillus*-dominant communities, whereas *L. crispatus*-dominant microbiomes rarely undergo this transition[56]. Additionally, *L. iners* produces only the L-isoform of lactic acid, which is produced by vaginal epithelial cells, as well as by some BV-associated microorganisms[57], whereas the other vaginal *Lactobacillus* spp. produce the D-isoform of lactic acid (*L. jensenii*) or both the D-isoform and L-isoform (*L. crispatus* and *L. gasseri*), which was confirmed in vitro[57]. Notably, the higher ratios of L-lactic acid to D-lactic acid in *L. iners*-dominant or diverse non-*Lactobacillus*-dominant communities have been shown to correlate with elevated levels of extracellular matrix metalloproteinase inducer and, consequently, matrix metalloproteinase 8 (MMP8) in vaginal secretions, which might alter the epithelial barrier integrity in these women[57]. Overall, these data suggest that dominance of the vaginal microbiome with *L. crispatus* might be optimal for vaginal health, whereas dominance with *L. iners* might be less beneficial.

Multiple factors have been shown to affect the vaginal microbiome. These can be behavioural (sexual orientation, sexual activity, number of sexual partners, use of lubricants and sex toys, contraception, feminine hygiene practices, smoking and vaping, alcohol consumption, diet and/or nutrition, obesity and physical activity), socioeconomic (education, income, structured racism and/or segregation, social policy and access to health care), genetic or host-related (age, genome and epigenome, hormonal status, pregnancy and altered immunity), other comorbidities (cardiometabolic, neuroendocrine and immuno-inflammatory) and environmental (STI status, human papilloma virus (HPV) vaccination, stress, antibiotics, probiotics, xenobiotics, toxins, carcinogens, geography and early life factors such as gestation, birth route and infancy)[28,36,37,55,58] (Fig. 2). For example, cigarette smoking has been strongly associated with an increased prevalence of BV and vaginal

microbiome alterations[48,55,59,60]. Women who douche are also at a higher risk of developing BV[61,62]. In addition, a diet rich in fat, with a high glycaemic load and nutritional density, and obesity have been linked to BV[55,63,64]. Additionally, in a human 3D in vitro model of vaginal epithelium, lubricants — particularly products with high osmolality — have been shown to alter the vaginal epithelial barrier[65] and might also affect the vaginal microbiota.

Oestrogen levels, in particular, have a profound effect on the composition of the vaginal microbiome. For instance, before puberty or in postmenopausal women, when circulating oestrogen levels are low, the vaginal microbiome is devoid of *Lactobacillus* spp. and consists of a diverse mixture of anaerobic bacteria[29,66]. By contrast, the vaginal microbiomes of pregnant women, which are subject to elevated levels of oestrogen, are more stable and typically dominated by *L. crispatus* or L. iners[67]. This phenomenon is thought to occur via the oestrogen-mediated production and secretion of glycogen by the vaginal epithelium; the high levels of free glycogen promote the growth of *Lactobacillus* spp., which are able to use glycogen breakdown products as an energy source through fermentation[46]. As a consequence of this metabolic process, *Lactobacillus* spp. produce lactic acid, which protects the vaginal microenvironment. The changes in the oestrogen levels during the menstrual cycle might also affect vaginal microbiome structure, particularly *Lactobacillus* dominance. Indeed, longitudinal studies revealed that the vaginal microbiome is a dynamic ecosystem, which can fluctuate over short periods of time in some women or be relatively stable in others[31,56,67]. Two key longitudinal studies showed that the changes in the vaginal microbiome composition are affected by the phase of the menstrual cycle, by the type of vaginal microbiome community and, to a certain extent, by sexual activity[31,56]. Another longitudinal study also demonstrated that the stability of the vaginal microbiome was significantly higher in pregnant women than that of non-pregnant women ($P < 0.001$)[67].

Microbiota of the upper FRT

The available data suggest that the microbiota of the upper FRT differ substantially from that of the vagina in both quantity and composition (Fig. 1). First, the upper FRT must be considered as a low-abundance site. For instance, the estimated number of bacteria in the uterus is ~10,000-fold lower than the bacterial load in the vagina[23]. Owing to this low bacterial biomass in the upper FRT microenvironment and the proximity to a microbial rich site, such as the vagina, sample contamination during collection and processing is a major hindrance in acquiring physiologically relevant data and, consequently, can lead to false biological conclusions[12,68,69]. In the majority of studies, samples for analysis of the uterine microbiome have been collected transcervically, which introduces a risk of cross-contamination with bacteria from the lower FRT[12]. An alternative would be to collect uterine samples directly from the bivalved uterus following hysterectomy, which would prevent contamination of specimens with bacteria from the cervix[12]. Other precautions, such as vaginal disinfection with povidone iodine, could further reduce cross-contamination[68]. Thus, careful sampling and rigorous controls during sample collection and processing are necessary in future studies to help identify possible contaminants[12,68,69]. Second, available data suggest that the upper FRT microbiota exhibit higher bacterial diversity than the vaginal microbiome[23] (Fig. 1). However, the microbiota compositions identified vary greatly across studies, so whether these bacterial species are genuine members of the

upper FRT microbiome or transient colonizers is unclear. Multiple studies have reported that *Lactobacillus* is found in the upper FRT[20–22,70,71]. Interestingly, the relative abundance of *Lactobacillus* gradually decreases throughout the upper FRT, with the highest levels in the vagina and cervix (100% and 97.6%, respectively), with notable, but no longer dominant, levels in the endometrium (30.6%) and with the lowest abundance in the Fallopian tubes (1.7%)[23] (Fig. 1).

An important limitation of upper FRT microbiome studies is the fact that the samples have not been from healthy individuals. In most studies, participants underwent hysterectomy for benign conditions, such as fibroids, uterine bleeding or chronic pelvic pain. These conditions are likely to affect the physical and biological barriers in the cervix, consequently allowing bacteria residing in the lower FRT (such as *Lactobacillus*) to ascend and misleadingly be considered a normal component of the upper FRT microbiome. As the collection of endometrial biopsies from healthy women is outside clinical practice guidelines, women undergoing in vitro fertilization procedures could be a more suitable population for future studies aiming to define the microbiota in the upper FRT under non-disease conditions[12]. However, the presence of infertility in these women might itself affect the FRT microbiome[72]; thus, the restriction of inclusion criteria to couples presenting with male factor infertility might provide a better control group for these studies[12]. However, microbial communities present in semen could potentially affect the composition of the endometrial microbiomes[25].

Genital and other microbiome interactions

Within individuals, the genital microbiota can interact with other body sites, both proximal (such as the urinary tract) and distal (such as the gut or oral cavity) (Fig. 3). Numerous studies have also shown that partners share and/or exchange members of the genital microbiota through sexual activity[73–83]. Thus, multiple sites can serve as potential reservoirs of genital microorganisms. Next-generation sequencing studies also demonstrated that common vaginal bacteria (such as *Lactobacillus*, *Sneathia*, *Prevotella*, *Gardnerella*, Atopobium, *Peptoniphilus*, *Gemella* and *Finegoldia*) are components of the urinary tract microbiota in both women and men[14–17,84,85], suggesting the interconnectivity of microbiota (bladder–vagina axis) within the urogenital tract. Furthermore, the predominant vaginal *Lactobacillus* spp. and other common vaginal bacteria, such as *G. vaginalis*, are commonly found in catheterized urine samples and can be cultured using an expanded quantitative urine culture (EQUC) protocol[85]. In a study using EQUC and whole-genome sequencing techniques, phylogenetically similar strains of *L. crispatus*, *L. iners*, *E. coli* and *Streptococcus anginosus* were found to be present in the bladder and vagina of the same individual[14]. Furthermore, the same study showed that vaginal and bladder microbiota exhibit similar functional capacities, which are distinct from those of the gut microbiota[14]. These findings strongly suggest microbial translocation within the urogenital tract, which is not limited to uropathogens but also includes health-associated bacteria. *Lactobacillus* spp. found in both the bladder and the vagina have been hypothesized to protect the urinary tract against invading uropathogens[14]. However, mechanisms related to the protective effect of urinary tract microbiota need to be determined in future studies[86]. Multiple studies showed that vaginal microbiome members (including *Lactobacillus* spp. and BV-associated

bacteria) can be detected in male penile skin, urethral, urine and semen specimens[25,73–77], which suggests that sexual partners share and exchange microorganisms inhabiting their urogenital tracts. Furthermore, a study that examined vaginal, penile and male urethral microbiota among monogamous heterosexual couples with or without BV revealed high similarity between the penile skin and urethral microbiota of the male partner and the vaginal microbiota of the female partner with BV, which supports sexual transmission of BV[24]. Furthermore, previous culture-based studies showed that the BV-associated organism *G. vaginalis* can be cultivated from male urethra or penile skin[78,79]. By contrast, in BV-negative couples, neither the urethral nor penile skin microbiota was similar to the vaginal microbiota[24], suggesting that the penile epithelial microenvironment might favour BV-associated bacteria colonization. In addition, multiple reports in women who have sex with women support the sexual transmission of BV-associated microorganisms[80–83]. Overall, this indicates that some vaginal bacteria can be shared between sexual partners and affect the species composition of each other's urogenital microbiota. In addition, other sexual activities, such as oral and anal sex, can affect the microbial continuum across distal mucosal sites, such as the gut and the oral cavity[55,87]. However, more studies, in particular using microbial culturomics and metagenomics approaches, are needed to assess relationships and dynamics among the microbiomes of urogenital and other extravaginal mucosal sites of sexual partners. In addition, the most prevalent vaginal *Lactobacillus* species (*L. crispatus*, *L. iners*, *L. jensenii* and *L. gasseri*) have also been shown to colonize the rectum; co-colonization of the vagina and rectum correlated with the lowest prevalence of BV[88,89]. These studies support the concept that the rectum is a key reservoir for vaginal lactobacilli and that rectal colonization with these microorganisms contributes to maintenance of health-associated vaginal microbiota. Finally, haematogenous spread of bacteria emanating from the distal mucosal sites, such as the gut or oral cavity, should be considered as a potential route of interplay between the genital microbiota, particularly that of the upper FRT, and distal mucosal sites[12].

Intriguingly, the gut microbiota can also indirectly influence the genital microbiota composition through oestrogen-mediated mechanisms (Fig. 3). In the lower FRT, oestrogen has a crucial role in homeostasis by facilitating growth of *Lactobacillus* spp. through induction of glycogen production[29]. However, studies have shown that circulating oestrogen levels in the human body are influenced by gut microbiota[90,91], leading to the concept of an oestrogen-mediated gut–vagina axis[92]. The interplay between these two distal mucosal sites involves enteric bacteria that are able to metabolize oestrogens; the collection of these microorganisms and their genes is termed the 'oestrobolome'[90]. These microorganisms secrete β-glucuronidase and β-glucosidase, which deconjugate hepatically conjugated oestrogens and promote their reabsorption to circulation[91]. After deconjugation, free oestrogen is transported to distal sites (including the FRT), where it binds to its receptors and triggers intracellular signalling, resulting in increased glycogen production and other physiological changes such as mucus production and thickening of the epithelium. Thus, a reduction in the gut microbiota diversity (resulting in a lack of oestrogen-metabolizing bacteria) could influence the vaginal microbiome composition (particularly *Lactobacillus* dominance) via the oestrobolome.

Microbial dysbiosis and carcinogenesis

Microorganisms, including viruses and bacteria, have been suspected to have a role in carcinogenesis for a long time. However, pinpointing specific bacterial species that can cause cancer has been challenging. The International Agency for Research on Cancer has classified only one bacterium, Helicobacter pylori, as a human carcinogen[93] — this common bacterium colonizes the human gastric mucosa and induces chronic inflammation and, consequently, gastric ulcers, which can progress to stomach cancer[94]. Additional malignancies presumed to be caused by single bacterial species include gallbladder cancer (associated with chronic *Salmonella enterica* serovar Typhi or Paratyphi infections)[95], immunoproliferative small intestine disease (associated with *Campylobacter jejuni* infections)[96] or certain lymphomas (associated with *Borrelia burgdorferi* or *Chlamydia psittaci* infections)[97,98]. Several viruses, including Epstein–Barr virus, HPV and hepatitis C virus, are also known causative agents of cancer[99]. These oncogenic bacteria and viruses exhibit the capacity to directly modulate carcinogenesis through specific toxins that can damage host DNA or the integration of oncogenes into host genomes, respectively[100]. However, clinical studies, as well as those performed in germ-free, gnotobiotic and antibiotic-treated mice, suggest that microbially driven carcinogenesis is frequently related to global changes in the microbiome, rather than attributable to single pathogens[101]. In contrast to gastric cancer, which is caused by infection with a specific pathogen, other malignancies, such as colorectal and liver cancer, seem to be caused by dysbiosis. In this case, carcinogenesis seems to be driven indirectly by the altered host defence responses to dysbiotic microbiota, pathobionts and/or pathogens[100]. Studies in mouse models of colorectal and liver cancer have demonstrated that antibiotic treatment and germ-free status lead to a substantial reduction in the number of tumours[101]. Additional studies also revealed that the transmission of dysbiotic gut microbiota triggered colorectal cancer development[102]. For example, a 2019 study demonstrated that transplanting faecal material from patients with colorectal cancer into germ-free mice caused lesions and epigenetic changes characteristic of the development of malignancy[103]. These reports provide strong evidence of the tumour-promoting effects of dysbiotic gut microbiota in several malignancies, particularly colorectal and liver cancers. Similar to the gut, microbial dysbiosis might also promote tumorigenesis in other organs inhabited by microorganisms, such as the skin, oral cavity, lungs and the genital tract1[04].

Considerable efforts have been made to understand the pathophysiological mechanisms underlying microbially driven carcinogenesis. Specific bacteria or dysbiotic bacterial communities are well documented to cause epithelial barrier failure, immune dysregulation and/or genotoxicity and, consequently, create a tumour-permissive microenvironment[100,101,105] (Fig. 4). In addition, chronic inflammation is a well-characterized mechanism modulating the hallmarks of cancer[106] and cancer-associated bacteria (for example, *Fusobacterium nucleatum* in colorectal cancer) have been shown to activate nuclear factor-κB (NF-κB), a key regulator of cancer-associated inflammation, through engagement of Toll-like receptors and nucleotide-binding oligomerization domain-like receptors[100]. Furthermore, the carcinogenic potential of intestinal bacteria leads to increased production of IL-6 and tumour necrosis factor (TNF), activation of signal

transducer and activator of transcription 3 (STAT3) and activation of IL-17–IL-23 pathways[100,107]. Collectively, these microbiota-induced innate and adaptive host immune responses can contribute to tumour development and progression by triggering cancer-promoting inflammation and promoting resistance to cell death[100–102,105,106]. Moreover, several bacteria, such as *H. pylori* (associated with gastric cancer), *F. nucleatum* and enterotoxigenic *Bacteroides fragilis* (both associated with colorectal cancer) produce proteins that can directly influence host Wnt–β-catenin signalling[108,109], which regulates cell proliferation, survival and migration, and angiogenesis, all of which are hallmarks of cancer[100,101] (Fig. 4). Interestingly, some bacteria, such as the sexually transmitted pathogen *C. trachomatis*, are able to induce epithelial-to-mesenchymal transition of infected cells, which might promote tumorigenesis through the loss of epithelial cell adhesion and downregulation of DNA damage responses[110]. Finally, bacteria can also promote carcinogenesis via a direct effect on cell transformation through production of DNA-damaging toxins. For example, colibactin (produced by *E. coli*) and cytolethal distending toxin (produced by Gram-negative bacteria such as *E. coli*, *C. jejuni*, *Helicobacter* spp. and *S.* Typhi) exert direct effects on DNA damage and genome instability, which are linked to the development of colorectal, gastric and gallbladder cancers[100,101]. In addition, toxins produced by other bacteria, such as *B. fragilis* toxin can indirectly damage DNA through induction of high levels of reactive oxygen species[101]. Members of the urogenital microbiota, particularly species associated with genital dysbiosis, are likely to use similar biological mechanisms, which could contribute to the development and progression of gynaecological cancers. Thus, mechanistic studies are urgently needed to investigate the functional effects of specific genital bacteria or their products on the hallmarks of cancer in the genital tract.

The microbiome and gynaecological cancers

A growing body of literature supports the concept that bacterial communities within the FRT might contribute to the aetiology, disease severity and/or treatment of gynaecological malignancies[111].

HPV, microbiota and cervical cancer

Cervical cancer is the most common HPV-related malignancy and the fourth most common cancer in women worldwide, with an estimated 570,000 new cases and 311,000 deaths in 2018[112]. Notably, the incidence of cervical cancer differs among racial and ethnic groups[113,114]. In the USA, Hispanic women are 60% more likely to be diagnosed with cervical cancer and 30% more likely to die from cervical cancer than non-Hispanic white women[113]. High-risk HPV genotypes, such as HPV16 or HPV18, are well-established oncogenic factors in cervical carcinogenesis. The squamocolumnar junction or transformation zone of the cervix is particularly susceptible to HPV infection and is the site at which cervical cancers arise[115,116]. In fact, 99.7% of cervical cancer biopsy samples contain the virus (confirmed by PCR)[117]. However, 85–90% of high-risk HPV infections are spontaneously cleared and only 10–15% persist, consequently leading to the development of precancerous cervical intraepithelial neoplasia (CIN) and subsequent progression to invasive cervical carcinoma (ICC)[118]. This discrepancy suggests that other factors in

the local cervicovaginal microenvironment might promote carcinogenesis in conjunction with HPV. Several factors, including age of sexual debut, multiparity, contraceptives and hormone treatments, other STIs (chlamydia, gonorrhoea, syphilis and herpes) and smoking, have been shown to increase the risk of progression of cervical neoplasia among HPV-infected women[119–124]. Over the past decade, emerging evidence suggests that the vaginal microbiome also has a role in cervical carcinogenesis[51,125–127]. Epidemiological studies have revealed associations between the diverse non-*Lactobacillus*-dominant vaginal microbiome and HPV infection and persistence[51,125,126,128–136]. Additionally, BV, which is characterized by depletion of *Lactobacillus* spp. and overgrowth of diverse obligate and strict anaerobic bacteria, has been associated with an increased risk of HPV acquisition and decreased clearance of HPV[128–130]. Initial cross-sectional studies involving a Korean twin cohort ($n = 68$ selected from 912 women participating in the Healthy Twin Study, part of the Korean Genome Epidemiology Study, which excluded postmenopausal women from the analysis owing to the effect of menopause on vaginal microbiome composition) or a Chinese cohort ($n = 70$) revealed that women infected with HPV without cervical dysplasia have a more diverse vaginal microbiome and had substantially higher abundance of *L. gasseri* and several BV-associated bacteria, such as *Gardnerella*, *Sneathia*, *Megasphaera* or *Dialister*, than HPV-negative women[66,126,131,132]. Furthermore, two small longitudinal 16S rRNA sequencing studies ($n = 32$ and $n = 72$) have identified that the abundance of vaginal species *L. gasseri* and *Atopobium* spp. is associated with HPV clearance and HPV persistence, respectively[133,134]. Only three microbiome studies have included women with cervical dysplasia and cancer and they are cross-sectional studies with modest sample sizes[51,125,126]. However, all of these reports consistently show depletion of *Lactobacillus* spp. and a substantial increase in vaginal microbiome diversity in women with CIN and ICC compared with healthy individuals (Fig. 1). These studies were also aimed at identifying particular bacterial taxa associated with cervical disease. The first ($n = 169$) identified that three BV-associated microorganisms — *Sneathia sanguinegens* ($P < 0.01$), *Anaerococcus tetradius* ($P < 0.05$) and *Peptostreptococcus anaerobius* ($P < 0.05$) — were considerably more abundant in the vaginal microbiome of patients with high-grade dysplasia than in that of patients with low-grade dysplasia[125]. A separate study ($n = 32$) also identified that *Sneathia* and *Fusobacterium* spp. were only present in women with cervical dysplasia or cancer, but not in women without neoplasia[126]. Furthermore, in a study of 100 women, decreased *Lactobacillus* dominance and increased rates of diverse vaginal microbiome were observed in patients with precancerous lesions and cervical cancer ($P < 0.05$)[51]. Notably, a significant increase in vaginal pH ($P = 0.01$) was also observed, which was related to the severity of cervical neoplasia and strongly correlated with the depletion of *Lactobacillus* spp.[51]. With regard to specific taxa, the majority of bacteria that were enriched in HPV-infected women or women with dysplasia or cancer are not only limited to microorganisms associated with BV (such as *Gardnerella*, *Atopobium*, *Prevotella*, *Megasphaera*, *Parvimonas*, *Peptostreptococcus*, *Anaerococcus*, *Sneathia*, *Shuttleworthia* and *Gemella*) but also include those that cause other forms of dysbiosis (including *Streptococcus agalactiae* and *Clostridium*). Intriguingly, *Sneathia*, a member of the phylum Fusobacteria, was the only microorganism that was enriched in women throughout the continuum of cervical carcinogenesis[51]. As these data are in accordance with previous reports that identified this microorganism to be associated with cervical dysplasia, the

presence of *Sneathia* in the vaginal microbiome might be a metagenomic marker for HPV persistence and progression of cervical neoplasm. Interestingly, *Sneathia* was enriched in women of Hispanic ethnicity in this study[51]. Taken together with the observation that Hispanic women exhibit decreased *Lactobacillus* dominance and elevated vaginal pH[51], these observations might begin to explain increased rates of cervical cancer incidence and cancer disparity in this ethnic group. Finally, a 2018 systematic review and meta-analysis of longitudinal studies supported a causal link between a diverse non-*Lactobacillus*-dominant vaginal microbiome and cervical carcinogenesis via the effect of the microbiome on HPV acquisition (overall RR 1.33; RR among young women 1.4; 95% confidence interval (CI); statistical heterogeneity (I^2) 0%) and persistence (RR 1.14; I^2 44.2%), as well as on the development of precancerous dysplasia (RR 2.01; I^2 0%)[137]. Another network meta-analysis of cross-sectional and longitudinal studies also revealed that women with a non-*Lactobacillus*-dominant vaginal microbiome or a vaginal microbiome dominated by *L. iners* had 2–3 times higher odds of high-risk HPV prevalence and cervical neoplasia and 3–5 times higher odds of any prevalent HPV (95% CI) than women with a vaginal microbiome dominated by *L. crispatus*[138]. Notably, a vaginal microbiome dominated by *L. gasseri* had the highest odds for high-risk HPV (odds ratio (OR), 3.3; 95% CI) compared with *L. crispatus*[138]. In addition, an independent meta-analysis validated these findings, showing that *L. crispatus*, but not *L. iners*, is associated with decreased detection of high-risk HPV (OR 0.49; 95% CI; I^2 10%) and dysplasia (OR 0.50; 95% CI; I^2 0%)[139].

Mechanisms of HPV and microbiome interaction.—To date, and to our knowledge, no published reports have investigated the mechanism of interaction between HPV and vaginal bacteria, besides those reported in clinical studies. HPV is difficult to cultivate in vitro, making these studies challenging to perform. In addition, mouse models for HPV-mediated cervical dysplasia and cancer[140–143] or BV using polymicrobial cocktails are limited[144,145]; however, future use of these mouse models might yield important insights into the role of vaginal bacteria in cervical carcinogenesis. In addition, human in vitro cell lines and 3D organotypic models can be used to study host-vaginal microbiota interactions[144,146]. Studies are ongoing in our laboratory to evaluate the role of individual, polymicrobial cocktails and clinical communities of vaginal bacteria using human 3D vaginal, cervical and endometrial epithelial models[147–153] on characteristic hallmarks of cancer (Fig. 4).

Effects on host response.—The functional effect of the vaginal microbiome on the host response during cervical carcinogenesis has not been comprehensively studied. One small cross-sectional study (n = 32) showed that elevated cervical expression of anti-inflammatory IL-4 and transforming growth factor β1 (TGFβ1) were associated with the presence of *Fusobacterium* spp., which is involved in the development of the immunosuppressive microenvironment[126]. In a cross-sectional study (n = 100), systematic examination of multiple immune mediators in the local cervicovaginal microenvironment in women with cervical cancer or dysplasia and women without neoplasia (infected or not with HPV) revealed that several proinflammatory (IL-36γ) and chemotactic (IFNγ-induced protein 10 (IP10), macrophage-inflammatory protein 1β (MIP1β) and RANTES), haematopoietic (FLT3 ligand) and adaptive immune response cytokines (IL-2, IL-4 and soluble CD40

ligand) were associated with non-*Lactobacillus* dominance ($P < 0.05$)[51]. Moreover, known circulating cancer biomarkers, including growth and angiogenic factors (such as basic fibroblast growth factor (FGF2), stem cell factor (SCF), apoptosis-related proteins (soluble Fas ligand, TNF-related apoptosis-inducing ligand (TRAIL)), hormones (prolactin), proinflammatory cytokines and chemokines (macrophage migration inhibitory factor (MIF) and TNF) and osteopontin were present in the local cervicovaginal microenvironment and correlated positively not only with genital inflammation but also with a dysbiotic vaginal microbiome (that is, non-*Lactobacillus* dominance) ($P < 0.05$)[154]. Additionally, integration of multiple datasets, including those of cervicovaginal immune mediators and cancer biomarkers, the vaginal microbiome and vaginal pH, revealed potentially 'high-risk' features of the local cervicovaginal microenvironment, such as a substantial increase in levels of proinflammatory cytokines and specific cancer biomarkers, abnormal vaginal pH and depletion of lactobacilli, that might directly or indirectly contribute to cervical carcinogenesis[154].

Previous metabolomic studies of the cervicovaginal microenvironment in women with BV suggested that the vaginal microbiome might drastically alter local metabolic profiles (mostly in amino acid, dipeptide, polyamine and ketone body pathways)[59,155], which could thereby affect cervical carcinogenesis. Indeed, a cross-sectional study ($n = 78$) from our own group, which to our knowledge was the first report on cervicovaginal metabolomes in HPV-mediated cervical neoplasia, demonstrated the functional interplay between HPV, neoplastic disease and the vaginal microbiome[127]. In this study, cervicovaginal metabolic profiles were strongly driven by cancer, followed by genital inflammation, HPV infection and vaginal microbiome composition[127]. Perturbations in lipids, an emerging hallmark of cancer[156], were observed in cancer metabolomes, whereas depletion in amino acid and nucleotide metabolites connected vaginal dysbiosis to HPV infection and precancerous cervical dysplasia. Specific metabolites, such as anti-inflammatory nucleotides, correlated with *Lactobacillus* dominance independently of the severity of the cervical neoplasm[127]. The multi-omics approach enabled the identification of specific sets of metabolites associated with *Lactobacillus* dominance involved mostly in peptide, amino acid and nucleotide pathways and genital inflammation involved in multiple lipid pathways[127].

We and others hypothesize that microenvironmental factors, including microbial[51,125,126,137,138], immune[51,125–127] and metabolic signatures[154], might favour HPV persistence in the local microenvironment and consequently increase the risk of neoplastic disease. Alternatively, the collective action of these conditions, including infection with high-risk HPV, might directly affect hallmarks of cancer (such as cell proliferation, evasion of apoptosis, senescence, DNA mutation and methylation, and angiogenesis) contributing to the development of precancerous lesions and progression to cancer (Fig. 4). Taken together, this increasing body of evidence suggests a complex relationship between the host, local microbiome and HPV during cervical carcinogenesis.

Microbiota axes and endometrial cancer

Endometrial cancer is the most common gynaecological cancer in developed countries and the fourth most common cancer affecting women in the USA[111]. Socioeconomic status, race

and/or ethnicity might place some women at an increased risk of developing endometrial cancer[157,158], as genetic alterations and hereditary factors underlie only 10–20% of cases[159]. Notably, in the USA, black and non-Hispanic white women have the highest incidence of endometrial cancer[160]; however, the mortality is twice as high in African American women compared with all other racial and ethnic groups[161]. Environmental factors, including obesity, inflammation, imbalances in oestrogen metabolism and oestrogen therapy after menopause, are major risk factors for the development of type I endometrial cancer[162,163]. Interestingly, these environmental factors are also associated with changes in the gut[164,165] and vaginal[51,166] microbiomes. A strong connection between the gut microbiota, oestrogen metabolism and obesity suggests a potential role of the microbiome in the aetiology of endometrial cancer[92,111,167].

Advances in sequencing technologies have shown that oestrogen compounds can shape vaginal and distal microbial communities[167–169] and that the oestrobolome can alter circulating oestrogen levels[90]. We and others postulate that the oestrobolome can influence the development and treatment of endometrial hyperplasia and cancer via its oestrogen modulatory effects[92,170]. Moreover, as oestrogen levels directly affect health and homeostasis in the vaginal microenvironment, oestrogen can also affect the gut–vaginal microbiome axis. Thus, the gut microbiome might indirectly promote endometrial carcinogenesis by altering genital microbial communities.

A 2016 study associated differences in the FRT microbiota with endometrial hyperplasia and cancer[19]. In this small (n = 31), cross-sectional study, several microbial taxa, including *Atopobium*, *Porphyromonas*, *Dialister*, *Peptoniphilus*, *Ruminococcus*, *Anaerotruncus*, *Anaerostipes*, *Treponema*, *Bacteroides* and *Arthrospira*, were reported to be enriched in women with hyperplasia and cancer along the reproductive tract, including the uterus, compared with benign uterine samples[19] (Fig. 1). Furthermore, the simultaneous presence of *Atopobium* and *Porphyromonas* combined with abnormal vaginal pH (>4.5) was strongly associated with disease status[19]. These bacteria or other disturbances in the microbiome could alter hallmarks of cancer, including the promotion of damaging local inflammation that contributes to carcinogenesis in the endometrium[92]. A study from our group that used a 3D human vaginal epithelium model showed that *A. vaginae* induces proinflammatory cytokines and antimicrobial peptides[148]. The association between proinflammatory *A. vaginae* and *Porphyromonas* spp. in this endometrial cancer cohort[19] supports a link between ascending BV-associated bacteria and other genital bacteria, inflammation and endometrial cancer. However, future studies with larger cohorts are needed to validate these initial findings. Mechanistic studies, employing in vitro[151] and animal models[171], will be also required to improve understanding of the functional role of genital microbiota and the oestrobolome in the aetiology of endometrial cancer.

Microbiota in tubal and ovarian cancer

Ovarian cancer is one of the deadliest malignancies in women — in 2018, an estimated 295,414 new cases of ovarian cancer were diagnosed and 184,799 women died of the disease worldwide[112] — owing to its asymptomatic clinical presentation. Thus, prevention strategies and early diagnosis are priorities[172]. In contrast to cervical cancer, risk of

developing ovarian cancer is 30% higher in non-Hispanic white women than in women of other races or ethnic groups[172]. However, the connection of this cancer disparity with the FRT microbiome is unknown. Additionally, multiparity, tubal ligation, use of oral contraceptives and oophorectomy are known to reduce the risk of developing ovarian cancer[172]. Conversely, dysbiosis in the genital microbiota has been associated with the development of ovarian cancer and proposed as a potential biomarker for the disease[173,174].

Similar to endometrial cancer, chronic infections with sexually transmitted pathogens and inflammation in the genital tract have been associated with the development of ovarian tumours[175]. Two cross-sectional studies with modest sample sizes (n = 119 and n = 127) have compared ovarian tissues in women with ovarian cancer and healthy participants and found a distinct ovarian microbiome in women with cancer[173,174]. Moreover, the microbiome of the malignant ovarian tissue had distinct microbial signatures compared with the healthy surrounding ovarian tissues within the same individuals[174]. In particular, the presence of potentially pathogenic intracellular microorganisms, such as *Brucella*, *Mycoplasma* and *Chlamydia* spp., was found in 60–76% of ovarian tumours[174,176,177] (Fig. 1). Additionally, an increase in *Proteobacteria*, especially in *Acinetobacter* spp., in the ovarian tumours was linked to inflammation-associated gene profiles in participants[173]. Finally, several pathogenic viruses and intracellular bacteria present in ovarian tissues, including HPV, cytomegalovirus and *C. trachomatis*, were identified as biosignatures of ovarian cancer[175].

Although these pilot studies have identified the presence of various bacteria in ovarian cancer tissues and suggested a link with inflammation, the causational link between microbiota and ovarian cancer remains unclear. These microorganisms might induce carcinogenesis through direct or indirect mechanisms; however, the highly anoxic tumour microenvironment might also favour the recruitment and growth of anaerobic microorganisms, such as *Chlamydia* spp.[174]. Similar to other upper FRT sites, the Fallopian tubes and ovaries are low-abundant sites, which makes sample collection without cross-contamination and interpretation of the results challenging[68]. Site-specific differences related to the ovarian tumour microbiome[173] warrant future comparative studies to improve understanding of the connection between microbiomes throughout the FRT and establish the role of the microbiome in this malignancy. Furthermore, the connection between the presence of pathogenic microorganisms, chronic inflammation and ovarian cancer needs further validation with epidemiological studies in large patient cohorts and/or longitudinal study designs.

Microbiome and therapy efficacy

Women with gynaecological cancers are typically treated with surgery (for example, hysterectomy or bilateral salpingo-oophorectomy), radiation, chemotherapy or a combination of chemoradiation and surgery depending on the site and stage of the cancer[178]. Immunotherapy has become an exciting new option for gynaecological cancer treatment, but it is currently in its early stages[178]. To date, the FDA has approved only a few immunotherapy options, including the immune checkpoint inhibitor targeting anti-programmed cell death 1 (PD1) and anti-angiogenic factor targeting VEGFR, for the

treatment of advanced gynaecological cancers[178]. These immunotherapeutics have been specifically used for the treatment of recurrent endometrial cancer with either mismatch repair protein-deficient or microsatellite stability index-high (MSI-H), ovarian cancer, and advanced cervical cancer with disease progression during or after chemotherapy[178]. However, other immunotherapy agents are being evaluated in multiple clinical trials as well as combinations of these therapies[178]. The microbiome has been demonstrated to affect the efficacy of cancer therapies, including chemotherapy and immunotherapy, for various tumour types (including melanoma, lung and kidney cancers)[179–182] (Fig. 5). A 2018 study reported a correlation between decreased efficacy of immunotherapy and survival in patients with melanoma who had received antibiotic treatment before immunotherapy, revealing that alteration of the gut microbiota via antibiotic treatment directly affects therapeutic responses[179]. These data have been reflected in several other studies in patients with cancer and in preclinical mouse models, which have demonstrated that the gut microbiome diversity and composition affect antitumour immunity and the efficacy of PD-1 immunotherapy for melanoma, lung and kidney cancers[179–181]. Notably, faecal microbiota transplantation (FMT) from human responders to antibiotic-treated or germ-free mice with xenograft tumours promoted the efficacy of PD-1 blockade against melanoma, lung and kidney cancers, whereas FMT from non-responders did not[179–181]. Particular bacterial taxa, for example, *Akkermansia muciniphila*, *Bifidobacterium longum* and *Ruminococcaceae*, were also found to be more abundant in faecal samples collected from PD-1-responding patients. Notably, the responsiveness to PD-1 or programmed cell death 1 ligand 1 (PD-L1) immunotherapy in mouse melanoma and lung cancer models could be induced through oral supplementation with *A. muciniphila*[179] and in a mouse melanoma model with Bifidobacterium[183]. These findings strongly suggest a mechanistic role for the gut microbiota in facilitating antitumour immunity[179,183].

The microbiota can alter therapeutic efficacy by a number of mechanisms, including translocation, immunomodulation, metabolism and enzymatic degradation, depending on the type of therapy[184,185]. For example, the composition of the gut microbiota has been shown to affect the level of efficacy for chemotherapeutics such as irinotecan via alterations in drug metabolism, including inhibited intestinal absorption and decreased activity of the irinotecan-activating enzyme carboxylesterase[186]. The gut microbiome has also been shown to affect immunotherapeutics including PD-1/PD-L1 blockade by modulating the host immune system, particularly by mediating T cell activation, increasing T cell priming and T cell accumulation at the tumour site[179–181]. Furthermore, early studies on microbiota in cancer demonstrated that interactions between the gut microbiota and the host immune system are bidirectional and that microbiota can also regulate the immune response to cancer cells[179,187] (Fig. 5). Microorganism-associated molecular patterns, such as cellular wall polysaccharides and microbial metabolic products — including short-chain fatty acids — have been shown to interact with pattern recognition receptors of the innate immune system to modulate therapy responses[188]. Moreover, many structural and metabolic features of bacteria, including mobility, cell toxicity, immunogenicity and their preferential accumulation within the tumour microenvironment, suggest that bacteria are potential targets for mediating the efficacy of cancer therapy[189]. The targeted interactions between bacteria and the host immune system are key factors in patient responsiveness

to immunotherapeutic agents[179]. Additionally, several reports have investigated the effect of the vaginal microbiome on the pharmacokinetics of topical antiretroviral drugs, including tenofovir gel and the dapivirine ring, with contradicting results[190–192]. One study demonstrated that vaginal microorganisms, such as *L. crispatus* and *G. vaginalis*, but not *A. vaginae*, decreased bioavailability of the drugs, resulting in decreased antiviral activity[190]. By contrast, another report showed no significant differences in tenofovir concentrations in cervicovaginal aspirates from women with *Lactobacillus*-dominant microbiome versus dysbiotic *Lactobacillus*-depleted microbiome ($P > 0.07$)[191]. However, the direct role of the vaginal microbiome in cancer therapies is unknown. Taking into account the connection between the gut and vaginal microbiomes[111], the effect of cancer therapeutics on oestrogen metabolism[92,111] and the known modulation of topical drug efficacies by the vaginal microbiome[190,191,193], the vaginal microbiome could have a role in the efficacy of cancer therapeutics. An improved understanding of the microbiota–drug interactions[185] and the vaginal–gut microbiota axis[92] should be further studied and might provide unique insights into the biology of gynaecological cancer and therapeutic efficacy.

IrAEs and the microbiome

In addition to mediating therapeutic responses, the microbiome can be an important target for the management of therapeutic toxic effects or immune-related adverse events (irAEs). Radiotherapy and chemotherapy for gynaecological cancers can induce urogenital, vaginal, rectal and skin-related toxicities[194–196]. In general, the connection between the microbiome and therapeutic toxicity is poorly understood[184]; however, preliminary studies in animal models have demonstrated that gut microbiota can mediate the gastrointestinal toxicities related to chemotherapy[197,198], which include diarrhoea, gastrointestinal pain and bleeding[199,200] (Fig. 5). These events were also associated with alterations in the gut microbiome[199] — a decrease in *Bifidobacterium* and *Lactobacillus* and an increase in *E. coli* and *Staphylococcus* in patients treated with chemotherapeutic agents such as cisplatin and carboplatin were associated with diarrhoea and increased levels of NF-κB, IL-1β and TNF[200]. Changes in the gut microbiome might also be related to adverse effects of poly(ADP-ribose) polymerase (PARP) inhibitors, which are standard maintenance therapeutics for the management of ovarian cancer[201] and are known to induce nausea, vomiting, diarrhoea and constipation[202]. Indeed, differences in gut microbiota composition in *Parp*-knockout mice and wild-type mice were associated with inflammation, diarrhoea and reduced gut microbiota diversity in the knockout mice[203]. Additionally, gut microbial activity can induce the toxic effects of common chemotherapeutic agents. For example, gut commensals that produce β-glucuronidase (an oestrobolome enzyme) can induce diarrhoea by converting irinotecan to its toxic form, which damages the intestinal epithelia[204]. Future studies characterizing the effect of chemotherapeutics on microbiota in the gut and at other sites will enhance our understanding of these microbiome-related toxicities[203] and could aid in the development of safe and effective therapies.

Radiotherapy and chemotherapies (for example, 5-fluorouracil) are also known to induce gastrointestinal mucositis, an inflammatory condition that affects the gastrointestinal tract caused by elimination of beneficial gut microorganisms and reduction of gut microbial diversity[205,206]. The damage to the proliferating epithelial barrier following administration

of cancer drugs provides opportunities for pathobionts, such as *Enterobacteriaceae* and *Corynebacterium*, to translocate and induce mucositis[206]. In addition to radiation and chemotherapy, mucositis has been observed in patients treated with PD-1 inhibitors[207], indicating that several commonly used cancer therapies have adverse effects on the gut microbiota. Animal studies in mice or rats treated with irinotecan have shown that the overgrowth of opportunistic microorganisms, such as *Enterobacteriaceae*, in the small intestine after chemotherapy is related to mucositis[198,206] and that dietary fibre supplementation after chemotherapy reduced the mucositis by strengthening the gut epithelial barrier[197]. As dietary fibre enhances butyrate production in the colon and butyrate enhances the gut epithelial barrier[197], prebiotic supplementation during cancer therapy might have the potential to reduce the severity of gastrointestinal symptoms of cancer therapeutics.

In addition to gastrointestinal toxicities, cancer treatment has adverse effects on the vagina and probably also the local microbiome[111]. Vaginal stenosis and vulvovaginal atrophy (VVA) are common adverse effects of pelvic radiotherapy or brachytherapy for the treatment of cervical and endometrial cancers[196,208,209]. Vaginal stenosis is defined as a shortening and narrowing of the vagina that causes dryness, itching and discomfort in patients and arises from cellular damage caused by radiotherapy, resulting in thinning of the vaginal walls[111]. Women with VVA have reduced levels of glycogen in their vaginal secretions, which can result in lower levels of health-associated *Lactobacillus* species[66]. Management of vaginal stenosis and VVA involves use of vaginal dilators, lubricants and moisturizers[210], and patients are advised to have sexual intercourse[211], all of which might also affect the vaginal microbiota composition and/or stability. Although the connection between vaginal stenosis and changes in the local cervicovaginal microenvironment and microbiome have not yet been investigated, one report has shown that radiation can adversely affect vaginal bacterial communities[212]. This pilot study (n = 19) characterizing vaginal microbiota profiles in women with gynaecological cancer before and after radiotherapy showed an increase in particular BV-associated bacteria, such as *Mobiluncus*, *Atopobium* and *Prevotella*, and a decrease in other BV-associated microorganisms, such as *Gardnerella* and *Peptostreptococcus*, as well as health-associated *Lactobacillus* spp. following treatment[212]. Acute and long-term toxic effects after gynaecological cancer therapy can profoundly affect the quality of life of survivors. Thus, investigation of microbiomes and their connection to therapeutic toxic effects is essential for the development of strategies to reduce these therapeutic toxicities or irAEs and enhance quality of life for these patients and survivors.

Modulating the microbiome

Gut microbiome modulation

Health benefits from the modulation of microbiomes have been successfully shown for many chronic and inflammatory diseases, including irritable bowel syndrome[213] and recurring *Clostridioides difficile* infections[214]. The accumulating evidence connecting gynaecological cancer and dysbiosis identifies microbiota as a target for cancer prevention and therapy. Notably, the investigation of microbiome editing, sometimes referred to as ‘bugs as drugs’[215], has been prompted by the observation that patients receiving antibiotics do

not respond to immunotherapy[179,216,217]. Microbiome modulation includes the use of bacterial therapeutics, probiotics and/or prebiotics, antibiotics or other drugs, and microbiota transplantation.

FMT has been widely used to determine the functional effect of gut microbiota on a wide a range of health conditions[218,219]. FMT from patients responding and not responding to immune checkpoint inhibitors to cancer xenograft mouse models showed that the gut microbiome dictates responsiveness to immunotherapy[179,181]. Furthermore, FMT has been shown to reduce toxic effects associated with radiotherapy and chemotherapy[220,221]. These innovative studies have led to FMT being proposed as a promising adjunct to cancer therapies[222]. Additional safety and testing regulations are also needed for the investigational use of FMT in human patients, especially those who are immunocompromised. As immunotherapy for gynaecological cancers is still in its infancy, the use of FMT to improve therapeutic efficacy has not been evaluated for any gynaecological cancers.

Notably, FMT studies have led to the identification of specific gut microorganisms that modulate therapeutic response[179,181]. Oral administration of *Bifidobacterium* species to mice with melanoma was as effective as treatment with the PD-L1 inhibitor alone and also improved PD-L1 inhibitor efficacy when administered adjunctively by increasing the number of primed T cells and their accumulation at the tumour site[183]. In a similar melanoma mouse model, the presence of *B. fragilis*-mediated and *Bacteroides thetaiotaomicron*-mediated T cell activation and responsiveness towards monoclonal antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4), another immune checkpoint inhibitor[187]. Similar to the experiments with *Bifidobacterium*, oral supplementation of *A. muciniphila*, a gut commensal that has been identified as a predictive biomarker of responsiveness to PD-1 inhibitors in a mouse FMT model, restored the PD-1 inhibitor response in mice that received FMT from PD-1 non-responder patients[179]. Considering that *Bifidobacterium* and *Bacteroides* species have been associated with immune modulation, immunotherapy efficacy[180,183] and oestrogen metabolism[167,223], investigation of their therapeutic potential in oestrogen-driven cancers, and particularly endometrial and ovarian cancer, is compelling.

Based on the beneficial effects on gut homeostasis[224], probiotics have also been shown to reduce therapeutic toxic effects. For example, in a wild-type mouse model, supplementation with *Lactobacillus lactis* engineered to secrete pancreatitis-associated protein, an antimicrobial peptide involved in gut homeostasis, following chemotherapy with 5-fluorouracil, reduced the abundance of pathobionts, such as *Enterobacteriaceae*, in the gut and alleviated mucositis severity[206]. Taken together, these data suggest that specific gut microbiota species induce antitumour responses and that associations between gut microbiota diversity and immunity indicate the exciting potential to develop microbiome-based or adjunct therapy regimens for various malignancies, including gynaecological cancers.

Vaginal microbiome modulation

Notably, vaginal probiotic lactobacilli, such as *L. crispatus* strain CTV-05 (as a vaginal suppository; LACTIN-V), have also been tested in clinical trials, mainly for the treatment

of BV[225,226] or urinary tract infection (UTI)[227] (Fig. 6). A randomized placebo-controlled phase IIa clinical trial, involving women with BV after standard metronidazole treatment (n = 24), showed that vaginal colonization with LACTIN-V was achieved in 44% participants (at day 28 following LACTIN-V treatment) and inhibited the growth of particular BV-associated bacteria, especially *Atopobium* (P = 0.04), compared with women not colonized with LACTIN-V[186]. Additionally, the study revealed that vaginal intercourse during treatment (P = 0.003) or pre-colonization of the vagina with endogenous *L. crispatus* (P = 0.018) reduced odds of successful colonization with probiotic *L. crispatus* strain[186]. In 2019, a multicentre phase IIb clinical trial (NCT02766023; n = 228) assessing the long-term efficacy of repeated doses of LACTIN-V in preventing BV recurrence was completed; however, the results have not yet been published (ClinicalTrials.gov). Another phase II trial (n = 100) evaluated LACTIN-V supplementation for the prevention of recurrent UTI and demonstrated a reduction in recurrent UTI compared with placebo treatment (P < 0.01)[227]. Overall, these studies demonstrate the feasibility of the use of vaginal probiotics to modulate the vaginal microbiome.

Other promising novel therapies against BV or vaginal dysbiosis include a boric acid-based anti-infective with enhanced biofilm disruptive activity (TOL-463)[228] and the nitroimidazole antibiotic secnidazole[229] (Fig. 6). A phase II clinical trial (n = 106) demonstrated that TOL-463 treatment in either vaginal gel or insert forms is safe and well tolerated and led to a 59% and 50% clinical cure rate of BV for insert and gel, respectively, at days 9–12 (95% CI)[228]. A novel formulation of secnidazole (single-dose oral granule) has also been shown to be well tolerated with a low number of treatment-emergent adverse effects in a phase III clinical trial (n = 283)[229]. In this study, 72.5% of women were also assessed by investigators not to require additional BV treatment[229].

Vaginal microbiota transplantation (VMT) from a donor with an optimal vaginal microbiome is a novel provocative treatment option under investigation for women with BV or vaginal disorders (Fig. 6). Currently, two phase I/II clinical trials in the USA (NCT03769688 and NCT04046900) and one clinical trial in Israel (NCT02236429) are recruiting participants (n = 10–126) to evaluate the safety and efficacy of this procedure in women with BV (ClinicalTrials.gov). A 2019 pilot study (n = 5) demonstrated the feasibility of using VMT from healthy donors as a therapeutic for women with intractable, antibiotic-unresponsive and recurrent BV[230]. Four of the five women treated with VMT had long-term remission, defined as marked improvement of symptoms and reconstitution of a *Lactobacillus*-dominant microbiome, at the follow-up 5–21 months after VMT[230]. To elicit a durable clinical response, repeat VMT was required in three of the five women in the exploratory study[230]. The authors did not observe adverse effects associated with VMT[230]; however, the long-term consequences of VMT remain unknown. The risks of this procedure, similar to other microbiome transplants, include transfer of antimicrobial-resistant microorganisms and undetected pathogens. Additionally, the transfer of sperm in vaginal fluid can result in unintended pregnancy. Thus, stringent inclusion/exclusion criteria and extensive testing of donor samples are imperative to minimize risks. A screening approach for universal VMT donors has been described and implemented in another 2019 pilot study (n = 20)[231]. Future studies with larger cohorts and randomized, placebo-controlled studies are required to determine the efficacy and durability of VMT.

In the future, vaginal probiotics, existing antimicrobials, live bacterial therapeutics, novel antimicrobials, biofilm disruptors and VMT have the potential to be used alone or in combination to modulate the vaginal microbiome by restoring homeostasis for prevention of gynaecological cancer and/or reduction of vaginal toxic effects related to gynaecological cancer therapies (Fig. 6).

Gaps, challenges and future directions

The clinical relevance of the urogenital microbiota in gynaecological and reproductive diseases, including cancer, has just begun to be explored. The advances in microbiomics and metagenomics have enabled us to start identifying microbial communities and/or particular bacterial species that might promote pathological states in the FRT and consequently contribute to tumorigenesis. Nevertheless, in the future, clinical studies with larger cohorts are necessary to validate and extend these initial findings for potential clinical use, a fact illustrated by a statement from the International Cancer Microbiome Consortium in 2019, which concluded that data from longitudinal cohort studies are needed to confirm the role of the human microbiome as a key driver in the aetiopathogenesis of cancer[232]. Furthermore, future epidemiological studies must include women of different races, ethnicities and socioeconomic backgrounds, as considerable differences have been reported in the genital microbiota compositions among women of different ethnic origins[26,48,50,51] and we hypothesize that these differences might contribute to disparities in cancer demographics, for example, the increased rate of cervical cancer in Hispanic, African American, and American Indian and Alaskan Native women. Other metadata collected from participants, such as behavioural, environmental, socioeconomic and genetic information (Fig. 2), might further help to identify possible covariates and confounders in association studies using cross-sectional study designs. In addition, longitudinal studies are required to determine the extent to which genital bacteria are causal factors, passengers or just a consequence of disease. Furthermore, rigorous and careful sample collection and standardized sample processing with appropriate controls must be employed in future clinical studies (particularly for studies evaluating low microbial biomass sites, such as the upper FRT) to be able to draw biologically meaningful conclusions. The 16S rRNA sequencing technique, which is commonly used for vaginal microbiome studies, has a number of limitations, particularly relating to the lack of absolute bacterial quantification, which is relevant not only for studies investigating low microbial biomass sites (upper FRT) but also those investigating vaginal dysbiosis in the lower FRT (characterized by overgrowth of anaerobic microorganisms). In future studies, other molecular techniques for bacterial quantification, for example, quantitative real-time PCR[233] or flow cytometry[234], could be used to determine absolute loads of specific bacteria associated with particular conditions. Integrated multi-omics approaches are also desirable in future studies to fully understand the complex relationships in the local microenvironment between the host, genital microbiota and the development and progression of neoplastic disease. These integrative analyses could lead to identification of new microbial and host signatures (such as bacterial communities and/or species, immune mediators and other proteins, and metabolites) to exploit as biomarkers for gynaecological cancers. Finally, mechanistic studies using in vitro and in vivo models are required to determine the role of the microbial communities and specific bacterial

species in triggering and/or promoting tumorigenesis in the FRT. Physiologically relevant 3D cell cultures, including organoids and bioreactor-derived models, can be successfully used to study the interactions between the host and genital bacteria, and future studies using these models should investigate the effect of these bacteria (alone or in polymicrobial milieu) on hallmarks of cancer[144,146,148–153]. Moreover, mouse models of cervical[143], endometrial[171] and ovarian cancers[235] could be infected with genital bacteria in order to further investigate the pathogenesis of clinically relevant bacteria and provide additional insights into microorganism-mediated mechanisms contributing to cancer development and/or progression.

Conclusions

Epidemiological studies suggest that the urogenital microbiota is linked to gynaecological cancers, particularly cervical cancer. Even so, the mechanisms of host defence to these microorganisms are still poorly understood. Further research is warranted to elucidate the functional effect of the microbial communities and/or particular bacterial species in the FRT on the local microenvironment related to tumorigenesis. Future studies using robust clinical datasets as well as in vitro human and animal models will help to define the influence of these microorganisms on homeostasis and disease and bring us closer to understanding how these microorganisms contribute to the development and progression of gynaecological malignancies. Finally, a clearer understanding of the complex host–microorganism interactions in the FRT might reveal new opportunities for cancer prevention, therapy and improving women's quality of life and overall health.

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Glossary

Microbiota
A community of microorganisms in a particular environment.

Microbiome
The entire habitat, which includes microorganisms, their genomes, and the surrounding environment.

Metagenome
The collection of genomes and genes from the members of a microbial community.

Pathobionts
Resident microorganisms with pathogenic potential, harmless to the host under normal conditions.

Osmolality
A concentration of osmotic solution expressed as the number of solute particles in 1 kg of solvent.

Microbial culturomics
An approach to identifying unknown bacteria that inhabit the human body utilizing bacterial culture techniques to provide unique insights into host–bacteria relationships.

Metagenomics
An approach to characterizing microbial communities at genome and gene level without requiring culturing.

Oestrobolome
The collection of microorganisms (and their genes) that are able to metabolize oestrogens.

Metabolomic studies
Studies of small molecules (metabolites), which are substrates, intermediates and products of metabolism within microorganisms, cells, tissues or body fluids.

Metabolomes
The collections of small molecules (metabolites) and interactions among these molecules within a biological system.

Faecal microbiota transplantation (FMT)
A process of transplantation of faecal material from a healthy individual to a recipient for restoration of the gut microbiota.

Probiotics
Live microorganisms that confer a health benefit on the host when taken as a dietary supplement in adequate amounts.

Biofilm
An assemblage of microbial cells that form on and coat various surfaces.

Vaginal microbiota transplantation (VMT)
A process of transplantation of vaginal secretions from a healthy individual to a recipient for restoration of the vaginal microbiota.

Microbiomics
The study of microbial communities inhabiting a particular environment (for example, the human body).

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Key points

- The majority of bacteria in the female reproductive tract (FRT) reside in the vagina and cervix; however, the upper FRT might have a distinct low-biomass microbiome and site-specific microenvironmental factors.
- A vaginal microbiome dominated by *Lactobacillus* species benefits the host, whereas a dysbiotic vaginal microbiome consisting of anaerobic bacteria is linked to numerous gynaecological and obstetric conditions, including gynaecological cancer.
- Multiple socioeconomic, behavioural, environmental, hormonal and genetic factors can affect the genital microbiome by disrupting homeostasis and promoting dysbiosis; the FRT microbiome is intimately interconnected with other mucosal sites.
- Emerging evidence suggests that microbial communities within the FRT might contribute to aetiology, disease severity and/or treatment of gynaecological cancers; however, further well-designed, large-cohort and mechanistic studies are needed.
- The gut microbiome can modulate oestrogen levels and thereby affect carcinogenesis of oestrogen-mediated cancers, might dictate therapeutic efficacy and toxicity for gynaecological cancer and, ultimately, influence quality of life.
- Vaginal microbiome modulation via probiotics, novel antimicrobials and/or vaginal microbiota transplantation might be a novel approach to the prevention of gynaecological cancers and/or the reduction of vaginal toxicities related to cancer treatment.

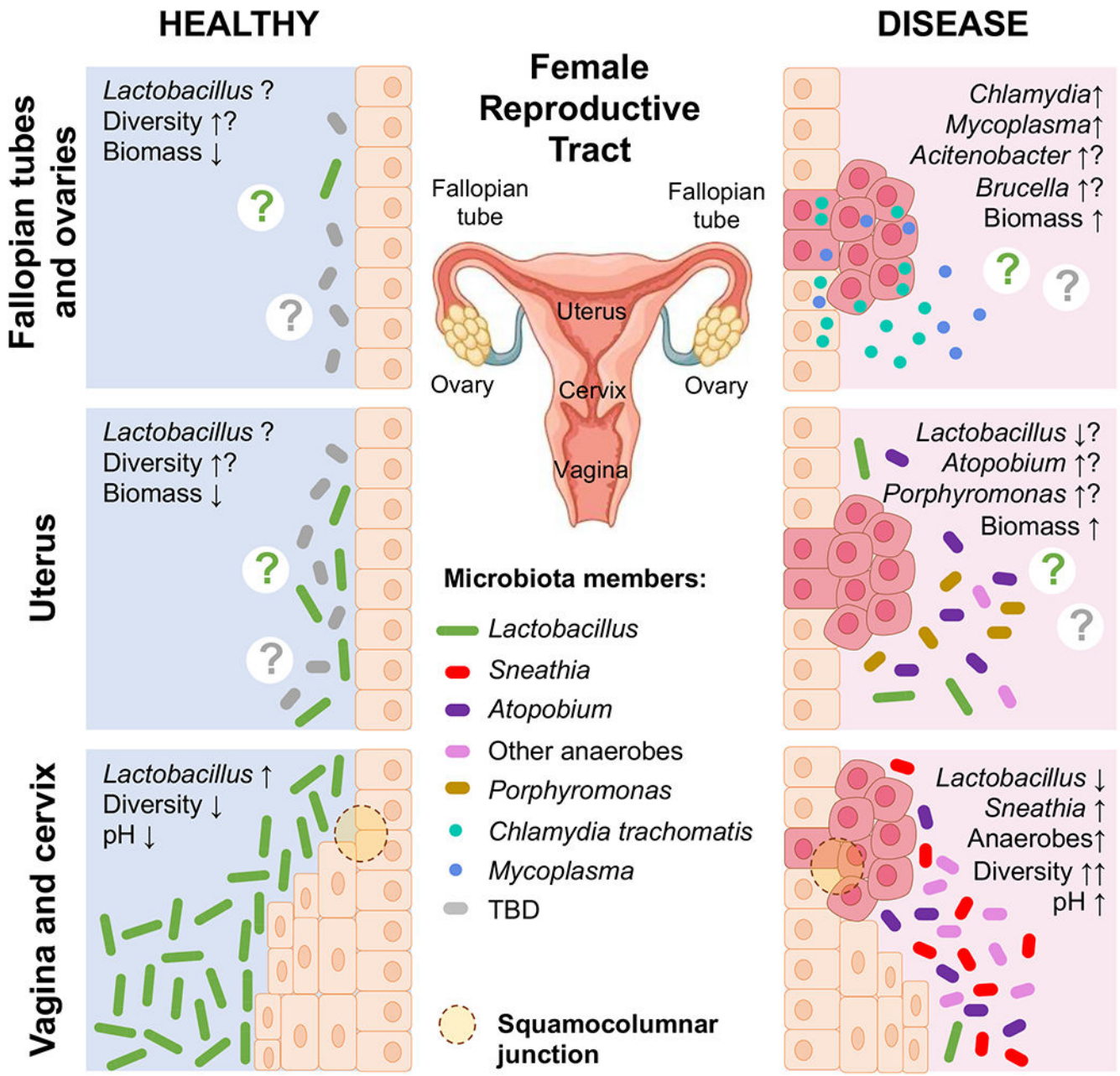

Fig. 1. Microbial communities associated with health and gynaecological cancers.

Microbiota composition and other features of the local microenvironment associated with health and gynaecological cancers across the female reproductive tract (FRT). In healthy reproductive-age women, the majority of bacteria reside in the lower FRT (vagina and cervix). The vaginal microbiota exhibits low microbial diversity and is dominated by *Lactobacillus* species, which acidify the local microenvironment through the production of lactic acid. By contrast, the upper FRT (endometrium, Fallopian tubes and ovaries) might contain no to low microbial biomass. The normal upper FRT microbiota is still not well characterized and is considered sterile by some groups. Available data suggest that the upper FRT microbiome exhibits higher microbial diversity than the lower

FRT microbiome, especially in diseased states. The presence of *Lactobacillus* species and other microorganisms has been reported in the endometrium, Fallopian tubes and ovaries. In women with gynaecological malignancies (cervical, endometrial and ovarian cancer), the local microbiota composition and biomass loads change considerably. In women with cervical cancer, the vaginal microbiome exhibits high microbial diversity and is characterized by an overgrowth of diverse anaerobic bacteria, particularly *Sneathia*. *Lactobacillus* depletion results in elevated vaginal pH. In women with endometrial cancer, the simultaneous presence of *Atopobium* and *Porphyromonas* has been associated with disease. In women with ovarian cancer, potential intracellular pathogens, such as *Brucella*, *Mycoplasma* and *Chlamydia*, and pathobionts, such as *Acinetobacter*, were reported. Overall, these dysbiotic microbiomes might contribute to the aetiology, disease severity and/or treatment of gynaecological cancers. TBD, to be determined.

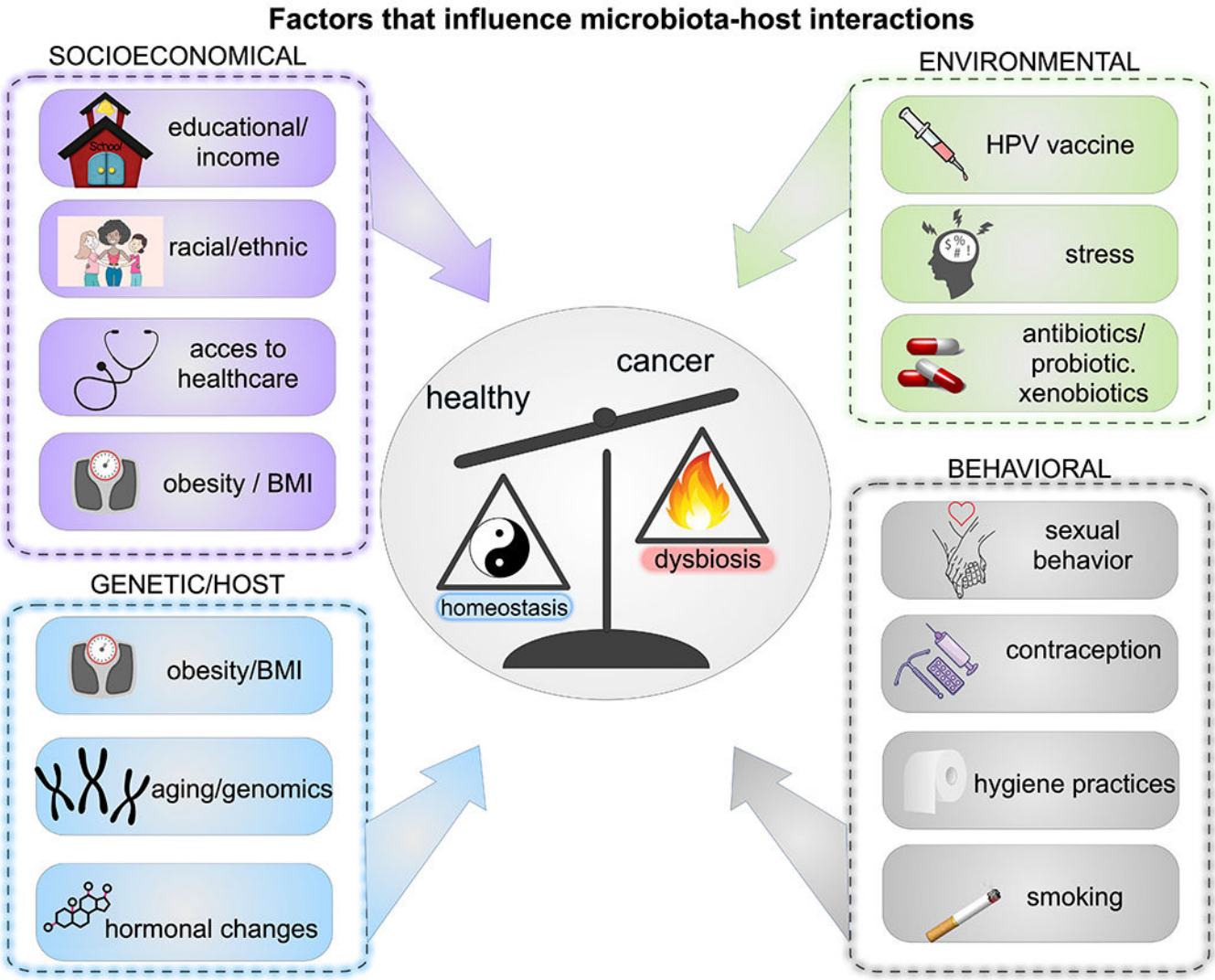

Fig. 2. Behavioural, socioeconomic, genetic and environmental factors contributing to genital dysbiosis and cancer.

Complex interactions between the microbiome and host that increase the risk of gynaecological cancer can be influenced by behavioural factors (sexual orientation, sexual activity, number of sexual partners, use of lubricants and sex toys, contraception, feminine hygiene practices, smoking and vaping, alcohol consumption, diet and nutrition, obesity and physical activity), socioeconomic factors (education, income, structured racism or segregation, social policy and access to health care), genetic and host factors (age, genome and epigenome, hormonal status, pregnancy, altered immunity or other comorbidities (cardiometabolic, neuroendocrine and immuno-inflammatory)) and environmental factors (sexually transmitted infection (STI) status (including bacterial, viral, fungal and parasitic infections), human papilloma virus (HPV) vaccination, stress, antibiotics, probiotics, xenobiotics, toxins, carcinogens, geography and early life factors such as gestation, birth route and infancy).

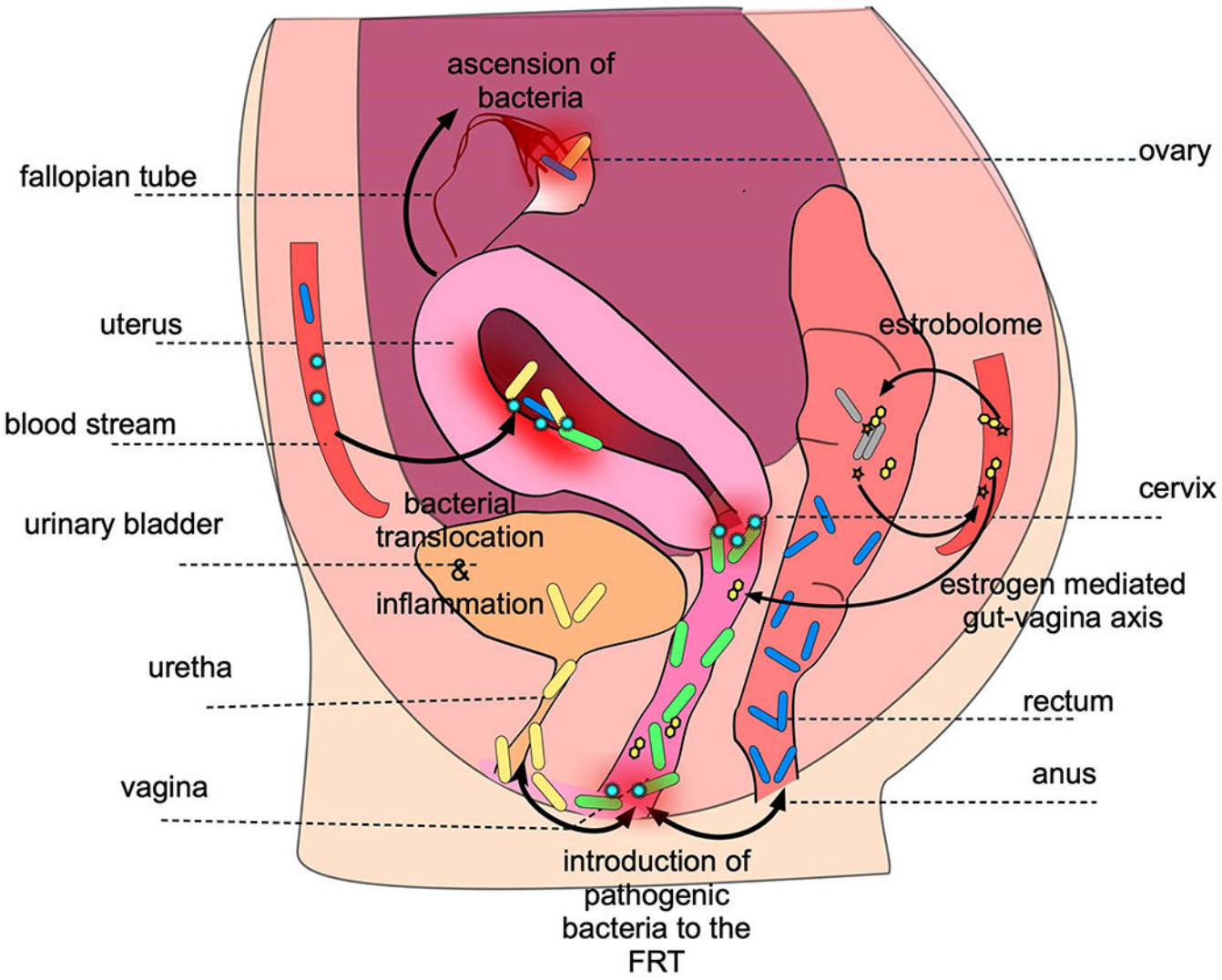

Fig. 3. Female microbiome axes.

The female reproductive tract (FRT) microbiota interacts with the gut (vagina–gut axis) and the urinary tract microbiota (vagina–bladder axis) and possibly other distal mucosal sites (for example, the oral cavity) through direct or oestrogen-mediated mechanisms. The bacteria residing in the lower FRT, including *Lactobacillus* species and dysbiotic anaerobes, can ascend to the upper FRT. Common vaginal bacteria, such as Lactobacillus species, are components of the urinary tract microbiota. Vaginal *Lactobacillus* species can also colonize the rectum. Furthermore, gut microbiota can indirectly influence genital microbiota through the oestrobolome. Finally, haematogenous spread of bacteria (for example, from the oral cavity) might be a putative seeding route for the upper FRT microbiome. Potential extravaginal reservoirs of genital microorganisms are depicted with arrows.

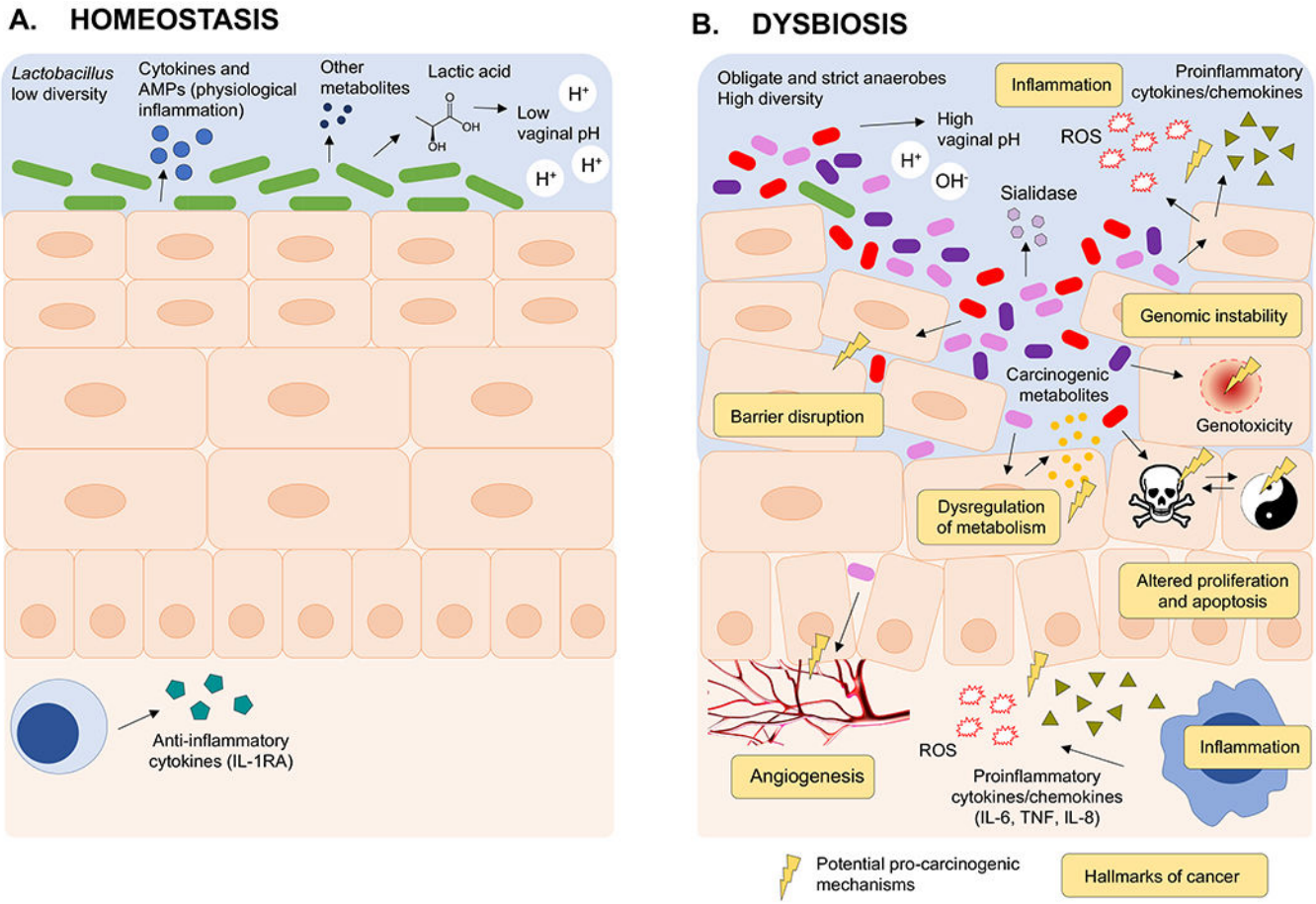

Fig. 4. Effect of microbiota on mucosal homeostasis and hallmarks of cancer.

a) Health-associated *Lactobacillus* spp. have a number of roles in homeostasis of the cervicovaginal microenvironment, including anti-inflammatory properties and improving barrier function. *Lactobacillus* species produce lactic acid, which acidifies the local microenvironment to pH <4.5 and protects the host from invading pathogens with a physiological level of inflammation. In addition, metabolites produced by *Lactobacillus* species can stimulate the host to produce antimicrobial peptides and anti-inflammatory cytokines. **b)** Dysbiotic genital bacteria might affect hallmarks of cancer, including chronic inflammation, barrier disruption, genomic instability, altered proliferation and/or apoptosis and angiogenesis. When dysbiosis occurs, *Lactobacillus* species are replaced with a diverse mixture of anaerobic bacteria (such as *Anaerococcus*, *Atopobium*, *Dialister*, *Fusobacterium*, *Gardnerella*, *Gemella*, *Prevotella*, *Megasphaera*, *Parvimonas*, *Peptoniphilus*, *Peptostreptococcus*, *Porphyromonas*, *Shuttleworthia* and *Sneathia*). These microorganisms induce the production of proinflammatory immune mediators and reactive oxygen species (ROS). Oxidative damage by ROS can exhibit genotoxic effects on epithelial cells or alter the proliferation of epithelial cells, which can consequently lead to cell apoptosis. Putative microbial products or metabolites might also directly affect cell proliferation and cause barrier disruption. Bacterial enzymes (for example, sialidase) can degrade the protective mucous layer. Finally, the vaginal bacteria might affect angiogenesis, for example, via stimulation of the Janus kinase (JAK)– signal transducer and activator of transcription

(STAT) pathway and the production of angiogenic factors such as vascular endothelial growth factor. TNF, tumour necrosis factor. AMPs, antimicrobial peptides.

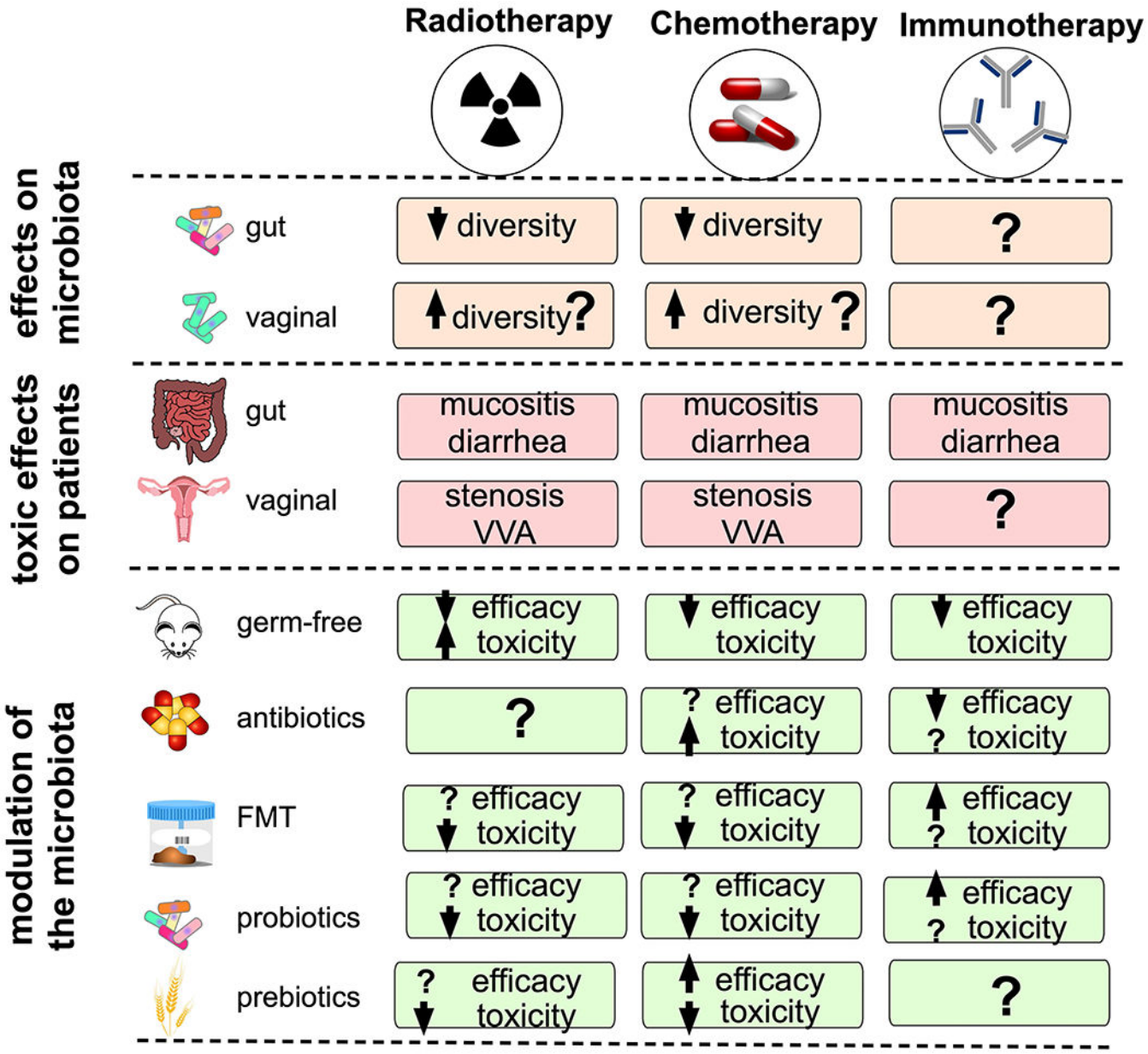

Fig. 5. An overview of microbiota–cancer therapy interactions.

a) Gynaecological cancer therapies (surgery, radiotherapy, chemotherapy and immunotherapy) can affect microbial diversity and composition. The first line of treatment for gynaecological cancers is surgery (for example, hysterectomy); however, data are lacking regarding hysterectomy and modulation of the gut microbiome. The adverse effects of radiation and chemotherapies on gut microbiota are well characterized, whereas the effects of immunotherapy need further characterization. Toxic effects of chemotherapy and radiotherapy on the gut and vaginal ecosystems include diarrhoea or constipation, nausea, vomiting, abdominal pain, gastrointestinal bleeding, mucositis, vaginal stenosis and vulvovaginal atrophy (VVA). **b)** Modulation of the gut microbiota has been shown to alter the toxic effects and efficacy of cancer treatments. Diminishing the gut microbiota via

antibiotics or other inhibitory agents, replenishing the microbiota via faecal microbiota transplantation (FMT) or vaginal microbiota transplantation (VMT) and supplementing the microbiota with prebiotics or probiotics might drive response to cancer therapy.

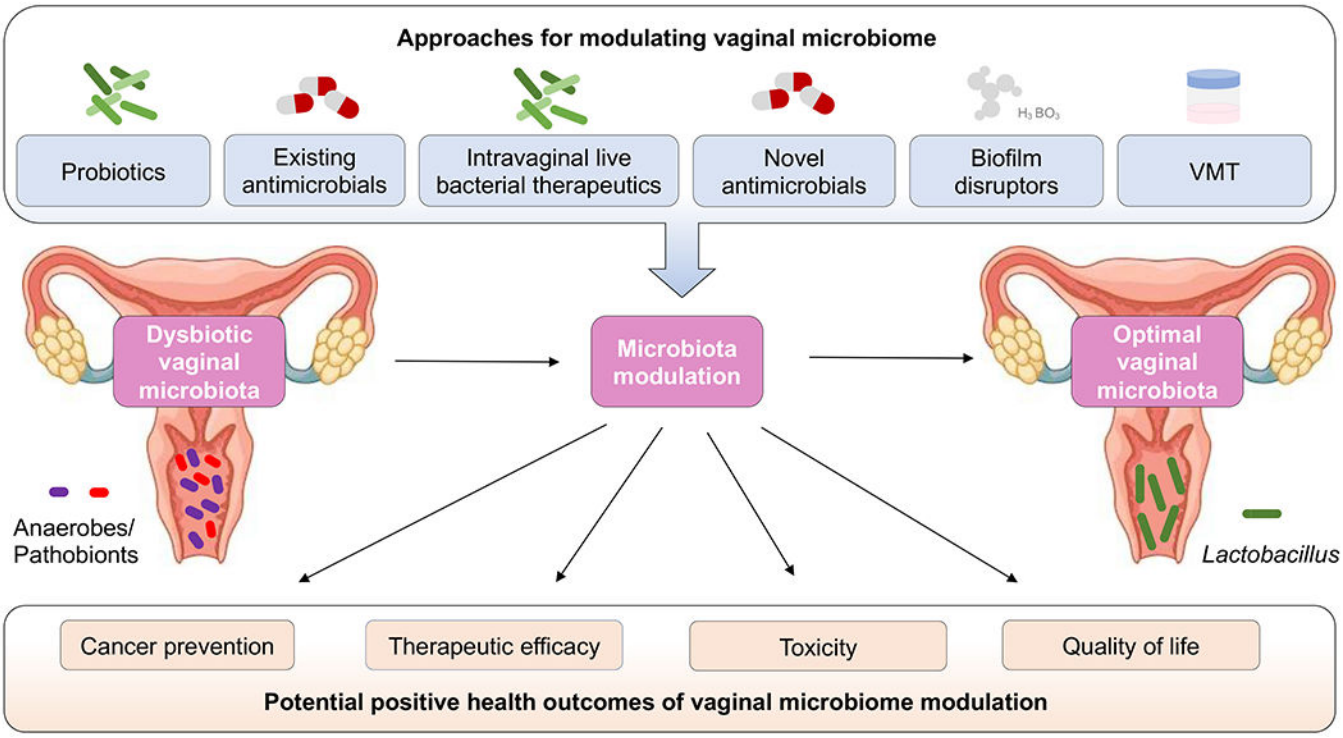

Fig. 6. Novel approaches for modulating vaginal microbiota.

Combining current and experimental protocols for modulating the vaginal microbiota from dysbiotic to optimal *Lactobacillus*-dominant community state could affect carcinogenesis, therapeutic efficacy, toxicity and quality of life for women. Current and experimental protocols include existing antimicrobials, probiotics, intravaginally delivered vaginal lactobacilli formulations, novel antimicrobials, biofilm disruptors and vaginal microbiota transplantation (VMT).