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

Kate R. Secombe, Janet K. Coller, Rachel J. Gibson, Hannah R. Wardill and Joanne M. Bowen

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 after 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. 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. This leads to a heightened risk of adverse events such as infection and diarrhoea. Symptoms such as abdominal bleeding and abdominal pain are common, and result in increased hospital stays and the need for parenteral nutrition. 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 $1,500 per episode of severe diarrhoea. 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. Severe complications of CIGT such as bacteraemia and sepsis cause chemotherapy dose reductions and in profound cases, treatment cessation, compromising remission and increasing mortality. Subsequently, CIGT presents as a major clinical and economic burden.

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. These types of damage signals are recognised by receptors in the innate immune system, present on gastrointestinal tract cells.

It is now known that cancer treatments cause a raft of changes to the microbiome and host innate immune system. 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. 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 after chemotherapy. A semi-structured search of PubMed for full-text articles in English found more than 1,000 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-kB), 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-3314–16) 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 (Fig. 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 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 after 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. TLRs are present in the gastrointestinal mucosa on basolateral and apical surfaces of epithelial cells, professional immune cells, enteric neurons and glia. There are eleven TLRs found in humans, recognising diverse ligands including RNA, DNA and LPS. TLR4 is a particular focus of recent research due to its known expression changes after chemotherapy, and its ability to recognise patterns released by chemotherapy-damaged cells and therefore will be the focus here. TLR4 can be activated by exogenous and endogenous danger signals such as LPS, high mobility group box chromosomal protein 1 and heat shock proteins, 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. However, there is some disparity surrounding the role of TLR4. One study where TLR4 was blocked using naloxone was unable to replicate irinotecan-induced gastrointestinal damage, whereas other research has shown that TLR4 agonist LPS can protect intestinal crypts from other insults such as radiation. Studies have shown protection and exacerbation, possibly due to differences between acute and chronic injury 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. However, there is growing evidence that the reverse is also true, and that the microbial environment is similarly influenced by the innate immune system. This bidirectional interaction between microbiome and immune system has been described by Hooper et al. 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. 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. Van den Abbeele et al. 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 microbiota, 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. The small intestine does not have these two distinct layers, and instead relies on antimicrobial peptides/receptors such as RegIIIγ and TLRs to minimise bacterial penetration of the mucus layer.

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. Gut microbiota dysbiosis has been shown to reduce infiltrating mature APCs. Additionally, commensal bacteria can regulate dendritic cell activity. When investigating the role of the microbiome in APC development, it was found that TLR4 agonist LPS can protect intestinal crypts from other insults such as radiation. Studies have shown protection and exacerbation, possibly due to differences between acute and chronic injury, 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.
### Table 1. Summary of studies investigating gut microbiome changes due to cancer treatment

<table>
<thead>
<tr>
<th>Study Type/Sample Size</th>
<th>Cancer Treatment</th>
<th>Study population age (years)</th>
<th>Detection Method</th>
<th>Increases</th>
<th>Decreases</th>
<th>Outcome/commentary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-clinical (tumour bearing rats) n = 30</td>
<td>Irinotecan</td>
<td></td>
<td>RT-PCR, PCR-denaturing gel electrophoresis</td>
<td>Clostridium cluster X1 (0.5 log) and Enterobacteriaceae (1.5 log) (p &lt; 0.05)</td>
<td>Total bacteria number (by day 3, 1 log), Clostridium cluster XIVa (1–3 log), Lactobacillus and Bifidobacterium (p &lt; 0.05)</td>
<td>Pathogenic bacteria increased</td>
<td>Lin et al. 65</td>
</tr>
<tr>
<td>Pre-clinical (mice) n = 100</td>
<td>Irinotecan</td>
<td></td>
<td>Differences in gastrointestinal toxicity response between germ-free and conventionalised mice. Conventionalised mice = increased inflammation, lesions of gastrointestinal epithelium and increased permeability. Conventionalisation of germ-free reversed phenotype to conventionalised.</td>
<td></td>
<td></td>
<td></td>
<td>Pedroso et al. 44</td>
</tr>
<tr>
<td>Pre-clinical (mice) n = 120</td>
<td>Irinotecan</td>
<td></td>
<td>Differences in digestive toxicity response between germ-free and holoxenic mice. Holoxenic mice = higher intestinal damage score, more diarrhea. Germ-free mice were more resistant to irinotecan than holoxenic (higher lethal dose).</td>
<td></td>
<td></td>
<td></td>
<td>Bandi et al. 45</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 81</td>
<td>Irinotecan</td>
<td></td>
<td>Microbiological culture</td>
<td>Escherichia spp., Clostridium, Enterococcus, Serratia, Staphylococcus (qualitative results)</td>
<td>Peptostreptococcus, Bifidobacterium (qualitative results)</td>
<td>Microbiome changes corresponded with diarrhoea incidence</td>
<td>Stringer et al. 66</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 81</td>
<td>Irinotecan</td>
<td></td>
<td>RT-PCR</td>
<td>E. coli, Staphylococcus spp. (p &lt; 0.05)</td>
<td>Lactobacillus spp. (p &lt; 0.05)</td>
<td>Species that decreased were beneficial, major components of gut microbiome</td>
<td>Stringer et al. 67</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 48</td>
<td>Irinotecan, 5-FU or oxaliplatin</td>
<td></td>
<td>16S pyrosequencing</td>
<td>Irinotecan: fusobacteria (relative abundance 13-fold) and Proteobacteria (relative abundance 17-fold)</td>
<td></td>
<td>Also noted changes in serum and urine metabolome</td>
<td>Forsgard et al. 58</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 27</td>
<td>Irinotecan</td>
<td></td>
<td>Microbiological culture and RT-PCR</td>
<td>E. coli (qualitative results)</td>
<td>Bifidobacterium (qualitative results)</td>
<td>Changes also observed in mucous secretion and release</td>
<td>Stringer et al. 69</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 75</td>
<td>5-FU</td>
<td></td>
<td>Microbiological culture</td>
<td>Large intestine: Gram-negative facultatives (1-fold change in proportion)</td>
<td>Large intestine: Gram-positive facultatives (9-fold change in proportion)</td>
<td>Small intestine: shift from domination by Gram-positive cocci to Gram-negative rods</td>
<td>Von Bultzingslowen et al. 69</td>
</tr>
<tr>
<td>Clinical n = 16</td>
<td>Various chemotherapies</td>
<td>Cohort 1: mean = 71 (range 36–82), Cohort 2: mean = 63 (range 40–77)</td>
<td>Microbiological culture, qRT-PCR</td>
<td>E. coli and Staphylococcus spp. (no statistics due to low patient number)</td>
<td>Lactobacillus spp., Bifidobacterium spp., Bacteroides spp., and Enterococcus spp.</td>
<td></td>
<td>Stringer et al. 53</td>
</tr>
<tr>
<td>Clinical n = 9</td>
<td>Various chemotherapies + antibiotics</td>
<td>Paediatric cohort, age not reported</td>
<td>PCR-denaturing gel electrophoresis fingerprinting and in situ hybridisation</td>
<td>Enterococci (100-fold)</td>
<td>Anaerobic bacteria (10,000-fold), Commensal species (Bacteroides spp., Clostridium cluster XIVa, Fae calbac terium prausnitzii and Bifidobacterium spp., 3,000–6,000-fold)</td>
<td>Prophylactic and therapeutic antibiotic use did not explain changes in microbiome composition</td>
<td>van Wiet et al. 64</td>
</tr>
</tbody>
</table>
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 after 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 Bacteroidetes, Clostridium cluster IV and

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Treatment</th>
<th>Sample Size</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zwielehner et al.</td>
<td>Clinical</td>
<td>Chemotherapy+/- antibiotics</td>
<td>n = 17</td>
<td>TaqMan qPCR and gel electrophoresis</td>
<td>Decrease in total number and abundance of bacteria</td>
</tr>
<tr>
<td>Montassier et al.</td>
<td>Clinical</td>
<td>Chemotherapy</td>
<td>n = 8</td>
<td>16S rRNA 454 high throughput—pyrosequencing</td>
<td>Fusobacteriaceae (6-fold) and Streptococcaceae (p &lt; 0.05)</td>
</tr>
<tr>
<td>Nam et al.</td>
<td>Clinical</td>
<td>Radiotherapy (chemotherapy in subset of patients)</td>
<td>n = 9</td>
<td>16S rRNA 454 high throughput—pyrosequencing</td>
<td>Firmicutes (10%) Number of species-level taxa significantly reduced after therapy</td>
</tr>
</tbody>
</table>

| Increase and decrease columns refer to bacterial species that changed after cancer treatment per qRT-PCR, qualitative real time polymerase chain reaction, 16S rRNA, 454 high throughput—pyrosequencing. |
Table 2. Summary of studies investigating probiotics and antibiotics in significantly modulating cancer treatment-induced gastrointestinal toxicity.

<table>
<thead>
<tr>
<th>Study Type/ Sample Size</th>
<th>Cancer treatment</th>
<th>Study population age (years)</th>
<th>Probiotic/ antibiotic</th>
<th>Administration</th>
<th>Endpoint</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-clinical (rats) n = 48</td>
<td>Irinotecan</td>
<td></td>
<td>VSL#3</td>
<td>Oral gavage, different schedules: 7, 21, 28 days pre-, pre- and post- or post-treatment</td>
<td>Clinical and histological injury markers</td>
<td>Probiotic reduced weight loss compared to irinotecan alone (5.3% vs 12.6%, p &lt; 0.05), increased crypt proliferation (p &lt; 0.05), inhibited apoptosis (p &lt; 0.05)</td>
<td>Bowen et al. 55</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 8</td>
<td>MTX</td>
<td></td>
<td>Cow or sheep milk yoghurt with probiotics</td>
<td>Oral gavages twice daily 7 days prior to MTX and for remainder of trial</td>
<td>Intestinal barrier function, lactulose/ mannitol ratio</td>
<td>Sheep yoghurt probiotic improved histological damage and small intestinal permeability (p &lt; 0.05)</td>
<td>Southcott et al. 54</td>
</tr>
<tr>
<td>Pre-clinical (rats) n = 27</td>
<td>MTX</td>
<td></td>
<td>Streptococcus thermophilus</td>
<td>7 oral gavages from 48 h prior to MTX to 72 h after</td>
<td>MPO levels, 13(C) sucrose breath test, histological injury markers</td>
<td>High-dose probiotic partially attenuated mucositis (</td>
<td>MPO</td>
</tr>
<tr>
<td>Pre-clinical (tumour bearing rats) n = 36</td>
<td>MTX</td>
<td></td>
<td>Streptococcus thermophilus</td>
<td>7 oral gavages from 48 h prior to MTX to 72 h after</td>
<td>MPO levels, 13(C) sucrose breath test, histological injury markers</td>
<td>No difference between groups</td>
<td>Tooley et al. 57</td>
</tr>
<tr>
<td>Clinical n = 15</td>
<td>Irinotecan + 5-FU/ leucovorin</td>
<td>Not reported</td>
<td>Neomycin + bacitracin</td>
<td>Oral, 3 x daily, from second chemotherapy cycle, days 2–5 and 16–19 of each cycle</td>
<td>Diarrhoea reduction or resolution</td>
<td>Antibiotic = complete resolution of diarrhoea from 2nd to 4th chemotherapy cycle in all patients</td>
<td>Alimonti et al. 60</td>
</tr>
<tr>
<td>Clinical n = 62</td>
<td>Irinotecan</td>
<td>Range = 36–80</td>
<td>Neomycin</td>
<td>Oral, 3 x daily for 3 days, starting 2 days before irinotecan</td>
<td>Grade 3 diarrhoea levels</td>
<td>No difference between groups</td>
<td>de Jong et al. 62</td>
</tr>
<tr>
<td>Clinical n = 7</td>
<td>Irinotecan</td>
<td>Median = 57, Range = 49–71</td>
<td>Neomycin</td>
<td>Oral, 3 x daily, if developed diarrhoea in first chemo course, oral, days –2 to 5 of other cycles</td>
<td>Diarrhoea reduction</td>
<td>Diarrhoea ameliorated in 6 of 7 patients (p = 0.033)</td>
<td>Kehrer et al. 61</td>
</tr>
<tr>
<td>Clinical n = 150</td>
<td>Adjuvant 5-FU (colorectal cancer patients)</td>
<td>Median = 60, Range = 31–75</td>
<td>Lactobacillus rhamnosus</td>
<td>Oral, 2 x daily, 24 weeks of treatment</td>
<td>Reduction of severe diarrhoea</td>
<td>Less severe diarrhoea (22 vs 37%, p = 0.027), less abdominal discomfort (22 vs 12%, p = 0.025), less dose reductions (22% vs 47%, p = 0.0008)</td>
<td>Osterlund et al. 58</td>
</tr>
<tr>
<td>Clinical n = 42</td>
<td>Various chemotherapies paediatric population</td>
<td>Range = 14 months –13 years 4 months</td>
<td>Bifidobacterium breve strain Yakult</td>
<td>Oral, 3 x daily, 2 weeks before treatment, 6 weeks after (8 weeks total)</td>
<td>Effect on infectious complications</td>
<td>Reduced need for antibiotics (3.2 vs 6.9 days, p = 0.04) and frequency of fever (44 vs 68%)</td>
<td>Wada et al. 72</td>
</tr>
</tbody>
</table>
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 preclinical 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 as 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. After 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 after chemotherapy.79 This could possibly occur via an altered capacity to mount an immune response. For example, commensal
<table>
<thead>
<tr>
<th>Study Type/ Sample Size</th>
<th>Cancer treatment</th>
<th>Study population age (years)</th>
<th>Detection method</th>
<th>Predictive factor</th>
<th>Pre-therapy significant differences compared to controls</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Pelvic radiotherapy</td>
<td>Median = 51 years, range = 41–64</td>
<td>16S rRNA pyrosequencing</td>
<td>Development of diarrhoea</td>
<td>Bacteroidetes, Dialister, Veillonella (p &lt; 0.05)</td>
<td>Patients who developed diarrhoea: lower bacterial diversity (Shannon’s index = 2.74 vs 3.78, p &lt; 0.01), higher Firmicutes/Bacteroidetes ratio (p &lt; 0.05)</td>
<td>Wang et al. 81</td>
</tr>
<tr>
<td>Clinical</td>
<td>Radiotherapy—head and neck cancers</td>
<td>Mean = 47.2, range = 22–75</td>
<td>16S rRNA pyrosequencing</td>
<td>Severe oral mucositis</td>
<td>Actinobacillus during erythema</td>
<td>Healthy controls: greater diversity of oropharyngeal bacteria. Built predictive model (AUC = 0.89) for mucositis aggravation i.e. mild progressing to severe. Could not construct model to predict aggravation prior to irradiation.</td>
<td>Zhu et al. 11</td>
</tr>
<tr>
<td>Clinical</td>
<td>Ipilimumab</td>
<td>Range = 28–85</td>
<td>16S rRNA pyrosequencing</td>
<td>Checkpoint blockade-induced colitis development</td>
<td>Bacteroidetes</td>
<td>Only study finding a protective phenotype. Model had sensitivity of 70% and specificity of 83%.</td>
<td>Dubin et al. 10</td>
</tr>
<tr>
<td>Clinical</td>
<td>Pelvic radiotherapy—gynaecological malignancies</td>
<td>Mean = 71.5</td>
<td>Used electronic nose and Field Asymmetric Ion Mobility Spectrometry</td>
<td>Gastrointestinal toxicity (Irritable Bowel Syndrome scale)</td>
<td>--</td>
<td>Clear separation between high and low toxicity groups after 4 weeks of treatment (90% accuracy of reclassification).</td>
<td>Covington et al. 82</td>
</tr>
</tbody>
</table>

Increase and decrease columns refer to differences in bacterial species from healthy controls or low toxicity groups that led to gastrointestinal toxicity symptoms. AUC, area under the curve.
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. It has been well established that *Bacteroides* genus levels are decreased by chemotherapy in both pre-clinical and clinical models. 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. 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. 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. Additionally, recent trials of ingestible electronic capsules have showed the ability to sense gases produced by the microbiome in the gastrointestinal tract. 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 immune-genetic factors relating to the *TNF-a* and TLR2 have been correlated with more severe toxicity. 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., and more recently by Wardill and Tissing. Use of the microbiome as a predictive marker is gaining support in a variety of fields, including recent prediction of chemotherapy-related bloodstream infection. 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.

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 after chemotherapy. One study of patients undergoing pelvic radiotherapy found that patients who went on to develop diarrhoea had lower bacterial diversity and a higher

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**Figure 1. Pathobiology of CIGT.** Chemotherapy treatment leads to direct DNA damage and release of Reactive Oxygen Species, causing transcription factor activation and release of pro-inflammatory cytokines. This leads to an amplification of apoptosis and epithelial damage, eventually causing intestinal ulceration and severe pain, in some cases caused by bacterial translocation through these ulcers. Removal of chemotherapy treatment will allow healing. Since the five-phase model was introduced in 2004, significant research has been done to improve this model. We now suggest that bacterial and immune cells not only play a role in the ulceration phase, but also have an important baseline role in setting up risk of severe toxicity. [Color figure can be viewed at wileyonlinelibrary.com]
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 study82 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 pretreatment 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,63 changes in diet84 or probiotics.85 Of these, diet modification has been shown to be one of the most consistent and predictable ways of remodel-ling 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.63,67 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 after 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.

**References**


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