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CURRENT OPINION

Pitfalls and novel experimental approaches to optimize microbial interventions for chemotherapy-induced gastrointestinal mucositis

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Purpose of review
There is a growing number of studies implicating gut dysbiosis in mucositis development. However, few studies have shed light on the causal relationship limiting translational potential. Here, we detail the key supportive evidence for microbial involvement, candidate mechanisms by which the microbiome may contribute to mucositis and emerging approaches to model host–microbe interactions with clinical relevance and translational potential.

Recent findings
Synthesis of existing clinical data demonstrate that modulating the microbiome drastically alters the development and severity of mucositis, providing a strong rationale for its involvement. Review of the literature revealed potential microbiome-dependent mechanisms of mucosal injury including altered drug metabolism, bile acid synthesis and regulation of the intestinal barrier. Current studies are limited in their mechanistic insight due to cross-sectional and would benefit from longitudinal analyses and baseline phenotyping.

Summary
The causative role of the microbiome in mucositis development remains unclear. Future studies must adopt comprehensive microbial analyses with functional assessment, and utilize emerging ex-vivo models to interrogate host–microbe interactions in mucositis.

Keywords
chemotherapy-induced gut toxicity, microbiome, microbiota, mucositis, probiotics, the human oxygen-bacteria anaerobic

INTRODUCTION

Cytotoxic chemo-radiation is notoriously nonselectively, resulting in off-target toxicity to a range of mucosal surfaces, clinically termed mucositis [1]. The alimentary tract, mouth to anus, is highly susceptible to mucositis due to its high proliferative turnover, strong immunological properties and intestinal excretion of some chemotherapeutic drugs [2]. While affecting the oral and gastrointestinal tract (GIT) to a comparable degree, oral mucositis has been studied in greater detail reflecting the ease at which the oral cavity can be assessed compared with the GIT, and the hesitancy of many patients to discuss gastrointestinal symptoms. This has resulted in significant under reporting of gastrointestinal mucositis (GI-M) and a subsequent dearth of GI-M focused research.

GI-M is a ubiquitous complication of anticancer therapy, with pelvic radiotherapy and high-dose chemotherapy associated with exceptionally high incidence rates. Clinically, GI-M manifests as diarrhea with associated abdominal pain and rectal

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KEY POINTS

- It has been shown that gastrointestinal mucositis (GI-M) drastically alters the composition of the gut microbiota with these changes overlapping with the development of severe GI-M.
- Studies attempting to dissect causative mechanisms have revealed some important aspects, however given the highly heterogenous landscape of GI-M, many remain reliant on cross section study designs and oversimplified experimental approaches.
- Candidate mechanisms for host microbe involvement include microbial impact on drug metabolism, bile acid synthesis and barrier function.
- Clinical phenomena should be deeply interrogated using sophisticated ex-vivo models to determine causation.
- Organoids, gut-on-a-chip and the human oxygen-bacteria anaerobic models provide an excellent alternative to study host–microbe interactions.

bleeding, requiring intensive supportive care and impacting patient quality of life. Mucosal barrier breakdown, including direct apoptosis and tight junction disruption, are through to drive diarrhea by impairing luminal absorption and leak-flux mechanisms [3]. Furthermore, these changes are considered critical initiating factors in the development of lethal secondary complications including blood stream infection and graft-versus-host disease (GvHD) [4]. Despite this, GI-M continues to be managed with therapeutic loperamide, which serves only to slow gastrointestinal transit and fails to address key mechanisms related to GI-M pathobiology [5]. This highlights the need to carefully characterise the factors that contribute to mucosal barrier injury to identify novel methods of intervention.

A growing body of research now supports the role of host–microbe interactions in the development of GI-M [6]. The development in DNA technology has showed that chemotherapeutic agents detrimentally affect the composition of the microbiome, either directly or indirectly, with losses in overall microbial diversity and a compositional shift toward a Gram-negative dominated pathogenic enterotype [7,8]. Most importantly, it has been suggested that these changes overlap with the development of severe GI-M and may therefore be involved in its symptomology [9]. Even though the scientific community firmly accepts disruption of the gut microbiota in GI-M, like many diseases associated with microbial dysbiosis, unraveling the causal relationship remains a daunting and hazardous task that to date has not been achieved. Studies attempting to dissect causative mechanisms have revealed some important aspects, however given the highly heterogenous landscape of GI-M, many remain reliant on cross section study designs and oversimplified experimental approaches. This hinders efforts to make significant advances in our fundamental understanding and impairs the development of novel microbial interventions.

In this review, we detail the key mechanisms by which the microbiome is likely to causally contribute to mucositis development, highlighting pitfalls in current experimental approaches and describing emerging approaches to model host–microbe interactions with clinical relevance and translational potential in the provision of supportive care.

MODULATING THE MICROBIOME CHANGES THE COURSE OF GASTROINTESTINAL MUCOSITIS

The most robust evidence supporting a causative role of the microbiome in GI-M development and symptomology comes from modulating its composition. An example of this causation is the use of antibiotics. Antibiotics are well recognized to negatively impact the composition of the microbiome, decreasing diversity and compositional changes. For example, broad spectrum antibiotics have been shown to increase relative abundance of Bacteroides, *Clostridium* cluster XI and *Escherichia coli* while decreasing commensal bifidobacteria and Clostridium cluster XI [10,11]. Antibiotics are largely attributed to poorer treatment outcomes in a variety of clinical scenarios relating to mucosal injury. For example, broad spectrum antibiotics increase the incidence and mortality of GvHD. This is hypothesised to occur via aggravation of acute mucosal injury, a key initiating factor of GvHD [12]. This is supported by preclinical data from our laboratory in which antibiotic-induced dysbiosis increased mucosal injury, impaired recovery and increased mortality [13]. This evidence also reiterates the long-standing belief that previous mucosal injury (from previous cycles of chemotherapy) predisposes to GI-M development, a phenomenon that may be driven by residual deficiencies in the microbiome.

It is important to note that these findings differ to those in which gut deleting protocols or germ-free mice are used. In these cases, the absolute absence of the gut microbiota results in protection, highlighting the importance of a stable and diverse microbiome. In a study performed by Pedroso *et al.*, the differences in the phenotypes between germ-free and conventional mice in the development of irinotecan treatment

were studied. Results showed that germ-free mice presence a resistance in the development of intestinal damage due after irinotecan administration [14]. Surprisingly, conventionalization of germ-free mice reversed the resistance phenotype previously observed in this model. The authors also confirmed the role of β-glucuronidase bacteria in the induction of mucositis. In fact, it was observed that the mono-association of germ-free with β-glucuronidase-producing bacteria increased permeability after irinotecan treatment [14].

Similarly, modulating the microbiome with pre and probiotics has been linked with less GI-M, although the results are conflicting and variable. The prebiotics fructose polysaccharide, inulin and short-chain fructo-oligosaccharide are commonly investigated in gastrointestinal disorders, largely inflammatory bowel disease, due to their ability to increase the amount of bifidobacteria, *Roseburia*, *Ruminococcaceae* and *Eubacterium* [15,16]. Despite emerging benefits in other benign inflammatory conditions of the gut, these have not been investigated in mucositis. However, the prebiotic properties of vitamins has been investigated, with ascorbic acid (vitamin C) recently shown to improve outcomes in a preclinical model of 5-fluorouracil (5-FU) mucositis [17]. Similarly, administration of the probiotic *Bifidobacterium infantis* in rats has resulted in higher body weight and villus height, reduced expression of Nuclear Factor- kappa B and increased production of IL-10 and reduced diarrhea in 5-FU-induced mucositis rats [18,19]. Commercialized probiotics such as VSL#3 have also shown prophylactic efficacy in reducing diarrhea following irinotecan administration [20]. Clinically, the efficacy of probiotics has also been demonstrated with several studies showing independent benefits of certain stains in mucositis/diarrhea prevention prompting the 2014 Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology guideline [5,21,22].

Although studies demonstrate benefits particularly in the oncological setting, there remains no wide-reaching recommendation for probiotics in the prevention of GI-M. This likely reflects the heterogeneity in studies included and variations in probiotic formulations [23▪▪]. Nonetheless, these studies show that modulating the composition of the gut microbiota can drastically alter the course of GI-M. Moreover, it also suggests that distinct baseline gut microbiota enterotypes may be associated with different toxicity responses. For example, people that go on to develop severe symptoms have a unique and identifiable microbial phenotypes at baseline that differ to those that do not develop those symptoms [24–26]. Taken together, these findings imply that baseline microbiome composition is critical in shaping toxicity outcomes, thus suggesting that the microbiome is causally involved in GI-M pathobiology.

CANDIDATE MICROBIOME-DEPENDENT MECHANISMS OF GASTROINTESTINAL MUCOSITIS

Preclinical and clinical studies now demonstrate a link between the composition of the gut microbiota and GI-M development [8,9,20,27–29,30▪▪]. Whilst these findings have undoubtedly shed new light into the pathogenesis of mucositis, our current understanding of the causative role that the microbiome plays in symptom development remains unclear. Fundamentally, the microbiome has the ability to modulate various aspects of mucositis pathogenesis via its intimate and bidirectional communication with the mucosal immune system, as elegantly described by Secombe *et al.* [30▪▪]. However, there remain several other candidate mechanisms by which the microbiome is likely to contribute to GI-M, namely via its impact on drug metabolism, mucosal barrier function and bile acid synthesis.

Drug metabolism

The most robust evidence mechanistically linking the microbiome to mucositis is its influence on chemotherapy drug metabolism. Irinotecan (CPT-11) is a prodrug that is converted by carboxylesterase enzymes to SN-38, a potent topoisomerase I inhibitor that is over 1000-fold more toxic than its prodrug predecessor. Metabolically, SN-38 is conjugated in the liver to SN-38 glucuronide (SN-38G), its inactive metabolite that is secreted into the gastrointestinal tract via bile for eventual excretion [31,32]. However, as SN-38G passes through the GIT, it acts as substrate for bacterial β-glucuronidases resulting in deconjungation of SN-38G to its active metabolite. This results in direct and extreme exposure of the gastrointestinal mucosa to SN-38 resulting in profound mucosal injury and the development of CPT-11-induced diarrhea [33]. In addition to the direct cytotoxic damage caused by SN-38, this active metabolite also acts as a ligand for the Toll-like receptor four (TLR4) coreceptor, MD2, resulting in TLR4-dependent immune activation and the initiation of an intense inflammatory response. Again, this is nicely demonstrated by Pedroso *et al.* [14], with germ-free mice protected from irinotecan-induced mucositis compared with wild-type. Authors concluded that this was due to germ-free animals being unable to reactive SN-38G, thus

eliminating its direct toxic effects in the intestinal lumen.

Comparable evidence also exists detailing the metabolic impact of the microbiome on other chemotherapy drugs. In a study performed by Lehouritis *et al.*, the effects of *E. coli* and *Listeria welshimeri* on the efficacy of a set of chemotherapeutic drugs were tested. Authors concluded that the cytotoxicity of cladribine, vidarabine and gemcitabine were decreased by bacteria. These alterations were most probably via enzymatic modifications which demonstrated the interaction between internal bacteria and drug therapy [34].

Barrier function

A simple mechanism used for antimicrobial protection is the presence of a two-tiered mucus layer which maintains the integrity of the intestinal microbiota and contributes to overall barrier function [35,36]. Mucins have been shown to have several beneficial properties including protection against bacterial translocation and might also serve as sources of carbohydrates and peptides [35,36], and are widely disrupted following chemotherapy [37,38▪]. Using a gnotobiotic model in which animals were colonized with a synthetic human gut microbiota composed by commensal bacteria, Desai *et al.,* investigated the link between the gut microbiota and colonic mucus barrier. Results show that, in cases of dysbiosis, a deficiency in nutrients leads to an increase in the population of mucin-depredating bacteria. These bacteria will in turn use mucin as a nutrient which results in barrier disruption. These results clearly demonstrate the crucial role of the gut microbiota on mucus integrity, and as such microbe-dependent mucin degradation is a clear candidate mechanism by which the microbiome causally contributes to mucositis [39].

In addition to the apical mucus layer, mucosal barrier integrity is maintained by intercellular junction complexes, in particular tight junctions. Tight junctions are highly dynamic structures, able to undergo rapid and reversible changes in response to a variety of physiological and pathological cues [40]. Changes in the molecular integrity and functional capacity of tight junctions is well described in the setting of GI-M, with both and clinical evidence demonstrating cytoplasmic translocation and downregulation of key tight junction proteins including claudin-1, occludin and zonular occludens-1 [41,42]. In a study by Feng *et al.*, antibiotic-treated mice showed severe alterations in the composition of the gut microbiota with paralleled changes in barrier integrity. Particularly, authors suggest that variations in Firmicutes and Bacteroides are responsible for the destruction of the intestinal barrier [43].

Bile acid metabolism

Bile acids are a family of steroid acids synthesized from cholesterol in the liver and secreted in the lumen of the intestine [44]. Intestinal microbes such as *Bacteroides intestinalis*, *Bacteroides fragilis* and *E. coli* have the necessary enzymes to convert bile acids into deoxycholic and lithotomic acid in the human colon [36,44]. When the composition of the microbiome is compromised, the ratio of primary/secondary bile acids is increased. A study by Fang *et al.* [45], suggests that CPT-11-induced metabolic disorders of bile acids potentially supress the production of IL-10, which in turns aggravates mucosal barrier hyper-permeability [45]. In-line with these anecdotal observations and mechanistic hypotheses, bile acid sequestrant colesevelam was shown to reduce diarrhea in a model of neratinib-induced GI-M [46]. Significantly, diarrhea induced by neratinib was unrelated to serum neratinib levels suggesting that modulation of secondary inflammatory processes (by bile acid modulation) may be more important in determining the clinical impact of toxicity than the effects of direct cytotoxicity.

FUTURE DIRECTIONS FOR EXPLOITING HOST–MICROBE INTERACTIONS IN MUCOSITIS

Despite anecdotal evidence supporting the role for the microbiome in mucositis development, it remains a significant challenge to identify causative and targetable mechanisms. Clinically, the heterogeneity in oncology cohorts, confounding variables and paralleled antibiotic use are significant obstacles. In-vitro models often lack the level of sophistication required to model the intimate and bidirectional pathways that join the host, immune system and microbes. As such, sensibly designed cohort studies which are integrated with novel ex-vivo platforms are critical to advance our understanding of the microbiome in GI-M.

Without an appreciation for the exact microbial signatures associated with GI-M, it is a difficult task to manipulate the microbiome in such a way that induces clinically relevant results. As such, we recommend that studies focus on comprehensively and longitudinally characterizing the microbiome, across various forms of cancer therapy (Fig. 1). This can be achievable by the implementation of biobanks for the high-frequency collection of stool samples before and during therapy. This will enable the identification of unique microbial factors that

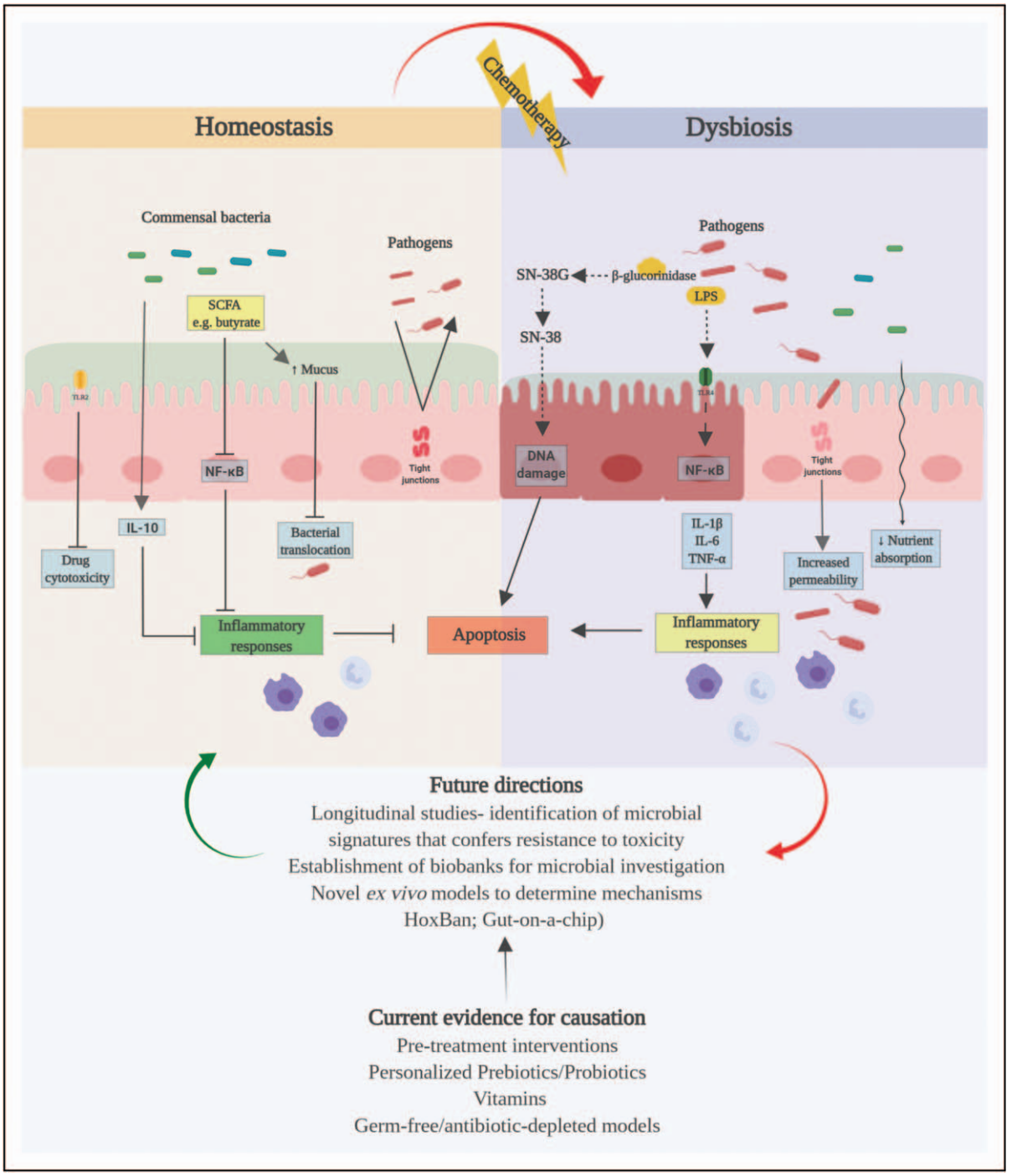

FIGURE 1. Schematic outlining mechanistic contribution of dysbiosis on pathobiology of mucositis. In a state of homeostasis, commensal bacteria are responsible for several functions, including maintenance of tight junctions and intestinal barrier function, promoting immune tolerance and stimulating mucus production, which ultimately prevent potentially harmful organisms from damaging the mucosa. Binding of commensal bacteria to Toll-like receptor's present on epithelial cells results in suppression of the Nuclear factor-kappa B pathway and consequent inhibition of proinflammatory production. Gut homeostasis can be disturbed by chemotherapeutic drugs such as irinotecan, methotrexate and 5-FU. Lipopolysaccharide produced by Gram-negative bacteria such as *Escherichia coli* activate the NF-κB pathway, resulting in exacerbated inflammation and consequently apoptosis. Reduced permeability also allows the entrance of pathogenic bacteria which aggravate the inflammatory state in the gut. Dysbiosis resulting in increased proteobacteria is also associated with increased β-glucuronidase production, which serves to amplify irinotecan reactivation and disrupt mucus production. Characterizing the dynamic shifts in the microbiota relative to baseline is critical in identifying appropriate microbial targets for therapeutic intervention design. These should be underpinned by novel ex-vivo models to dissect causative mechanisms.

Table 1. Summary of the advantages/disadvantages of the different approaches to understand the contribution of the microbiome in gastrointestinal mucositis

Approach	Advantage	Disadvantage	References
Antibiotic-depleted mice	Low costs of maintenance Applicability to any genotype No specialized equipment is necessary	Difficult to control the number and composition of the gut microbiota Promotion of fungal outgrowth due to selection for resistant bacteria	[51–53]
Germ-free/ gnotobiotic mice	Bacteria free in all tissues Exclusive colonization with defined microbes	Maintenance costs Specialized equipment and training are needed Developmental defects	[14,51,54]
Gut-on-a-chip	Controlled study of host–microbial interactions All the dynamic physical and functional features of the human intestine Ability to integrate different sensors	Absence of an immune system Costs of maintenance	[55]
3D-organoids	3D architecture of the tissue culture Possibility to study different diseases	Ability to propagate for a long time Challenging to culture Absence of an immune system	[56,57]
Prebiotics	Stimulation of mucosal and immune responses Demonstrated to increase the amount of bifidobacteia, *Roseburia*, *Ruminococcaceae* and *Eubacterium*	Not assessed in the setting of GI-M Not all prebiotics have resulted in clinical improvements	[15,16]
Probiotics	Promotion of mucus production Modulation of epithelial barrier function Activation of immune responses	Inconsistent results Fail to improve cancer-therapy-induced diarrhea	[7,20,23▪▪]

GI-M, gastrointestinal mucositis.

may be critical in shaping an individual response to treatment as well as the dynamic changes that occur throughout mucositis development. Bio banking efforts must be paired with the comprehensive collection of outcome measures, including objective biomarkers of GI-M, clinician reported outcomes and patient reported outcomes [47]. Unique response phenotypes can then be interrogated in an ex-vivo manner to understand the microbial contribution to GI-M development, thus allowing cause and effect to be dissected.

Although financially burdensome, germ-free and antibiotic-depleted mice are powerful systems of preclinical models aimed at dissecting causative roles [14]. However, these models come with certain limitations (Table 1). For example, depletion of the gut microbiota by antibiotics use in animals has revealed to be challenging due to inability to control the exact composition and number of organisms that remain in the gut, with expansion of antibiotic-resistant microbes a significant problem. Germ-free mice also pose significant limitations largely related to their lack of oral tolerance and hypersensitivity to microbial products [48]. Despite these limitations, gnotobiotic mice, which are germ-free mice colonized with selected known populations of bacteria, have shown success in the field of Inflammatory Bowel Disease and oncology, with particular success in understanding individual response to immunotherapies [49,50].

Alternative ex-vivo models have also been developed to better study host–microbe interactions. The human oxygen-Bacteria anaerobic coculturing system, developed in our lab by Sadabad *et al.*, is a novel approach that allows researchers to analyze cell growth, transcriptome and exo-metabolome of cocultured cells. This approach allows the study of host–microbiome interactions, particularly the investigation of anaerobic bacteria in the gut under oxidative stress conditions [51]. Other innovating systems such as gut-on-a-chip and three-dimensional organoid models have gained relevance in the field of the gut microbiome [52–54]. The gut-on-a-chip device for instance provides a controlled study of host–microbial interactions with all the dynamic physical and functional features of the human intestine [52]. Organoids have been successfully generated from different regions of the GIT. Although challenging to culture, they offer several advantages including the ability to propagate for a long time and the possibility to culture both tissue and microbes from an individual patient, and evaluate their unique response(s). The use of organoids colonized with microbes, and cocultured with

immune cells would also enable more robust interrogation of host–microbe interactions and their relevance to mucosal inflammation (Table 1).

It is becoming increasingly evident that an individual's unique pretreatment microbiome may be critical in determining their response to treatment, both in terms of its efficacy and toxicity [24,59▪]. This hypothesis is supported by a growing body of research in which distinct differences are observed in the pretreatment microbiome of people that go on to develop severe mucositis compared with those that do not. For example, Esfahani *et al.* [60] demonstrated distinct olfactory signatures, detected using an e-nose, of pretreatment stool samples in patients receiving pelvic radiotherapy. Similar results have been shown in patients with malignant melanoma treated with Programmed cell death-1 checkpoint inhibitors in which the presence of bifidobacteria and *Clostridiales* enabled a more efficient response to PD-1 blockade. These results highlight the importance of the pretreatment microbiome in driving treatment response, and thus demonstrate its potential in risk-prediction strategies, as well as risk mitigation approaches.

Regardless their limitations, these innovative systems could provide us with crucial information on host–microbe interactions. Unraveling this interactions will help us to dissect the causation mechanisms therefore guiding us to novel approaches to prevent GI-M (Fig. 1).

CONCLUSION

Several studies have been reporting the crucial role of the gut microbiota in the development of mucositis [9,35]. Indeed, both preclinical and clinical studies show that anticancer treatment is associated with a decrease in microbial diversity and a decrease in the number of anaerobic bacteria [9,35]. Furthermore, this decrease usually coincided with the development of severe mucositis. We now need to adequately characterise the microbial populations unique to different chemotherapeutic agents, to design and develop the ideal microbiota protectant (Fig. 1). Once the most favorable microbiota composition for each clinical condition has been identified, the next challenge will be how to modify the patient's microbiota. The resilience and stability of the gut microbiota and its responsiveness to physiological, pathological and environmental changes are characteristics that would enable us to use the microbiota composition as a biomarker, a diagnostic tool and possibly a therapeutic target. However, we believe that a preinterventional characterization of the gut microbiota will help us to develop sophisticated approaches to reduce mucositis, thus offering a better quality of life to cancer patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Sonis ST. Pathobiology of mucositis. Semin Oncol Nurs 2004; 20:11–15.
2. Sonis ST, Elting LS, Keefe D, *et al.* Perspectives on cancer therapy-induced mucosal injury. Cancer 2004; 100(S9):1995–2025.
3. Kwon Y. Mechanism-based management for mucositis: option for treating side effects without compromising the efficacy of cancer therapy. Onco Targets Ther 2016; 9:2007–2016.
4. Naymagon S, Naymagon L, Wong SY, *et al.* Acute graft-versus-host disease of the gut: considerations for the gastroenterologist. Nat Rev Gastroenterol Hepatol 2017; 14:711–726.
5. Lalla RV, Bowen J, Barasch A, *et al.* MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. Cancer 2014; 120:1453–1461.
6. Bowen J, Al-Dasooqi N, Bossi P, *et al.* The pathogenesis of mucositis: updated perspectives and emerging targets. Support Care Cancer 2019; 27:4023–4033.
7. Touchefeu Y, Montassier E, Nieman K, *et al.* Systematic review: the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis – current evidence and potential clinical applications. Aliment Pharmacol Ther 2014; 40:409–421.
8. van Vliet MJ, Harmsen HJM, de Bont ESJM, Tissing WJE. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS Pathog 2010; 6:1–7.
9. Fijlstra M, Ferdous M, Koning AM, *et al.* Substantial decreases in the number and diversity of microbiota during chemotherapy-induced gastrointestinal mucositis in a rat model. Support Care Cancer 2015; 23: 1513–1522.
10. Stringer AM, Al-Dasooqi N, Bowen JM, *et al.* Biomarkers of chemotherapy-induced diarrhoea: a clinical study of intestinal microbiome alterations, inflammation and circulating matrix metalloproteinases. Support Care Cancer 2013; 21:1843–1852.
11. Zwielehner J, Lassl C, Hippe B, *et al.* Changes in human fecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting. PLoS One 2011; 6:e28654.
12. Nishi K, Kanda J, Hishizawa M, *et al.* Impact of the use and type of antibiotics on acute graft-versus-host disease. Biol Blood Marrow Transplant 2018; 24:2178–2183.
13. Dc W. Abstracts of the MASCC/ISOO 2017 annual meeting. Support Care Cancer 2017; 25:21–266.
14. Pedroso SHSP, Vieira AT, Bastos RW, *et al.* Evaluation of mucositis induced by irinotecan after microbial colonization in germ-free mice. Microbiology 2015; 161:1950–1960.
15. Gori A, Rizzardini G, van't Land B, *et al.* Specific prebiotics modulate gut microbiota and immune activation in HAART-naive HIV-infected adults: results of the 'cOPA' pilot randomized trial. Mucosal Immunol 2011; 4:554–563.
16. Langlands SJ, Hopkins MJ, Coleman N, Cummings JH. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. Gut 2004; 53:1610–1616.
17. Al-Asmari AK, Khan AQ, Al-Qasim AM, Al-Yousef Y. Ascorbic acid attenuates antineoplastic drug 5-fluorouracil induced gastrointestinal toxicity in rats by modulating the expression of inflammatory mediators. Toxicol Rep 2015; 2:908–916.

18. Yuan KT, Yu HL, Feng WD, *et al.* Bifidobacterium infantis has a beneficial effect on 5-fluorouracil-induced intestinal mucositis in rats. Benef Microbes 2015; 6:113–118.

19. Mi H, Dong Y, Zhang B, *et al.* Bifidobacterium infantis ameliorates chemotherapy-induced intestinal mucositis via regulating T cell immunity in colorectal cancer rats. Cell Physiol Biochem 2017; 42:2330–2341.

20. Bowen JM, Stringer AM, Gibson RJ, *et al.* VSL#3 probiotic treatment reduces chemotherapy-induced diarrhoea and weight loss. Cancer Biol Ther 2007; 6:1445–1450.

21. Blanarova C, Galovicova A, Petrasova D. Use of probiotics for prevention of radiation-induced diarrhea. Bratislava Med J 2009; 110:98–104.

22. Mego M, Chovanec J, Vochyanova-Andrezalova I, *et al.* Prevention of irinotecan induced diarrhea by probiotics: a randomized double blind, placebo controlled pilot study. Complement Ther Med 2015; 23:356–362.

23. ■■ Wardill HR, Van Sebille YZA, Ciorba MA, Bowen JM, *et al.* Prophylactic probiotics for cancer therapy-induced diarrhoea: a meta-analysis. Curr Opin Supportive Palliative Care 2018; 12:187–197.

Recent review providing insight on the efficacy of probiotics in the setting of mucositis.

24. Montassier E, Al-Ghalith GA, Ward T, *et al.* Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. Genome Med 2016; 8:1–11.

25. Lavelle A, Hoffmann TW, Pham HP, *et al.* Baseline microbiota composition modulates antibiotic-mediated effects on the gut microbiota and host. Microbiome 2019; 7:1–13.

26. Chaput N, Lepage P, Coutzac C, *et al.* Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. Ann Oncol 2017; 28:1368–1379.

27. Li HL, Lu L, Wang XS, *et al.* Alteration of gut microbiota and inflammatory cytokine/chemokine profiles in 5-fluorouracil induced intestinal mucositis. Front Cell Infect Microbiol 2017; 7:1–14.

28. Lin XB, Dieleman LA, Ketabi A, *et al.* Irinotecan (CPT-11) chemotherapy alters intestinal microbiota in tumour bearing rats. PLoS One 2012; 7:3–10.

29. Stringer AM, Gibson RJ, Logan RM, *et al.* Faecal microflora and β-glucuronidase expression are altered in an irinotecan-induced diarrhoea model in rats. Cancer Biol Ther 2008; 7:1919–1925.

30. ■■ Secombe K, Coller J, Gibson RJ, Wardill HR. The bidirectional interaction of the gut microbiome and the innate immune system: implications for chemotherapy-induced gastrointestinal toxicity. Int J Cancer 2019; 144:2365–2376.

Review providing evidence for the role of the innate immunity in the setting of chemotherapy-induced gastrointestinal toxicity.

31. Wardill HR, Gibson RJ, Van Sebille YZA, *et al.* Irinotecan-induced gastrointestinal dysfunction and pain are mediated by common TLR4-dependent mechanisms. Mol Cancer Ther 2016; 15:1376–1386.

32. Swanson HI. Special section on drug metabolism and the microbiome – commentary drug metabolism by the host and gut microbiota: a partnership or rivalry? Drug Metab Dispos 2015; 43:1499–1504.

33. Panebianco C, Andriulli A, Pazienza V. Pharmacomicrobiomics: exploiting the drug-microbiota interactions in anticancer therapies. Microbiome 2018; 6:92.

34. Lehouritis P, Cummins J, Stanton M, *et al.* Local bacteria affect the efficacy of chemotherapeutic drugs. Sci Rep 2015; 5:1–12.

35. Stringer AM, Gibson RJ, Logan RM, *et al.* Gastrointestinal microflora and mucins may play a critical role in the development of 5-fluorouracil-induced gastrointestinal mucositis. Exp Biol Med 2009; 234:430–441.

36. Jandhyala SM, Talukdar R, Subramanyam C, *et al.* Role of the normal gut microbiota. World J Gastroenterol 2015; 21:8836–8847.

37. Thorpe D, Stringer A, Butler R. Chemotherapy-induced mucositis: the role of mucin secretion and regulation, and the enteric nervous system. Neurotoxicology 2013; 38:101–105.

38. ■ Thorpe D. The role of mucins in mucositis. Curr Opin Support Palliat Care 2019; 13:114–118.

New insights into the role of mucins during mucositis.

39. Hofmann AF, Eckmann L. How bile acids confer gut mucosal protection against bacteria. Proc Natl Acad Sci U S A 2006; 103:4333–4334.

40. Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp Mol Med 2018; 50:103.

41. Wardill HR, Bowen JM, Al-Dasooqi N, *et al.* Irinotecan disrupts tight junction proteins within the gut. Cancer Biol Ther 2014; 15:236–244.

42. Wardill HR, Bowen JM, Van Sebille YZA, *et al.* TLR4-dependent claudin-1 internalization and secretagogue-mediated chloride secretion regulate irinotecan-induced diarrhea. Mol Cancer Ther 2016; 15:2767–2779.

43. Feng Y, Huang Y, Wang Y, *et al.* Antibiotics induced intestinal tight junction barrier dysfunction is associated with microbiota dysbiosis, activated NLRP3 inflammasome and autophagy. PLoS One 2019; 14:1–19.

44. Ridlon J, Kang D, Hylemon P, Bajaj J. Bile acids and the gut microbiome. Curr Opin Gastroenterol 2014; 30:332–338.

45. Fang ZZ, Zhang D, Cao YF, *et al.* Irinotecan (CPT-11)-induced elevation of bile acids potentiates suppression of IL-10 expression. Toxicol Appl Pharmacol 2016; 291:21–27.

46. Secombe KR, Ball IA, Shirren J, *et al.* Targeting neratinib-induced diarrhea with budesonide and colesevelam in a rat model. Cancer Chemother Pharmacol 2019; 83:531–543.

47. Van Vliet MJ, Tissing WJE, Rings EHHM, *et al.* Citrulline as a marker for chemotherapy induced mucosal barrier injury in pediatric patients. Pediatr Blood Cancer 2009; 53:1188–1194.

48. Lundberg R, Toft MF, August B, *et al.* Antibiotic-treated versus germ-free rodents for microbiota transplantation studies. Gut Microbes 2016; 7:68–74.

49. Nagao-Kitamoto H, Shreiner AB, Gillilland MG, *et al.* Functional characterization of inflammatory bowel disease-associated gut dysbiosis in gnotobiotic mice. Cell Mol Gastroenterol Hepatol 2016; 2:468–481.

50. Llewellyn SR, Britton GJ, Contijoch EJ, *et al.* Interactions between diet and the intestinal microbiota alter intestinal permeability and colitis severity in mice. Gastroenterology 2018; 154:1037–1046.e2.

51. Kennedy EA, King KY, Baldridge MT. Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. Front Physiol 2018; 9:1–16.

52. Alimonti A, Satta F, Pavese I, *et al.* Prevention of irinotecan plus 5-fluorouracil/leucovorin-induced diarrhoea by oral administration of neomycin plus bacitracin in first-line treatment of advanced colorectal cancer. Ann Oncol 2003; 14:805–806.

53. de Jong FA. Prophylaxis of irinotecan-induced diarrhea with neomycin and potential role for UGT1A1*28 genotype screening: a double-blind, randomized, placebo-controlled study. Oncologist 2006; 11:944–954.

54. Al-Asmakh M, Zadjali F. Use of germ-free animal models in microbiota-related research. J Microbiol Biotechnol 2015; 25:1583–1588.

55. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 2012; 12:2165–2174.

56. Gjorevski N, Sachs N, Manfrin A, *et al.* Designer matrices for intestinal stem cell and organoid culture. Nature 2016; 539:560–564.

57. Leushacke M, Barker N. Ex vivo culture of the intestinal epithelium: strategies and applications. Gut 2014; 63:1345–1354.

58. Sadabad MS, Von Martels JZH, Khan MT, *et al.* A simple coculture system shows mutualism between anaerobic faecalibacteria and epithelial Caco-2 cells. Sci Rep 2015; 5:1–9.

59. ■ Alexander JL, Wilson ID, Teare J, *et al.* Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol 2017; 14:356–365.

New mechanistic approach for the role of the gut microbiota in citoxicity

60. Esfahani S, Sagar NM, Kyrou I, *et al.* Variation in gas and volatile compound emissions from human urine as it ages, measured by an electronic nose. Biosensors 2016; 6:1–11.