ACCEPTED VERSION

The bidirectional interaction of the gut microbiome and the innate immune system: implications for chemotherapy-induced gastrointestinal toxicity

Kate R. Secombe [a], Janet K. Coller [b], Rachel J. Gibson [a, c], Hannah R. Wardill [a, d] and Joanne M. Bowen [a]

[a] Cancer Treatment Toxicities Group, Discipline of Physiology, Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia

[b] Cancer Treatment Toxicities Group, Discipline of Pharmacology, Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia

[c] Division of Health Sciences, University of South Australia, Adelaide, South Australia, Australia

[d] Department of Pediatric Oncology/Hematology, University of Groningen, Beatrix Children's Hospital, University Medical Center Groningen, Groningen, The Netherlands.

Corresponding Author: **Kate Secombe**

kate.secombe@adelaide.edu.au

+61 8 8313 1537

Level 2 Helen Mayo Building South

University of Adelaide, Frome Rd

Adelaide 5005 AUSTRALIA

The bidirectional interaction of the gut microbiome and the innate immune system: implications for chemotherapy-induced gastrointestinal toxicity

Abstract

Chemotherapy-induced gastrointestinal toxicity (CIGT) occurs in up to 80% of all patients undergoing cancer treatment, and leads to symptoms such as diarrhoea, abdominal bleeding and pain. There is currently limited understanding of how to predict an individual patient's risk of CIGT. It is believed the gut microbiome and its interactions with the host's innate immune system plays a key role in the development of this toxicity and potentially other toxicities, however comprehensive bioinformatics modelling has not been rigorously performed.

The innate immune system is strongly influenced by the microbial environment and vice-versa. Ways this may occur include the immune system controlling composition and compartmentalisation of the microbiome, the microbiome affecting development of antigen-presenting cells, and finally, the NLRP6 inflammasome orchestrating the colonic host-microbiome interface. This evidence calls into question the role of pre-treatment risk factors in the development of gastrointestinal toxicity following chemotherapy.

This review aims to examine evidence of a bidirectional interaction between the gut microbiome and innate immunity, and how these interactions occur in CIGT. In the future, knowledge of these interactions may lead to improved personalised cancer medicine, predictive risk stratification methods and the development of targeted interventions to reduce, or even prevent, CIGT severity.

Introduction

Gastrointestinal toxicity is a significant and often dose-limiting adverse event of many chemotherapeutic agents used in cancer treatment, and is currently without a widely effective preventative or treatment strategy. Chemotherapy-induced gastrointestinal toxicity (CIGT) covers a constellation of cancer treatment-related adverse events often referred to as mucositis, as inflammation of the mucosa is a key aspect of tissue injury [1]. Characterised by painful ulcerative lesions along the entire gastrointestinal tract from mouth to anus, CIGT affects up to 80% of patients, depending on treatment regimen [1]. This leads to a heightened risk of adverse events such as infection and diarrhoea [2]. Symptoms such as abdominal bleeding and abdominal pain are common, and result in increased hospital stays and the need for parenteral nutrition [1]. These interventions, as well as use of pain management medication, results in a significantly increased economic cost, with Medicare data from Australia suggesting a cost of $1500 per episode of severe diarrhoea [3]. Available economic evidence substantially underestimates the larger societal burden and loss of quality of life of CIGT, caused by loss of productivity, need for informal care arrangements and increases in anxiety and depression levels [4]. Severe complications of CIGT such as bacteraemia and sepsis cause chemotherapy dose reductions and in profound cases, treatment cessation, compromising remission and increasing mortality [5]. Subsequently, CIGT presents as a major clinical and economic burden [2].

Activation of transcription factors and upregulation and release of pro-inflammatory cytokines, in response to initiating events, are integral in the pathobiology of CIGT. More recent reports have focussed on an altered gut microbiome and damaged epithelial cells that produce cellular damage signals, causing activation of the innate immune system [6, 7]. These types of damage signals are recognised by receptors in the innate immune system, present on gastrointestinal tract cells [8].

It is now known that cancer treatments cause a raft of changes to the microbiome and host innate immune system [9]. There is significant heterogeneity in the microbiome and in immune function, and as such, this emergent data represents a chance to personalise cancer treatment, and allow the identification of patients at risk of severe symptoms [10, 11]. However there is a clear gap in knowledge in

translating these results from studies in radiotherapy- and immunotherapy-based patients into those with chemotherapy. The symptoms of CIGT and other cancer-treatment induced toxicities (including radiotherapy) are similar, and while occur from different initiating events, have a similar timeline of pathogenesis [12]. These radiotherapy studies are beyond the scope of this review, however chemotherapy and radiotherapy are often concurrently used and these studies have been included in this review, in order to best assess available evidence. In addition to translating results into chemotherapy studies, it is not yet known if there is a specific gut microbiome profile that establishes risk of acute CIGT, and the role of the host immune system in maintaining or changing that profile. This review will provide evidence for an updated mechanistic hypothesis of CIGT wherein the bidirectional interaction of the host innate immune system and native microbiome may predict the severity of gastrointestinal toxicity a patient will suffer following chemotherapy. A semi-structured search of PubMed for full-text articles in English found more than 1000 studies investigating the microbiome and/or innate immune system in chemotherapy-induced gastrointestinal toxicity. Search terms included: 'microbiome', 'chemotherapy-induced gastrointestinal toxicity', 'chemotherapy-induced mucositis', 'cancer treatment diarrhoea' and 'chemotherapy microbiome'. Findings of key studies are summarised in tables 1-3.

Pathobiology of CIGT

Although CIGT is a significant concern in the treatment of cancer, the underlying mechanisms remain unclear. Sonis introduced a model in 2004, consisting of five continuous and overlapping phases. This model elegantly showed the integral role of transcription factor activation and subsequent upregulation and release of pro-inflammatory cytokines, in response to initiating events [12]. These initiating events may be the innate immune system's recognition of a Pathogen-Associated Molecular Pattern (PAMP) (e.g. lipopolysaccharide (LPS)), Damage-Associated Molecular Pattern (DAMP) (e.g. high mobility group box chromosomal protein 1, heat shock proteins) or ChemoRadiotherapy-Associated Molecular Pattern (CRAMP) [13]. There are several cellular mediators critical in these developmental events. These include nuclear factor kappa B (NF-κB), which causes the upregulation of up to 200 genes possibly involved in CIGT development [12]. Pro-inflammatory cytokines (primarily

tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-18 and IL-33 [14-16]) are significantly elevated, leading to an amplification of apoptosis and epithelial damage [12].

Since this model was introduced, more recent research suggests that the large population of bacteria resident in the small and large intestines plays a fundamental role in CIGT development (Figure 1). With recent advances in 'omics' technology, our ability to understand the unique idiosyncrasies of the microbiome is vastly improving. This onslaught of information highlights the need for improved translational integration of the role of gut bacteria in the pathobiological model.

The gut microbiome

The gut microbiome, defined here as the collection of bacteria and other microorganisms present in and around tissues from the mouth through to the anus, is made up of almost one hundred trillion microorganisms [17]. Functions include protective and immunological actions, supporting energy metabolism and triggering mucous barrier formation [8, 18]. The importance of the microbiome in normal development and functioning can be observed in germ-free mice, which are completely devoid of a microbiome raised under special conditions so they have no bacterial population. As a consequence of this, they have a variety of developmental differences including immature immune systems and altered digestive enzyme development, and are thus extremely susceptible to infection [19].

Although the composition of the human gut microbiome is dependent on many factors such as diet, sex and ethnicity, much of its composition is determined in birth and infancy [19]. New findings also suggest that microbiome composition is not significantly associated with genetics, and is often more associated with environment [20]. While each individual's gut microbiome is unique; the overall framework is often similar with the *Firmicutes* and *Bacteroidetes* phyla comprising over 90% of the gut microbiome [21]. Most bacteria belong to the *Clostridium* and *Bacteroides* genera, with major commensal (species which cohabitate with mutual benefit) species being *Lactobacillus* spp. and *Bifidobacterium* spp. [18]. Additionally, there are marked differences between the oral, gastric, small and large intestinal bacterial populations [22], reflecting physiological differences such as oxygen gradient, pH and presence

of antimicrobial peptides. The oral microbiome is dominated by *Streptococcus* spp., the small intestine by *Lactobacillaceae* and *Enterbacteriaceae*, and the colon by species such as *Bacteriodaceae* and *Prevotellaceae* [22, 23].

The gastrointestinal epithelium is constantly in contact with adherent bacteria. A sensitive balance exists, with continual cross talk between the microbiome, immune cells and the mucosal barrier to maintain homeostasis [24]. A disruption in this balance, known as dysbiosis, has been shown to have a role in multiple autoimmune diseases such as multiple sclerosis [25]. Additionally, the inter-individual differences in the gut microbiome are now considered to be one of the key contributors to immune response in humans. This is thought to be via influence of an individual's cytokine response (with strongest effects on interferon gamma (IFN-γ) and TNF-α production), and therefore disease susceptibility and overall immune function [26].

The innate immune system

The innate immune system is the first line of response to bacterial invasion or an aseptic tissue injury. It responds to danger signals by recruiting immune cells to the injury site, inducing inflammation and activating the adaptive immune system. The innate immune system is vital in the gastrointestinal tract, with the luminal gastrointestinal surface of being one of the largest common surface areas between host and environment. With a diverse range of microbes living so close to the host, the innate immune system is critical in maintaining immune tolerance to commensal microbes, whilst ensuring the rapid initiation of an immune response following invading pathogens.

The innate immune system utilises many systems in the gastrointestinal tract in order to maintain homeostasis, including separation mechanisms including epithelial and mucosal layers, and compounds such as antimicrobial peptides and antibodies. Additionally, the innate immune system utilises a system of pattern recognition receptors that recognise microbial molecular patterns.

One type of pattern recognition receptor, Toll-Like Receptors (TLRs), are particularly important in sensing molecular patterns from gut microbes. TLRs are highly conserved transmembrane receptors and are members of the Toll-Interleukin 1

Receptor signalling pathway [27]. TLRs are present in the gastrointestinal mucosa on basolateral and apical surfaces of epithelial cells, professional immune cells, enteric neurons and glia [28, 29]. There are eleven TLRs found in humans, recognising diverse ligands including RNA, DNA and LPS [27]. TLR4 is a particular focus of recent research due to its known expression changes following chemotherapy, and its ability to recognise patterns released by chemotherapy-damaged cells and therefore will be the focus here [6]. TLR4 can be activated by exogenous and endogenous danger signals such as LPS, high mobility group box chromosomal protein 1 and heat shock proteins [27], causing a downstream signalling pathway of transcription factor (e.g. NF-κB) upregulation and pro-inflammatory cytokine release.

The role of TLR4 in CIGT development is now well known, with TLR4 knockout mice having less diarrhoea, weight loss and histological damage in response to irinotecan treatment [6]. However, there is some disparity surrounding the role of TLR4. One study where TLR4 was blocked using naloxone was unable to reduce irinotecan-induced gastrointestinal damage [30], whereas other research has shown that TLR4 agonist LPS can protect intestinal crypts from other insults such as radiation [31]. Studies have shown protection and exacerbation, possibly due to differences between acute and chronic injury [32] or chemotherapeutic agents, and therefore the role of TLR4 appears to be complicated and context-specific. Immunologic cell death, a form of cell death where dendritic cell activation leads to a specific T cell response, is caused by some anti-cancer agents such as oxaliplatin and is characterised by release of DAMPs. As TLR4 is of vital importance in binding DAMPs, it is possible that immunogenic cell death may contribute to modulating the role of TLR4 in CIGT.

A bidirectional interaction: innate immunity and the gut microbiome

The innate immune system is strongly influenced by the microbial environment [26]. However, there is growing evidence that the reverse is also true, and that the microbial environment is similarly influenced by the innate immune system [8, 33]. This bidirectional interaction between microbiome and immune system has been described by Hooper et al. [34] as 'inside-out' and 'outside-in' interactions, and is observed in a number of ways.

The immune system controls composition and compartmentalisation of the microbiome [34]. There is ample evidence to suggest that the host immune system is integral in selecting mucosal and luminal bacterial populations, ensuring there is minimal direct contact between the gastrointestinal surface and bacteria, keeping penetrant bacteria constrained to the lumen [8, 21]. Van den Abbeele et al. [21] hypothesised that a distinct mucosal-associated microbial community has many immune-regulating effects with large potential biological outcome. Conversely, bacteria likely to be targeted by host defences are restricted to the lumen. This paper also suggested an outer colonic mucus layer, situated between the inner mucus layer and the lumen, may contain a 'backup' of microorganisms, which could act as an inoculum to restore the initial microbial balance after a perturbation, ensuring continual stability. In contrast, the colonic inner mucus layer is effectively devoid of bacteria [35]. The small intestine does not have these two distinct layers, and instead relies on antimicrobial peptides / receptors such as RegIIIɣ and TLRs to minimise bacterial penetration of the mucus layer [22].

An alternative mechanism explaining the interaction between the microbiome and immune system is through the development of antigen-presenting cells (APCs) such as dendritic cells and macrophages. Gastrointestinal dysbiosis has been shown to reduce infiltrating mature APCs [36]. Additionally, commensal bacteria can regulate dendritic cell activity. When investigating the role of the microbiome in APC development, it was found there were less gastrointestinal, but not systemic, dendritic cells in germ-free animals, and subsequent mono-colonisation of the gastrointestinal tract with *E. coli* caused gastrointestinal dendritic cell recruitment [37, 38]. Additionally, microbe-derived adenosine triphosphate has been shown to stimulate CD70 and CX3CR1 expressing dendritic cells, which can then go on to induce differentiation of Th17 cells [39].

The NLRP6 inflammasome is key in orchestrating the colonic host-microbiome interface [40], and is important in production of pro-inflammatory IL-18. Mice deficient in NLRP6 have been shown to be more susceptible to enteric infection [40], have an altered microbial biogeography and to have a dysbiotic bacterial profile more likely to cause colitis-like symptoms [41]. A recent paper has shown microbiome-modulated metabolites are able to regulate the NLRP6 inflammasome and subsequent IL-18

production [42]. These microbial metabolic products are also known to regulate the role of colonic regulatory T cell homeostasis among a raft of other effects [43].

The gut microbiome in CIGT

The gut microbiome plays a variety of roles in the development of CIGT. One study showed that compared to germ-free mice, conventional mice treated with the same dose of irinotecan had more lesions within the jejunal intestinal epithelium and higher gastrointestinal permeability [44]. This was also reflected in another study, which showed that diarrhoea was more common in conventional mice compared to germ-free mice, which also had a lowered intestinal damage score [45]. Rigby et al. [46] also showed the role of gastrointestinal bacteria in mediating doxorubicin-induced gastrointestinal damage by showing that germ-free mice did not display the changes in crypt depth and proliferative cell numbers that conventional mice treated with doxorubicin showed. Closely linked are the inflammatory pathways that are markedly upregulated in all cases of CIGT. It is now known that microbiome-host interactions modulate inflammatory cytokine production capacity [26]. Dysbioses of gut microbes are often linked to aberrant immune responses, often complemented by abnormal production of inflammatory cytokines. Additionally, commensal bacteria have protective effects on the integrity of the gastrointestinal mucosal barrier, including interactions with tight junctions and regulation of mucous layer [47].

Research in pre-clinical models conducted over the past decade has shown a variety of changes to microbiome composition and diversity in the gastrointestinal tract due to chemotherapy treatment. There are some limitations to using pre-clinical microbiome models with 85% of bacterial sequences seen in a mouse representing genera not detected in humans [48]. However, there is also significant similarity in the distal gut microbiome between human and mice at a divisional level, and both have the same two most abundant bacterial divisions (*Firmicutes* and *Bacteroidetes*). Therefore pre-clinical models are often routinely used in this field.

Pre-clinical studies show a decrease in commensal species following chemotherapy, which causes reduced protective effects and decreased resistance to pathogenic colonisation (table 1). This increase in pathogenic species also corresponds to an increase in gram negative species, which release LPS that is known to initiate the

inflammatory pathways involved in CIGT development [27]. These pre-clinical studies generally show decreases in *Lactobacillus* and *Bifidobacterium* and increases in *Escherichia coli* (*E. coli*) and *Staphylococcus* [49]. However, many of these studies used polymerase chain reaction (PCR) or microbial culture techniques to delineate species and changes. While these methods were standard at the time, with more sophisticated pyrosequencing techniques now the norm, these results may have limited reproducibility.

A small number of clinical studies have also been conducted with patients undergoing chemotherapy, often replicating what has been shown in pre-clinical studies (table 1). However, due to a focus on clinical outcomes (e.g. diarrhoea severity), these studies have failed to conclusively link pre-treatment to post-treatment microbiome composition. Results overall have included a decrease in total bacteria numbers and diversity [50, 51]. At a species specific level, findings have shown increases in *Bacteriodetes*, *Clostridium* cluster IV and *E. coli* [51, 52] and decreases in *Lactobacillus*, *Bifidobacterium* and *Clostridium* cluster XIV [52, 53].

Attempts to ameliorate chemotherapy-induced changes to the microbiome profile have been variably successful in lowering damage severity. Administration of probiotics in pre-clinical models have attenuated gastrointestinal damage from chemotherapy by preventing apoptosis, reducing barrier disruption and promoting crypt survival (table 2) [54, 55]. However there have been inconsistencies, with level of diarrhoea reduction varying due to probiotic strains, dosing and treatment plan. For example in pre-clinical studies using *Streptococcus thermophiles*, one study showed promising results, with non-tumour bearing rats treated with methotrexate having attenuation of gastrointestinal damage [56], while another study utilising the same probiotic and chemotherapeutic in tumour bearing rats showed no benefit [57]. These studies additionally demonstrate a potential role of the tumour itself in regulating CIGT and gut microbiome changes, however further research is required to more fully understand this.

Multiple clinical trials have used probiotics in patients undertaking chemotherapy, with *Lactobacillus* species have been a particular focus (table 2). For example, Osterlund et al. [58] showed that *Lactobacillus* supplementation led to less severe diarrhoea, less abdominal discomfort and fewer dose reductions after 5-fluorouracil

(5-FU) treatment for colorectal cancer. Subsequently, the Multinational Association for Supportive Care in Cancer (MASCC) released new clinical guidelines in 2014 suggesting the use of "probiotic agents containing *Lactobacillus* species for the prevention of chemotherapy and radiation-induced diarrhoea in patients with a pelvic malignancy" [1]. Despite this, a recent meta-analysis found insufficient current evidence to support widespread implementation of probiotics after chemotherapy [59].

Clinical trials have also investigated the impact of the antibiotic neomycin in combination with irinotecan, and results have shown less diarrhoea [60, 61]. However, a larger study conducted by de Jong et al. [62] did not find a substantial role for neomycin in reducing diarrhoea severity. Following inadequate / conflicting evidence, a 2013 systematic review was conducted and concluded that no clinical guideline for the use of neomycin was possible [63]. Another study administering antibiotics showed compromised anti-tumour efficacy of chemotherapy and an increase in potentially pathogenic bacteria [64]. These results may suggest that the removal of the entire microbiome with broad spectrum antibiotics does more harm than good, and that restoring microbial diversity is more important in maintaining damage-defence mechanisms.

Gastrointestinal toxicity, the microbiome and the innate immune system

Modifications to both immune function and bacterial profile may influence severity of gastrointestinal injury following chemotherapy [75]. This could possibly occur via an altered capacity to mount an immune response. For example, commensal bacteria are able to induce CD4+ T cell differentiation. Specifically, *Bacteroides fragilis* can induce the development of a systemic Th1 response through polysaccharide A molecules [76]. It has been well established that *Bacteroides* genus levels are decreased by chemotherapy in both pre-clinical and clinical models [49, 64, 65]. This therefore leads to a potential decreased ability to mount a Th1 response after chemotherapy, which may affect the severity of gastrointestinal injury.

The TIMER (translocation, immunomodulation, metabolism, enzymatic degradation, reduced diversity) model was recently proposed by Alexander et al. [9] to show the various ways the gut microbiome can influence chemotherapy efficacy and toxicity. It is possible that each part of this model is reliant on the microbiome's interaction with the innate immune system, and thus the gut microbiome and innate immune system henceforth should be investigated together as much as practicable.

There are a variety of ways this interaction could be studied in future. Currently, much CIGT research is undertaken in animals or relatively rudimentary cell cultures. Additionally, there are limitations to using animal pre-clinical models for microbiome models, with many strains present in the mouse microbiome not present in humans as discussed previously [48]. Reproducible and scientifically robust *in vitro* and *ex vivo* models are therefore needed to effectively study the microbiome in intestinal models. This may include using human tissue samples or stem cells to grow organoids, or the development of gut-on-a-chip technology to incorporate microbiome changes [77]. Additionally, recent trials of ingestible electronic capsules have showed the ability to sense gases produced by the microbiome in the gastrointestinal tract [78]. This may represent a real-time method of monitoring gut microbiome changes that could be paired with other analyses.

Future opportunities for risk prediction and modification

A personalised approach could soon be taken to manage CIGT, where a patient's individual risk of toxicity could be managed early in their treatment plan. This

approach is supported by observations relating genetic testing on DNA extracted from saliva to severe CIGT, in which key immunogenetic factors relating to the *TNF-a* and *TLR2* have been correlated with more severe toxicity [79]. Whether the patient's gut microbiome profile pre-cancer treatment could also predict toxicity severity is largely unknown, particularly in the setting of chemotherapy-induced damage. However, this idea was first postulated by Touchefeu et al. [50], and more recently by Wardill and Tissing [75]. Use of the microbiome as a predictive marker is gaining support in a variety of fields, including recent prediction of chemotherapy-related bloodstream infection [80]. Studies may employ methods such as machine learning or metabolomics techniques to create an algorithm or predictive model in which a patient's microbiome profile can be input to test risk of disease [80].

There is some initial evidence that such an idea could also prove true in relation to other cancer treatment-induced toxicities (table 3), although as yet no study has investigated this following chemotherapy. One study of patients undergoing pelvic radiotherapy [81] found that patients who went on to develop diarrhoea had lower bacterial diversity and a higher *Firmicutes* / *Bacteroidetes* ratio. A study of patients suffering from oral mucositis after radiotherapy for head and neck cancers was also able to build a predictive model for the aggravation of oral mucositis (similar to CIGT in the oral cavity) [11]. Only one study has identified a protective phenotype of the gut microbiome for gastrointestinal toxicity [10]. This study measured development of checkpoint blockade-induced colitis in melanoma patients receiving the immunomodulatory therapy ipilimumab. Using 16S rRNA pyrosequencing techniques, it was shown that an increased representation of bacteria in the *Bacteroidetes* phylum before treatment was associated with resistance to development of colitis.

Finally, one study [82] did not use a bacterial sequencing approach, but rather used the relatively novel method of an electronic nose and the Field Asymmetric Ion Mobility Spectrometry method, to analyse stool samples. Gases and other metabolic by-products of microbiome fermentation emitted from the samples were analysed. The patient pre-radiotherapy samples were successfully separated, using principal component analysis and linear discriminant analysis, into those who suffered from gastrointestinal toxicity and those who did not. This method represents a translatable

clinical test, where pre-treatment samples could be analysed, and future cancer treatment and supportive care measures adapted to suit.

This emergent data on the role of the microbiome and the immune system in determining severity of cancer treatment-induced toxicities represents an additional opportunity to personalise cancer treatment, and allow the identification of patients at risk of severe symptoms. Alexander et al. [9] summarised evidence of being able to adapt the microbiome profile of the gastrointestinal tract using an enzyme inhibitor such as a β-glucuronidase inhibitor [83], changes in diet [84] or probiotics [55]. Of these, diet modification has been shown to be one of the most consistent and predictable ways of remodelling the microbiome, able to induce rapid shifts in composition and subsequent function [85]. An enzyme inhibitor may be particularly useful for patients undergoing irinotecan treatment, as the enzyme β-glucuronidase is crucial in irinotecan toxicity. SN-38, the active form of irinotecan, is conjugated in the liver to a less toxic metabolite, SN-38G. When excreted to the gastrointestinal tract via bile, it is hydrolysed back to the toxic SN-38 form by microbe-derived β-glucuronidase [61, 67]. As discussed above, probiotics have previously been used in CIGT studies, with mixed results (table 2). There is currently a renewed call for carefully planned studies with new types of probiotics to better understand the potential benefits they could play in modulating CIGT risk [86]. Also required is better characterisation of the microbial profiles associated with toxicities caused by different agents to properly identify and develop the ideal microbial protectant. Finally, the developing area of faecal microbiota transplants and even faecal capsules represent methods of directly modifying the gut microbiome yet to be investigated in this context.

Conclusion

Effective treatments or predictive strategies for gastrointestinal toxicity caused by chemotherapy are urgently required. This under-reported but common adverse event has a substantial effect on quality of life and economic burden. Understanding the effect the gut microbiome and the innate immune system has on CIGT is key to developing treatment and prevention strategies. This review has summarised the key interactions between the gut microbiome and the innate immune system, and how these interactions may adversely affect gastrointestinal toxicity following

chemotherapy. We have also drawn emphasis to new data suggesting that the bidirectional interaction between microbiome and immune system is distinct to each patient and how we may be able to predict high-risk patients, thereby adjusting supportive care measures to each person. It is hoped that through pragmatic and rigorous scientific investigation, effective CIGT treatment may be within reach.

1. Lalla RV, Bowen J, Barasch A, Elting L, Epstein J, Keefe DM, McGuire DB, Migliorati C, Nicolatou-Galitis O, Peterson DE, Raber-Durlacher JE, Sonis ST, Elad S, and The Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. Cancer 2014; 120(10): 1453-61.

2. Carlotto A, Hogsett VL, Maiorini EM, Razulis JG, and Sonis ST. The economic burden of toxicities associated with cancer treatment: review of the literature and analysis of nausea and vomiting, diarrhoea, oral mucositis and fatigue. Pharmacoeconomics 2013; 31(9): 753-66.

3. Vouk K, Benter U, Amonkar MM, Marocco A, Stapelkamp C, Pfersch S, and Benjamin L. Cost and economic burden of adverse events associated with metastatic melanoma treatments in five countries. J Med Econ 2016; 19(9): 900-12.

4. Tarricone R, Abu Koush D, Nyanzi-Wakholi B, and Medina-Lara A. A systematic literature review of the economic implications of chemotherapy-induced diarrhea and its impact on quality of life. Crit Rev Oncol Hematol 2016; 99: 37-48.

5. Di Fiore F and Van Cutsem E. Acute and long-term gastrointestinal consequences of chemotherapy. Best Pract Res Clin Gastroenterol 2009; 23(1): 113-24.

6. Wardill HR, Gibson RJ, Van Sebille YZ, Secombe KR, Coller JK, White IA, Manavis J, Hutchinson MR, Staikopoulos V, Logan RM, and Bowen JM. Irinotecan-Induced Gastrointestinal Dysfunction and Pain Are Mediated by Common TLR4-Dependent Mechanisms. Mol Cancer Ther 2016; 15(6): 1376-86.

7. Vasconcelos RM, Sanfilippo N, Paster BJ, Kerr AR, Li Y, Ramalho L, Queiroz EL, Smith B, Sonis ST, and Corby PM. Host-Microbiome Cross-talk in Oral Mucositis. J Dent Res 2016; 95(7): 725-33.

8.   Jacobs JP and Braun J. Immune and genetic gardening of the intestinal microbiome. FEBS Lett 2014; 588(22): 4102-11.

9.   Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, and Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nat Rev Gastroenterol Hepatol 2017; 14(6): 356-65.

10.   Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, No D, Gobourne A, Littmann E, Huttenhower C, Pamer EG, and Wolchok JD. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun 2016; 7: 10391.

11.   Zhu XX, Yang XJ, Chao YL, Zheng HM, Sheng HF, Liu HY, He Y, and Zhou HW. The Potential Effect of Oral Microbiota in the Prediction of Mucositis During Radiotherapy for Nasopharyngeal Carcinoma. EBioMedicine 2017; 18: 23-31.

12.   Sonis ST. The pathobiology of mucositis. Nat Rev Cancer 2004; 4(4): 277-84.

13.   Sonis ST. New thoughts on the initiation of mucositis. Oral Dis 2010; 16(7): 597-600.

14.   Logan RM, Stringer AM, Bowen JM, Gibson RJ, Sonis ST, and Keefe DM. Serum levels of NFkappaB and pro-inflammatory cytokines following administration of mucotoxic drugs. Cancer Biol Ther 2008; 7(7): 1139-45.

15.   Lima-Junior RC, Freitas HC, Wong DV, Wanderley CW, Nunes LG, Leite LL, Miranda SP, Souza MH, Brito GA, Magalhaes PJ, Teixeira MM, Cunha FQ, and Ribeiro RA. Targeted inhibition of IL-18 attenuates irinotecan-induced intestinal mucositis in mice. Br J Pharmacol 2014; 171(9): 2335-50.

16.   Guabiraba R, Besnard AG, Menezes GB, Secher T, Jabir MS, Amaral SS, Braun H, Lima-Junior RC, Ribeiro RA, Cunha FQ, Teixeira MM, Beyaert R, Graham GJ, and Liew FY. IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice. Mucosal Immunol 2014; 7(5): 1079-93.

17.   Ley RE, Peterson DA, and Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 2006; 124(4): 837-48.

18. Purchiaroni F, Tortora A, Gabrielli M, Bertucci F, Gigante G, Ianiro G, Ojetti V, Scarpellini E, and Gasbarrini A. The role of intestinal microbiota and the immune system. Eur Rev Med Pharmacol Sci 2013; 17(3): 323-33.

19. Roy S and Trinchieri G. Microbiota: a key orchestrator of cancer therapy. Nat Rev Cancer 2017; 17(5): 271-85.

20. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, and Segal E. Environment dominates over host genetics in shaping human gut microbiota. Nature 2018; 555(7695): 210-5.

21. Van den Abbeele P, Van de Wiele T, Verstraete W, and Possemiers S. The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. FEMS Microbiol Rev 2011; 35(4): 681-704.

22. Donaldson GP, Lee SM, and Mazmanian SK. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 2016; 14(1): 20-32.

23. Lloyd-Price J, Abu-Ali G, and Huttenhower C. The healthy human microbiome. Genome Medicine 2016; 8(1): 51.

24. Wells JM, Rossi O, Meijerink M, and van Baarlen P. Epithelial crosstalk at the microbiota-mucosal interface. Proc Natl Acad Sci U S A 2011; 108 Suppl 1: 4607-14.

25. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim SW, Morita H, Hattori M, and Yamamura T. Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. PLoS One 2015; 10(9): e0137429.

26. Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, Jansen T, Jacobs L, Bonder MJ, Kurilshikov A, Fu J, Joosten LA, Zhernakova A, Huttenhower C, Wijmenga C, Netea MG, and Xavier RJ. Linking the

Human Gut Microbiome to Inflammatory Cytokine Production Capacity. Cell 2016; 167(4): 1125-36 e8.

27.   Akira S and Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004; 4(7): 499-511.

28.   Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat Rev Immunol 2010; 10(2): 131-44.

29.   Barajon I, Serrao G, Arnaboldi F, Opizzi E, Ripamonti G, Balsari A, and Rumio C. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. J Histochem Cytochem 2009; 57(11): 1013-23.

30.   Coller JK, Bowen JM, Ball IA, Wardill HR, van Sebille YZ, Stansborough RL, Lightwala Z, Wignall A, Shirren J, Secombe K, and Gibson RJ. Potential safety concerns of TLR4 antagonism with irinotecan: a preclinical observational report. Cancer Chemother Pharmacol 2017; 79(2): 431-4.

31.   Riehl T, Cohn S, Tessner T, Schloemann S, and Stenson WF. Lipopolysaccharide is radioprotective in the mouse intestine through a prostaglandin-mediated mechanism. Gastroenterology 2000; 118(6): 1106-16.

32.   Zheng B, Morgan ME, van de Kant HJG, Garssen J, Folkerts G, and Kraneveld AD. Transcriptional modulation of pattern recognition receptors in chronic colitis in mice is accompanied with Th1 and Th17 response. Biochem Biophys Rep 2017; 12: 29-39.

33.   Thaiss CA, Zmora N, Levy M, and Elinav E. The microbiome and innate immunity. Nature 2016; 535(7610): 65-74.

34.   Hooper LV, Littman DR, and Macpherson AJ. Interactions between the microbiota and the immune system. Science 2012; 336(6086): 1268-73.

35.   Hansson GC and Johansson ME. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Gut Microbes 2010; 1(1): 51-4.

36. Xu C, Ruan B, Jiang Y, Xue T, Wang Z, Lu H, Wei M, Wang S, Ye Z, Zhai D, Wang L, and Lu Z. Antibiotics-induced gut microbiota dysbiosis promotes tumor initiation via affecting APC-Th1 development in mice. Biochem Biophys Res Commun 2017; 488(2): 418-24.

37. Haverson K, Rehakova Z, Sinkora J, Sver L, and Bailey M. Immune development in jejunal mucosa after colonization with selected commensal gut bacteria: a study in germ-free pigs. Vet Immunol Immunopathol 2007; 119(3-4): 243-53.

38. Williams AM, Probert CS, Stepankova R, Tlaskalova-Hogenova H, Phillips A, and Bland PW. Effects of microflora on the neonatal development of gut mucosal T cells and myeloid cells in the mouse. Immunology 2006; 119(4): 470-8.

39. Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, Yagita H, Ishii N, Evans R, Honda K, and Takeda K. ATP drives lamina propria T(H)17 cell differentiation. Nature 2008; 455(7214): 808-12.

40. Wlodarska M, Thaiss CA, Nowarski R, Henao-Mejia J, Zhang JP, Brown EM, Frankel G, Levy M, Katz MN, Philbrick WM, Elinav E, Finlay BB, and Flavell RA. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. Cell 2014; 156(5): 1045-59.

41. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, and Flavell RA. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 2011; 145(5): 745-57.

42. Levy M, Thaiss CA, Zeevi D, Dohnalova L, Zilberman-Schapira G, Mahdi JA, David E, Savidor A, Korem T, Herzig Y, Pevsner-Fischer M, Shapiro H, Christ A, Harmelin A, Halpern Z, Latz E, Flavell RA, Amit I, Segal E, and Elinav E. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. Cell 2015; 163(6): 1428-43.

43. Shapiro H, Thaiss CA, Levy M, and Elinav E. The cross talk between microbiota and the immune system: metabolites take center stage. Curr Opin Immunol 2014; 30: 54-62.

44. Pedroso SH, Vieira AT, Bastos RW, Oliveira JS, Cartelle CT, Arantes RM, Soares PM, Generoso SV, Cardoso VN, Teixeira MM, Nicoli JR, and Martins FS. Evaluation of mucositis induced by irinotecan after microbial colonization in germ-free mice. Microbiology 2015; 161(10): 1950-60.

45. Brandi G, Dabard J, Raibaud P, Di Battista M, Bridonneau C, Pisi AM, Morselli Labate AM, Pantaleo MA, De Vivo A, and Biasco G. Intestinal microflora and digestive toxicity of irinotecan in mice. Clin Cancer Res 2006; 12(4): 1299-307.

46. Rigby RJ, Carr J, Orgel K, King SL, Lund PK, and Dekaney CM. Intestinal bacteria are necessary for doxorubicin-induced intestinal damage but not for doxorubicin-induced apoptosis. Gut Microbes 2016; 7(5): 414-23.

47. Reinoso Webb C, Koboziev I, Furr KL, and Grisham MB. Protective and pro-inflammatory roles of intestinal bacteria. Pathophysiology 2016; 23(2): 67-80.

48. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, and Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005; 102(31): 11070-5.

49. Stringer AM, Gibson RJ, Bowen JM, Logan RM, Ashton K, Yeoh AS, Al-Dasooqi N, and Keefe DM. Irinotecan-induced mucositis manifesting as diarrhoea corresponds with an amended intestinal flora and mucin profile. Int J Exp Pathol 2009; 90(5): 489-99.

50. Touchefeu Y, Montassier E, Nieman K, Gastinne T, Potel G, Bruley des Varannes S, Le Vacon F, and de La Cochetiere MF. 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(5): 409-21.

51. Montassier E, Gastinne T, Vangay P, Al-Ghalith GA, Bruley des Varannes S, Massart S, Moreau P, Potel G, de La Cochetiere MF, Batard E, and Knights D. Chemotherapy-driven dysbiosis in the intestinal microbiome. Aliment Pharmacol Ther 2015; 42(5): 515-28.

52. Zwielehner J, Lassl C, Hippe B, Pointner A, Switzeny OJ, Remely M, Kitzweger E, Ruckser R, and Haslberger AG. Changes in human fecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting. PLoS One 2011; 6(12): e28654.

53. Stringer AM, Al-Dasooqi N, Bowen JM, Tan TH, Radzuan M, Logan RM, Mayo B, Keefe DM, and Gibson RJ. Biomarkers of chemotherapy-induced diarrhoea: a clinical study of intestinal microbiome alterations, inflammation and circulating matrix metalloproteinases. Support Care Cancer 2013; 21(7): 1843-52.

54. Southcott E, Tooley KL, Howarth GS, Davidson GP, and Butler RN. Yoghurts containing probiotics reduce disruption of the small intestinal barrier in methotrexate-treated rats. Dig Dis Sci 2008; 53(7): 1837-41.

55. Bowen JM, Stringer AM, Gibson RJ, Yeoh AS, Hannam S, and Keefe DM. VSL#3 probiotic treatment reduces chemotherapy-induced diarrhea and weight loss. Cancer Biol Ther 2007; 6(9): 1449-54.

56. Tooley KL, Howarth GS, Lymn KA, Lawrence A, and Butler RN. Oral ingestion of streptococcus thermophilus diminishes severity of small intestinal mucositis in methotrexate treated rats. Cancer Biol Ther 2006; 5(6): 593-600.

57. Tooley KL, Howarth GS, Lymn KA, Lawrence A, and Butler RN. Oral ingestion of Streptococcus thermophilus does not affect mucositis severity or tumor progression in the tumor-bearing rat. Cancer Biol Ther 2011; 12(2): 131-8.

58. Osterlund P, Ruotsalainen T, Korpela R, Saxelin M, Ollus A, Valta P, Kouri M, Elomaa I, and Joensuu H. Lactobacillus supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. Br J Cancer 2007; 97(8): 1028-34.

59. Wardill HR, Van Sebille YZA, Ciorba MA, and Bowen JM. Prophylactic probiotics for cancer therapy-induced diarrhoea: a meta-analysis. Curr Opin Support Palliat Care 2018. 10.1097/spc.0000000000000338.

60. Alimonti A, Satta F, Pavese I, Burattini E, Zoffoli V, and Vecchione A. 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(5): 805-6.

61.   Kehrer DF, Sparreboom A, Verweij J, de Bruijn P, Nierop CA, van de Schraaf J, Ruijgrok EJ, and de Jonge MJ. Modulation of irinotecan-induced diarrhea by cotreatment with neomycin in cancer patients. Clin Cancer Res 2001; 7(5): 1136-41.

62.   de Jong FA, Kehrer DF, Mathijssen RH, Creemers GJ, de Bruijn P, van Schaik RH, Planting AS, van der Gaast A, Eskens FA, Janssen JT, Ruit JB, Verweij J, Sparreboom A, and de Jonge MJ. 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(8): 944-54.

63.   Gibson RJ, Keefe DM, Lalla RV, Bateman E, Blijlevens N, Fijlstra M, King EE, Stringer AM, van der Velden WJ, Yazbeck R, Elad S, and Bowen JM. Systematic review of agents for the management of gastrointestinal mucositis in cancer patients. Support Care Cancer 2013; 21(1): 313-26.

64.   van Vliet MJ, Tissing WJ, Dun CA, Meessen NE, Kamps WA, de Bont ES, and Harmsen HJ. Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis 2009; 49(2): 262-70.

65.   Lin XB, Dieleman LA, Ketabi A, Bibova I, Sawyer MB, Xue H, Field CJ, Baracos VE, and Ganzle MG. Irinotecan (CPT-11) chemotherapy alters intestinal microbiota in tumour bearing rats. PLoS One 2012; 7(7): e39764.

66.   Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, Burns J, and Keefe DM. Chemotherapy-induced diarrhea is associated with changes in the luminal environment in the DA rat. Exp Biol Med (Maywood) 2007; 232(1): 96-106.

67.   Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, and Keefe DM. Faecal microflora and beta-glucuronidase expression are altered in an

irinotecan-induced diarrhea model in rats. Cancer Biol Ther 2008; 7(12): 1919-25.

68. Forsgard RA, Marrachelli VG, Korpela K, Frias R, Collado MC, Korpela R, Monleon D, Spillmann T, and Osterlund P. Chemotherapy-induced gastrointestinal toxicity is associated with changes in serum and urine metabolome and fecal microbiota in male Sprague-Dawley rats. Cancer Chemother Pharmacol 2017; 80(2): 317-32.

69. Von Bultzingslowen I, Adlerberth I, Wold AE, Dahlen G, and Jontell M. Oral and intestinal microflora in 5-fluorouracil treated rats, translocation to cervical and mesenteric lymph nodes and effects of probiotic bacteria. Oral Microbiol Immunol 2003; 18(5): 278-84.

70. Montassier E, Batard E, Massart S, Gastinne T, Carton T, Caillon J, Le Fresne S, Caroff N, Hardouin JB, Moreau P, Potel G, Le Vacon F, and de La Cochetiere MF. 16S rRNA gene pyrosequencing reveals shift in patient faecal microbiota during high-dose chemotherapy as conditioning regimen for bone marrow transplantation. Microb Ecol 2014; 67(3): 690-9.

71. Nam YD, Kim HJ, Seo JG, Kang SW, and Bae JW. Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. PLoS One 2013; 8(12): e82659.

72. Wada M, Nagata S, Saito M, Shimizu T, Yamashiro Y, Matsuki T, Asahara T, and Nomoto K. Effects of the enteral administration of Bifidobacterium breve on patients undergoing chemotherapy for pediatric malignancies. Support Care Cancer 2010; 18(6): 751-9.

73. Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, and Lorvidhaya V. Randomized controlled trial of live lactobacillus acidophilus plus bifidobacterium bifidum in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. Radiat Oncol 2010; 5: 31.

74. Giralt J, Regadera JP, Verges R, Romero J, de la Fuente I, Biete A, Villoria J, Cobo JM, and Guarner F. Effects of probiotic Lactobacillus casei DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter,

randomized, placebo-controlled nutritional trial. Int J Radiat Oncol Biol Phys 2008; 71(4): 1213-9.

75. Wardill HR and Tissing WJE. Determining risk of severe gastrointestinal toxicity based on pretreatment gut microbial community in patients receiving cancer treatment: a new predictive strategy in the quest for personalized cancer medicine. Curr Opin Support Palliat Care 2017; 11(2): 125-32.

76. Mazmanian SK, Liu CH, Tzianabos AO, and Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005; 122(1): 107-18.

77. Arnold JW, Roach J, and Azcarate-Peril MA. Emerging Technologies for Gut Microbiome Research. Trends Microbiol 2016; 24(11): 887-901.

78. Kalantar-Zadeh K, Berean KJ, Ha N, Chrimes AF, Xu K, Grando D, Ou JZ, Pillai N, Campbell JL, Brkljača R, Taylor KM, Burgell RE, Yao CK, Ward SA, McSweeney CS, Muir JG, and Gibson PR. A human pilot trial of ingestible electronic capsules capable of sensing different gases in the gut. Nat Electronics 2018; 1(1): 79-87.

79. Coller JK, White IA, Logan RM, Tuke J, Richards AM, Mead KR, Karapetis CS, and Bowen JM. Predictive model for risk of severe gastrointestinal toxicity following chemotherapy using patient immune genetics and type of cancer: a pilot study. Support Care Cancer 2015; 23(5): 1233-6.

80. Montassier E, Al-Ghalith GA, Ward T, Corvec S, Gastinne T, Potel G, Moreau P, de la Cochetiere MF, Batard E, and Knights D. Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. Genome Med 2016; 8(1): 49.

81. Wang A, Ling Z, Yang Z, Kiela PR, Wang T, Wang C, Cao L, Geng F, Shen M, Ran X, Su Y, Cheng T, and Wang J. Gut microbial dysbiosis may predict diarrhea and fatigue in patients undergoing pelvic cancer radiotherapy: a pilot study. PLoS One 2015; 10(5): e0126312.

82. Covington JA, Wedlake L, Andreyev J, Ouaret N, Thomas MG, Nwokolo CU, Bardhan KD, and Arasaradnam RP. The detection of patients at risk of

gastrointestinal toxicity during pelvic radiotherapy by electronic nose and FAIMS: a pilot study. Sensors (Basel) 2012; 12(10): 13002-18.

83.   Wallace BD, Wang H, Lane KT, Scott JE, Orans J, Koo JS, Venkatesh M, Jobin C, Yeh LA, Mani S, and Redinbo MR. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 2010; 330(6005): 831-5.

84.   Faber J, van Limpt K, Kegler D, Luiking Y, Garssen J, van Helvoort A, Vos AP, and Knol J. Bacterial translocation is reduced by a specific nutritional combination in mice with chemotherapy-induced neutropenia. J Nutr 2011; 141(7): 1292-8.

85.   David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, and Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505(7484): 559-63.

86.   Ciorba MA, Hallemeier CL, Stenson WF, and Parikh PJ. Probiotics to prevent gastrointestinal toxicity from cancer therapy: an interpretive review and call to action. Curr Opin Support Palliat Care 2015; 9(2): 157-62.