Review

Impact of the microbiome on checkpoint inhibitor treatment in patients with non-small cell lung cancer and melanoma

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ABSTRACT

The microbiome is increasingly recognized for its role in multiple aspects of cancer development and treatment, specifically in response to checkpoint inhibitors. While checkpoint inhibitors have revolutionized cancer treatment by producing durable anti-tumor responses, only a minority of patients respond to the available immunotherapy drugs and accurate, sensitive and specific microbiome predictors of response to treatment remain elusive. Additionally, the specific mechanisms linking the microbiome and host immunological responses remain unclear. In this review, we examine the evidence for the gut microbiome's association with anti-tumor responses to checkpoint inhibitors in the treatment of melanoma, non-small cell lung cancer, and renal cell carcinoma. Furthermore, we discuss the current evidence available from murine models seeking to explain the immunological mechanisms that may drive this process. While this work is promising in defining the impact of gut microbiota in cancer treatment, many unanswered questions indicate the need for additional human and experimental studies.

1. Introduction

The microbiome is increasingly being recognized for its role in multiple aspects of cancer development and treatment. While specific bacteria and biofilms have been linked to the development of gastrointestinal malignancies, the gut microbiome has also been linked to the response to cancer immunotherapy in malignancies occurring outside the GI tract [1]. However, the specific members or communities within the microbiome and mechanisms that drive these associations remain unknown. In this review, we will focus on the role of the microbiome in non-small cell lung cancer (NSCLC) and melanoma responses to immune checkpoint inhibitors (ICIs). ICIs block tumors from activating inhibitory checkpoint pathways on immune cells, and thereby unleash the host immune response. Given an expanding knowledge of the microbiome's impact (and specific microbes) on normal immune system development and in drug action, the hypothesis that therapeutic tumor responses are influenced by the microbiome warrants further investigation [2].

Use of checkpoint blockade has revolutionized cancer treatment across multiple cancer types and has received the first ever FDA-approval of a tumor agnostic agent in tumors with microsatellite instability (MSI) [3]. The most widely used ICIs are monoclonal antibodies that target the programmed cell death protein (PD-1), its ligand (PD-L1), or the cytotoxic T-lymphocyte antigen 4 protein (CTLA-4). Checkpoint blockade is highly effective for a subset of patients, yet only ~10–30% of all tumors respond to treatment. Increasing response rates and, in parallel, toxicity are observed when ICIs are used in combination [4], although data on combination ICI therapy is limited. Multiple mechanisms of ICI resistance have been implicated in the poor response rates, including low tumor mutational burden (TMB), low PD-L1 expression, poor antigenicity of tumor cells, local immunosuppression, functional exhaustion of tumor-infiltrating lymphocytes (TILs), and absence of priming or defective antigen presentation during priming [5].

In addition, the gut microbiome has been implicated as a possible factor in the efficacy of ICIs. The human intestinal tract (primarily colon) contains more than 100 trillion bacteria with 500–1000 individual species thought to engage the mucosal immune system and to be crucial for the functioning of the immune system in both healthy and disease states [6]. (This subject is well characterized in other reviews and will not be discussed here). Gut

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commensal bacteria can have beneficial or inflammatory effects and interact with the host immune system within gut-associated lymphoid tissues. Multiple factors affect the healthy gut microbiome, including age, sex, diet, environment, host genetics, and early microbial exposure. However, diet and medications seem to be two of the most significant. Oral antibiotics rapidly yield major microbiome shifts however other medications can do so as well (e.g., metformin, proton pump inhibitors, etc) [7–9]. In contrast, the host genetic impact on the microbiome now appears to be limited despite the many murine studies demonstrating that single gene mutations trigger gut microbiome changes and disease phenotypes [10]. Studies from The Human Microbiome Project Consortium found significant variability in microbiome diversity and abundance in healthy subjects. Most studies have examined stool composition using 16S rRNA gene sequencing to preferentially examine and identify bacterial genomes, while a few studies have taken a metagenomics approach by performing whole genome shotgun sequencing (WGS) [11]. In the following sections, we will review microbiota changes in the setting of malignancy at baseline and during treatment with ICIs.

2. Melanoma

The initial association between the gut microbiome and response to ICIs was first reported by Vetizou et al. in 2015, who examined the gut microbiota of metastatic melanoma patients treated with ipilimumab ($n = 25$), a checkpoint inhibitor targeting CTLA-4 and the first checkpoint inhibitor approved for therapeutic use in metastatic melanoma in 2011 [12] (Table 1). Using antibiotic-treated mouse models and patient fecal microbiota transfer to mouse models, the authors showed that specific *Bacteroides* (*B. fragilis* and/or *B. thetaiotaomicron*) and *Burkholderiales cepacia* in the gut were associated with anti-tumor responses [13]. In a simultaneous report, Sivan et al. used a melanoma mouse model to show inoculation with a commercially available cocktail of *Bifidobacterium* species, which included *B. breve* and *B. longum*, improved tumor control by a mouse anti-PD-L1 antibody with accumulation of CD8$^+$ T cells in the tumors [14]. This study did not utilize human specimens. Subsequently, in metastatic melanoma patients treated with ipilimumab ($n = 26$), Chaput et al. reported increased progression-free survival (PFS) and overall survival (OS) in subjects with a baseline microbiota enriched in the *Faecalibacterium* genus and other Firmicutes, as opposed to those with a microbiota enriched in *Bacteroides* [15]. Of note, the role of *Bacteroides* in ICI therapeutic responses in Chaput et al. [15] contrasts the findings of Vetizou et al. [13]. At baseline, the specific species identified by Vetizou et al., *B. fragilis* and *B. thetaiotaomicron*, were not abundantly represented in the Chaput et al. dataset, and additional experiments are needed to identify which Bacteriodes species may modulate ICI efficacy.

Additional clinical trials have since examined the gut microbiome in cancer patients being treated with ICIs. Frankel et al. used metagenomic shotgun sequencing to study pre-treatment samples from patients with metastatic melanoma ($n = 39$) who underwent treatment with single or a combination of ICIs. The stools from all ICI responders were enriched in *Bacteroides caccae*. Those undergoing combination treatment with ipilimumab and nivolumab were enriched with *Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron*, and *Holdemania filiformis* while treatment with pembrolizumab was associated with higher levels of *Dorea formicogenerans* [17]. Matson et al. analyzed the baseline stools of patients with metastatic melanoma who received either anti-PD-1 ($n = 38$) or anti-CTLA-4 ($n = 4$) regimens using both 16S rRNA gene sequencing and metagenomic approaches. Eight species were enriched in responders: *Enterococcus faecium, Collinsella aerofaciens, Bifidobacterium adolescentis, Klebsiella pneumoniae, Veillonella*

Table 1
Characteristics of the gut microbiome in cancer patients treated with immune checkpoint inhibitors (ICIs).

Authors	Patient population	N	Bacteria associated with response to ICI therapy
Vetizou et al. 2015	Metastatic melanoma	$n = 25$	*B. fragilis* and/or *B. thetaiotaomicron, Burkholderiales* species
Chaput et al. 2017	Metastatic melanoma	$n = 26$	*Faecalibacterium* genus and other Firmicutes
Frankel et al. 2017	Metastatic melanoma	$n = 39$	*Bacteroides caccae, Faecalibacterium prausnitzii, Bacteroides thetaiotaomicron, Holdemania filiformis, Doreaformico generans*
Matson et al. 2018	Metastatic melanoma	$n = 42$	*Enterococcus faecium, Collinsella aerofaciens, Bifidobacterium adolescentis, Klebsiella pneumoniae, Veillonella parvula, Parabacteroides merdae, Lactobacillus* species, and *Bifidobacterium longum*
Gopalakrishnan et al. 2017	Metastatic melanoma	$n = 112$	*Ruminococcaceae, Faecalibacterium*
Routy et al. 2017	Advanced NSCLC, RCC	NSCLC $n = 60$ RCC $n = 40$	*Ruminococcaceae, Faecalibacterium,* specifically *Ruminococcae, Alistipes* and *Eubacterium* species

NSCLC, non small cell lung carcinoma; RCC, renal cell carcinoma

parvula, Parabacteroides merdae, Lactobacillus species, and *Bifidobacterium longum.* In contrast, nonresponders were associated with *Ruminococcus obeum* and *Roseburia intestinalis* [18]. Finally, Gopalakrishnan et al. examined the microbes present in patients with metastatic melanoma receiving anti-PD-1 treatment ($n = 112$), using both 16S rRNA gene and metagenomic approaches, to show that high alpha diversity estimated by the inverse Simpson index, a measure of the species richness and evenness within a specific region or sample, and abundance of *Ruminococcaceae* and *Faecalibacterium* in the gut corresponded with a favorable response to checkpoint blockade, while low alpha diversity and a high abundance of Bacteroidales associated with a lack of response [19].

To date, these studies implicate a range of bacteria in facilitating a response or non-response to ICIs in melanoma patients. Some taxa appear to associate with response to immunotherapy across multiple studies. For example, *Faecalibacterium* was identified in 3 studies as associated with response to ICIs, although the role of other taxa diverges between studies [15,17,19]. Three studies also suggest a contribution of Bacteroidetes to ICI responses in melanoma [13,17,18], while two studies suggest that members of the Bacteroidetes phylum are detrimental [15,19]. Another example is the Ruminococcaceae family has been implicated in both responses and non-response to ICIs [18–21]. Discrepancies in study design, technical and computational methods, timing of sample collection, and antibiotic use are among variables that may account for the differences. Hence, rigorous prospective and adequately powered clinical studies accompanied by mechanistic studies are required to better understand the contribution of the microbiome to ICI therapy in melanoma.

3. Non-small cell lung cancer

In addition to the work in melanoma, Routy et al. examined microbial associations in epithelial tumors in a cohort of patients with NSCLC ($n = 60$) and renal cell carcinoma (RCC, $n = 40$). This study found that greater metagenomic species richness in the gut correlated with clinical response, and *Akkermansia muciniphila* was the most highly correlated species with a response to ICIs. Enrichment of *Ruminococcae, Alistipes* and *Eubacterium* species was also noted in responders with a diminished presence of *Bifidobacterium* and *Parabacteroides* [21]. Zhang et al. also examined the baseline gut microbiome of patients with lung cancer ($n = 41$) and found a lower abundance of *Firmicutes* and *Proteobacteria*, along with relatively higher levels of *Bacteroidetes* and *Fusobacteria*, compared to healthy controls. The ratio of *Firmicutes* to *Bacteroidetes* in lung cancer patients was also low, which has been linked to a lower concentration of circulating short-chain fatty acids (SCFA) and thereby could influence host immune responses [22].

Moreover, ongoing study of the lung microbiome suggests the hypothesis that the organ-specific microbiome may play a causal role in lung cancer, although the data, below, are only associations and mostly with late stage disease [23,24]. An initial study by Lee et al. examined fluid from bronchoalveolar lavage (BAL) from patients with lung cancer ($n = 20$) and found two phyla, Firmicutes and TM7, and two genera, *Veillonella* and *Megasphaera* (Firmicutes), associated with disease state [25]. TM7 (Saccharibacteria) is a poorly understood candidate phylum, detected in environmental 16S rRNA sequences. Two additional studies used bronchial brushing specimens from patients with NSCLC, finding that decreased alpha diversity, *Streptococcus*, *Neisseria*, and *Veillonella* associated with cancerous sites compared to a noncancerous site from patients or healthy controls [26,27]. Microbiome shifts have been further demonstrated using 16S rRNA amplicon sequencing of lung tumor and paired normal tissue. Yu et al. demonstrated reduced alpha diversity in lung tumor tissue ($n = 31$) [28], while Greathouse et al. showed *Acidovorax* (phylum Proteobacteria) was enriched in smokers and in squamous cell carcinoma with TP53 mutations ($n = 143$) [29]. Finally, in a study of surgically-resected early stage NSCLC that examined microbiota from tumors and paired remote normal lung tissues ($n = 19$), increased alpha diversity in the normal lung tissue associated with reduced disease-free and recurrence-free survival (DFS, RFS). Specifically, greater abundance of families Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae, and genera *Bacteroides, Faecalibacterium, Roseburia,* and *Ruminococcus* in normal lung tissue were associated with reduced DFS/RFS, whereas greater abundance of *Koribacteraceae* (aka, Coriobacteriaceae, phylum Actinobacteria) and *Sphingomonadaceae* (phylum Proteobacteria) were associated with improved DFS/RFS. Two points from this study are: 1) notably, genera such as *Bacteroides, Faecalibacterium* and *Ruminococcus* associated with improved outcomes in some melanoma studies, are proposed as harmful in NSCLC [20]; and 2) most often, lower alpha diversity has been associated with disease and higher alpha diversity with 'health'. Thus, these preliminary results in early stage NSCLC suggest the unexpected hypothesis that a diverse lung microbiome in normal lung tissue from lung cancer patients may enhance disease recurrence after resection. Lastly, a recent study by Jin et al. provides initial mechanistic insight. Using germ free (GF) and antibiotic-treated (ABX-TX) mouse models, these investigators found that local microbiota provokes inflammation associated with lung adenocarcinomas by activating lung-resident $\gamma\delta$ T cells which produce IL-17 and thereby promote inflammation and tumor cell proliferation [30]. This is consistent with previous data in colon cancer murine models where IL-17, regardless of producing cell type, is procarcinogenic [31].

Overall, NSCLC patients appear to have diminished alpha diversity of their lung microbiota, and in a few studies, an enrichment of *Veillonella* species. Whether bacteria contribute directly to tumorigenesis, however, remains uncertain and will require dedicated study. Because all lung cancer characterization studies to date have utilized 16S rRNA amplicon sequencing, further study with metagenomic approaches may provide functional data from the microbiota.

4. Antibiotic treatment: effect on tumor growth and efficacy of checkpoint blockade

Limited data have begun to examine how antibiotic exposure may impact cancer therapeutic responses. Routy et al. presented data suggesting that recent antibiotic use (within 60 days) correlated with a negative impact on overall survival (OS) in a cohort of advanced NSCLC, RCC, or urothelial carcinoma patients ($n = 249$) that received anti-PD-1 alone or combined with anti-CTLA-4 [21]. In a follow-up study with larger numbers of RCC ($n = 121$) and NSCLC ($n = 239$) patients, Derosa et al. used a multivariate model to control for classic prognostic features and found that antibiotic use within 1 month of immunotherapy was associated with a shorter overall survival (OS) in NSCLC and a shorter progression-free survival (PFS) in RCC [32]. A portion of the data analyzed in Derosa et al. [32] was derived from Routy et al. [21]. These findings are consistent with two other studies. Ahmed et al. showed broad spectrum antibiotic use within 2 weeks before or after the first dose of ICIs was associated with a lower response rate and shorter PFS ($n = 60$, advanced cancers) and Zhao et al. showed antibiotic use concomitant with anti-PD-1 therapy associated with worse progression-free and overall survival ($n = 109$, NSCLC) [33,34]. These studies contrast with a prior study published by Kaderbhai et al. [35] in which no change in PFS was found in metastatic NSCLC patients treated with antibiotics 3 months prior to, or during treatment, with nivolumab monotherapy ($n = 74$). However, several of the authors of Kaderbhai et al. [35] contributed to Routy et al. [21] where a significant effect on OS in larger NSCLC

cohorts ($n = 140$–239) was reported using an antibiotic exposure interval of 2 months before to 1 month after the first anti-PD1 dose.

Together the impact of antibiotic exposure and timing on ICI therapy is uncertain. Reported discrepancies may be due, for example, to the interval between antibiotic exposure and first dose of immunotherapy (up to 3 months prior to ICI), the clinical outcome evaluated (PFS vs OS) as well as variable study designs and power, monotherapy versus combination ICIs, and confounding by other medications that may affect the gut microbiome [7–9,35]. Critical antibiotic features such as route and duration of exposure, class of antibiotics among others have not yet been examined. To date, mouse tumor models support the concept that antibiotic exposure diminishes anti-tumor immune responses to treatments such as anti-CTLA4 [13], cisplatin [37], or platinum and CPG oligonucleotides [38] In contrast, the impact of antibiotics on tumor growth is more mixed; broad-spectrum antibiotics promoted tumor growth in implanted syngeneic mouse tumor models [36,39], but protected against tumor growth in a transgenic lung Kras^{mt}/P53$^{-/-}$ model [30]. However, these murine models use broad oral anti-aerobic and anti-anaerobic antibiotic combinations that differ substantively from patient antibiotic exposure. First and foremost, carefully-designed human studies are needed to clarify antibiotic interactions with ICI therapy. Ideally, hypotheses derived from human studies will foster experimental studies to define potential mechanisms by which antibiotic exposure may impact disease outcomes.

5. Immunotherapy-related toxicities

While ICIs have provided a means to overcome immunological tolerance to tumors, risk of autoimmunity in normal tissues is a significant limitation in their use. Immune-related adverse events (irAEs) occur more commonly in patients on anti-CTLA-4 as compared with those taking anti-PD-1/PD-L1, and the incidence of irAEs seems to increase accordingly when these agents are used in combination [40]. Specific microbiota have been associated with risk of developing immune-related toxicity. In a study by Dubin et al., abundance of species within the Bacteroidetes phylum, specifically *Bacteroidaceae*, *Rikenellaceae* and *Barnesiellaceae*, was associated with resistance to colitis in patients with metastatic melanoma treated with ipilimumab ($n = 34$), whereas diminished detection of genetic pathways involved in polyamine transport and vitamin B synthesis in the gut associated with an increased risk of colitis [16]. A protective role of Bacteroidetes was also reported by Chaput et al. using samples from metastatic melanoma patients treated with ipilimumab ($n = 26$). In the subset of patients who developed colitis ($n = 7$), the baseline gut microbiota shifted at the time of toxicity to having decreased alpha diversity with a proportionate reduction in the Firmicutes phylum, specifically *Ruminococcus, Lachnospiracea incertae sedis, Blautia, Clostridium IV, Eubacterium,* unclassified *Lachnospiraceae* and *Pseudoflavonifractor*, while a high proportion of Bacteroidetes was observed in patients who remained colitis-free [15].

6. Mechanisms by which the gut microbiome can influence anti-tumor responses by checkpoint inhibitors

The primary microbiome associations discussed above have in some instances been complemented by correlative murine tumor and immune cell studies using human samples to discern immunological mechanisms corresponding to human ICI responses. Along with greater tumor infiltration by CD8$^+$ T cells, Gopalakrishnan et al. reported that responder patients (on anti-PD1 therapy), enriched in *Faecalibacterium* genus, the Ruminococcae family and the Clostridiales order, had more circulating CD4$^+$ and CD8$^+$ T cells with preserved cytokine responses. In contrast, non-responder patients showed a higher abundance of *Bacteroidales* in the gut and had a higher frequency of circulating regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) with a blunted cytokine response [19]. Chaput et al. found that responders (*Faecalibacterium*-dominant) had low baseline circulating Tregs, low $\alpha4^+\beta7^+$ T cells and higher serum CD25, compared to patients that did not respond (*Bacteroides*-dominant). The integrin $\alpha4\beta7$ plays a crucial role in the intestinal homing of T cells and may provide insight into mechanisms mediating colitis in irAEs while serum CD25 serves as a soluble marker for inflammation [15].

Complementary to these studies, mouse models have proven useful to examine mechanisms relevant to the microbiome. To assess immunological responses, germ-free (GF) and antibiotic-treated (ABX-TX) conventional mice have been used along with syngeneic tumors and human fecal microbiota transfer (FMT) of patient stools, to compare the impact of gut microbiota from patients who responded (R-FMT), to those who did not respond (NR-FMT) on ICI therapy. Using melanoma patient stool samples, R-FMT into syngeneic mouse tumor models has shown increased efficacy of checkpoint blockade with corresponding increased tumor-infiltrating lymphocytes (TILs) consisting of CD8$^+$ T cells not seen in NR-FMT mice [18,19]. Tumors from R-FMT mice were also enriched in innate effector cells (CD45$^+$CD11b$^+$Ly6G$^+$) with a corresponding depletion in myeloid cells (CD11b$^+$CD11c$^+$) while tumors from NR-FMT mice had higher RORγ+T helper (Th) 17 cells [19]. IL17 is a proinflammatory cytokine known for its role in host pathogen defense that has also implicated in chronic inflammation and tumorigenesis [41]. Using NSCLC patient stool samples, R-FMT mouse models show an accumulation of intratumoral CXCR3$^+$CD4$^+$ T cells as well as up-regulation of PD-L1 in splenic T cells [21]. Interestingly, inoculation of mice with *Akkermansia muciniphilia* and *Enterococcus hirae (*enriched in responders)*, caused accumulation of central memory CD4$^+$ T cells expressing the small intestine-associated chemokine receptor CCR9 and/or the Th1-associated chemokine receptor CXCR3 in mesenteric lymph nodes, tumor-draining lymph nodes, and tumors. Further, R-FMT mice revealed dendritic cells induced to secrete IL-12, a Th1 cytokine previously shown to be involved in the immunogenicity of PD-1 blockade [21]. More recently, Tanoue et al. examined healthy human stools and identified a consortium of 11 strains, 7 Bacteroidales and 4 non-Bacteroidales species, that when inoculated in mice increased IFNγ$^+$CD8$^+$ T cells in the intestines along with upregulation of IFNγ-regulated genes, specifically CXCL9 and CXCL10, in the colonic epithelium. These microbes also facilitated anti-tumor responses to anti-PD1 or anti-CTLA4 in a syngeneic mouse colon cancer model in which tumors showed infiltration of IFNγ+CD8+ T cells expressing granzyme B, a key effector molecule of cytotoxic T cells, and dendritic cells with high expression of major histocompatibility class I [42].

Overall, these mouse studies show that microbiota associated with response to checkpoint inhibitors can induce changes in the tumor microenvironment consistent with favorable outcomes in humans (i.e. increasing the number of intratumoral CD8$^+$ T cells, increasing the CD4/FoxP3 ratio). There are at least 3 ways the gut microbiome could be influencing the response to immunotherapy. First, innate or adaptive immune cells, educated by gut microbes, could travel to impact the tumor microenvironment. Specific gut microbes can be immunosuppressive or immunostimulatory [6,43] and can elicit chemotactic factors that cause immune cell trafficking, as supported by findings by Tanoue et al. (increased CXCL9 and CXCL10 in intestines) and Routy et al. (central memory CD4+ T cells expression CCR9 and/or CXCR3) [21,42,44]. However, more work is required to demonstrate movement of immune cells from the gut to a peripheral tumor in the setting of an altered

microbiome. Second, a similarity or "molecular mimicry" may exist between gut microbial and tumor neoantigens, which is supported by identification in healthy subjects of circulating T memory cells with reactivity to previously-encountered pathogens and to gut commensals [45]. In fact, T cells from NSCLC/RCC patients on ICIs showed a recall response when stimulated with autologous monocytes preincubated with responder microbes, and significantly, T cells stimulated with *A. muciniphila* produced IFNγ and were associated with a prolonged PFS [21]. Several other studies have also shown the presence of T cells with reactivity to gut bacteria in the circulation of patients with cancer or in the tumor microenvironment [46,47]. Finally, a metabolic effect of the microbiome may influence the anti-tumor immune response at extraintestinal sites [48]. Certain microbes produce SCFAs and secondary bile acids that may have significant effects on the immune system [49]. Specifically, anacardic acid was shown to be elevated in ICI responder melanoma patients along with 82 other stool metabolites [17], and mevalonate and dimethylglycine were increased in the cecal contents of mice receiving the 11 species consortium defined by Tanoue et al. [42].

7. Summary and outstanding questions

The recent discovery of the microbiome's impact on the efficacy of checkpoint inhibitors and the subsequent work to elucidate the immunological mechanisms driving these effects have revolutionized microbiome research in oncology. While this work is promising, unanswered questions are abundant and the sources of the diversity in the reported results unclear. As demonstrated, many different bacteria and multiple metabolites have been associated with response to ICIs, but only a few have been found across multiple studies and across tumor types. Moreover, many of these studies do not utilize methods to correct for false discovery rate or multiplicity of observations, which affects the statistical significance reached for specific species in each of these studies. Therefore, continued human and mouse studies are necessary to determine whether ICI responses linked to the microbiome are microbe-, tumor-, patient- and/or mechanism specific. Further, to date, nearly all microbiome data are cross-sectional limiting data interpretation. Longitudinal studies are required to assess microbiome causality in disease genesis and therapeutic response or non-response [50,51].

Notwithstanding, the potential for clinical impact is clear. As a noninvasive test, assessment of the gut microbiome as a biomarker for response to ICIs could serve to enhance the precision of therapeutic choices. Moreover, modification of the baseline microbiome and/or application of FMT as a supportive therapy may offer modulation of treatment efficacy and a means by which to mitigate irAEs [51–53]. Several clinical trials are underway in patients with advanced malignancies resistant to checkpoint inhibitors, as well as in patients undergoing stem cell transplant or as treatment for refractory graft versus host disease [51]. While FMT may be a promising therapeutic option, the risk of bacterial translocation (including antibiotic resistant bacteria) and sepsis in patients remains a significant safety concern [52] as illustrated by recent reports of sepsis and a fatality following FMT leading to a FDA safety alert [54]. These and future studies are crucial to determine the feasibility of the microbiome to improve the response rate of cancer patients treated with checkpoint inhibitors.

Selection criteria

Pubmed search of articles published before 2/1/2019: "Microbiome + melanoma" and "Microbiome + lung + cancer", "oral microbiome + lung cancer", "lung microbiome + lung cancer". Additional articles selected for review were based on articles in these searches and prior review of the literature by the authors (published before 6/12/19 and as suggested by reviewers).

Author contributions

All authors contributed to literature search, manuscript draft, and writing. FYS made the table. All authors have approved the final version of this manuscript.

Declaration of Competing Interest

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