

# The role of gut microbiome in immune modulation in metastatic renal cell carcinoma

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**Abstract:** Treatment of metastatic renal cell carcinomas (mRCC) has drastically improved since the advent of immunotherapy with immune checkpoint inhibitors (ICIs), with a significant proportion of patients achieving durable responses. While this has revolutionized treatment and improved outcomes for mRCC patients, a large subset of patients still does not respond to treatment with ICIs. Moreover, ICIs can induce various immune-related adverse events, limiting their use in many patients. Therefore, there is a need to identify the predictive biomarkers of both efficacy and toxicity associated with ICIs, which would allow for a more personalized approach and help with clinical decision-making. This review aims to explore the role of the gut microbiome in RCC to overcome primary resistance and predict response to treatment with ICIs. First, current therapeutic strategies and mechanisms of action of ICI therapies for RCC treatment will be reviewed. With the technological development of shotgun whole-genome sequencing, the gut microbiome has emerged as an exciting field of research within oncology. Thus, the role of the microbiome and its bidirectional interaction with ICIs and other drugs will be explored, with a particular focus on the microbiome profile in RCC. Lastly, the rationale for future clinical interventions to overcome resistance to ICIs using fecal microbiota transplantation in patients with RCC will be presented.

**Keywords:** immunotherapy, microbiome, predictive biomarker, renal cell carcinoma, tumor microenvironment

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## Introduction

Immunotherapies with immune checkpoint inhibitors (ICIs) have revolutionized the treatment of advanced cancers in the last decade, particularly in melanoma, non-small-cell lung cancer (NSCLC), and renal cell carcinoma (RCC).<sup>1</sup> More recently, ICIs have been used in triple-negative breast cancers, head and neck malignancies, and investigated in new settings such as adjuvant treatment of urothelial carcinoma and RCC.<sup>2,3</sup> RCC accounts for 2–3% of cancers with an annual incidence of 338,000 cases worldwide.<sup>4</sup> At diagnosis, roughly 30% of these patients present with de novo metastatic disease. Additionally, approximately one-third of those who initially receive curative-intent treatment eventually progress to advanced disease.<sup>1</sup> The median overall

survival (mOS) in patients with metastatic disease was about 22 months, prior to the advent of ICIs, at a time when multi-targeted receptor tyrosine kinase inhibitors (TKIs) were the standard of care first-line treatment.<sup>5</sup> However, recent advances in the treatment of metastatic RCC (mRCC) with either combination of ipilimumab and nivolumab or anti-programmed cell death-1 (PD-1) inhibitors plus a TKI agent have significantly improved efficacy, at the cost of risk of immune-related adverse events (irAEs).<sup>6–10</sup> Furthermore, particularly impressive is that a subset of patients seems to obtain durable responses. Although response rates are higher with combination therapies than single-agent TKI, unfortunately, many patients still progress on treatment. For example, the objective response rates (ORRs) in patients with

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mRCC treated with combined immunotherapy with dual ICIs were only 42% in the checkmate 214 trial.<sup>6</sup> This suggests primary resistance occurring in more than half of the patients. Researchers have been exploring ways to overcome primary resistance by several mechanisms, including utilizing combination treatments, identifying potential molecular biomarkers, and modulating the gut microbiome to enhance response to treatment. Current challenges include translating RCC heterogeneity into individualized treatment plans, identifying and utilizing biomarkers that predict survival and/or treatment response, and identifying optimal tools to help guide precision medicine. The landscape of biomarker-driven targeted therapy in RCC is rapidly changing. There are several ongoing clinical trials with the potential to personalize the standard of care treatment for this heterogeneous disease. This review aims to describe an overview of the mechanism of immunotherapy in RCC treatment, describe our current knowledge of the microbiome in cancer, and how it may modulate the immune system. Moreover, given the emerging role of the microbiome in modulating response to ICIs, we discuss the literature around the microbiome and mRCC, specifically highlighting the implications of response to treatment with ICIs.

### Methods

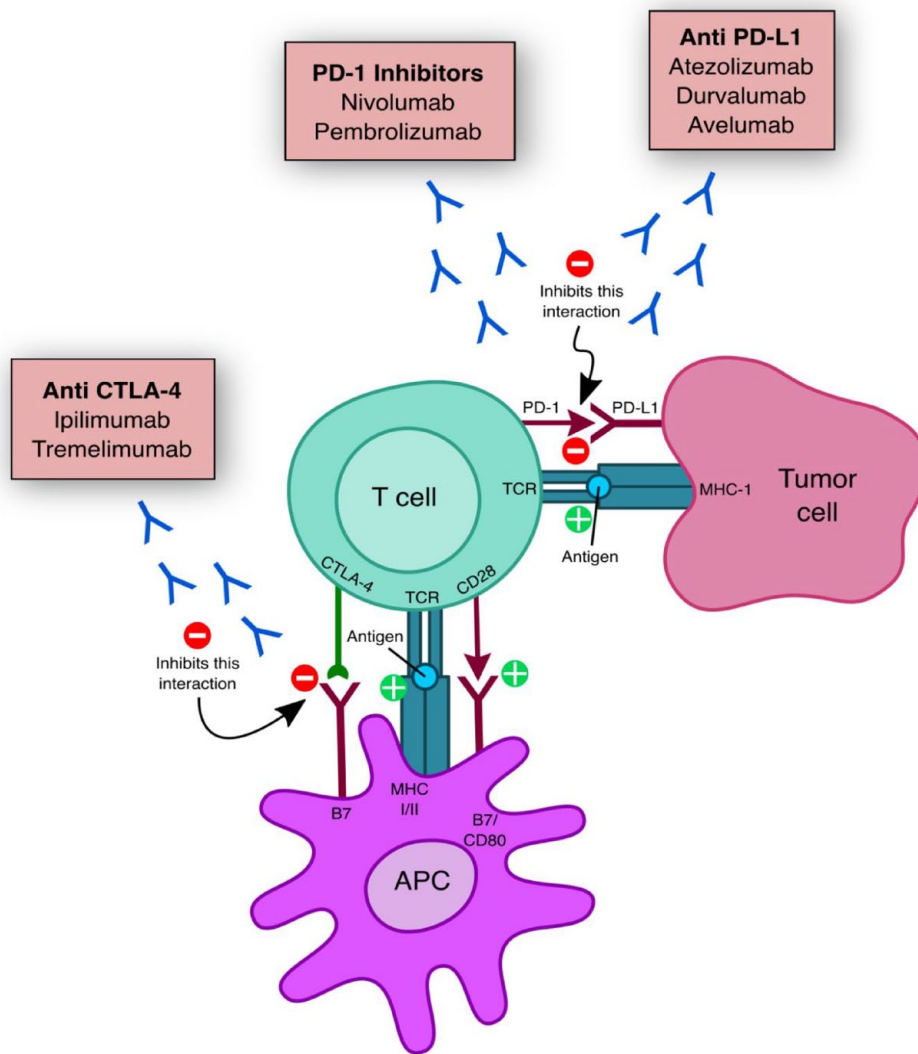
A literature search was conducted on PubMed using the terms ‘advanced/metastatic renal cell carcinoma’ and ‘microbiome’ from January 2000 to July 2021. The same search terms were used for the ClinicalTrials.gov registry of clinical trials. In addition, abstracts from the annual meetings for the American Society of Clinical Oncology and the European Society for Medical Oncology were included. Only English studies were included. Since there were very few primary articles with a specific focus on the microbiome in RCC, articles including characterizations of the microbiome in other solid cancer types were also included for discussion and review articles.

### ICIs in RCC

Immune checkpoints are self-recognition proteins inherent in the host, which function to suppress the immune response to prevent tissue damage to the host in response to inflammation.<sup>11</sup> Examples of these self-recognition proteins include cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and PD-1 and its ligands (PD-L1 and PD-L2),

which help the body evade its immune system to prevent any exaggerated immune response against normal tissues. Figure 1 describes the mechanism of action of these ICIs. CTLA-4 is a receptor homolog to cluster of differentiation (CD) 28 and expressed on T lymphocytes. CTLA-4 binds to B7 receptors (CD80/CD86) expressed on antigen-presenting cells with higher affinity than CD28, thereby inhibiting the activation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, mitigating the T-cell response. Thus, when CTLA-4 is inhibited with therapeutic monoclonal antibodies such as ipilimumab, it activates T cells.<sup>11</sup> PD-1, on the other hand, is a co-signaling receptor part of the B7/CD28 family, expressed on activated T cells, B cells, and natural killer cells, and particularly exhausted T cells. Its ligands PD-L1 and PD-L2 are expressed in T cells, B cells, dendritic cells, macrophages, and mast cells. When bound to its ligand, PD-1 reduces T-cells’ activity and effector functions in peripheral tissues.<sup>11</sup> PD-L1 is often expressed on many tumor cells, resulting in inhibition of T-cell response in the tumor microenvironment (TME), thus inhibiting T-cell-mediated cytokine production and tumor cell killing. Blocking this interaction allows the resumption of T-cell activity in the periphery. When either PD-1 or its ligands are blocked, the result is the enhancement of the immune response. While immune checkpoints have a role in protecting host tissue from an auto-immune response, tumors have exploited these mechanisms as a way to camouflage from the immune system by disguising themselves as ‘self’ by increasing the expression of PD-L1 on their surface to escape immune surveillance.<sup>12</sup>

The treatment landscape of mRCC has been rapidly changing with the advent of immunotherapy-based combinations. The role of immunotherapy as first-line systemic therapy for mRCC has become quite evident with either doublet immunotherapy or in combination with a vascular endothelial growth factor receptor TKI (VEGFR-TKI) based on phase III studies that demonstrated improved survival compared to single-agent VEGFR-TKI (sunitinib).<sup>6,9,10</sup> In patients with international metastatic RCC database consortium intermediate or poor-risk group patients, ipilimumab and nivolumab combined treatment in mRCC led to shrinkage of cancer metastases, with improved ORR of 42% of patients responding compared to 27% in the sunitinib group, leading to improved OS and less grade 3–4 toxicity, particularly in patients whose immunohistochemistry showed PD-L1 expression in more than 1%



**Figure 1.** Mechanism of action of ICIs. CTLA-4 binds to B7, and PD-1 binds to PD-L1. Both of these interactions function to inhibit T-cell activation and immune response. Inhibiting the interaction between these receptors with ICIs allows the T cell to remain in an activated state, thus enhancing the host's immune response.

CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte-associated protein-4; ICIs, immune checkpoint inhibitors; MHC, major histocompatibility complex; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; TCR, T-cell receptor; +, activating interaction; -, inhibitory interaction.

of sampled cells.<sup>6</sup> Notably, 11% of patients achieved a complete response to treatment without any radiographic evidence of disease. However, despite the clinical efficacy, more than half of patients did not seem to benefit from these therapies due to the tumor's primary (innate) resistance. Subsequent studies have combined treatment strategies to overcome such resistance by combining ICI with targeted agents.<sup>8–10,13,14</sup>

Due to the inactivation of the von Hippel–Lindau tumor suppressor gene, which occurs in an

estimated 46–82% of sporadic RCC tumors, VEGF is commonly overexpressed in these tumors.<sup>15</sup> Interestingly, VEGF also alters the TME by enhancing T-cell regulation, enhancing myeloid-derived suppressor cells, both of which inhibit the immune response. Therefore, by blocking this pathway with TKIs or monoclonal antibodies, there is less inhibition of the immune response.<sup>16</sup> In theory, the removal of immune response suppression could work additively, or perhaps even synergistically, in combination with immunotherapy by enhancing the anti-tumor

activity of the immune system. Combining these two types of treatments could help overcome primary resistance. Several combinations with anti-PD1/PD-L1 plus TKI have been investigated in phase III trials.<sup>9,10</sup> Using these combination strategies, there was a significant improvement in ORR from 25–35% to 55–71% and survival benefit compared with single-agent sunitinib.

### The gut microbiome: the guardian

The microbiome is the collective genome that is found within an ecosystem, and microbiota is the specific community of microorganisms inhabiting the surface of an organism.<sup>1</sup> Microbial pathogens are suspected of driving tumorigenesis in 15–20% of cancers by modulating the immune system *via* gut cells and immune signaling pathways.<sup>1</sup> With the microbiome accounting for roughly 1000 times the number of cells in the human body and 10,000 times the amount of DNA, there is a high statistical likelihood that peptide products of gut microbiota could mirror neoantigens created by tumors. This could potentially lead to the generation of T-cell epitopes that more swiftly or accurately recognize the otherwise foreign tumor cells due to molecular mimicry.<sup>17,18</sup> This can partly explain why some patients experience more favorable responses to ICIs and others do not benefit at all.

Perhaps more critical than molecular mimicry is the microbiota's critical role in modulating immune response by producing metabolites that induce immune responses or promote the development of anti-TMEs.<sup>12,19</sup> Of the metabolites produced, short-chain fatty acids (SCFAs) such as butyrate and propionate have been implicated in anti-tumor activity in colorectal cancer.<sup>12</sup> SCFAs have been found to inhibit transcription factors involved in tumorigenesis by inhibiting histone deacetylase and can indirectly regulate tumor development by modulating inflammation.<sup>12</sup> Pyridoxine is produced by a broad group of bacteria and can stimulate anti-tumor immune responses.<sup>12</sup> Lactic acid-producing bacteria can control tumor cell growth by stimulating anti-tumor immune responses and decreasing the production of myeloperoxidases and tumor necrosis factor-alpha (TNF- $\alpha$ ).<sup>12</sup> High levels of TNF- $\alpha$  have been implicated in a number of different malignancies, and also seem to play a role in augmenting the expression of PD-L1 on tumors.<sup>20</sup>

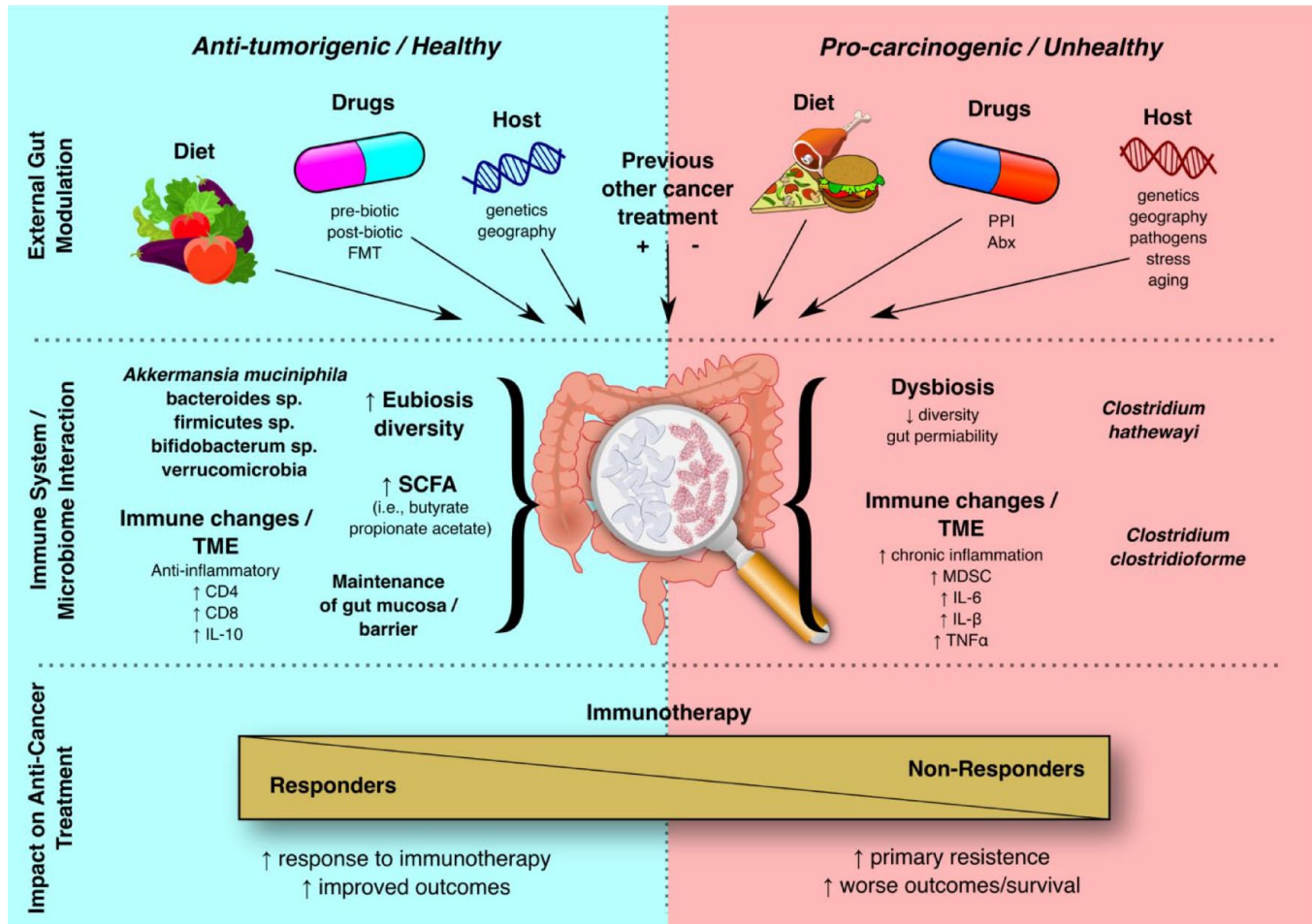
A number of factors can alter the gut microbiome composition, which could potentially alter how

the microbiome can interact with the immune system and whether the host is more susceptible or resistant to treatments (Figure 2). This includes host factors (race, ethnicity, pregnancy, hormonal changes, sexual activity, hygiene, vaginal birth *versus* cesarean section, breast *versus* bottle feeding, and genetics) as well as environmental factors (geographic location, exposure to antibiotics or proton pump inhibitors (PPIs), lifestyle, religion, and culture).<sup>1</sup> Some of these factors are potentially modifiable by changing behavior or by exogenous manipulation. For example, plant-based diets have also been examined as a modifiable factor affecting microbiome composition favoring healthy immune responses.<sup>21</sup>

Diversity appears to be a vital component of a healthy gut microbiome to maintain homeostasis. Metagenomic analysis of feces from cohorts of NSCLC and RCC patients treated with ICIs was analyzed, and authors found a correlation between bacterial diversity with favorable 6-month progression-free survival (PFS).<sup>22</sup> While it is helpful to identify specific species that may confer particular health benefits, their impacts are difficult to emulate as the organisms exist as a community and exhibit symbiotic relationships. Metabolites from one microorganism may be prebiotic fuel for other types of bacteria, working together to create a rich TME. Conversely, the relative abundance of one type of organism could easily disrupt the homeostasis, creating a more or less hostile environment for tumors. The host and environmental factors above can all alter this balance within the microbiome, creating dysbiosis.

Microbial dysbiosis has been associated with the development of cancer in several ways. Some of the metabolites can regulate the permeability of the intestinal wall as a form of protection against carcinogens and by regulating homeostasis among pro- and anti-inflammatory immune cytokines.<sup>19</sup> The gut microbes interact with the host immune system by inducing T-cell responses by their antigens and their metabolism and production of small molecules that regulate the cell microenvironment.<sup>19</sup> For example, *Bacteroides fragilis* is involved in the differentiation of CD4<sup>+</sup> cells into regulatory T cells and secrete cytokines such as interleukin (IL)-10.<sup>23</sup> The increased cytokines may also enhance the autoimmune effects of ICIs. Recent reports demonstrate that patients on ICI who experience autoimmune-related effects also have better anti-tumor responses than those who do not.<sup>24,25</sup>





**Figure 2.** Modulation of the gut microbiome. The gut microbiome can be modulated by external environmental factors such as diet and drugs. This causes changes in the microbiota composition, and thus the downstream metabolites and cytokines. This leads to an impact on response to treatment with immunotherapy.

Abx, antibiotics; CD, cluster of differentiation; FMT, fecal microbiota transplantation; IL, interleukin; MDSC, myeloid-derived suppressor cells; PPI, proton pump inhibitors; SCFA, short-chain fatty acids; sp., species; TME, tumor microenvironment; TNF, tumor necrosis factor.

Researchers have attempted to reverse gut dysbiosis. This can be accomplished by administering prebiotics, which are fibers or foods that feed particular taxa and promote their development. Administering prebiotics such as insulin and mucin alone does not enhance anti-tumor activity in gut-free mice, suggesting their role is dependent on the interaction between those prebiotics and the microorganisms. Prebiotics can enhance the development of particular taxa that enhance anti-tumor immunity.<sup>23</sup> Probiotics are specific microbes thought to have a positive impact on microbiome health. By administering or replenishing prebiotics, probiotics, and even fecal microbiota transplantation (FMT), whereby stool from a healthy donor or treatment responder is transplanted orally into the recipient host, the

hope is that this will restore homeostasis and improve the health of the host. This approach has already been utilized in other diseases such as inflammatory bowel disease or infectious colitis and diabetes.<sup>26–28</sup> There has been a growing interest in similar interventions in cancer, both to prevent tumorigenesis and as a way of augmenting response to treatments, particularly immunotherapies. A recent review was published describing the specifics of pharmacomicrobiomics, targeting microbiota to optimize cancer therapy outcomes.<sup>29</sup> The authors reviewed several studies on solid tumors and provide detailed mechanisms by which bacteria may influence or modulate activity of chemotherapy or immunotherapy, and the potential to translate this impact into clinical outcomes.<sup>29</sup>

### The microbiome and solid cancers: a new adventure world

With the advent of metagenomics utilizing shotgun sequencing approaches or sequencing of 16S ribosomal ribonucleic acid, stool samples from patients with cancer can be analyzed for intestinal microbiota composition, identifying microbe signatures at baseline or after treatment with ICIs, potentially distinguishing between responders and non-responders.<sup>30–32</sup> The microbiome seems to have a bidirectional relationship with various cancer treatments, including surgery, radiation, and drug therapies such as chemotherapy, targeted agents, and immunotherapy, whereby treatment may alter the microbiome's composition. However, the microbiome may also confer sensitivity or resistance to treatments based on its composition.<sup>33</sup> Mechanisms of overcoming resistance to immunotherapies include increasing tumor immunogenicity and T-cell priming, enhancing the TME, overcoming T-cell exhaustion, and combining therapies.<sup>34</sup> Modulating the gut microbiome by administering microbes and microbial metabolites such as butyrate and other SCFAs have also been implicated in orchestrating anti-tumor responses through induction of CD8 T cells and have been investigated as supplemental treatments to augment immune response and enhance response to treatment.<sup>35,36</sup>

Conversely, depleting the microbiome and decreasing diversity have negatively impacted response to treatment with ICIs in various cancers.<sup>1</sup> Treatment modalities that disrupt the homeostasis of immune-stimulating and suppressing signals can alter the response to immunotherapies. Specifically, antibiotic use has been studied in patients with NSCLC, RCC, and urothelial cancer that showed antibiotic use within 30 days of initiating ICI therapy was associated with increased risk of early progression, lowered response rates, and reduced both PFS and OS,<sup>1,30,37</sup> particularly true of broad-spectrum antibiotics.<sup>23</sup> A separate study found that the negative impact was maximal within 6 weeks after initiating immunotherapy.<sup>38</sup> Interestingly, this negative association between antibiotic use and lower efficacy of ICIs only seems to apply to antibiotics administered around the time that immunotherapy was initiated.<sup>30</sup> The inverse association between baseline use of antibiotics and response to immunotherapy was still significant when controlling for the patient's baseline functional status and comorbidities that may also have had a negative impact on response to treatment or survival.<sup>39</sup>

Several meta-analyses conducted examining patients with solid cancers receiving ICI treatment found antibiotic administration was significantly correlated with worse OS, most likely to occur within 60 days before or after initiating ICI treatment,<sup>40</sup> as well as negatively associated with ORR, PFS.<sup>41–43</sup> This has been shown specifically in mRCC patients treated with ICIs and other targeted agents.<sup>43</sup> Another retrospective study specific to patients with mRCC in Turkey found that patients with antibiotic exposure 3 months before or 3 months after initiating immunotherapy with nivolumab had shorter OS.<sup>44</sup> Another study with pooled results on 44 cohorts showed in a subgroup analysis that patients treated with ICIs exposed to antibiotics had worse ORR in patients with RCC compared to other cancers.<sup>41</sup>

Diet can also alter the microenvironment. Meat-based diets may enhance gut colonization with microbiota, such as *Bacteroides* and *Prevotella* sp., whereas plant-based diets may increase the presence of SCFAs and enhance the anti-tumor environment.<sup>21</sup> Researchers are beginning to recognize the specific ways in which we can take advantage of modulating our own immune system by altering our diet or taking oral supplementation as a strategy to overcome resistance to immunotherapy and improve patient outcomes.<sup>45,46</sup>

Administration of PPIs also seems to be linked to resistance to treatments and negative outcomes due to microbial dysbiosis; however, the link is not quite as strong.<sup>47,48</sup> One retrospective study examined the impact of antibiotic use and/or PPI use on efficacy and safety of ICIs in patients with NSCLC, melanoma, upper airway and digestive tract carcinoma or RCC.<sup>49</sup> Patients having received antibiotics within 60 days of initiation or PPIs within 30 days of initiation of ICI were included.<sup>49</sup> They found use of either antibiotics or PPIs in these time frames negatively impacted PFS and OS, but the combination of both did not add to the magnitude of the effect.<sup>49</sup> No impact on toxicity was observed. Another study, however, did not find an association between PPI use and efficacy of single or combination ICIs in patients with metastatic RCC.<sup>50</sup>

While some microbes may augment immune response, some may degrade beneficial prebiotics such as mucin, lead to changes in the microenvironment, or lead to T-cell exhaustion. There is some evidence that the microbes may also alter drug metabolism.<sup>51</sup> Researchers have begun to

analyze commensal microbiota in stool samples to identify stool signatures in various cancers and their relation to response to ICIs.<sup>52</sup> In one recent review, 10 studies evaluating patients with various cancer types examined the effects of the gut microbiome on clinical outcomes of ICI and related toxicity.<sup>53</sup> Their findings were that baseline gut microbiome consistently was related to response to ICIs in terms of PFS and OS.<sup>53</sup> Specifically, two of the studies included reported that *bacteroides* spp. Was associated with colitis, and two studies showed FMT from patients with anti-PD1 responsive disease given to patients with anti-PD-1 refractory disease showed improved response rates without added toxicity in the anti-PD-1 refractory patients.<sup>53</sup> In NSCLC, higher microbiota diversity at baseline was associated with response to PD-1 inhibitors and prolonged PFS.<sup>54</sup> They also identified certain microbes in responders and non-responders that could potentially serve as predictive biomarkers for favorable/unfavorable response by measuring microbes at baseline as well as during treatment.<sup>30,54</sup> It is also possible that treatment itself could be altering the TME or microbiome and associated with treatment efficacy. Given the heterogeneity in response to ICIs across tumor sites, it would be important to characterize the microbiome for each cancer type individually to identify signature similarities or differences that could account for some of this difference in efficacy of treatment. Unfortunately, many studies examining the gut microbiome in solid tumors have combined results across several tumor sites and biologies, and very few have looked specifically at RCC alone.

A systematic review that identified cohort studies evaluating gut microbiome in relation to ICI efficacy and toxicity in various solid tumors showed an association of higher proportions of *Firmicutes* and *Verrucomicrobia* and response to treatment with ICIs, whereas *proteobacteria* were associated with negative outcomes. *Bacteroidetes* seemed to have mixed correlations. *Firmicutes* were also associated with a higher incidence of adverse events, whereas *Bacteroidetes* were associated with less toxicity.<sup>55</sup> Another study identified higher abundance of *Bacteroides intestinalis* in patients with advanced melanoma treated with ICIs who experienced toxicity, and further demonstrated upregulation of mucosal IL-1 $\beta$  in patient samples of colitis in pre-clinical models.<sup>56</sup> As mentioned previously, many of these bacteria affect the immune system *via* the production of SCFAs.

SCFAs can act as probiotics to other bacteria and also result in the recruitment of various cytokines that either augment or inhibit the anti-tumor immune response. This could explain why supplementation with single microbes is a simple solution to a complex problem. The fine balance of gut homeostasis and its impact on health likely depends on microbial diversity rather than on singular microbes to exert its benefits. It would also explain why there can be conflicting reports on whether particular bacteria are associated with improved efficacy or resistance to treatment. Perhaps it is not the microbial species that is important, but rather the complex interactions within a community of microbes, which is very difficult to replicate artificially with therapeutics. This could perhaps, in part, also explain why different tumor types with differing TMEs may have differing efficacy with treatment with ICIs.

FMT is a mechanism whereby feces and its associated microbiome are taken from a donor and transplanted into a recipient, often by oral ingestion. This has been investigated in several diseases as a mechanism of restoring homeostasis due to dysbiosis or by colonizing the recipient with beneficial bacteria in the hopes of enhancing the impact of their microbiota on their immune system and response to treatment. Unfortunately, there is still some stigma of providing or accepting stool samples, which creates a barrier to use in clinical practice.<sup>1</sup> The benefit of this approach is that it accounts for the complexity of interactions between a whole community of microbes and the delicate balance with metabolites and immune cells, which is difficult to replicate with the administration of single metabolites or microbes.<sup>31</sup>

Several ongoing trials are investigating administering oral probiotics in combination with immunotherapy in patients with melanoma, bladder cancer, RCC, and NSCLC to assess safety, clinical response, as well as change in gut microbiota composition (NCT03817125; NCT03637803).

### The microbiome and RCC: time for loaded guns

In recent years, there has been enormous interest in identifying and validating biomarkers associated with treatment response to ICIs, mainly if it captures the dynamic interplay of the TME and treatment response.<sup>57</sup> Identifying which patients are more likely to respond to treatment with immunotherapy is essential because the benefit of

potentially achieving a complete or durable response must be balanced with the risk of toxicity. In the checkmate 214 trial, nearly 50% of participants with mRCC receiving ipilimumab and nivolumab experienced grade 3 or 4 toxicity, which led to treatment discontinuation in over 20% of participants.<sup>6</sup> In combination treatments with an anti-angiogenic drug and an ICI to treat mRCC, grade 3 or 4 toxicities occurred between 63% and 82% of the time.<sup>8-10,14,16</sup> In treating RCC, there is also the unique opportunity to capitalize on the synergy between ICI and the indirect immunological effects of TKIs.<sup>57</sup> Profiling the microbiome in patients with mRCC receiving ICI has shown that higher diversity was associated with improved efficacy of treatment.<sup>58</sup> Furthermore, treatment response was associated with a change in microbial species composition throughout treatment.<sup>58</sup> Similar to other cancers, administration of antibiotics to patients with mRCC just prior to or soon after starting ICI was associated with reduced efficacy of treatment.<sup>38</sup> Interestingly, *Bacteroides* have also been associated with toxicity, specifically diarrhea, in patients who have received TKI therapy in RCC. This is important as diarrhea was found to be dose-limiting toxicity and prevented patients from tolerating full-dose treatment.<sup>59</sup> Administration of antibiotics targeting *Bacteroides* actually improved PFS in these patients in a duration response manner.<sup>59</sup>

The microbiome may also play a role in drug metabolism, as particular microbes have been identified that may metabolize and inactivate chemotherapy drugs such as gemcitabine, resulting in resistance to treatment.<sup>60</sup> It has also been found that there is a reciprocal relationship between cancer drugs and the gastrointestinal microbiome such that the microbes may alter drug metabolism, but the drugs may also alter microbial composition.<sup>60</sup> This has been described in patients with RCC on oral TKIs such as anti-VEGF treatments.<sup>61</sup> Derosa *et al.*<sup>62</sup> found a significant difference in baseline microbiota in patients that had received previous treatment with TKIs and was felt to enhance the immune response and anti-tumor environment. This may have important implications for treatment sequencing or utilization of treatment combinations.

Several researchers have begun attempts to profile the microbiome of patients with metastatic RCC both at baseline and throughout the course of treatment with ICIs in an attempt to identify

microbes that may be predictive of response or non-response to ICIs. Table 1 summarizes the results of these studies. *Akkermansia muciniphila* was associated with response to ICIs, and this was described in each of the four studies summarized in Table 1.<sup>22,58,63,64</sup> Over the course of treatment, the relative abundance of *Akkermansia* spp. generally increased.<sup>58</sup> The most significant correlation was shown by *Akkermansia muciniphila* and was validated across multiple cohorts.<sup>51</sup> *Akkermansia* recruits CD4+ cells and dendritic cells, mediated by IL-12. In a review by Cimadamore *et al.*<sup>65</sup>, *Akkermansia muciniphila* was the microbial commensal in RCC and NSCLC most strongly associated with good clinical outcomes with treatment with ICIs, felt to be related to the production of SCFAs such as propionate and acetate, which cascaded an immunomodulatory effect. Interestingly, the urinary microbiome has been discovered to be distinct from the gut microbiome and may represent an untapped potential resource in our understanding of the interplay between the immune system and RCC.<sup>60</sup>

*Bacteroides*, *Firmicutes*, and *Bifidobacterium* were also among microbial species identified in RCC profiling associated with response to ICIs. *Bacteroides* species have also been associated with augmented response to anti-CTLA-4 therapy in melanoma.<sup>19,23</sup> *Bacteroides* species are known to produce capsular polysaccharides that induce adaptive T-cell-mediated immune responses.<sup>66</sup> *Bifidobacterium* spp. also correlated to improved efficacy of anti-PD-L1 treatments as oral administration was correlated to enhanced tumor control mediated by an increased number of CD8+ T cells in a mouse model of melanoma.<sup>23,30</sup> *Bifidobacterium* induces immune responses by increasing tumor-infiltrating lymphocytes, activating dendritic cells, and enhancing the proliferation of tumor-specific CD8+ T cells.<sup>30,51</sup> Patients with lower levels of CD8+ T cells have been shown to have significantly shorter survival than those with high levels in patients with cutaneous melanoma.<sup>67</sup> *Faecalibacterium* spp. and *Firmicutes* spp. in enriched hosts had improved clinical outcomes with ipilimumab, but at the expense of higher rates of immune-related colitis.<sup>68</sup> Gut microbiota capable of producing SCFAs such as *Eubacterium*, *Lactobacillus*, and *Streptococcus* were positively associated with improved response to anti-PD-1 and PD-L1 treatment across various types of GI cancers.<sup>19</sup> *Firmicutes* spp. is another microbe associated with response to ICI in patients with NSCLC and



**Table 1.** Microbiome profile of ICI responders and non-responders in participants with metastatic RCC.

Study	N	Microbiome diversity in responders after treatment with ICI	Bacteria with higher relative abundance in responders	Bacteria with higher relative abundance in non-responders
Agarwal <i>et al.</i> <sup>64</sup>	22	No significant difference	<i>Akkermansia muciniphila</i>	<i>Unspecified</i>
			<i>Verrucomicrobiae</i> bacteria	
Derosa <i>et al.</i> <sup>62</sup>	69	Increased	<i>Akkermansia muciniphila</i>	<i>Clostridium clostridioforme</i>
			<i>Bacteroides salyersiae</i>	<i>Clostridium hathewayi</i>
			<i>Eubacterium siraeum</i>	<i>Erysipelotrichaceae</i> bacterium
Routy <i>et al.</i> <sup>22</sup>	40	Increased	<i>Akkermansia muciniphila</i>	<i>Bacteroides nordii</i>
			<i>Alistipes</i> sp.	<i>Parabacteroides distasonnis</i>
			<i>Eubacterium</i> sp.	<i>Proteobacteria</i>
			<i>Firmicutes</i> sp.	
			<i>Intestinihomonas</i>	
Salgia <i>et al.</i> <sup>58</sup>	31	Increased	<i>Akkermansia muciniphila</i>	<i>Bacteroides ovatus</i>
			<i>Bacteroides eggerthii</i>	<i>Eggerthelia lenta</i>
			<i>Barnesiella intestine hominis</i>	<i>Flavonifractor plautii</i>
			<i>Bifidobacterium adolescentis</i>	<i>Fusicatenibacter saccharivorans</i>
			<i>Faecalibacterium</i> sp.	
			<i>Firmicutes</i> bacterium	
			<i>Odoribacter splanchnicus</i>	
			<i>Prevotella copri</i>	
<i>Prevotella</i> sp.				
<i>Ruminococcus torques</i>				

ICI, immune checkpoint inhibitor; N, number of participants; RCC, renal cell carcinoma.

RCC, also thought to be related to the production of SCFAs.<sup>30</sup> Thus, it seems there are indeed at least some similarities across tumor sites in the types of gut microbiota associated with response to immunotherapy.

What was also interesting was that the same bacteria in abundance in non-responders were the same bacteria in abundance in patients treated with antibiotics.<sup>63</sup> Given the fact that the use of

antibiotics was also associated with lower efficacy of ICIs, it raises the question of whether the shared commonality in the abundance of these species of bacteria plays an important role in drug resistance. What is quite notable is that the bacteria associated with the poor response across tumor sites seems to be much less well characterized.

Derosa *et al.*<sup>62</sup> evaluated the predictive value of bacterial composition in stool in a cohort of

patients with mRCC by prospectively collecting fecal samples of patients treated with Nivolumab in both human and pre-clinical animal studies. Whole-genome sequencing was utilized to identify bacterial fingerprints associated with prior antibiotic or tyrosine kinase exposure in relation to therapeutic response to ICIs.<sup>62</sup> They found that recent antibiotic use significantly reduced response rates and markedly affected gut microbiota composition, with the dominance of *Clostridium hathewayi*. The *C. Clostridioforme* and *C. hathewayi* bacteria were associated with primary resistance and enriched by antibiotic use, as well as mRCC status (as opposed to healthy volunteers). Antibiotic treatment in mice created significant dysbiosis, decreasing bacterial diversity, and blunted the response to ICIs in murine mouse models hosting orthotopic tumors. They also identified commensals that were associated with more favorable prognoses, such as *E. rectale*, *E. siraeum*, *D. longicatena*, *A. mucimiphila*, and the *Bacteroides* family.<sup>62</sup> These bacteria were associated with adaptive immune responses beneficial against murine cancer and involved in homeostasis.

Recent evidence has shown that specific bacterial species and microbiome diversity can enhance the response to immunotherapy with ICIs in mRCC.<sup>22,58</sup> A recent phase I randomized, prospective, open-label clinical trial to investigate whether oral administration of *Clostridium butyricum* (through the constituent of CBM-588) could modulate the gut microbiome of patients with intermediate or poor-risk mRCC receiving first-line combination ICIs with ipilimumab and nivolumab, and whether this resulted in improved clinical outcomes.<sup>69</sup> CBM588 is a bacterial strain that increases abundance of *Bifidobacterium* spp.<sup>70</sup> Stool was analyzed with metagenomic sequencing at multiple timepoints to assess relative abundance of *Bifidobacterium* spp., but no significant difference was found.<sup>71</sup> Results of this study showed patients who received CBM-588 had an eightfold increase in *Bifidobacterium bifidum* and a sixfold increase in *Bifidobacterium adolescentis*. *Escherichia coli* and *Klebsiella* spp. were more prevalent in patients not receiving CBM-588.<sup>69</sup> As discussed previously in this review, *Bifidobacterium* spp. has been implicated in several pre-clinical and clinical studies with improved immune response in various types of advanced cancers. Furthermore, this study found that the addition of CBM-588 significantly increased PFS and a trend to improve ORR.<sup>69</sup> There was no observed difference in toxicities. A similar ongoing phase I

clinical trial, NCT05122546, is evaluating the effects on the microbiome of CBM588 in combination with nivolumab and cabozantinib in patients with metastatic RCC.<sup>70</sup>

An emerging area of research is now also examining intra-tumoral microbiota, and its possible implications with response to ICIs. Wang *et al.*<sup>71</sup> collected tumor tissue samples from 24 patients with mRCC and matched adjacent tissue samples and utilized 16S rRNA gene sequencing to identify bacteria taxa. They found that compared with the adjacent healthy tissue, 25 taxa increased in abundance and 47 reduced in abundance in the RCC tissue.<sup>71</sup> The class Chloroplast and the order Streptophyta were highly specific to discriminate RCC tissue from healthy tissue.<sup>71</sup> Another study looked at the association of intra-tumoral microbiome and response to ICIs in patients with mRCC and showed several intra-tumoral bacteria had clinical relevance such as *Corynebacterium* spp. and *Stenotrophomonas maltophilia*.<sup>72</sup> Other research is also focusing on profiling the urinary microbiome in urological cancers to investigate its prognostic role in urinary cancers.<sup>73</sup>

FMT in patients with RCC showed that transplanting feces from patients who responded to ICIs into germ-free mice resulted in the mice exhibiting higher response rates to anti-PD-1 treatment, and this response was diminished with the administration of antibiotics.<sup>60</sup> Derosa *et al.*<sup>62</sup> also found in pre-clinical studies with mouse models of RCC that were sterilized with antibiotics and received FMT from patients with RCC had high concordance rates in their response to ICIs. Interestingly, the non-responding mice could be rescued by FMT from responding donors, as well as by oral administration of immunostimulatory microbes.<sup>62</sup> An early phase I trial in patients with melanoma examined the use of FMT in patients who were refractory to ICI, and observed clinical responses in 3 out of the 10 patients included, and also found favorable changes in their immune cell infiltrates and gene expression in the TME.<sup>74</sup> Taking it one step further, researchers are now also examining stool post-FMT for donor and recipient microbiota, to explain variability in response to FMT.<sup>75</sup> These researchers propose perhaps clearing the recipients microbiota in advance of FMT may allow for optimal colonization from the donor microbiota.<sup>75</sup> Ongoing trials are looking at the role of FMT in patients with RCC receiving ICIs, either evaluating whether FMT may help to prevent

**Table 2.** Selected clinical trials with FMT and ICIs in genitourinary tumors.

Agent	Pathway	Setting	Phase	Sample size	Duration	Identifier
Ipilimumab + nivolumab	Anti-CTLA-4 and anti-PD-1	Metastatic RCC	I	20	January 2020–November 2028	NCT04163289
ICI	Unknown	Metastatic RCC	I/II	50	February 2021–February 2022	NCT04758507
ICI	Anti-PD-1 and anti-PD-L1	All solid tumors	I	65	November 2018–December 2023	NCT03686202
Pembrolizumab, enzalutamide	Anti-PD-1, ARAT agent	Metastatic castrate resistant prostate cancer	II	32	October 2019–October 2023	NCT04116775
ICI	Anti-CTLA-4 and anti-PD-1	Advanced genitourinary cancers	I	40	February 2021–January 2021	NCT04038619
ICI	Unknown	All solid tumors	Pilot	10	May 2021–May 2023	NCT04883762

ARAT, androgen receptor axis targeted; CTLA-4, cytotoxic T-lymphocyte-associated protein-4; ICI, immune checkpoint inhibitor; FMT, fecal microbiota transplantation; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; RCC, renal cell carcinoma.

immune-related toxicities or improve treatment efficacy (NCT04163289; NCT04758507). Table 2 lists selected clinical trials with FMT and ICIs in genitourinary tumors. An excellent review on gut microbiota influence on immunotherapy responses in various tumors was recently published, which includes proposed mechanisms and therapeutic strategies including FMT and other probiotics.<sup>76</sup>

### Conclusion

In summary, the clinical use of ICIs in mRCC has improved survival outcomes in the last decade; however, there are still a significant number of patients whose tumors have primary resistance to ICIs, and response rates are not as high as in other types of immunogenic cancers. Identifying ways to overcome primary resistance is an unmet need and of utmost importance in improving survival for these patients. While combination strategies with targeted anti-angiogenic agents plus immunotherapy have added improvement compared to a single treatment modality, likely due to changes in the TME that result in augmented immune responses, the vast majority of patients still do not respond. Unfortunately, a more personalized analysis with multi-modal arsenal of biomarker-driven targets to identify patients who would benefit from treatment is lacking.

This review discussed the mechanism of action of immunotherapy drugs used to treat RCC and the need for more specific biomarkers. Several

promising markers have been described, but there remains a need for further validation in prospective clinical studies. The role of the gut microbiome in maintaining immune homeostasis and either augmenting or inhibiting the body's immune response was reviewed. Gut microbes may have the potential to serve as additional biomarkers to identify the patients that may or may not benefit from treatment with ICIs, as we begin to characterize each microbe's role in interacting with the body's immune system. This interaction is complex and may be unique depending on interactions among microbes as well as with specific tumor cell types. This will require ongoing research to characterize the cellular effects of the microbes as well as specific microbiome composition in patients who respond or do not respond to immunotherapy.

While some studies have examined the relationship of the gut microbiome to the efficacy of treatment with ICIs, much of the research has been limited to animal studies and cell lines or has been non-homogeneous in that the participants had various tumor types that may individually interact in different ways with the microbiome and TME. Some solid tumors, such as melanoma, have better outcomes than others, such as RCC, suggesting that biology plays a role in the immunogenicity of the tumors. Future research should focus on examining more homogeneous groups of patients with RCC to characterize baseline microbial populations and during and after treatment to identify any differences that could account for variability

in response. While supplementing treatments with prebiotics or probiotics may potentially augment immune responses, it is very likely that FMT may more easily replicate dynamic, complex interactions among the microbiome community from donors who responded to treatment with immunotherapy or from healthy donors with a diverse population of microbes. Future research to understand and explore these dynamic interactions between the gut microbiome and response or resistance to immunotherapy in RCC is a promising direction for potentially overcoming resistance to treatment and improving survival outcomes for patients. Ultimately, prospective studies will be key to providing validation of novel biomarkers to be used and integrated into clinical practice.

## Declarations

### *Ethical approval and consent to participate*

Not applicable.

### *Consent for publication*

Not applicable.

### *Author contribution(s)*

**Jasna Deluce:** Conceptualization; Writing – original draft; Writing – review & editing.

**Saman Maleki Vareki:** Conceptualization; Funding acquisition; Supervision; Writing – review & editing.

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RF has the following disclosures: Advisory Board or Honoraria: Merck, Novartis, Ipsen, Janssen,

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### *Availability of data and materials*

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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