S Table 1. Recent trials conducted to investigate the effects of probiotics on clinical outcomes of cancer patients

ClinicalTrials.gov Identifier	Location	Disease	Number of Participants	Intervention/Treatment	Duration	Outcome	Reference
NCT03358511	USA	Breast cancer	20	Primal Defense Ultra® Probiotic (*Saccharomyces boulardii*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *lactobacillus casei*, *Lactobacillus salivarius*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Bifidobacterium longum*, and *Lactobacillus paracasei*)	2-4 weeks	Recruiting subjects. This study examines if pre-operative probiotics will help the body's immune system react to breast cancer.	N/A
NCT03290651	Canada	Women at risk of breast cancer	40	*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14	90 days	Recruiting subjects. This study examines if probiotics reduce harmful bacteria, as well as inflammation, in the breast, and lower the chances of developing breast cancer. Changes in breast microbiota will be determined by nextgeneration sequencing.	N/A
NCT02819960	Slovakia	Colon cancer	100	PROBIO-FIX INUM® (*Lactobacillus rhamnosus* GG, *Bifidobacterium animalis subsp. lactis* BB-12)	6 weeks	Recruiting subjects. This trial evaluates potential of probiotics to prevent grade 3-4 diarrhea in patients treated with irinotecan.	N/A

NCT03705442	Croatia	Metastatic colorectal cancer	76	OMNi-BiOTiC® 10AAD (*Lactobacillus acidophilus*, *Lactobacillus acidophilus*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Enterococcus faecium*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Bifidobacterium longum*)	84 days	Recruiting subjects. This study examines if probiotics can be used as adjuvant therapy to reduce diarrhea in cancer patients receiving chemotherapy.	N/A
NCT03782428	Malaysia	Colon cancer	52	*Lactobacillus acidophilus*, *Lactobacillus lactis*, *Lactobacillus casei subsp*, *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Bifidobacterium infantis*	6 months	Postoperative probiotics reduced the level of proinflammatory cytokines, including TNF-α, IL-6, IL-10, IL-12, IL-17A, IL17C and IL-22, in patients.	Ref. (113)
NCT03072641	Sweden	Colon cancer	36	ProBion Clinica (*Bifidobacterium lactis* Bl-04, *Lactobacillus acidophilus* NCFM and inulin)	31±28 days	Probiotics increased level of butyrate-producing bacteria,including *Faecalibacterium* and *Clostridiales* spp in the tumor, nontumor mucosa and faecal microbiota of patients while reducing the level of *Fusobacterium* and *Peptostreptococcus* in the faecal microbiota of patients.	Ref. (106)
NCT01468779	Brazil	Colon cancer	91	*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Bifidobacterium* and fructooligosaccharide	19 days	Perioperative administration of symbiotics significantly reduced postoperative infection rates in patients.	Ref. (100)

NCT01609660	Brazil	Colon cancer	33	*Saccharomyces boulardii*	7 days	Preoperative probiotics downregulated proinflammatory cytokines IL-1β and IL-23A, as well as anti-inflammatory cytokine IL-10, in the intestinal colonic mucosa, with no statistical impact on postoperative infection rates.	Ref. (105)
NCT01479907	Greece	Colon cancer	75	Synbiotic Forte™ (*Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei ssp. paracasei*, *Lactobacillus plantarum*, b-glucan, inulin, pectin and resistant starch)	15 days	Postoperative synbiotics ameliorated postcolectomy gastrointestinal function and diarrhea in patients.	Ref. (101)
NCT01410955	Slovakia	Colon cancer	46	Colon Dophilus™	12 weeks	Probiotics reduced the incidence of irinotecaninduced diarrhea in colorectal cancer patients.	Ref. (102)
NCT01790035	USA	Gastrointestinal, abdominal, or pelvic cancer	23	*Lactobacillus rhamnosus* GG (LGG)	LGG administered at least 3 days prior, during, and 2 weeks following radiation treatment	*In vitro* and *in vivo models* were used to identify the mechanism of LGG radioprotection. Preliminary result suggested LGG releases radioprotective lipoteichoic acid (LTA), which primes the protection of epithelial stem cells by triggering an immune signaling cascade involving macrophages and PGE2 secreting mesenchymal stem cells.	Ref. (112)
NCT02654652	Brazil	Head and neck cancer	40	LactoFos (*Lactobacillus paracasei* LPC-31, *L. rhamnosus* HN00l, *L. acidophilus* NCFM, *and Bifidobacterium lactis* HN019 plus fructo-oligosaccharides)	5-7 days	Postoperative symbiotics did not affect intestinal function and postoperative outcomes of head and neck surgical patients.	Ref. (104)

NCT03829111	USA	Kidney cancer	30	*Clostridium butyricum* CBM 588	Treatment every 21 days for 4 cycles; treatment every 28 days beginning cycle 5	Recruiting subjects. This phase I trial examines if CBM 588 and immunotherapy (nivolumab and ipilimumab) exert a synergistic effect on patients with advanced kidney cancer.	N/A
NCT02771470	China	Lung cancer	41	*Clostridium butyricum*	3 weeks	*Clostridium butyricum* promoted beneficial genera, including *Blautia*, *Clostridium*, *Faecalibacterium* and *Lactobacillus*, while decreasing the abundance of pathogenic bacteria *Escherichia-Shigella*. *C butyricum* reduced not only chemotherapyinduced diarrhea but also systemic inflammatory response in patients. However, there was no significant alteration in lymphocyte subsets, immunoglobulin and albumin expression levels.	Ref. (103)
NCT03574051	China	Thyroid cancer	30	*Bifidobacterium infantis*, *Lactobacillus acidophilus* and *Enterococcus faecalis*	30 days	Recruiting subjects. This study examine if probiotics prevent adverse effects of hypothyroidism and improve side effects caused by 131I treatment.	N/A

Legend for S Table 1: Several trials report an improved clinical outcome in cancer patients receiving probiotics whereas other trials deny the clinical benefits of probiotics. Probiotics have been shown to modulate the production of pro-inflammatory cytokines, to lower the incidence of chemotherapy-induced diarrhea, as well as postoperative infection, and to protect the intestine from radiation by releasing lipoteichoic acid. On the contrary, strands of evidence indicate that probiotic treatment does not improve intestinal function of cancer patients. Patients receiving the probiotics and those receiving the placebo had similar rate of postoperative infection and displayed comparable level of inflammatory markers and diamine oxidase, an indicator of gut permeability. The majority of the trials assesses the effect of probiotics on cancer treatment-induced toxicities rather than their direct impact on mediating the efficacy of anti-cancer drugs. Ongoing and future trials that examine the interactions between probiotics and cancer therapy will predict patient's response to treatment and provide new insights into personalized medicine tailored according to patient's microbial profile.

S Table 2. Limitations of current microbiome research and efforts to address these concerns

Manipulating the microbiome to prevent/ treat cancer	Current state	Ongoing work and future trend
Conception	• The genomic content and functions of bacterial strains can be changed during the lifespan of individuals by forces of evolution, including host-associated factors, environmental factors or microbe-microbe interactions • Microbial profiles are determined by 16S rRNA gene amplicon and metagenomic sequencing, with limited resolution of bacterial species at strainlevel	• Efforts are directed to identify bacterial strains that play a significant role in cancer, and to elucidate the associated mechanisms • Functions of bacterial strains should be examined in the context of the entire microbiota as bacteria interact with one another, as well as the host and other microorganisms, in this complex ecosystem • Findings are to be validated extensively with preclinical models and large-scale, cross-cohort clinical trials
Research approach	• Microbial profiles are determined by 16S rRNA gene amplicon and metagenomic analysis of stool and mucosa samples of cancer patients • Inferred functions of microbes based on association analysis • Correlation-based	• The role of bacteria in cancer should be examined by integrative analysis involving host genome/metabolome/ immune profile, oral/gut/faecal microbiome, fungal and viral communities • The role of exogenous factors, such as diet and medications should be included to elucidate the complex interactions between the host, the microbes, and anti-cancer drugs • Causation/Mechanism-based
Clinical translation	• Candidate bacteria related to cancer diagnosis or prognosis can potentially be used as novel biomarkers for cancer • Modulating the microbiota via FMT improves the outcome of patients with *Clostridium difficile* infection, inflammatory bowel disease or other diseases • Probiotic treatment improves cancer treatment-induced toxicities, including diarrhea and infection	• Precision therapy to be developed based on host and microbiome characteristics • Diet modification or probiotic/prebiotic supplementation may augment the anti-tumor effect of chemotherapy/ immunotherapy and improve patient outcomes

Safety	• The majority of the clinical studies on probiotics shares limitations including small sample size, short duration of treatment, and lack of follow-up to examine the long-term effect of probiotics on patients. Detailed mechanisms to elucidate the functions of probiotics in cancer patients remain elusive • Reports of long- term safety outcome of probiotics in cancer patients are lacking	• Nanotechnologies have been exploited in drug delivery to target cancer-associated bacterial species, minimizing the perturbation to commensal bacteria • Large-scale, cross-cohort clinical studies are required to examine the long-term effect and safety of FMT, probiotics or other therapies that modulate the microbiota in cancer patients

Legend for S Table 2: Various aspects of microbiome research and their implications in cancer are examined. With advances in sequencing technology and development of powerful computational tools, the research paradigm has shifted from association-based approach to mechanism-based approach. Unravelling causal links between bacteria and cancer has become an area of active research. In an attempt to generate a holistic view of the microbiota-host interaction in the context of cancer, researchers examine the interactions between the gut microbiome, the host, anti-cancer drugs and other exogenous factors through integrative analysis of multiple types of sequencing data. Regardless of the previous findings, it remains challenging to assess the clinical benefits of FMT/probiotics in cancer patients. The majority of the clinical trials on probiotics shares limitations, such as small sample size, short duration of treatment and lack of follow-up to examine the long-term effect of probiotics on patients. As such, well-designed studies that explore the direct interactions between the microbiota, tumor cells and anti-cancer drugs are critical for the evaluation of FMT/probiotic treatment in cancer patient. FMT, fecal microbiota transplant.