Platinum Priority – Review – From Lab to Clinic
Editorial by Dick A.W. Janssen on pp. 647–648 of this issue

The Microbiome and Genitourinary Cancer: A Collaborative Review

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Article info

Abstract

Context: The recent discovery of the existence of a human genitourinary microbiome has led to the investigation of its role in mediating the pathogenesis of genitourinary malignancies, including bladder, kidney, and prostate cancers. Furthermore, although it is largely recognized that members of the gastrointestinal microbiota are actively involved in drug metabolism, new studies demonstrate additional roles and the potential necessity of the gastrointestinal microbiota in dictating cancer treatment response.
Objective: To summarize the current evidence of a mechanistic role for the genitourinary and gastrointestinal microbiome in genitourinary cancer initiation and treatment response.
Evidence acquisition: We conducted a literature search up to October 2018. Search terms included microbiome, microbiota, urinary microbiome, bladder cancer, urothelial carcinoma, renal cell carcinoma, kidney cancer, testicular cancer, and prostate cancer.
Evidence synthesis: There is preliminary evidence to implicate the members of the genitourinary microbiota as causative factors or cofactors in genitourinary malignancy. Likewise, the current evidence for gastrointestinal microbes in dictating cancer treatment response is mainly correlative; however, we provide examples where therapeutic agents used for the treatment of genitourinary cancers are affected by the human-associated microbiota, or vice versa. Clinical trials, such as fecal microbiota transplant to increase the efficacy of immunotherapy, are currently underway.
Conclusions: The role of the microbiome in genitourinary cancer is an emerging field that merits further studies. Translating microbiome research into clinical action will require incorporation of microbiome surveillance into ongoing and future clinical trials as well as expansion of studies to include metagenomic sequencing and metabolomics.
Patient summary: This review covers recent evidence that microbial populations that reside in the genitourinary tract—and were previously not known to exist—may influence the development of genitourinary malignancies including bladder, kidney, and prostate cancers. Furthermore, microbial populations that exist at sites outside of the genitourinary tract, such as those that reside in our gut, may influence cancer development and/or treatment response.

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1. Introduction

1.1. The human microbiome—what and where are the microbes?

We are roughly 10% human. The number of microbes that inhabit our bodies greatly outnumbers the human cells, and these commensal relationships exist at all body sites that are exposed to the outside world, including the gastrointestinal tract, skin, oral cavity, and genitourinary tract. Each body site that harbors microbial ecosystems differs in microbial composition as well as metabolic functions [1,2]. Each person's microbiome is unique, rapidly developing throughout early childhood and then with differing rates of variability in adulthood [2,3]. Variations in microbial composition can be influenced by both genetic and environmental factors, including diet, geographic location, toxin/carcinogen exposure, and hormones.

The recent discovery of microbial populations that reside in the urinary tract, termed the "urinary microbiome," is of particular relevance to genitourinary cancers. Whereas urine was traditionally thought to be sterile, recent evidence has challenged this dogma with studies showing both culture- and molecular-based detection of microbes in the urinary tract and the bladder [4–7]. As the majority of these studies have been performed with clean catch and/or catheterized urine, the lingering questions are how much of the urinary tract is colonized and which species are coming from where. Urine samples may contain microbes (or microbial fragments and DNA) that originated from the urethra, bladder, ureter, and/or kidney [8]. Clean catch urine samples may also be contaminated by microbes residing in or on the vagina or on the penis. *In situ* localization of bacteria at any of these anatomic sites is limited. In addition, most studies have focused exclusively on bacteria, with the contribution of other microbial populations such as fungus and viruses to the urinary microbiome yet to be elucidated.

The urinary microbiome may change with aging, as age-specific genera are reported in the urinary microbiome community structure [9]. It is not known if these age-related changes in the urinary microbiota contribute to the risk of genitourinary cancer later in life. Sex-related disparities have been better studied, and distinct differences in the composition of the urinary microbiome by sex have been identified. The female urinary microbiota is largely predominated by the genera *Lactobacillus* and *Gardnerella*, whereas *Corynebacterium*, *Staphylococcus*, and *Streptococcus* are the majority genera of the urinary microbiota in males [10,11]. As a result, it may be postulated that the sex disparity in the commensal genitourinary flora might contribute to the well-known sex disparity of genitourinary cancer incidence in men.

This review summarizes the current evidence of a mechanistic role for the genitourinary and gastrointestinal microbiome in genitourinary cancer initiation and treatment response.

2. Evidence acquisition

We conducted a literature search for research studies and review articles related to the microbiome and genitourinary cancers (bladder, kidney, and prostate) up to October 2018. Our search included peer-reviewed publications available through PubMed as well as preprints available through *bioRxiv*. Keywords in our search included microbiome, microbiota, urinary microbiome, bladder cancer, urothelial carcinoma (UC), renal cell carcinoma (RCC), kidney cancer, testicular cancer, and prostate cancer. We did not find reported evidence linking the urinary or gastrointestinal microbiome to testicular cancer risk or biology; therefore, testicular cancer is not covered in this review.

3. Evidence synthesis

3.1. Mechanisms by which the microbiome impacts human cancer etiology/pathology

3.1.1. Infections and the urinary microbiome in genitourinary cancer etiology/pathology

Pathogenic infections are not synonymous with the presence of a "microbiome," as the latter implies a commensal relationship between microbes and human cells. However, a history of genitourinary tract infections is a risk factor for the development of certain malignancies, such as squamous cell bladder cancer following parasitic infection [12,13]. Many infectious agents elicit a chronic inflammatory response upon resolution of the acute infection, and this chronic inflammatory response may be a cofactor in driving carcinogenesis [12,14,15]. Certain commensal strains of bacteria in the urinary microbiome may control pathogenic outgrowth of bacteria in the genitourinary tract similar to the beneficial effect of vaginal *Lactobacillus* species in controlling vaginal infections in women [16]. Thus, further study of the genitourinary microbiome is warranted to fully elucidate its role in regulating pathogenic infections and mediating cancer development.

3.1.2. Bladder cancer

The association between bladder schistosomiasis infections, inflammation, and bladder cancer risk has been well established [12]. The archetypical example of this is in the areas of the world where schistosomiasis is endemic. There is a long-recognized association of chronic *Schistosoma haematobium* bladder infection and the subsequent development of bladder squamous cell carcinoma [17]. Interestingly, there is evidence that members of the urinary microbiome may influence schistosomiasis-induced bladder cancer. Specifically, Adebayo and colleagues [18] studied urine samples from 70 participants from southwestern Nigeria and reported that several urinary taxa such as *Fusobacterium*, *Sphingobacterium*, and *Enterococcus* distinguished urogenital schistosomiasis-induced bladder pathologies from urogenital schistosomiasis infection alone and from healthy persons. Strains of bacteria that can

mediate the formation of N-nitrosamines have been proposed to contribute to schistosomiasis-induced bladder cancer as well (Fig. 1) [13]. Finally, given that epidemiological evidence is inconsistent, the idea that chronic urinary tract infections (UTIs) may be a proposed risk factor for bladder cancer development deserves additional investigation [19].

Whether the urinary microbiome influences the development or progression of bladder cancer or alternatively whether bladder cancer impacts the composition, diversity, or abundance of bladder-associated microorganisms remains to be determined. One hypothesis is that the bladder microbiome may alter the extracellular matrix, which may promote or inhibit urothelial carcinogenesis [20]. Likewise, as the intestinal microbiome has been shown to influence the development of cancer and the response to therapeutics [21,22], it might not be unexpected to find similar links in the context of the urinary microbiome and bladder cancer. Preliminary studies have identified differences in the bladder urinary microbiome of UC patients compared with healthy cancer-free individuals, with *Streptococcus*, *Pseudomonas*, or *Anaerococcus* genera appearing more frequently in UC patients [23]. In another small series, no differences in microbial alpha diversity (interindividual diversity) or composition of the urinary microbiota were observed in 12 patients with bladder cancer and 11 age- and sex-matched healthy donors [24]. The only differences found were in the relative abundance of specific taxa between bladder cancer patients and healthy donors,

in which a subset of cancer patients had an enrichment of taxa belonging to the genera *Fusobacterium*, *Actinobaculum*, *Facklamia*, and *Campylobacter*, whereas *Veillonella*, *Streptococcus*, and *Corynebacterium* species were more prevalent in healthy human urine [24]. These findings are at odds with other studies of gastrointestinal microbiota that have reported a decrease in diversity with disease state in the gut [25].

Another study, in which urine samples of 31 bladder cancer patients and 18 non-neoplastic patients were sequenced, determined that bacterial richness was increased in cancer patients compared with the control group, whereas diversity was not [26]. The relative abundance of organisms at taxonomic levels from phylum to genus was not different between bladder cancer patients and the control group, with the exception of the families of *Thermoactinomycetaceae* and *Sphingobacteriaceae*, which were increased in cancer patients or control patients, respectively [26]. Additional significant differences were observed in the abundance of several organisms at the genus level using linear discriminant analysis effect size, an analysis tool based on relative abundance between two or more populations, which in this case were those facilitating identification of microbial populations associated with cancer [26]. Of note, bacterial richness was increased in groups classified at a higher risk of recurrence or progression [26], suggesting that microbial composition may provide clues to patient outcome. Nevertheless, studies involving larger numbers of patients, with various stages of
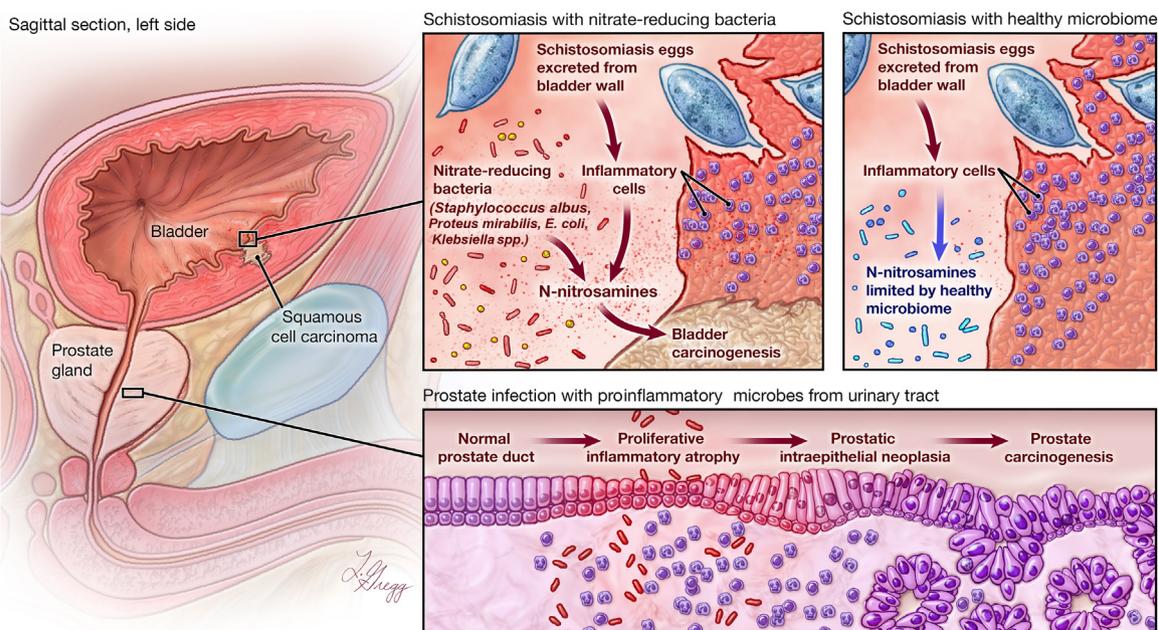
Fig. 1 – Examples of potential direct interactions between the urinary microbiota and the development of genitourinary cancer. There is a long-recognized association between chronic bladder infection with *S. haematobium* and the subsequent development of bladder squamous cell carcinoma in both men and women. However, not everyone afflicted with schistosomiasis develops bladder cancer. The presence of species of bacteria such as *Staphylococcus albus*, *Proteus mirabilis*, *Escherichia coli*, and *Klebsiella* spp. that can mediate the formation of carcinogenic N-nitrosamines as part of the urinary microbiota may contribute to schistosomiasis-induced bladder cancer. Likewise, in prostate cancer, infection of the prostate with proinflammatory bacteria originating from the urinary tract is proposed to contribute to the development of a prostate cancer risk factor lesion termed proliferative inflammatory atrophy.
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disease, will be needed to properly evaluate whether urinary microbiota differences exist between bladder cancer patients and healthy individuals. Indeed, as our understanding of molecular subtypes in bladder cancer deepens [27], it is conceivable that correlations in microbiome composition or diversity and specific disease manifestation may arise.

Notably, all the bladder cancer patients enrolled in the studies of Popović et al. [24] and Wu et al. [26] were male. However, as the composition of the urinary microbiome has been shown to differ between men and women, it may be that the microbiome in women—in particular the composition, diversity, or abundance of microorganisms—provides a level of protection against or facilitates the development of bladder cancer. As such, further studies in female bladder cancer patients and healthy controls are needed as well.

3.1.3. Kidney cancer

The epidemiology of RCC has been extensively studied and described [28]. However, the contribution of prior UTIs to the incidence of RCC is of unclear significance. UTIs have been described as a modifiable risk factor for the development of RCC [14]. In one retrospective study, a self-reported history of a physician-diagnosed kidney or bladder infection conferred a significantly higher risk of RCC (odds ratio 1.9 [95% confidence interval: 1.5–2.5]) compared with no prior infection [29]. This risk was further increased in males (vs females) and smokers (vs nonsmokers) [29]. Moreover, the recent discovery of a distinct microbiome of the urinary tract [4] sheds new light on the possibility that there is an underappreciated degree of direct interaction between microbiota and the kidney, although this interaction has not yet been studied in relation to RCC pathogenesis. As the technology to identify specific bacteria on a species level is now available, this may prompt further investigation of the urine microbiome with respect to RCC incidence.

3.1.4. Prostate cancer

Although an infectious etiology to prostate cancer has long been discussed, the evidence for such a potential causal relationship has been conflicting [15,30]. For example, while several bacterial species are known to infect the prostate and cause acute and/or chronic bacterial prostatitis, studies to identify prostatitis as a risk factor for prostate cancer development are contradictory and may be flawed by potential biases such as detection bias (reviewed by Sfanos et al. [15]). Likewise, epidemiological studies on single infectious agents such as those associated with sexually transmitted infections (STIs) are inconclusive, and almost all have focused on one infectious agent or a few specific infectious agents and typically on known pathogens. It has been proposed that one potential reason why it has been so difficult to settle on a single infectious origin for prostate cancer development is that there may be multiple different types of microorganisms that contribute to prostate cancer risk via eliciting persistent chronic inflammation [15]. In support of this concept, Shrestha et al. [11] reported that the urinary microbiome of men with biopsy-proven prostate cancer may be enriched for proinflamma-

tory bacterial species that are associated with genitourinary disease such as prostatitis, UTIs, bacterial vaginosis, and STIs. The composition and prevalence of each of these species differed among individuals, and several were associated with the degree of acute or chronic inflammation in the prostate biopsy [11]. A potential hypothesis is that these proinflammatory species, if introduced into the prostate, could elicit chronic inflammation that mediates the development of proliferative inflammatory atrophy, a suspected risk factor lesion for prostate cancer development (Fig. 1) [15]. One major limitation to this study is that it was not longitudinal, and therefore it was hard to determine a causative role of the species versus their presence as a consequence of prostate cancer.

Extensive studies have also been performed to detect pathogens in prostate cancer tissues, as was recently reviewed by Porter et al. [31]. Although microbial DNA is detectable in prostate cancer tissues [32–34], similar to studies of the urinary microbiome, few studies have visualized or localized the detected bacteria in the respective tissue samples from which they were identified. It is unlikely that the prostate contains a ubiquitous commensal microbiota or that there is a "prostate microbiome" per se (reviewed by Porter et al. [31]), but instead microbial presence is likely pathogenic in nature and may present in focal regions, and perhaps in regions associated with foci of acute or chronic inflammation or "fossilized" in prostatic corpora amylacea that are thought to be remnants of past infections [32,35]. Thus, in the absence of epidemiological studies linking infections to prostate cancer risk or studies that have visualized bacteria or other microorganisms in prostate tissues, the contributions of prostate infections to prostate cancer risk remains unknown.

3.1.5. Impact of the microbiome on genitourinary cancer treatment

Many members of the gastrointestinal microbiota are known to influence the metabolism, pharmacokinetics, and toxicity of drugs and xenobiotics (Fig. 2) [36]. Specifically with regard to cancer therapies, examples of this relationship include the finding that intratumoral *Mycoplasma hyorhinis*, as well as species of *Proteobacteria*, may metabolize and inactivate the chemotherapy drug gemcitabine, resulting in drug resistance [37]. Likewise, reactivation of the excreted, inactive metabolite of the topoisomerase I inhibitor irinotecan can occur with β-glucuronidases produced by bacterial species in the gastrointestinal tract, causing adverse drug toxicities, including severe diarrhea [38]. Finally, although the mechanism is not fully understood, there is emerging evidence that the gastrointestinal microbiota can influence the efficacy of immunotherapy [39–45].

There is also a reciprocal relationship between cancer therapies and the gastrointestinal microbiome, whereby cancer therapies may alter the gastrointestinal microbial composition (see Table 1). Such alterations could conceivably impact factors such as the local inflammatory environment in the intestinal tract, systemic inflammatory effects, and/or the efficacy of any subsequently adminis-
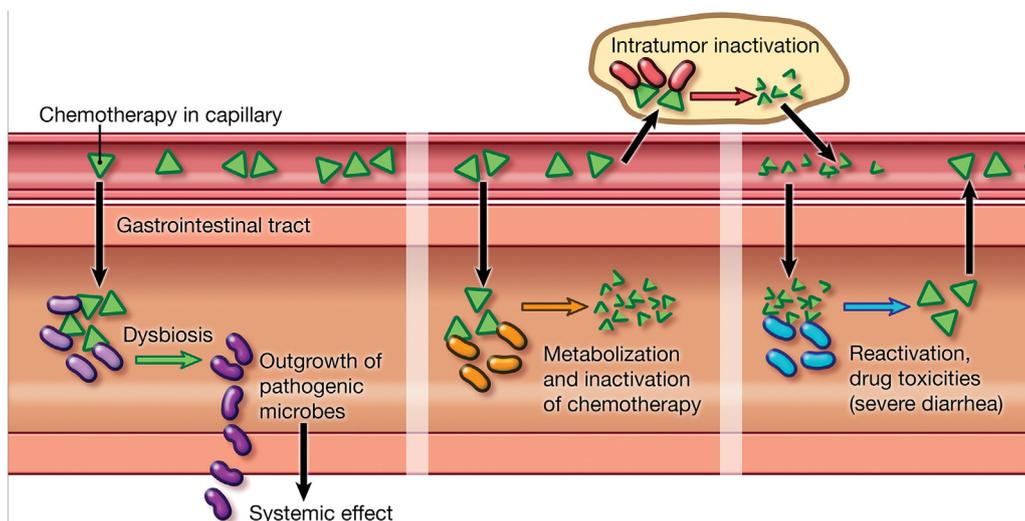
Fig. 2 – Examples of interactions between the gastrointestinal (GI) microbiota and cancer therapies. Cancer therapies, such as chemotherapy, can have a bacteriostatic effect on the GI microbiota, leading to GI microbial dysbiosis and the outgrowth of pathogenic species. This pathogenic outgrowth can lead to systemic effects, such as the production of proinflammatory cytokines that influence tumor growth. The GI microbiota is likewise implicated in the metabolism and inactivation of cancer therapies both in the GI tract and locally within the tumor if there is a bacterial presence. Finally, reactivation of therapies by the GI microbiota locally within the GI tract can lead to treatment-related drug toxicities, including severe diarrhea. Copyright 2018, *Johns Hopkins University*, by Lydia Gregg.

tered cancer therapies. A screen of >1000 marketed nonantibiotic drugs against 40 representative gastrointestinal bacterial strains found that nearly a quarter of the drugs with human targets exhibited bacteriostatic properties [46]. Chemotherapy and immunotherapy induce dysbiosis of the gastrointestinal microbiota in rodent models [41,47], and bone marrow transplant conditioning chemotherapy in non-Hodgkin's lymphoma patients induces pathogenic shifts in the gastrointestinal microbiota that are associated with treatment toxicities [48,49]. Finally, chemotherapy given to pediatric acute myeloid leukemia

patients demonstrated direct bacteriostatic effects on the gastrointestinal microbiota, as well as outgrowth of pathogenic enterococci that could not be explained fully by concurrent use of antibiotics [50].

Indirect effects of the microbiome on tumorigenesis are not well understood, and could include modulation of antitumor immune responses and effects on host metabolism [51]. Exciting evidence is emerging to indicate that the gastrointestinal microbiota can exert a profound influence on the efficacy of certain cancer treatments, including chemotherapy and immunotherapy [39–42,44,45]. Recent

Table 1 – Examples of interactions between human-associated bacteria and therapeutic agents used in the treatment of genitourinary cancers

Therapeutic agents altered by bacteria	Therapeutic influence	Reference(s)
Gemcitabine	Efficacy decreased by select bacteria	PMID: 26416623, PMID: 28912244
Doxorubicin	Efficacy decreased by select bacteria	PMID: 26416623
Etoposide phosphate	Efficacy decreased by select bacteria	PMID: 26416623
Mitoxantrone	Efficacy decreased by select bacteria	PMID: 26416623
Floxuridine	Efficacy increased by select bacteria	PMID: 28431244
α-PD-1 immunotherapy	Efficacy increased by select bacteria	PMID: 29097493, PMID: 29302014, PMID: 29097494

Therapeutic agents that inhibit bacteria	Relationship	Reference(s)
Gemcitabine	Bacteriostatic	PMID: 29555994, PMID: 27575730
Methotrexate	Bacteriostatic	PMID: 29555994, PMID: 6704313
Doxorubicin hydrochloride	Bacteriostatic	PMID: 29555994, PMID: 27575730
Paclitaxel	Bacteriostatic	PMID: 29555994
5-Fluorouracil	Bacteriostatic	PMID: 29555994, PMID: 2416271, PMID: 6704313, PMID: 6704313, PMID: 27575730
Gefitinib	Bacteriostatic	PMID: 29555994
Etoposide	Bacteriostatic	PMID: 29555994, PMID: 2416271, PMID: 3196009
Floxuridine	Bacteriostatic	PMID: 29555994, PMID: 27025633
Cisplatin	Bacteriostatic, cytotoxic	PMID: 14287410, PMID: 8637503
Carboplatin	Cytotoxic	PMID: 8637503
Mitomycin	Bacteriostatic	PMID: 2416271, PMID: 6704313

studies in animal models have demonstrated that members of the intestinal microbiota are essential for therapeutic efficacy of agents such as cyclophosphamide [41], platinum chemotherapy (oxaliplatin) [40], and both CTLA-4 blockade [39] and anti-PD-L1 [42] immunotherapies. In these studies, eradication of the commensal intestinal microbiota by antibiotic treatment or via deriving specific pathogen-free and/or germ-free animals eliminated the therapeutic efficacy of these agents in multiple different tumor models. Intriguingly, a study using a melanoma model showed that anti-PD-L1 immunotherapy could be bolstered by feeding animals a strain of *Bifidobacterium*—a species commonly used in probiotic supplements—prior to initiating therapy [42].

In addition, three separate recent studies, including one in patients with RCC, demonstrated that transplanting fecal material from patients who responded to immune check-point inhibitors into germ-free mice recapitulated the phenotype of anti-PD-1 response in the animal model [43–45]. This response was attenuated with antibiotics, suggesting an influence of the microbiome—in the gut or at other sites—on the response to anti-PD-1 treatment. Furthermore, Routy et al. [44] studied a cohort of patients that included 67 patients with RCC and 42 patients with UC, in addition to 140 patients with advanced non-small cell lung cancer (NSCLC), 28% of whom were prescribed antibiotics (β-lactam ± inhibitors, fluoroquinolones, or macrolides) within 2 mo before or 1 mo after the first administration of anti-PD-1/PD-L1 immunotherapy. These investigators reported that progression-free survival (PFS) and overall survival (OS) were significantly shorter in the antibiotic-treated group. Antibiotic treatment predicted resistance to anti-PD-1 immunotherapy in univariate and multivariate Cox regression analyses, independently of classical prognostic markers in both NSCLC and RCC. This study was recently expanded to include additional RCC patients and fully reanalyzed for multivariate analysis. Antibiotics use within 1 mo before the first dose of anti-PD-L1 antibody was associated with shorter PFS and shorter OS in patients with advanced RCC and NSCLC, even after multivariate analyses adjusted for known risk factors in each tumor type [52].

With regard to the specific gastrointestinal species that might mediate the relationship between immunotherapy and treatment response, Routy et al. [44] identified *Akkermansia muciniphila* as a relevant bacterium. This species restored efficacy of PD-l blockade when provided as a probiotic supplement to murine models where immunotherapy efficacy had been abrogated via antibiotic treatment. In a similar study, the composition of the gut microbiome was examined in melanoma patients, with the finding that gastrointestinal alpha diversity and relative abundance of *Ruminococcaceae* species correlated with clinical responses to checkpoint inhibition [45].

In addition to immunotherapy, other advanced RCC treatments, such as oral anti-vascular endothelial growth factor (anti-VEGF) therapy, may be influenced by the gastrointestinal microbiota. A common toxicity of anti-VEGF therapy is diarrhea, which can be dose limiting. In a pilot study of RCC patients on anti-VEGF therapy, stool microbiota profiling studies identified high levels of *Bacteroides* spp. and low levels of *Prevotella* spp. in patients experiencing diarrhea [53]. Interestingly, in patients with poor-risk RCC, the use of antibiotics with *Bacteroides* spp. coverage resulted in improved PFS, suggesting that alterations of the gut microbiome can impact outcome following non-immune-based treatments [54]. With multiple clinical trials investigating the effect of the combination of anti-VEGF and immunotherapies, understanding how the microbiome can be altered and how it may influence treatment responses is of paramount importance.

Prostate cancer is the least mature in terms of data assessing the potential impact of the gastrointestinal microbiome on cancer treatment response. Sfanos et al. [55] recently profiled the fecal microbiota in a cross-sectional study of patients that included healthy male volunteers and men with different clinical states of prostate cancer (ie, localized, biochemically recurrent, and meta-static disease). They reported compositional differences in the gastrointestinal microbiota of men taking oral androgen receptor axis-targeted therapies (such as enzalutamide and abiraterone acetate), including a greater abundance of species previously linked to the response to anti-PD-1 immunotherapy, such as *A. muciniphila* and *Ruminococcaceae* spp. Of interest, these authors reported an enriched representation of bacteria predicted to contain gene pathways involved in steroid hormone biosynthesis in the fecal microbiota of men taking oral androgen receptor axis-targeted therapies. This finding led to the speculation that alterations to the gastrointestinal microbiome driven by androgen axis-targeted therapies might represent a mechanism for potential alternative pathways of production of steroid metabolites that could, in turn, influence treatment response to these drugs [55].

3.2. Translating microbiome research to clinical action and relevance

Not only can microbes be present within cancer tissue, they also densely populate the intestinal tract where we deliver many therapeutic drugs for host absorption. The presence of bacteria at both oncological and drug delivery sites should drive us to have a better understanding of their broad functional roles in disease, diagnosis, and therapy (Table 2).

3.2.1. The microbiome and screening/risk identification
The etiological role of microbiota in some cancers has been determined, such as malignancies in the stomach and colon, and as discussed above, similar interactions are starting to be uncovered in the urinary and reproductive systems [26,31,56,57]. Although the ability to relate specific microbes with tumor pathogenesis and treatment response will be crucial, challenges remain as microorganism composition depends upon how biological fluids such as urine are collected. Indeed, urine may have microorganisms originating from various sites in the urinary (urethra, bladder, and kidneys) and reproductive systems (vagina and prostate). Even microbes from urine obtained directly from the bladder may not be entirely representative of the focal

Table 2 – Proposed mechanisms of interaction between the microbiome and genitourinary cancer

Direct Interactions

Urinary microbiome

Control of pathogen outgrowth in the urinary tract

Cofactor for cancer development (eg, inciting chronic inflammation, production of N-nitrosamines and schistosomiasis, etc.)

Intratumoral cancer drug metabolism

Intratumoral cancer drug efficacy (eg, competition for fibronectin binding with BCG therapy)

Indirect Interactions

Gastrointestinal microbiome

Cancer drug metabolism

Cancer drug efficacy (eg, dictating response to immunotherapy)

Xenobiotic metabolism (dietary carcinogens, hormones, pro- or anti-inflammatory compounds, etc.)

Control of systemic immunity (chronic inflammation, immunotherapy response, etc.)

BCG = Bacillus Calmette-Guerin.

site where the tumor may be present unless a considerable field change has occurred [8]. While there are technical hurdles to overcome to evaluate this technology for "liquid biopsy," noninvasive diagnostic and prognostic tools would be of great clinical value.

Utilizing the urinary microbiome to predict cancer risk and response to treatment is an intriguing possibility with technological advancements. Bacterial 16S rDNA gene sequencing in particular has advanced our understanding of the compositional characterization of the bacterial populations occupying urinary and reproductive sites. However, future broad metagenomic sequencing and other nucleic acid technologies, coupled with metabolomic studies examining the small molecules produced through metabolism may provide extensive additional information about the functional abilities of these microbes, and might in time yield useful biomarkers, both prognostic and predictive. For instance, Liss et al. [57] discovered 10 gastrointestinal microbiome-derived metabolic pathways that were found to be differentially present in men with prostate cancer versus those without (ie, folate, arginine) in addition to enrichments in bacterial species. Likewise, multiple studies have examined metabolomic biomarkers in the urine in relation to genitourinary cancer risk [58–61], and future studies linking the presence of these metabolites to the urinary microbiome structure are warranted. Ultimately, it may be that specific metabolites, rather than specific species of microbes, drive pathological changes, tumor development, or therapy response.

3.2.2. Therapeutic possibilities

If microorganisms are responsible for cancer initiation and/or progression, or if they impact cancer treatment, then we need to consider how to modulate the microbiota for potential benefit in the urinary tract. Researchers in the "pre-microbiome" era understood this concept, which led them to use probiotic bacteria such as the intestinal *Lactobacillus casei* Shirota strain, among others, to potentially reduce recurrence of bladder cancer. Nitrosamine-induced bladder carcinogenesis was inhibited in animal

models [62–68]. Probiotics prevented secondary tumor growth and modulated cytokine production, and lower grades of disease were observed in the probiotic-treated animals. This naturally led to studies in humans where results initially looked promising, but confounding factors such as high discontinuation rates and study design have stalled further clinical investment [69–73]. With the invigoration of the microbiome field and new tools for its investigation, these lactobacilli-based studies should be reinvestigated.

Perhaps the most exciting area at present time is how the microbiome, in both the urinary system and the intestinal tract, may alter drug therapies and influence urological oncological outcomes (see Tables 1 and 2, and Fig. 2). For example, Bacillus Calmette-Guerin (BCG), the *Mycobacterium bovis*-derived vaccine strain for tuberculosis, is widely used to prevent recurrence of bladder cancer by direct bladder instillation. BCG induces an immune response, in part through the potential binding of fibronectin in the bladder, which increases in intensity over the treatment period to induce tumor-specific immunity [74]. However, we now understand that commensal microorganisms are also present in the bladder and may potentially interact with BCG, influencing the development of immunity to bladder cancer. At other mucosal sites, the microbiome plays a role in barrier homeostasis and moderates inflammatory events induced by potentially opportunistic microorganisms and other insults. Different species of *Lactobacillus* have various abilities to bind fibronectin [75,76]. McMillan et al. [77] demonstrated that urinary-specific lactobacilli, such as *Lactobacillus iners*, are potent binders of fibronectin, and this species is also present in the bladder [78]. This finding may have consequences for BCG therapy if BCG is competing for fibronectin-binding positions. Therefore, it is imperative that we assess the microbiome at the target site, before, during, and following therapy, and with subsequent outcome following BCG therapy in future clinical trials.

The microbiome may also have direct influences on treatments used for urological cancers. For example, abiraterone acetate, an androgen deprivation (CYP17A inhibitor) agent used for the treatment of advanced prostate cancer, is poorly absorbed and spends considerable time in the intestinal tract. Abiraterone and other agents may potentially undergo xenobiotic metabolism and also alter the microbiome, subsequently affecting the drug's biological availability and activity [55]. In addition, the microbiome may influence systemic treatments that never actually come into direct contact with it, such as chemotherapy or immunotherapy. One of the most rapidly emerging areas is that of the role of the intestinal microbiome on a patient's response to immunotherapy, especially to immune checkpoint inhibitors. These agents potentially have application in prostate cancer and are approved for use in RCC and bladder cancer. We are rapidly becoming aware that the host's microbial factors may significantly influence the outcome of these agents, as observed for other cancers as described in previous sections. Notably, as mentioned above, antibiotic treatment of patients with RCC, NSCLC,

and UC for common indications (dental, pulmonary, and urinary infections) significantly reduced the PFS and OS of immunotherapy-treated patients [44]. Therefore, it may be possible to alter the immune response by modifying the patient's microbiome using fecal microbiota transplant (FMT) for a beneficial immunotherapeutic outcome. This concept is currently being tested in clinical studies (NCT03341143 and NCT03353402), with additional studies likely following. FMT is not the only way to alter the microbiome response or composition through the intestinal tract, but it has shown to dramatically shift bacterial populations in patients with severe intestinal infectious disease such as *Clostridium difficile* infection or ulcerative colitis.

4. Conclusions

We are only now beginning to appreciate the potential influence of the microbiome at sites in the urinary tract on the pathobiology of genitourinary cancer. We have much to learn regarding its composition and function during homeostasis and in disease states. Perhaps with further study, we can develop the means to manipulate the urinary and/or gastrointestinal microbiome to improve patient outcome. Translating microbiome research into clinical practice requires the joint efforts of multidisciplinary teams of microbiome researchers, infectious disease experts, tumor immunologists, and medical oncologists to successfully translate laboratory-based research to relevant patient care.

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