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## Gut microbiome modulation via fecal microbiota transplant to augment immunotherapy in patients with melanoma or other cancers

Jennifer L McQuade, MD<sup>1</sup>, Gabriel O Ologun, MD<sup>2</sup>, Reetakshi Arora, PhD<sup>2</sup>, Jennifer A Wargo, MD, MMSc<sup>3</sup>

<sup>1</sup>Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 0430, Houston, 77030, TX, USA

<sup>2</sup>Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Unit Number 1484, Houston, TX, 77030, USA

<sup>3</sup>Department of Surgical Oncology and Genomic Medicine, The University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Unit Number 1484, Houston, TX, 77030, USA

### Abstract

**Purpose of Review:** We review emerging evidence regarding the impact of gut microbes on anti-tumor immunity, and ongoing efforts to translate this in clinical trials.

**Recent Findings:** Pre-clinical models and human cohort studies support a role for gut microbes in modulating overall immunity and immunotherapy response, and numerous trials are now underway exploring strategies to modulate gut microbes to enhance responses to cancer therapy. This includes the use of fecal microbiota transplant (FMT), which is being used to treat patients with *Clostridium difficile* infection among other non-cancer indications. The use of FMT is now being extended to modulate gut microbes in patients being treated with cancer immunotherapy, with the goal of enhancing responses and/or to ameliorate toxicity. However significant complexities exist with such an approach, and will be discussed herein.

**Summary:** Data from ongoing studies of FMT in cancer will provide critical insights for optimization of this approach.

### Keywords

fecal microbiota transplant; microbiome; immunotherapy; immune checkpoint blockade; cancer; clinical trials

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**Corresponding author:** Dr. Jennifer L. McQuade, Departments of Medical Oncology, The University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Unit Number 1484, Houston, TX 77030, USA. (jmcquade@mdanderson.org).

#### Conflict of Interest

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#### Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

## Introduction

The development and approval of immune checkpoint blockade (ICB) has dramatically changed the landscape of cancer therapy and improved outcomes in many malignancies [1]. However, outcomes with immunotherapy are heterogeneous and there remains a critical need to identify predictors of response and resistance and develop synergistic strategies to enhance response. There is now strong evidence that the gut microbiome can shape response to immunotherapy. Importantly, unlike tumor genomics, the gut microbiome is modifiable, and thus, modulation of the gut microbiome to enhance response to immunotherapy is an attractive therapeutic strategy. Herein, we review the potential role for fecal microbiota transplant (FMT) in the context of immunotherapy, including ongoing clinical trial efforts.

## Background and rationale for FMT in immunotherapy

The human microbiome refers to the trillions of microorganisms (and their genomes) that live on and in human bodies in a symbiotic relationship. The microbes that inhabit our gut aid in harvesting nutrients from our diet and maintaining gut mucosal integrity, among other functions. The gut microbiome has also been shown to play a key role in shaping the development of mucosal and systemic immunity [2]. There is considerable cross-talk between the gut microbiome and our immune system, with a homeostatic balance of tolerance of beneficial commensal microbes and defense against pathogenic bacteria.

Dysbiosis, i.e., an imbalance in the gut microbiome, has been implicated in the pathogenesis of multiple diseases, including autoimmune diseases, gastrointestinal disorders, and luminal malignancies [3,4,5,6]. Initial evidence that the gut microbiome could play a role in therapeutic outcomes in cancer came from work in allogeneic hematopoietic stem cell transplant (HSCT) and graft-versus-host disease (GVHD) [7, 8, 9]. In preclinical studies, it had been demonstrated that the gut microbiome can influence the immune response to chemotherapeutics such as cyclophosphamide [10].

A role for the gut microbiome in response to immunotherapy was first suggested in two preclinical studies published in *Science* in 2015 where it was shown that the gut microbiota influenced response to ICB in mice [11, 12]. Multiple studies in human cohorts subsequently demonstrated strong associations between the gut microbiome and response to ICB in various types of cancer [13, 14, 15, 16, 17]. Importantly, it was further shown in preclinical models that the gut microbiome could be modulated to enhance therapeutic response [11, 12, 13, 14, 15]. Thus, the gut microbiome is not only a biomarker of response to immunotherapy, but also an interventional target.

The gut microbiome can be targeted with a variety of modalities including via diet and the provision of putative beneficial organisms as either single-strain probiotics or bacterial consortia [18]. However, the most well-established method of microbiome modulation is through FMT, whereby a donor microbiome is transferred to a recipient in the form of a stool suspension given either endoscopically or in capsule format. FMT is a guideline-recommended therapy for recurrent *Clostridium difficile* infection, where it has been shown

to correct the dysbiotic state and result in clinical resolution of symptoms in randomized clinical trials [19,20,21], and FMT is being investigated in multiple other diseases.

FMT is the most direct method of microbiome modulation and results in the transfer of the entire donor microbial ecosystem. This approach has two distinct advantages over the introduction of single putatively pro-immunotherapy response bacteria. First, colonization of single bacteria introduced into a complex host microbiome ecosystem with an established homeostasis can be challenging [22]. Second, the transfer of an entire ecosystem includes both the putative pro-response bacteria and the many other organisms which may either support or have redundant roles with these candidate bacteria.

## Lessons learned from non-cancer FMT studies

Though investigations of FMT have only recently been initiated in the context of immunotherapy, FMT has been well-studied for other indications with important lessons to be learned from these investigations in terms of approach. The most established indication for FMT is for recurrent/refractory *Clostridium difficile* infection (CDI). CDI is a clear dysbiosis associated with antibiotic use disrupting the normal gut microbiome allowing outgrowth of this toxin-producing spore-forming obligate anaerobe [23]. CDI can be treated by metronidazole or oral vancomycin but recurrence is high, in part due to collateral damage to the commensal microbiota by these antibiotics [24]. Randomized clinical trials have established that FMT given as a single dose is a highly effective treatment for refractory CDI with systematic reviews demonstrating efficacy rate of 80–90%, and importantly a much lower rate of recurrence than standard-of-care, and consensus guidelines support the use of FMT for this indication [19,20,21]. Longitudinal microbiome profiling has demonstrated that in this setting, a single-dose colonoscopically delivered FMT from unselected healthy donors leads to rapid and reliable engraftment of donor stool, with normalization of community structure and diversity, and durability (at least up to 6 months which is longest time period which has been surveilled) [25]. In an effort to improve FMT accessibility and safety, more recent studies have utilized oral capsules of frozen donor stool specimens, with a randomized study demonstrating non-inferiority compared to colonoscopic FMT [26].

The success of FMT in CDI has prompted the investigation of this modality in multiple other indications, spanning from gastrointestinal to metabolic to neuropsychiatric disorders [27]. The most well-studied indication beyond CDI is inflammatory bowel disease (IBD), a disease in which the gut microbiome has also been implicated, though direct causality is less clear [28]. FMT within this context has shown significant promise, though results have been more mixed than in CDI with variability of engraftment and clinical response when single-dose unselected FMT is used in this setting [28, 29]. However, more recently, an RCT of an intensive FMT protocol in ulcerative colitis published in *The Lancet* demonstrated a significant improvement in steroid-free clinical remission rates for the FMT group vs placebo (26% vs 8%) [30]. In this study, patients received a single FMT by colonoscopic infusion followed by FMTs via enema, 5 times per week for 8 weeks. Interestingly, despite meeting the primary outcome of clinical response, there was no significant benefit detected in terms of quality of life which may reflect the intensity of the treatment regimen. While single infusion healthy donor FMTs have repeatedly been shown to be effective in CDI,

these data suggest that less profoundly dysbiotic disease states/hosts may be more resistant to therapeutic microbiota modulation and require more intensive/ongoing interventions [31]. Donor selection may also be important, with “superdonor” patterns emerging in some trials in IBD where clinical response is enriched by specific donors [32]. Specific donor microbiota features associated with response have been described, including taxonomic and metabolic profiles, as well as overall richness and diversity emerging as a key characteristic [32,33,34,35]. Again, this is in contrast to CDI where response does not appear to vary by donor. As the optimal donor microbial characteristics in IBD are being established, others have instead pursued a donor “pooling” approach to minimize donor-dependent effects [30]; however, how pooling effects on the overall ecology of the infused stool and associated engraftment is unclear and there are potential risks associated with donor pooling, as well as issues in understanding the contribution of specific microbes/donor profiles to potential therapeutic response.

FMT has also been studied in metabolic syndrome bolstered by preclinical studies demonstrating that obesity and metabolic syndrome are transferable by FMT in mice [36]. Two clinical studies have suggested that insulin resistance can be improved by FMT from lean donors to patients with metabolic syndrome, though these responses were variable and not durable. Of note, these studies also employed a single FMT infusion and no preceding antibiotic ablation [37, 38]. In this setting, baseline fecal microbiota composition of the recipient predicted response to FMT, with those with lower baseline diversity exhibiting better response [38]. These findings along with those from the IBD studies have important potential implications for optimizing recipient and donor selection and FMT frequency and duration to promote engraftment into less dysbiotic and potentially more resistant host states. Studies in these other indications have shown that FMT is quite safe, with the most common adverse event being abdominal discomfort [19]. Over the many patients treated in trials and now in clinical practice with FMT, infection transmission has not been a substantial problem with only 4 cases of gram-negative bacteremia reported, three of which had alternative explanations [39]. However, recently, there were two cases (and one resulting death) reported of systemic infections with extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli* which was traced back to donor stool [39]. Obviously, this has placed renewed attention on the established guidelines to test donors for potential pathogens [40] and the Food and Drug Administration (FDA) issued a safety alert and has mandated additional screening following these events [41].

Overall, experience with FMT for recurrent CDI supports that FMT can be a highly effective and safe approach to modulate the microbiome, with more variable results in other indications suggesting that host and donor characteristics and timing and duration of intervention may influence therapeutic efficacy and are important considerations in the design of clinical trials in the immunotherapy space (Fig. 1)—as this patient population is more reminiscent of a highly heterogeneous group of individuals with relative dysbiosis (as opposed to the profound dysbiosis observed in CDI).

## Current efforts of FMT in immunotherapy:

Knowledge gained from FMT for other indications as well as from studies of FMT combined with immunotherapy in the preclinical setting has informed the design of several clinical trials of FMT in the context of ICB in patients with cancer (Table 1). In a search of the U.S. National Library of Medicine Clinical Trial database, 8 clinical trials of FMT in patients receiving ICB were identified, with relevant features summarized in Table 1. Of these, six studies are aimed at using FMT as a strategy to improve response to ICB, while two are targeting ICB-induced colitis.

As the malignancy with the most established indication for immunotherapy, melanoma is the most common cancer type for which FMT is being investigated. Other cancer types studied include gastrointestinal and genitourinary cancers. Notably, this field is still relatively nascent; thus, these studies are all relatively small phase I/II studies, and the primary endpoint of the majority of these trials is safety. Heterogeneity in the design of these studies reflects many ongoing questions in the optimal study population and intervention for FMT in the setting of ICB (Fig. 1) [18]. For example, the majority of studies are enrolling patients refractory to anti-PD1, while some are enrolling ICB-naïve patients. Of the studies enrolling patients in the anti-PD1-exposed setting, some studies are enrolling patients who are truly refractory to anti-PD1 (i.e., verified progressive disease), whereas others enrich for patients who do not achieve a response at 12 weeks. Methods of FMT delivery also vary, either via colonoscopy (as a one-time FMT) or frozen stool capsules which can be delivered over a longer period of time.

Another factor to be considered is effect of pretreatment antibiotic ablation. Peri-immunotherapy antibiotic use has been associated with diminished response to immunotherapy in retrospective cohorts [15••, 42]. However, antibiotic ablation seems to improve rates of engraftment in other indications, and the antibiotic used for this purpose (oral vancomycin) may spare the beneficial pro-response bacteria based on its coverage [43]. However, the impact of antibiotic use must be carefully evaluated in the context of treatment with FMT and immunotherapy, and it is likely that more targeted approaches may be prudent in optimizing such regimens in the future.

Early preliminary data from five patients from the Israeli study ([NCT03353402](#)) were presented at the 2019 AACR meeting [44], demonstrating safety and efficacy of this approach in a subset of treated patients. In this trial, patients with metastatic melanoma were considered eligible for enrollment if they progressed on at least one line of anti-PD-1 therapy—with BRAF-V600E-positive patients also having progressed on BRAF-targeted therapy. Several FMT donors were used in this trial and were chosen on the basis of a durable (> 1 year) complete response to anti-PD-1 monotherapy and also passed all standard FMT safety screening. Microbiome modulation was performed via colonoscopy (single dose) and oral capsules (every 2 weeks with anti-PD-1 administration) after a preparative regimen using oral antibiotics and polyethylene glycol orally prior to colonoscopy. The trial is not fully accrued yet; however, early results demonstrate at least 3 responses of 10 treated patients (with one complete response and 2 partial responses observed). The treatment thus far has been well-tolerated in the group with no severe adverse events related to FMT and no

grade 2 or above immune-related adverse events. Microbiome analysis demonstrated a change in the microbiome of treated patients with increase in immune infiltrates in tumors of treated patients.

The Canadian group presented initial safety data from the first two patients treated on their clinical trial ([NCT03772899](#)) at The Society of Immunotherapy of Cancers (SITC) 34th Annual meeting in 2019 [45], with no AEs attributed to FMT and one patient with a mixed clinical response and one partial response, though of note this study is being conducted in PD1-naïve patients.

FMT is also being studied in the treatment of ICB-related colitis/diarrhea with two trials ([NCT03819296](#), [NCT04038919](#)) evaluating safety, toxicities, and rate of diarrhea resolution (Table 1). ICB-colitis is a common immune-related adverse event, especially with anti-CTLA-4-based therapy. The standard treatment is steroids and other immunosuppressive medications, each of which has its own potential side effects, and some patients have recurrent/refractory colitis despite these treatments. Wang et al. recently published results from the first reported case series of two patients with refractory ICB-associated colitis successfully treated with FMT from healthy donors [46]. Both patients reportedly had complete resolution of clinical symptoms following treatment with FMT, as well as marked endoscopic and histologic improvement. Building on this experience, prospective clinical trials of FMT for refractory colitis are now being conducted.

### Future of FMT: Influence on tumor microbiome

As treatment with ICB is being investigated and approved in more and more malignancies, there is a growing interest in investigating the effect of microbiota (and potential for microbiome modulation via FMT) in these cancers. Provocative recent data has further suggested that modulation of the gut microbiome may impact the intratumoral microbiome in gastrointestinal malignancies. Recent studies have demonstrated that, far from being sterile, there are intratumoral bacteria in the majority of pancreatic cancers (and not in adjacent normal pancreatic tissue) [47]. Studies in murine models have further suggested that this likely represents translocation of these bacteria from the gut [5]. In a recent *Cell* publication, long-term survivors of pancreatic adenocarcinoma were found to have distinct tumor microbiomes compared to short-term survivors, with an intratumoral microbiome signature highly predictive of survival [48]. Interestingly, FMT from humans to mice using fecal microbiome specimens from long-term vs short-term demonstrated that FMT could modulate not just the gut microbiome in the mice but also the tumor microbiome, tumor growth, and tumor immune infiltrate. This study provides a strong rationale to study the impact of FMT on tumor microbiome in patients.

Another malignancy where this is a strong rationale to examine the role of the intestinal (and tumor) microbiome and FMT is hepatocellular carcinoma. Studies have shown that the liver is exposed to intestinal microbiota through the portal vein which delivers gut-derived bacterial products or toxins, such as lipopolysaccharide and deoxycholic acid [49]. FMT has shown some initial promise in small studies in the treatment of chronic liver disease, including alcoholic hepatitis [50], hepatic encephalopathy [51], and viral hepatitis clearance

[52]. Given the recent approval of ICB in HCC, the potential of FMT in patients with hepatocellular carcinoma deserves investigation.

### Next Steps:

Valuable lessons will be gained from these initial trials of FMT in immunotherapy and will be used to further optimize this modality in the effort to improve therapeutic outcomes. Intensive characterization and analysis of the host and donor features predictive of benefit will be critical to efforts to further personalize microbiota modulation efforts. Early data has suggested both healthy donors and complete responder gut microbiota profiles differ in expression of pro-response signatures identified in retrospective cohorts suggesting that donor profiling may be an important consideration [53]. In studies that utilize multiple donors, it will thus be important to examine microbiota characteristics associated with better engraftment (though studies will be too small at this point to examine associations with therapeutic response). Similarly, though no studies at this point appear to be using baseline microbiota profile as an inclusion criteria, at least one study is stratifying patients based on previously identified pro-response microbiota signature (NCT03353402), and again, these early studies may suggest microbiota features to be used as selection criteria for future studies to enrich for patients most likely to benefit from FMT. Beyond donor and recipient microbiota characteristics, underlying genetic and immunological differences could influence both engraftment and downstream physiological and clinical outcomes. External factors may also be critical—medications can strongly influence the gut microbiota [54], and antibiotic use has been associated with impaired response to immunotherapy [15•]. Antibiotic ablation prior to FMT may be critical to allow engraftment of the donor microbiota; however, medication and antibiotic use (and relationship to microbiota profile) should be carefully tracked following FMT. Interestingly, many of the candidate pro-immunotherapy response bacteria have well-described dietary associations and known functions in the metabolism of specific nutrients such as fiber, a key prebiotic [18]. Diet is a key determinant of the gut microbiota, with nutrients ingested by the host providing the commensal microbes with substrates required for their proliferation and survival and the microbes in turn digesting nutrients otherwise indigestible to their hosts [55, 56]. The effects of pre- or post-FMT diet on engraftment has not been well-studied to date [57], but dietary assessment at baseline and after FMT would be a useful adjunct to understand this potential interaction which could plausibly inform future patient guidance on how to best sustain a donor microbiota. However, this also begs the question of how important maintenance of the donor microbiota is in this setting. Is an initial shift in the gut microbiota triggering an immune response sufficient or is sustainment of the transplanted gut microbiota necessary to sustain response? Longitudinal profiling of the fecal microbiota and integration with response and clinical variables from these early trials will provide rich data on these interactions.

### Conclusions:

There is compelling evidence from preclinical models and human cohorts that the gut microbiota shapes response to immunotherapy. FMT is a proven method of modulating the gut microbiota and is currently being investigated in multiple clinical trials in the setting of immunotherapy to either enhance response or treat toxicity. Key considerations in the design

of FMT interventions based on trials for other indications include patient selection, donor profile, timing, FMT modality, and post-FMT follow-up (Fig. 1). The results from these early studies, and analysis of correlative fecal, blood, and tissue biospecimens, will yield important insights into the mechanism whereby microbiome modulation might impact antitumor immunity, as well as help optimize interventional approaches.

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•• Of major importance

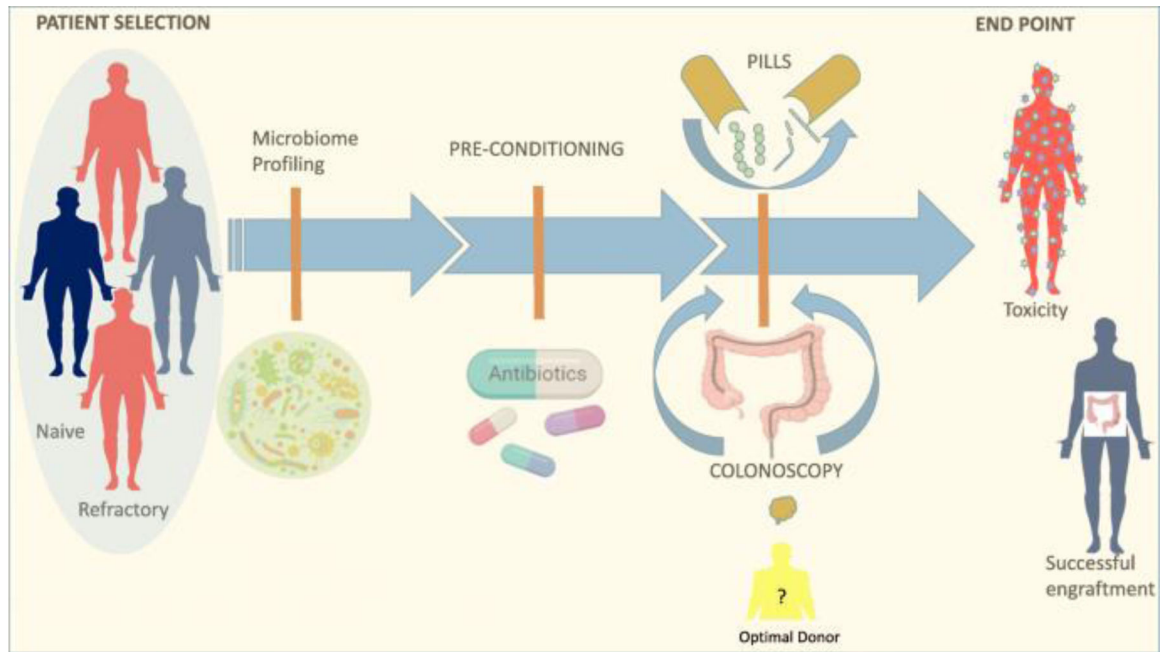
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**Figure 1.**

Fecal microbiota transplant to enhance immunotherapy in cancer patients: overview of the potential role of fecal microbiota transplant (FMT) as a therapeutic strategy to modulate the gut microbiome. Through FMT, donor microbiome is transferred to a recipient in the form of a stool suspension given either endoscopically or via pills. Key considerations in the design of FMT interventions include patient selection, donor profile, timing, FMT modality, and post-FMT follow-ups.

**Table 1.**

Trials of FMT in different cancer types

NCT Number	Patient population	Patients (n)	Intervention	Study Phase	Treatment Setting (PD1 naïve vs Refractory)	Randomized vs Single Arm	FMT Donor (Healthy vs ICB responder)	FMT Donor (Pooled vs single donor)	FMT Modality (Route of Administration)	FMT Frequency
<a href="#">NCT03353402</a>	Anti-PD-1 refractory melanoma	40	FMT+ Pembrolizumab/ Nivolumab	1	Refractory	Single arm	Responder	Pooled	Colonoscopy & Capsule	Not specified
<a href="#">NCT03341143</a>	Anti-PD-1 refractory melanoma	20	FMT + Pembrolizumab/ Nivolumab	2	Refractory (non-response at 12 weeks)	Single arm	Responder	Not Specified	Colonoscopy	Single dose
<a href="#">NCT03772899</a>	Metastatic melanoma	20	FMT + Pembrolizumab/ Nivolumab	1	Naive	Single arm	Healthy	Single	Capsule	Not specified
<a href="#">NCT03817125</a>	Metastatic melanoma	30	FMT + Nivolumab	1b	Naive	Randomized	Not specified	Single	Capsule	Capsule taken QOD x3 Then Qweekly x8
<a href="#">NCT04130763</a>	Anti-PD1-refractory luminal GI cancer	5	FMT + Pembrolizumab/ Nivolumab	1	Refractory	Single arm	Not specified	Not specified	Capsule	Capsule taken QOD x3 Then Qweekly x2
<a href="#">NCT04116775</a>	Metastatic castrate-resistant prostate cancer	32	FMT + Pembrolizumab + Enzalutamide + Androgen deprivation	2	Refractory CRPC patients initiated on Pembrolizumab + Enzalutamide. who fail to respond at 12 weeks	Single arm	Responder	Pooled	Colonoscopy	Once
<a href="#">NCT04056026</a>	Metastatic mesothelioma	1	FMT+ Pembrolizumab/ Nivolumab	1	Naïve	Single arm	Healthy	Pooled	Colonoscopy	Single dose
<b>FMT Directed</b>	<b>for Toxicity</b>									
<a href="#">NCT03819296</a>	Patients with melanoma or GU cancer who develop ICB-related colitis	100	FMT	1	ICI treated	Single arm	Healthy	Not specified	Colonoscopy	Not specified
<a href="#">NCT04038619</a>	Patients with GU cancer who develop ICB-related colitis	40	FMT + Loperamide	1	Prior ICI therapy	Single arm	Healthy	Pooled	Colonoscopy	Once, 15 to 30 minutes

PSA= Prostate specific antigen, RECIST= Response criteria in solid tumor, iRECIST= Immune-related response criteria in solid tumor, ORR= objective response rate, ICB= Immune checkpoint blockade, CTCAE= Common Terminology Criteria for Adverse Events version 5, CRCP= castrate-resistant prostate cancer, QOD= every other day, QWeekly= Every week, RCT= randomized control trial, FMT= fecal microbiota transplant.

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