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REVIEW ARTICLE

Toll-like receptor/interleukin-1 domain innate immune signalling pathway genetic variants are candidate predictors for severe gastrointestinal toxicity risk following 5-fluorouracil-based chemotherapy

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Abstract

Purpose Severe gastrointestinal (GI) toxicity is a common adverse effect following 5-fluorouracil (5-FU)-based chemotherapy treatment. The presence of severe GI toxicity leads to treatment revisions, sub-optimal therapy outcomes, and decreases to patients' quality of life. There are no adequate predictors for 5-FU-induced severe GI toxicity risk. The Toll-like receptor/interleukin-1 (TIR) domain innate immune signalling pathway is known to be a mediating pathway in the development of GI toxicity. Hence, genetic variability in this signalling pathway may alter the pathophysiology of GI toxicity and, therefore, be predictive of risk. However, little research has investigated the effects of TIR domain innate immune signalling pathway single nucleotide polymorphism (SNPs) on the risk and development of severe GI toxicity.

Methods This critical review surveyed the literature and reported on the in vitro, ex vivo and in vivo effects, as well as the genetic association, of selected TIR domain innate immune signalling pathway SNPs on disease susceptibility and gene functioning.

Results Of the TIR domain innate immune signalling pathway SNPs reviewed, evidence suggests interleukin-1 beta (*IL1B*) and tumour necrosis factor alpha (*TNF*) SNPs have the greatest potential as predictors for severe GI toxicity risk. These results warrant further research into the effect of *IL1B* and *TNF* SNPs on the risk and development of severe GI toxicity.

Conclusions SNPs of the TIR domain innate immune signalling pathway have profound effects on disease susceptibility and gene functioning, making them candidate predictors for severe GI toxicity risk. The identification of a predictor for 5-FU-induced severe GI toxicity will allow the personalization of supportive care measures.

Keywords 5-Fluorouracil (5-FU) · Gastrointestinal (GI) toxicity · Toll-like receptors (TLRs) · Proinflammatory cytokines · Single nucleotide polymorphisms (SNPs) · Genetic variant

Abbreviations

5-FU	5-fluorouracil
DAMPs	Damage associated molecular patterns
DPD	Dihydropyrimidine dehydrogenase enzyme
DPYD	Dihydropyrimidine dehydrogenase gene
GI	Gastrointestinal
HSCT	Hematopoietic stem cell transplantation
IKK	Inhibitor of NF-κB-kinase complex
IRAK1	Interleukin-1 receptor-associated kinases 1
IRAK4	Interleukin-1 receptor-associated kinases 4
MYD88	Myeloid differentiation primary response protein 88
NCI CTCAE v5.0	The National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0

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PAMPs	Pathogen associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
SNPs	Single nucleotide polymorphisms
TAB1	Tak-1 binding protein 1
TAB2	Tak-1 binding protein 2
TAK1	Transforming growth factor beta factor-β activated kinase 1
TIR	Toll-like receptor/interleukin-1
TLR	Toll-like receptor
TRAF6	TNF receptor associated factor 6

Introduction

5-fluorouracil (5-FU) is a commonly administered chemotherapy drug used for the treatment of breast, colorectal and upper gastrointestinal (GI) tract solid tumours [1, 2]. Although highly effective, with response rates for 5-FU-based regimens between 40 and 50% in patients with advanced colorectal cancer [3, 4], 5-FU causes severe damage to mucosal membranes of the GI tract [5–7]. This damage results in apoptosis, altered histopathology and an increase in proinflammatory cytokine expression giving rise to inflammation [5–7]. This in turn leads to the clinical manifestation of GI toxicities including but not limited to, mucositis, diarrhea, nausea and vomiting [8].

The development of GI toxicity is variable amongst patients, making it difficult to predict which patients will develop severe toxicity. Although supportive care measures are recommended and suggested, these are limited and are for use in highly specific patient cohorts [9]. Additionally, supportive care is often administered therapeutically not prophylactically, which in some patients is too late for the management and relief of severe GI toxicity symptoms [9, 10]. In these patients, further interventions are required to improve symptom management and relieve severe GI toxicity symptoms [11].

Currently, there are no adequate predictors for 5-FU-induced severe GI toxicity risk. It is imperative predictors are identified and translated to clinical practice to identify "at risk" patients prior to 5-FU treatment. This will facilitate proactive delivery and personalization of supportive care measures aimed at reducing the severity of GI toxicity experienced.

Following administration of 5-FU, an innate immune inflammatory response is initiated, mediated by the Toll-like receptor/interleukin-1 domain innate immune signalling pathway. Toll-like receptor/interleukin-1 is commonly abbreviated to TIR. Activation of the TIR domain innate immune signalling pathway leads to the upregulation of potent transcription factors and secretion of proinflammatory cytokines [7, 12, 13]. Increased levels of these proinflammatory cytokines, in particular tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), have been significantly associated with severe GI toxicity in both preclinical and clinical studies [5, 7, 14]. Due to the importance of the TIR domain innate immune signalling pathway in inflammatory signalling and subsequent severe GI toxicity development, single nucleotide polymorphisms (SNPs) in this pathway may be potential predictors for severe GI toxicity risk. However, these SNPs are largely understudied in the context of GI toxicity.

This critical literature review will firstly provide an overview of 5-FU-based chemotherapy and the pathophysiology of GI toxicity; then outline and summarize previous research on the influence and function of TIR domain innate immune signalling pathway SNPs and how this can be applied to GI toxicity risk.

Background

5-FU mechanism of action

5-FU is an antimetabolite drug that inhibits DNA and RNA synthesis [1]. The uracil analogue was developed in the 1950s following identification of uracil metabolism as a potential target for therapy due to the rapid use of uracil by rat hepatomas compared to normal tissues [15]. In the liver, 5-FU is metabolised to three active metabolites, fluorodeoxyuridine monophosphate (FdUMP), fluoro-deoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) [16, 17]. The oral prodrug of 5-FU, capecitabine, travels unaltered through the gut wall where it is converted to 5-FU by liver carboxylesterase (CES) 1 and CES2, and cytidine deaminase [18, 19]. The principal mechanism of action of 5-FU is inhibition of thymidylate synthase (TS, encoded by the gene *TYMS*), a critical enzyme necessary for conversion of precursor deoxyribonucleotides required for purine and pyrimidine synthesis. Its principal active metabolite, FdUMP, inhibits TS, whilst metabolites FdUTP and FUTP directly misincorporate into DNA and RNA, disrupting DNA and RNA synthesis, RNA processing and protein synthesis [16, 20, 21]. 5-FU catabolism is governed by the rate determining enzyme dihydropyrimidine dehydrogenase (DPD, encoded by the *DPYD* gene), catabolising 80% of administered 5-FU to dihydroflurouracil, which is then excreted in the urine [16, 22].

Current 5-FU regimens

5-FU is administered intravenously as either a bolus dose or continuous infusion over a 24–48 h period to treat solid tumours of the breast, colon and upper GI tract [23]. In breast cancer regimens, 5-FU is generally administered in

conjunction with epirubicin and cyclophosphamide [23]. For colorectal and upper GI tract cancers, 5-FU is administered as either a monotherapy in synergistic combination with leucovorin or in combination with other chemotherapeutics such as irinotecan, oxaliplatin and cisplatin [23]. 5-FU is used in both a curative and palliative care setting. Additional therapies including radiation therapy, monoclonal antibodies (bevacizumab, cetuximab), taxanes (docetaxel and paclitaxel) and tyrosine kinase inhibitors (lapatinib) may also be administered in conjunction with or following 5-FU treatment in an attempt to further reduce tumour activity [23]. Capecitabine, the oral and more selective prodrug of 5-FU, may also be substituted in replacement of 5-FU in the before-mentioned regimens [24].

5-FU administration induces GI toxicity

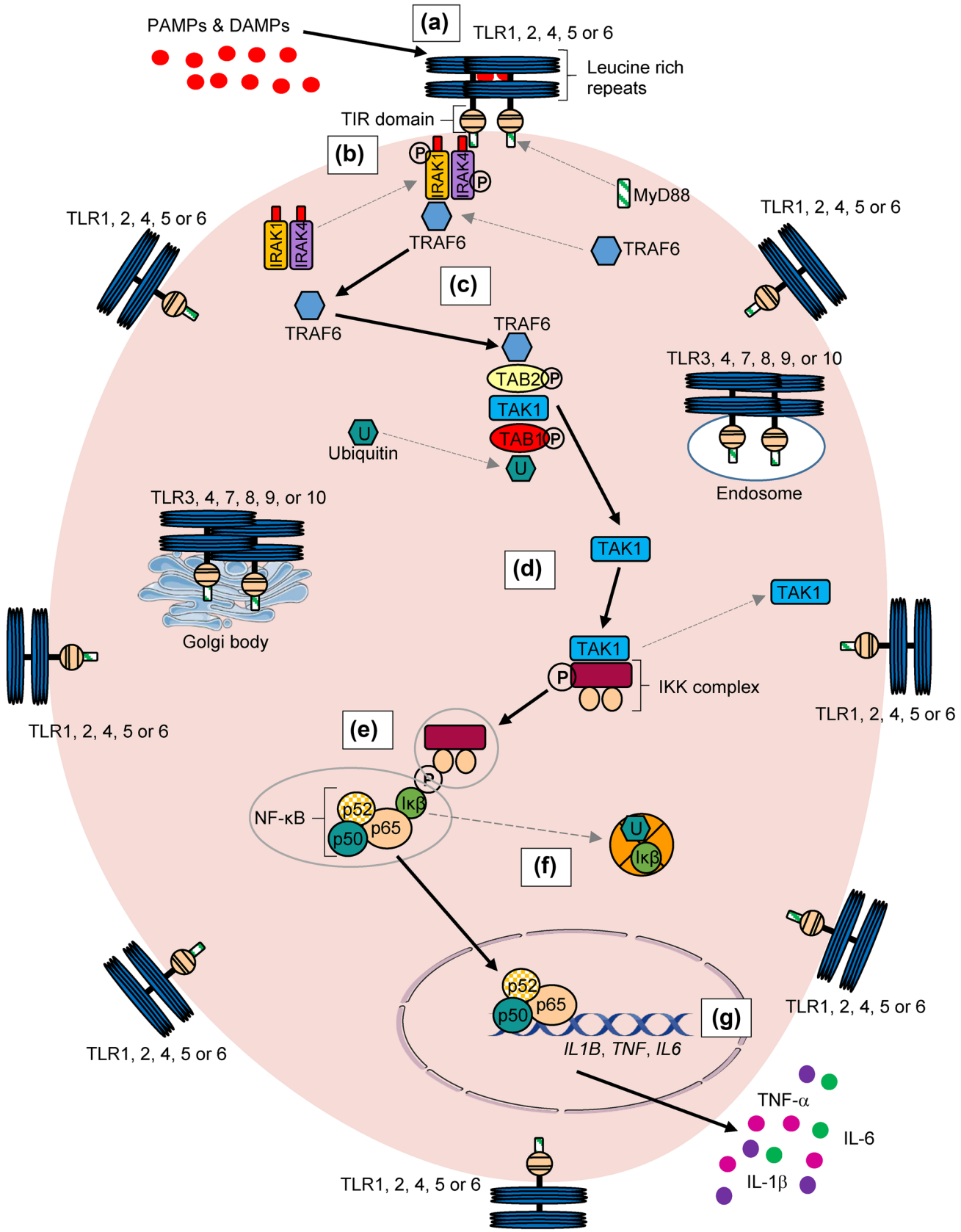
Of all patients receiving 5-FU-based therapies, 25–50% will experience GI toxicity [25]. Recording the prevalence and incidence of GI toxicity is vital for symptom management. However, this is often difficult and inconsistent due to a lack of standardised scoring criteria [26]. A number of toxicity grading scales exist, each with their own toxicity grading criteria. Among the most commonly used toxicity grading scale is The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v5.0) [27]. The NCI CTCAE was developed during the late 1980s and uses clinical symptoms and number of events to grade GI toxicity on a scale of 1 to 5 [27, 28]. GI toxicity graded as 1 or 2 is classified as 'mild' toxicity whilst GI toxicity graded 3 or 4 is classified as 'severe' toxicity [27]. Additionally, patients can also be categorized as suffering from a grade 3 or 4 GI toxicity on the NCI CTCAE v5.0 if they receive a dose reduction, treatment delay, cease treatment prematurely and/or are hospitalized as a direct result of GI toxicity [27, 28].

There is currently no effective approach to prevent GI toxicity [7]. The Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO) provide world-wide clinical practice guidelines for management of GI toxicity secondary to cancer therapy [9, 29, 30]. Table 1 lists the current MASCC/ISOO recommendations and suggestions for the management and relief of oral mucositis, diarrhea, nausea and vomiting in adults [9, 10, 31].

In addition to GI toxicity, 5-FU can also induce hand and foot syndrome, peripheral neuropathy and hematological toxicities including leukopenia, thrombocytopenia and neutropenia [16]. This is due to off-target effects of 5-FU; 5-FU metabolites non-specifically target highly proliferative basal epithelium and hematopoietic progenitor cells, as well as highly proliferative cancer cells [7, 32]. Bolus administration of 5-FU increases the risk of hematological toxicities whilst continuous infusion of 5-FU increases the likelihood of hand and foot syndrome [16, 33]. In contrast, treatment modality does not affect the risk of GI toxicity [33]. Furthermore, patients with GI toxicity may also develop additional toxicities and experience 'toxicity clusters' [34]. In a cohort of patients with colorectal cancer receiving 5-FU-based treatment, diarrhea was strongly linked with the presence of bloating, constipation and cystitis, whilst vomiting was strongly linked with the presence of nausea, dehydration and chills [34]. No links were identified between GI toxicities and hematological or neurological toxicities [34]. Although research is still in its infancy, symptoms grouped in

Table 1 MASCC/ISOO clinical practice guidelines for the management of GI toxicity

Oral Mucositis [9]
Recommended
Thirty min of oral cryotherapy (sucking on ice chips) to prevent mucositis in patients receiving 5-FU bolus regimens
Suggested
Good oral hygiene (regular tooth brushing, flossing and mouth rinsing) to prevent mucositis across all treatment modalities
Diarrhea [9]
Recommended
Loperamide use to treat moderate diarrhea induced by standard or high-dose chemotherapy
Octreotide use to treat diarrhea induced by standard or high-dose chemotherapy if loperamide is ineffective
Suggested
Probiotics such as *Lactobacillus* use prior to and during chemotherapy treatment to maintain gut homeostasis and reduce diarrhea occurrence
Nausea and vomiting [10, 31]
Recommended
Prophylactic administration of antiemetics such as aprepitant (substance P antagonist), ondansetron (5-HT_3 receptor antagonist), dexamethasone (corticosteroid) or metoclopramide (dopamine-receptor antagonist) for patients receiving low emetogenic or high-dose chemotherapy
No prophylactic administration of antiemetics for patients receiving minimal emetogenic chemotherapy
Patients whom suffer vomiting should be treated as if they were receiving low emetogenic chemotherapy
Breakthrough nausea and vomiting (categorized as nausea or vomiting that occurs within 5 days of chemotherapy administration following the use of antiemetic agents) treated with olanzapine (antipsychotic medication)


PAMPs & DAMPs
(a)
TLR1, 2, 4, 5 or 6
Leucine rich repeats
TIR domain
(b)
P
IRAK1
IRAK4
P
MyD88
TLR1, 2, 4, 5 or 6
IRAK1
IRAK4
TRAF6
TRAF6
TLR1, 2, 4, 5 or 6
(c)
TRAF6
TRAF6
TAB2 P
TAK1
TAB1 P
U
Ubiquitin
U
TLR3, 4, 7, 8, 9, or 10
Endosome
TLR3, 4, 7, 8, 9, or 10
Golgi body
(d)
TAK1
TAK1
TAK1
P
IKK complex
TLR1, 2, 4, 5 or 6
TLR1, 2, 4, 5 or 6
(e)
P
NF-κB
p52
Iκβ
p50
p65
U
Iκβ
(f)
TLR1, 2, 4, 5 or 6
TLR1, 2, 4, 5 or 6
p52
p65
p50
(g)
IL1B, TNF, IL6
TNF-α
IL-6
IL-1β
TLR1, 2, 4, 5 or 6

◂**Fig. 1** The TIR domain innate immune signalling pathway: **a** following 5-FU administration, DAMPs are released from injured and apoptotic cells whilst PAMPs are produced from colonized bacteria and microorganisms. DAMPs and PAMPs are recognized by TLR2 and TLR4 and on binding, stimulate the homodimerization of TLR4, and heterodimerization of TLR2 with either TLR1 or TLR6; **b** dimerization prompts the recruitment and binding of MyD88, which in turn recruits IRAK1 and IRAK4. Phosphorylation of IRAK1 and IRAK4 enables binding of TRAF6; **c** TRAF6 disengages from the newly formed IRAK complex and forms a new complex with TAK1, TAB1 and TAB2. Phosphorylation of TAK1 and TAB2 occurs, stimulating ubiquination of TRAF6 and subsequent activation of TAK1; **d** activated TAK1 disengages and phosphorylates the IKK complex; **e** phosphorylated IKK complex disengages from TAK1 and phosphorylates the Iκβ subunit of NF-κB; **f** following phosphorylation, Iκβ is ubiquinated and degraded from NF-κB by the 26S proteosome. Activated NF-κB translocates to the nucleus; **g** in the nucleus, NF-κB upregulates gene transcription and subsequent production and secretion of proinflammatory cytokines such as IL-1β, TNF-α and IL-6

clusters may share a common biological mechanism meaning that, a predictive marker for severe GI toxicity risk may also have the ability to predict the risk of additional toxicities.

The issue at hand: severe GI toxicity

Of the 20–25% of patients who experience GI toxicity, a subset of these patients will develop severe GI toxicity, graded as ≥ 3 on the NCI CTCAE [27]. Supportive care measures are ineffective for patients with severe GI toxicity and treatment delays, dose reductions or premature treatment cessation will be required to manage and relieve symptoms [7]. Furthermore, severe GI toxicities can induce secondary symptoms such as pain, dehydration and malnutrition requiring opioid analgesics, intravenous fluids and parenteral nutrition [7, 8]. Treatment interruption may negatively influence prognosis and potentially impact long-term survival. In addition, the presence of severe GI toxicity can decrease patient quality of life whilst receiving treatment and increase health costs, with inpatient hospitalization as a direct result of severe GI toxicity estimated to cost the US health care system US$15,500 per episode [35]. Currently, there is no way to identify patients at most risk of severe GI toxicity prior to treatment.

How does 5-FU induce GI toxicity?

Inhibition of DNA and RNA synthesis by 5-FU results in significant cell injury and death

Following inhibition of DNA and RNA synthesis by 5-FU, base-excision repair is initiated to remove precursor deoxyribonucleotides and misincorporated 5-FU metabolites, leading to excessive DNA fragmentation resulting in mucosal injury and cell apoptosis [1, 36]. Consequently, injured and apoptotic cells release damage associated molecular patterns (DAMPs) including DNA, heat shock proteins and intracellular components [7, 37]. The injury and death of intestinal epithelial cells leads to the breakdown of the mucosal barrier, allowing entry of microorganisms and the colonization of bacteria [7]. Microorganisms and bacteria release their own endogenous danger signals such as lipopolysaccharide, lipoteichoic acid and single-stranded mRNA, termed pathogen-associated molecular patterns (PAMPs) [7, 37].

The release of DAMPs and PAMPs initiates a signalling cascade mediated by Toll-like receptors (TLRs) of the TIR domain innate immune signalling pathway. Activation of the TIR domain innate immune signalling pathway leads to the recruitment of leukocytes and subsequent secretion of proinflammatory cytokines such as TNF-α and IL-1β. These molecular events, particularly TLR signalling, underlie the pathophysiology of GI toxicity [7, 37–40].

The TIR domain innate immune signalling pathway underpins the pathophysiology of GI toxicity

TLRs are a family of highly conserved type 1 integral membrane glycoproteins containing an extracellular motif of leucine-rich repeats and cytoplasmic TIR domain (Fig. 1) [41–43]. To date, ten TLRs have been identified in humans and are present on either the cell surface of innate immune cells (TLR1, 2, 4, 5 and 6) or localized in intracellular compartments such as the Golgi bodies and endosomes (TLR3, 7, 8 and 9) [44, 45]. TLRs respond to a variety of PAMPs, DAMPs or synthetic compounds [46]. TLR2 and TLR4 are key TLRs which mediate mucosal destruction and protection against GI chemotoxicity [44, 47]. They are also abundantly present on a variety of cells throughout the GI tract including goblet cells, cells of the lamina propria and enterocytes [44, 47]. Activation of TLR2 and TLR4 initiates the innate immune signalling pathway via the TIR domain and leads to the subsequent activation and translocation of NF-κB [46]. The TIR domain innate immune signalling pathway is detailed in Fig. 1.

Following recognition and binding of DAMPs and PAMPs, TLR2 dimerizes with TLR1 or 6, whilst TLR4 homodimerizes [46]. TLR dimerization prompts recruitment and binding of adapter protein myeloid differentiation primary response protein 88 (MyD88) to the TIR domain [46]. Binding of MyD88 in turn recruits interleukin-1 receptor-associated kinases (IRAK) 1 and IRAK4, resulting in phosphorylation of IRAK4 and subsequent activation and auto-phosphorylation of IRAK1 [46]. Phosphorylation of IRAK1 and IRAK4 enables binding of TNF receptor associated factor (TRAF) 6 to the TLR/MyD88/IRAK complex. Once bound, TRAF6 disengages from the TLR/MyD88/IRAK complex and associates with

transforming growth factor beta factor-β activated kinase (TAK) 1, TAK1-binding protein (TAB) 1 and TAB2 to form a new complex in the cytosol [46]. TAB1 enhances TAK1 kinase activity whilst TAB2 is an adapter protein responsible for linking TAK1 with TRAF6. Phosphorylation of TAB2 and TAK1 then occurs and the remaining complex associates with ubiquitin enzymes leading to the ubiquitylation of TRAF6 and activation of TAK1 [46]. TAK1 then phosphorylates the IKK complex (inhibitor of NF-κβ-kinase complex).

Transcription factor NF-κB exists as a heterodimer of three subunits, NF-κβ1 (p50/p105), NF-κβ2 (p52/p100) and p65 (Rel A) bound to the inhibitor kappa beta (Iκβ) subunit, responsible for maintaining NF-κB in an inactive state [46, 48, 49]. Once phosphorylated, the IKK complex phosphorylates the Iκβ subunit of NF-κβ, leading to its ubiquitination and subsequent degradation from the NF-κB complex by the 26S proteasome. Degradation of Iκβ permits the translocation of activated NF-κB to the nucleus (Fig. 1) [48].

In the nucleus, NF-κB regulates nearly 200 target genes, many of which are implicated in mucosal injury and cell death [50]. Of particular importance is the upregulation of genes including interleukin-1 beta (*IL1B*) and tumour necrosis factor alpha (*TNF*) encoding the proinflammatory cytokines IL-1β and TNF-α, respectively [12]. Inactive precursor IL-1β is cleaved by caspase1/IL-1β-converting enzyme (ICE) to its active form and is released from peripheral blood mononuclear cells and tissue macrophages [51]. TNF-α is a pleiotropic cytokine produced by tissue macrophages, natural killer cells and T lymphocytes [52, 53]. The production of IL-1β and TNF-α mediates an inflammatory response reducing epithelial oxygenation, initiating mesenchymal-epithelial signalling and stimulating further injury and death in cells of the epithelium and submucosa [7, 13, 38]. In addition, these cytokines can amplify primary damage by degrading Iκβ, further activating NF-κB and instigating its translocation to the nucleus [7, 13, 38].

These changes have been reported in both preclinical and clinical studies following 5-FU administration. For example, in Dark Agouti rats given 5-FU, TNF-α levels were highly elevated in the oral mucosa, jejunum and colon whilst IL-1β levels were highly elevated in the oral mucosa [5]. Additionally, TNF-α and IL-1β levels were associated with severe GI damage including reduced epithelial thickness, blunting and fusion of villi and obliteration of crypts [5]. Elevated TNF-α and IL-1β are also observed in the peripheral blood of patients whom experience GI toxicity following chemotherapy [7, 14].

To summarise, following damage instigated by 5-FU administration, the TIR domain innate immune signalling pathway is activated initiating a signalling cascade leading to the activation of proinflammatory cytokines such as IL-1β and TNF-α. These proinflammatory cytokines are consistently elevated in severely damaged mucosal tissue and associated with GI toxicity symptoms such as diarrhea and mucositis.

Have any predictors for severe GI toxicity risk been identified?

Sex and age

Patient characteristics such as sex and age are important facets that must be considered when identifying patients at risk of severe GI toxicity [54]. However, evidence defining the relationship between sex, age and severe GI toxicity is highly contradictory, with sex and age more adequately serving as co-contributors rather than predictors for severe GI toxicity risk. In patients receiving 5-FU-based chemotherapy for colorectal, gastric and upper GI tract cancers, women have been identified at being of higher risk for developing mucositis ($P = 0.04$) [55]. No significant relationship was identified between patients of advanced age and GI toxicity risk in the cohort [55]. Likewise, in patents receiving 5-FU and leucovorin for colorectal cancer, females developed higher counts of severe diarrhea ($P < 0.01$) and vomiting ($P = 0.03$) compared to their male counterparts [56]. Univariate analysis identified sex ($P < 0.0001$) as an independent predictor for severe GI toxicity in addition to advanced age ($P = 0.001$) [55], contradictory to the findings by Schwab et al. [55]. In similar studies, no significant relationship between the development of GI toxicity and sex was identified in patients receiving 5-FU-based chemotherapy [57], whilst advanced age was identified as protective against severe GI toxicity, with decreases in diarrhoea and nausea/vomiting episodes in advanced age patients ($P = 0.01$) [58]. Although sex and age cannot solely predict severe GI toxicity risk and the utility of these associations is not understood [54], it is known that certain SNPs, such as those found in dihydropyrimidine dehydrogenase (*DPYD*), are more commonly identified in one sex than the other [55] and, this may be true for other SNPs that are identified as predictors for severe GI toxicity risk. Therefore, factors such as sex and age are important co-contributors for severe GI toxicity risk prediction.

SNPs in the 5-FU rate determining enzyme

SNPs in *DPYD*, responsible for encoding the 5-FU rate determining enzyme DPD, have been thoroughly investigated for their role in the development of severe GI toxicity. During 5-FU-based therapies, 60–100% of patients

carrying one or multiple *DPYD* SNPs develop grade 3–4 toxicities (non-hematological and hematological) compared to 10–20% of patients carrying no *DPYD* SNPs [11, 55, 59, 60]. In particular, two recent meta-analyses identified the presence of *DPYD* SNPs, IVS14 + 1G > A, 2846A > T, 1679T > G***, and 1236G > A, increased the risk of grade 3 GI toxicities such as mucositis and diarrhoea ($P \leq 0.05$) [61, 62]. In addition, a clinical study of patients with colorectal cancer receiving FOLFOX (fluorouracil and oxaliplatin) or FOLFIRI (fluorouracil and irinotecan), showed *DPYD* SNPs D949V and 2*A increased the risk of diarrhea ($P = 0.003$) and nausea/vomiting ($P = 0.007$), respectively [63]. However, all of these aforementioned SNPs occurred in less than 5% of the populations studied and did not account for the majority of severe GI toxicity events [61–63]. Consequently, the clinical usefulness of routine genotyping in Caucasians for these *DPYD* SNPs prior to 5-FU treatment hasn't been established and current literature regarding the clinical sensitivity and specificity of diagnostic testing of the *DPYD* gene in this population is yet to be demonstrated [61]. This is further complicated by inconsistencies in the literature regarding the association of particular *DPYD* variants and GI toxicity between different ethnic populations [64, 65], such that there is the possibility that a rare variant in one ethnicity may be more frequent in another ethnicity and, therefore, have a greater impact on GI toxicity prevalence. Nevertheless, the US Food and Drug Administration (FDA) and The European Medicines Agency (EMA) warns about the increased risk of severe toxicity in patients carrying at least one decreased function *DPYD* allele on 5-FU and capecitabine drug labels [66–68] and, the Clinical Pharmacogenomics Implementation Consortium recommends altered 5-FU and capecitabine dosing based on *DPYD* genotype and the resulting phenotype [54]. Interestingly though, neither agency requires genetic testing for *DPYD* SNPs prior to treatment [63, 64]. As a result, new genetic predictors for severe GI toxicity risk need to be identified.

Candidate predictors for severe GI toxicity may lie within the TIR domain innate immune signalling pathway

SNPs in key TIR domain innate immune signalling pathway genes are uniquely positioned to influence gene functioning

The TIR domain innate immune signalling pathway is known to play a pivotal role in the development of GI toxicity; therefore, SNPs in key TIR domain innate immune signalling pathway genes may alter the pathophysiology and subsequent severity of GI toxicity. Hence, these mutations are candidate predictors for severe GI toxicity risk. A subset of mutations in TIR domain innate immune signalling pathway genes *TLR2, TLR4, MyD88, IRAK1, IRAK4, TRAF6, NFKB, IL1B* and *TNF* are summarized in Table 2.

The *TLR2* and *TLR4* genes are located on chromosomes 4q31.3 and 9q33.1, respectively, with many *TLR* SNPs resulting in either synonymous or missense mutations [69]. Synonymous mutations do not alter the primary amino acid sequence. However, they may have indirect effects on gene functioning by influencing mRNA splicing and subsequent mRNA translation [70]. The *MYD88, IRAK1, IRAK4* and *TRAF6* genes are located on chromosomes 3q22.2, Xq28, 12q12, and 11p12, respectively [69]. Many of the *MYD88, IRAK1, IRAK4* and *TRAF6* mutations are located within the intronic regions of their respective genes. It is currently thought intronic mutations influence mRNA stability and translation, and may cause alternative splicing sites [41, 71, 72].

The *NFKB* gene is located on chromosome 4q24, with the *NFKB* mutation rs38362491 resulting in a premature stop codon [69]. Dependent on location in the gene, premature stop codons can lead to early termination of gene transcription and subsequent changes in protein function [73, 74]. The *IL1B and TNF* genes are located on chromosomes 2q14.1 and 6q21.33, respectively [69]. Key *IL1B* and *TNF* mutations lie within the promoter region and, therefore, are in prime location to alter transcription factor binding and subsequent gene transcription [69].

Evidence suggests TIR domain innate immune signalling pathway SNPs alter gene functioning and influence disease susceptibility

The in vitro, ex vivo and in vivo effects of key TIR domain innate immune signalling pathway SNPs as well as their association with disease susceptibility, are summarized in Table 3. It should be noted that for some SNPs, there is a lack of knowledge regarding functional impacts: a current limitation that warrants further investigation.

A pilot study conducted by Coller et al. was the first to investigate the association between innate immune receptor genetic variability and severe GI toxicity risk following 5-FU-based chemotherapy [77]. *TLR2* rs384100 and *TNF* rs1800629 SNPs (in conjunction with colorectal and gastric cancer types) were identified to be predictive of severe GI toxicity risk [77]. However, no relationship was identified with *TLR4, MyD88* and *IL1B* SNPs [77].

Other studies have focussed on association of SNPs with disease susceptibility and impact of the SNPs on protein expression. Patients with *TLR2* and *TLR4* SNPs were identified to have a higher risk of developing bloodstream infections and sepsis, and higher circulating levels of PAMPs such as lipoteichoic acid (as described in Table 3) [75, 78]. On a

Table 2 Summary information about TIR domain innate immune signalling pathway SNPs [69]

ID	Type of variant	Base pair change	Amino acid change	MAF
TLR2				
rs11938228	Intronic	C > A	–	CEU: 0.30, HCB: 0.35 JPT: 0.48, YRI: 0.10
rs1898830	Intronic	A > G	–	CEU: 0.31, HCB: 0.36 JPT: 0.49, YRI: 0.08
rs3804099	cds-synon	T > C	–	CEU: 0.45, HCB: 0.37 JPT: 0.27, YRI: 0.63
rs3804100	cds-synon	T > C	–	CEU: 0.08, HCB: 0.33 JPT 0.22, YRI: 0.06
rs4696480	Intronic	T > A	–	CEU: 0.00, HCB: 0.00 JPT 0.00, YRI: 0.00
rs5743708	Missense	G > A	Arginine > glutamine	CEU: 0.05, HCB:0.00 JPT:0.00, YRI:0.00
TLR4				
rs10759930	Upstream	C > T	–	CEU: 0.37, HCB: 0.62 JPT: 0.65, YRI: 0.05
rs10759932	Upstream	T > C	–	CEU: 0.14, HCB: 0.26 JPT: 0.25, YRI: 0.25
rs4986790	Missense	A > G	Aspartate > glycine	CEU: 0.04, HCB: 0.00 JPT: 0.00, YRI: 0.04
rs4986791	Missense	C > T	Threonine > isoleucine	CEU: 0.05, HCB: 0.01 JPT: 0.00, YRI: 0.00
rs5030710	cds-synon	T > C	–	CEU: 0.00, HCB: 0.00 JPT: 0.00, YRI: 0.18
rs7044464	Upstream	T > A	–	CEU: 0.14, HCB: 0.07 JPT: 0.07, YRI: 0.29
rs7856729	Upstream	G > T	–	CEU: 0.15, HCB: 0.05 JPT: 0.06, YRI: 0.41
MYD88				
rs6853	3′-UTR	A > G	–	CEU: 0.12, HCB: 0.03 JPT: 0.01, YRI: 0.33
rs7744	3′-UTR	A > G	–	CEU: 0.15, HCB: 0.41 JPT: 0.28, YRI: 0.01
IRAK1				
rs1059701	cds-synon	C > T	–	HCB: 0.14, JPT: 0.18, YRI: 0.15
rs1059702	Missense	T > C	Phenylalanine > serine	CEU: 0.79, HCB: 0.15 JPT: 0.24, YRI: 0.98
rs1059703	Missense	C > T	Serine > leucine	CEU: 0.77, HCB: 0.17 JPT: 0.20, YRI: 0.62
rs2239673	Intronic	C > T	–	GMAF: 0.48
rs3027898	Downstream	C > A	–	CEU: 0.74, HCB: 0.12 JPT: 0.22, YRI: 0.52
rs5945174	Intronic	G > A	–	GMAF: 0.48
rs7061789	Intronic	G > A	–	GMAF: 0.48
rs731642	Intronic	G > A	–	GMAF: 0.44
IRAK4				
rs1141168	3′-UTR	A > G	–	CEU: 0.53, HCB: 0.47 JPT: 0.63
rs1461567	Intronic	C > T	–	CEU: 0.03, HCB: 0.41 JPT: 0.54, YRI: 0.02
rs3794262	Intronic	T > A	–	CEU: 0.93, HCB: 0.83 JPT: 0.91, YRI: 0.38
rs4251429	Intronic	G > C	–	CEU: 0.02, HCB: 0.07 JPT: 0.06, YRI: 0.35

Table 2 (continued)

ID	Type of variant	Base pair change	Amino acid change	MAF
rs4251431	Intronic	G > T	–	CEU: 0.05, HCB: 0.14 JPT: 0.14, YRI: 0.16
rs4251466	Intronic	C > T	–	CEU: 0.08, HCB: 0.09 JPT: 0.06, YRI:0.22
rs4251513	Intronic	C > G	–	CEU: 0.50, HCB: 0.42 JPT: 0.32, YRI: 0.05
rs4251532	Intronic	C > T	–	CEU: 0.07, HCB: 0.16 JPT: 0.09, YRI: 0.62
rs4251545	Missense	G > A	Alanine > threonine	CEU: 0.08, HCB: 0.09 JPT: 0.06, YRI: 0.31
rs4251569	5′-UTR	C > T	–	CEU: 0.09, HCB: 0.17 JPT: 0.12, YRI: 0.00
TRAF6				
rs16928973	Intronic	C > T	–	CEU: 0.19, HCB: 0.01 JPT: 0.12, YRI: 0.00
rs331449	Intronic	T > C		CEU: 0.00, HCB: 0.00 JPT: 0.00, YRI: 0.20
rs3740961	3′-UTR	A > G	–	CEU: 0.11, HCB: 0.51 JPT: 0.55, YRI: 0.01
rs5030411	Intronic	C > T	–	CEU: 0.60, HCB: 0.71 JPT: 0.72, YRI: 0.06
rs5030416	Intronic	A > C	–	CEU: 0.17, HCB: 0.10 JPT: 0.14, YRI: 0.16
rs5030445	Intronic	A > T		GMAF: 0.24
NFKB				
rs28362491	Insertion/deletion	– > ATTG	Delete codon	GMAF: 0.42
IL1B				
rs1143623	Upstream	C > G	–	CEU: 0.67, HCB: 0.61 JPT: 0.67, YRI: 0.94
rs1143627	Promoter	T > C	–	CEU: 0.37, HCB: 0.47 JPT: 0.46, YRI: 0.64
rs1143634	cds-synon	T > C	–	CEU: 0.02, HCB: 0.01 JPT: 0.05, YRI: 0.09
rs16944	Promoter	C > T	–	CEU: 0.35, HCB: 0.45 JPT: 0.47, YRI: 0.58
rs4848306	Promoter	G > A	–	CEU: 0.46, HCB: 0.52 JPT: 0.46, YRI: 0.30
TNF				
rs1799964	Downstream	T > C	–	CEU: 0.21, HCB: 0.23 JPT: 0.14, YRI: 0.12
rs1800629	Promoter	G > A	–	CEU: 0.17, HCB: 0.03 JPT: 0.02, YRI: 0.09
rs1800750	Promoter	G > A	–	CEU: 0.01, HCB:0.00 JPT: 0.00, YRI: 0.01
rs361525	Promoter	G > A	–	GMAF: 0.06
rs4248158	Downstream	C > T	–	GMAF: 0.02

> change, *cds-synon* coding synonymous mutation, *CEU* Caucasian population Utah, USA, *GMAF* global minor allele frequency, *HCB* Asian population Beijing, China, *JPT* Asian population Tokyo, Japan, *MAF* minor allele frequency, *UTR* untranslated region, *YRI* Sub-African population Yoruba, Nigeria

molecular level, *TLR2* and *TLR4* SNPs have been linked to altered interferon gamma secretion, altered specific antibody responses and reduced NF-κB circulating serum levels and activation (as described in Table 3) [75, 76, 81]. No relationship was identified between *MYD88* SNPs and TNF-α and IL-6 concentrations post-stimulation of ex vivo PBMCs

Table 3 In vitro, ex vivo, in vivo and genetic association studies investigating the effects of TIR domain innate immune signalling pathway SNPs on disease susceptibility, gene transcription, protein binding and cytokine secretion

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
TLR2	Ex vivo				
	rs4696480	Stimulated TNF-α, IL-6 and IL-10 secretion from isolated PMNs in patients with cirrhosis	$n=114$	↓ stimulated TNF-α secretion in HE and HM vs WT ($P<0.01$) ↓ stimulated IL-6 secretion in HE and HM vs WT ($P<0.01$) ↑ stimulated IL-10 secretion in HE and HM vs WT ($P<0.01$)	[75]
	In vivo				
	rs3804099 rs3804100	Association of SNPs with measles vaccine-induced immune responses	$n=745$	Changes in antibody titer rs3804100 ↓ HE and HM vs WT ($P=0.002$) Changes in cytokine secretion rs3804099 ↓ IFNλ-1 HE ($P=0.002$) and HM ($P=0.009$) vs WT	[76]
	rs4696480	Circulating LTA, LPS, TNF-α and IL-6 levels; stimulated secretion of TNF-α, IL-16 and IL-10 from cultured PMNs from patients with cirrhosis	$n=114$	↓ circulating TNF-α in HM vs WT ($P<0.05$) ↓ circulating IL-6 in HE and HM vs WT ($P<0.05$)	[75]
	Genetic association				
	rs3804100	Association of SNPs with severe CIGT incidence following 5-FU-based treatment	$n=34$	Predictive of severe CIGT incidence in conjunction with *TNF* rs1800629 and colorectal and gastric cancer types ($P=0.033$, ROC AUC = 87.3%)	[77]
	rs5743708	Association of SNPs with the severity and course of sepsis in critically ill patients	$n=145$	↑ sepsis HE vs WT ($P=0.03$) ↑ number of infections HE vs WT ($P=0.012$) ↑ difficult-to-treat pathogens HE patients vs WT ($P=0.045$)	[78]
	rs5743708	Association of SNPs with sepsis and pneumonia in patients with AML following induction chemotherapy	$n=155$	↑ pneumonia HE vs WT ($P=0.006$, OR: 10.78 95% CI = 2–23)	[79]
	rs11938228 rs1898830 rs3804099 rs3804100	Association of SNPs with HCC susceptibility	$n=443$	rs3804099 and rs3804100 were in LD ($R^2>0.9$) ↓ HCC rs3804099 HE vs WT ($P<0.001$, OR = 0.49, 95% CI = 0.3–0.7) and rs3804100 HE vs WT ($P<0.001$, OR = 0.509, 95% CI = 0.3–0.8) rs11938228 & rs1898830: no significant associations	[80]
TLR4	In vitro				
	rs4986790 rs4986791	FEV1 decline following inhalation of LPS	$n=83$	SNPs were in LD (data not provided) Dose–response decline in FEV1: WT 1.86% vs HE 0.59% ($P=0.037$)	[81]
	Ex vivo				
	rs4986790	Stimulated TNF-α, IL-6 and IL-10 secretion from isolated PMNs in patients with cirrhosis	$n=114$	↓ stimulated IL-6 secretion in HE vs WT ($P<0.01$) ↑ stimulated IL-10 secretion in HE vs WT ($P<0.01$)	[75]
	rs4986790 rs4986791	LPS-stimulated response in isolated airway epithelial cells	$n=83$	SNPs were in LD (data not provided) ↓ IL-1α secretion by airway epithelial cells of HE vs WT patients ($P<0.01$)	[81]

Table 3 (continued)

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
	In vivo				
	rs10759930 rs10759932 rs16906053 rs5030710 rs7044464 rs7856729	Association of SNPs with measles vaccine-induced immune responses	$n=745$	Changes in antibody titer rs5030710 and rs16906053 ↑ HE ($P=0.001$) and HM ($P=0.005$) vs WT rs16906053 ↓ IFNλ-1 HE ($P=0.002$) and HM ($P=0.009$) vs WT rs7044464 and rs7856729 ↓ IL-10 HE and HM vs WT ($P=0.001$) rs10759932 ↑ IFN-α HE and HM vs WT ($P=0.001$) rs10759930 ↓ IFN-γ HE and HM vs WT ($P=0.001$) ↑ IFNλ-1 HE and HM vs WT ($P=0.006$)	[76]
	rs4986790	Circulating LTA, LPS, TNF-α and IL-6 levels; stimulated secretion of TNF-α, IL-16 and IL-10 from cultured PMNs from patients with cirrhosis	$n=114$	↓ stimulated IL-6 secretion in HE vs WT ($P<0.01$) ↑ stimulated IL-10 secretion in HE vs WT ($P<0.01$) No significant differences in circulating TNF-α, IL-6 and IL-10 levels between WT, HE and HM ($P>0.05$)	[75]
	rs4986790 rs4986791	LPS-stimulated response in THP-1 cells	–	↓ NF-κB activation by THP-1 cells with rs4986790 allele vs WT ($P<0.01$)	[81]
	Genetic association				
	rs4986790	Association of SNPs with the severity and course of sepsis in critically ill patients	$n=145$	↑ endocarditis HE vs WT ($P<0.05$) ↑ bloodstream infections HE vs WT ($P<0.05$) ↑ progression of sepsis to septic shock HE vs WT ($P=0.014$)	[78]
	rs4986790 rs4986791	Association of SNPs with severe CIGT incidence following 5-FU-based treatment	$n=34$	No significant associations	[77]
	rs4986790 rs4986791	Association of SNPs with sepsis and pneumonia in patients with AML following induction chemotherapy	$n=155$	SNPs were in LD (data not provided) ↑ sepsis development ($P=0.021$, OR: 3.6 95% CI = 1.2–10.4) ↑ pneumonia HE vs WT ($P=0.014$, OR: 3.6 95% CI = 1.3–9.9)	[79]
MyD88	Ex vivo				
	rs6853 rs7744	TRAF6 expression and stimulated TNF-α and IL-6 secretion from PBMCs of patients with sepsis-induced ALI	$n=90$	No significant associations	[71]
	Genetic association				
	rs6853	Association of SNPs with severe CIGT incidence following 5-FU-based treatment	$n=34$	No significant associations	[77]
	rs6853 rs7744	Association of SNPs with T2DM and its vascular complications	$n=1106$	rs6853 2.9 ↑ T2DM HE and HM vs WT ($P=0.01$, OR: 2.9 95% CI = 1.3–6.7)	[82]
	rs6853 rs7744	Association of SNPs with sepsis-induced ALI susceptibility	$n=548$	No significant associations	[71]

Table 3 (continued)

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
	rs6853 rs7744	Association of SNPs with SIPD risk and 2° SIPD symptoms	$n=200$	rs6853 Genotype analysis ↑ SIPD WT vs HE and HM ($P<0.0001$, OR = 2.1 95% CI = 1.8–2.5) ↑ death from SIPD HE and HM vs WT ($P=0.005$, OR = 16.1, 95% CI = 3.3–77.6) Allele analysis ↑ SIPD in patients carrying variant allele ($P<0.0001$, OR = 1.9, 95% CI = 1.5–2.6) ↑ association of death in patients carrying variant allele ($P=0.0064$, OR = 8.4, 95% CI = 2.5–28.5) rs7744: no significant associations	[83]
IRAK1	Ex vivo				
	rs1059703	TRAF6 expression and stimulated TNF-α and IL-6 secretion from PBMCs of patients with sepsis-induced ALI	$n=90$	No significant associations	[71]
	Haplotype rs1059701 rs1059702 rs1059703 rs2239673 rs3027898 rs5945174 rs7061789 rs731642	Association of IRAK-1 haplotype with stimulated NF-κB activation from peripheral blood neutrophils	$n=30$	↑ NF-κB nuclear accumulation post-LPS in HE and HM vs WT ($P=0.001$)	[84]
	Genetic association				
	rs1059703	Association of SNPs with sepsis-induced ALI susceptibility	$n=548$	No significant associations	[71]
	rs1059702 rs1059703	Association of SNPs with SIPD risk and 2° SIPD symptoms	$n=200$	rs1059701 ↑ SIPD WT vs HE and HM ($P=0.0067$, OR = 1.4 95% CI = 1.1–1.8) rs1059702 ↑ leukocytosis HE and HM vs WT ($P=0.046$, OR = 7.5, 95% CI = 1.9–30.2)	[83]
	Haplotype rs1059701 rs1059702 rs1059703 rs2239673 rs3027898 rs5945174 rs7061789 rs731642	Association of IRAK-1 haplotype with 2° symptoms in patients with sepsis	$n=30$	2.9 ↑ likelihood of developing septic shock in HE and HM vs WT ($P=0.047$) 2.6 ↑ likelihood of death from sepsis in HE and HM vs WT ($P=0.05$)	[84]

Table 3 (continued)

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
	Meta-analysis				
	rs1059702 rs1079703 rs3027898	Susceptibility of ADs in patients with *IRAK1* SNPs	–	rs1059702 ↑ ADs WT vs HE and HM ($P=0.000$, OR = 0.8, 95% CI = 0.7–0.8) ↑ SLE WT vs HE ($P=0.000$, OR = 0.7, 95% CI = 0.6–0.7) ↑ SSc WT vs HE and HM ($P=0.032$, OR = 0.8, 95% CI = 0.6–0.9) rs1079703 ↑ SLE variant vs WT ($P=0.000$, OR = 1.5, 95% CI = 1.3–1.6) rs3027898 ↑ ADs WT vs HE and HM ($P=0.034$, OR = 0.7, 95% CI = 0.6–0.9) ↑ SLE WT vs HE ($P=0.001$, OR = 0.8, 95% CI = 0.6–0.9) ↑ RA WT vs HE ($P=0.021$, OR = 0.8, 95% CI = 0.7–0.9)	[85]
IRAK4	Ex vivo				
	rs1461567 rs3794262 rs4251429 rs4251431 rs4251466 rs4251513 rs4251545 rs4251569	TRAF6 expression and stimulated TNF-α and IL-6 secretion from PBMCs of patients with sepsis-induced ALI	$n=90$	No significant associations	[71]
	Genetic association				
	rs1141168 rs1461567 rs4251513	Association of SNPs with SIPD risk and 2° SIPD symptoms	$n=200$	rs4251513 Genotype analysis ↑ SIPD WT vs HE and HM ($P<0.0001$, OR = 2.2, 95% CI = 1.6–3.0) ↑ SIPD sequelae HE and HM vs WT ($P=0.001$ OR = 7.1, 95% CI = 2.6–18.9) Allele analysis ↑ presence of SIPD in patients carrying variant allele ($P<0.0001$, OR = 1.5, 95% CI = 1.4–1.5) rs1461567 ↑ SIPD variant allele vs WT ($P=0.016$, OR = 1.5, 95% CI = 1.1–1.9) rs1141168: no significant associations	[83]
	rs1461567 rs4251513 rs4251532 rs4251569	Association of SNPs with T2DM and its vascular complications	$n=1106$	1.66 ↑ T2DM HE vs WT and HM ($P=0.03$, OR = 1.7, 95% CI = 1.1–2.6)	[82]
	rs1461567 rs3794262 rs4251429 rs4251431 rs4251466 rs4251513 rs4251545 rs4251569	Association of SNPs with sepsis-induced ALI susceptibility	$n=548$	No significant associations	[71]

Table 3 (continued)

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
TRAF6	Ex vivo				
	rs4755453 rs5030493 rs540386	TRAF6 expression and stimulated TNF-α and IL-6 secretion from PBMCs of patients with sepsis-induced ALI	$n=90$	rs4755453 ↑ TRAF6 baseline mRNA expression WT vs HE ($P=0.012$) and vs HM ($P=0.003$) ↑ TRAF6 post-LPS mRNA expression WT vs HE ($P=0.009$) and vs HM ($P=0.005$) ↑ TNF-α post-LPS secretion WT vs HE and HM ($P=0.015$) ↑ IL-6 post-LPS secretion WT vs HE and HM ($P=0.009$) rs5030493 & rs540386: no significant associations	[71]
	In vivo				
	rs331449	Association of SNPs with measles vaccine-induced immune responses	$n=745$	↑ HE and HM vs WT ($P=0.007$)	[76]
	Genetic association				
	rs16928973 rs5030445	Association of SNPs with T2DM and its vascular complications	$n=1106$	No significant associations	[82]
	rs4755453 rs5030493 rs540386	Association of SNPs with sepsis-induced ALI susceptibility	$n=548$	↓ % variant allele in sepsis-induced ALI vs sepsis alone groups ($P=0.003$,OR: 0.5, 95% CI = 0.4–0.7)	[71]
	rs3740961 rs5030411 rs5030416 rs5030445	Association of SNPs with susceptibility and severity of sepsis	$n=510$	No significant associations	[86]
NFKB	In vitro				
	rs28362491	Transient transfection luciferase reporter gene assay; and EMSA to determine protein binding in HeLA and HT-29 cells	–	↓ activity at baseline with variant vs WT allele in HeLA cells ($P=0.05$) ↓ activity following stimulation with variant vs WT allele in HeLA cells ($P=0.02$) ↓ activity following stimulation with variant vs WT allele in HT-29 cells ($P=0.02$)	[74]
	Genetic association				
	rs28362491	Association of SNP with IBD risk	$n=822$	No significant associations	[87]
	rs28362491	Association of SNPs with pathological response in patients with rectal cancer treated with PCRT	$n=159$	↑ pathological response HE and HM vs WT ($P=0.03$, OR = 6.4, 95% CI = 0.8–52.7)	[88]
IL1B	In vitro				
	rs1143634	Association of SNP with IL-1β serum concentration following infliximab treatment	$n=47$	↑ IL-1β with variant vs WT allele ($P=0.026$)	[89]

Table 3 (continued)

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
	rs1143623 rs1143627 rs16944 rs4848306	Transient transfection reporter gene assay and gel mobility shift assay in THP-1 cells	–	rs4848306 ↓ nuclear protein binding with variant vs WT allele ($P<0.01$) rs1143623 ↑ nuclear protein binding with variant vs WT allele ($P<0.01$) ↓ transcriptional activity variant vs WT allele ($P<0.01$) rs1143627 ↑ nuclear protein binding of complex 1 with variant vs WT allele ($P<0.01$) ↓ binding of complex 2 and 3 with variant vs WT allele ($P<0.01$) ↓ transcriptional activity with variant vs WT allele ($P<0.01$) rs16944 ↑ transcriptional activity with variant vs WT allele ($P<0.05$)	[90]
	Ex vivo				
	rs1143627 rs1143634 rs16944	Stimulation of isolated human monocytes and EMSA of nuclear extracts	$n=442$	rs1143627 and rs16944 in LD (no data given) rs1143627 Fivefold ↑ DNA binding post-LPS stimulation in HE vs WT	[91]
	Genetic association				
	rs16944	Association of SNPs with toxicity in patients undergoing 5-FU and cisplatin chemotherapy	$n=100$	↑ thrombocytopenia HE and HM vs WT ($P=0.015$, OR = 2.9, 95% CI = 1.2–7.0) predictive of stomatitis ($P<0.01$) and thrombocytopenia ($P=0.02$) in conjunction with *TNF* rs1799964	[92]
	rs1143627 rs16944	Association of SNPs with pathological response in patients with rectal cancer treated with PCRT	$n=159$	No significant associations	[88]
	rs1143627 rs1143634 rs16944	Association of SNPs with risk of gastric cancer	$n=442$	rs1143627 and rs16944 in LD (0.99) rs1143627 HE associated with gastric cancer risk OR = 1.9 (95% CI = 1.5–2.6) rs16944: no significant association	[91]
	rs1143627 rs1143634 rs16944	Association of SNPs with severe CIGT incidence following 5-FU-based treatment	$n=34$	No significant associations	[77]
TNF	In vitro				
	rs1800629	Functional analysis of SNP using CAT reporter gene in Jurkat and Raji cells	–	No significant associations	[93]
	rs1800629	Transient transfection luciferase reporter gene assay in Jurkat and U937 cells	–	Twofold ↑ transcription with variant vs WT allele in both Jurkat and U937 cells ($P<0.05$)	[94]
	Ex vivo				
	rs1800629 rs1800750 rs361525	Association of SNPs with endotoxin-induced TNF-α secretion from PBMCs	$n=129$	No significant differences	[95]

Table 3 (continued)

Gene	SNPs	Study details	Number of participants	Impact of SNPs	Refs.
	rs1800629 rs1800750 rs361525	Stimulated TNF-α secretion from whole blood	$n=179$	No significant associations	[96]
	rs1800629 rs1800750 rs361525 rs4248158	Association of SNPs with TNF-α serum concentration following infliximab treatment	$n=47$	No significant associations	[89]
	Genetic association				
	rs1800629	Association of SNP with clinical outcome, incidence and severity of toxic complications and GvHD in HSCT patients	$n=70$	↑ severe toxicity HE vs WT ($P=0.014$, OR = 17.2 95% CI = 1.8–168.1)	[73]
	rs1800629	Association of SNPs with severe CIGT incidence following 5-FU-based treatment	$n=34$	*TLR2* and *TNF* SNPs were predictive of severe CIGT incidence in conjunction with colorectal and gastric cancer types ($P=0.033$, ROC AUC = 87.3%)	[77]
	rs1799964	Association of SNPs with toxicity in patients undergoing 5-FU and cisplatin chemotherapy	$n=100$	↑ stomatitis HE and HM vs WT ($P=0.02$, OR = 3.1 95% CI = 1.2–8.3) predictive of stomatitis ($P<0.01$) and thrombocytopenia ($P=0.02$) in conjunction with *IL1B* 16,944	[92]
	rs1800629 rs1800750 rs361525	Association of SNPs with MS	$n=179$	No significant associations	[96]

↑ increase, ↓ decrease, *2°* secondary, *AD* autoimmune disease, *ALI* acute lung injury, *AML* acute myeloid leukaemia, *CAT* chloramphenicol acetyltransferase, *EMSA* electromobility shift assay, *GvHD* graft vs host disease, *HCC* hepatocellular carcinoma, *HE* heterozygous, *HM* homozygous, *HSCT* hematopoietic stem cells, *IBD* irritable bowel syndrome, *LD* linkage disequilibrium, *LPS* lipopolysaccharide, *LTA* lipoteichoic acid, *MS* multiple sclerosis, *OR* odds ratio, *PCRT* primary chemoradiation therapy, *PBMCs* peripheral blood mononuclear cells, *PMNs* peripheral polymorphonuclear cells, *RA* rheumatoid arthritis, *ROC AUC* receiver operator characteristic, area under the curve, *SLE* systemic lupus erythematosus, *SSC* systemic sclerosis, *SIPD* invasive pneumococcal disease, *T2DM* type 2 diabetes, *WT* wild-type

and the effect of *MYD88* SNPs on MyD88 protein expression is variable (as described in Table 3) [71, 97]. *IRAK1* and *IRAK4* SNPs have been associated with an increased risk of autoimmune disease, with *IRAK1* SNPs also identified to increase NF-κB activation (as described in Table 3) [84, 85]. *TRAF6* SNPs were found to increase TNF-α and IL-6 secretion at baseline and post-stimulation of ex vivo PBMCs, but no association was identified between *TRAF6* SNPs with sepsis susceptibility and severity (as described in Table 3) [71, 86]. *NFKB* SNP rs28362491 was associated with severe toxic complications in hematopoietic stem cell transplantation (HSCT) patients and decreased NF-κB activity (as described in Table 3) [73, 74].

IL1B and *TNF* SNPs show the greatest potential as predictors for severe GI toxicity risk

Of the TIR domain innate immune signalling pathway SNPs discussed in Table 3, *IL1B* and *TNF* SNPs show the greatest potential as predictors for severe GI toxicity as they have been identified to not only alter gene function but also increase cancer risk and predict toxic side effects following chemotherapy. The *IL1B* SNP rs16944 was shown to increase transcriptional activity of IL-1β and was identified as being predictive of stomatitis (in conjunction with *TNF* 1799964) in patients receiving 5-FU and cisplatin chemotherapy treatment [90, 92] (Table 3). *IL1B* rs1143634 was identified to increase the risk of gastric cancer, with an increase in nuclear protein binding identified in reporter gene assays [90, 91] (Table 3). This, in addition to further evidence presented in Table 3, demonstrates *IL1B* SNPs are candidate predictors for severe GI toxicity risk. However, using individual *IL1B* SNPs as predictors for severe GI toxicity risk may be complicated, as many SNPs in *IL1B* are in linkage disequilibrium; therefore, SNPs with opposing effects may 'cancel' one another out [91]. With regards to *TNF* SNPs, rs1800629 influences gene functioning, with a twofold increase in transcriptional activity identified in patients carrying the variant [94] In addition, a small pilot study of patients receiving 5-FU-based chemotherapy, rs1800629 was found to be predictive of severe GI toxicity risk in a multivariate logistic regression model [77].

Conclusion

Severe GI toxicity is a debilitating side effect following 5-FU-based chemotherapy. It is essential predictors for severe GI toxicity risk are found to allow patients at most risk of severe GI toxicity to be identified prior to treatment, allowing the personalization of supportive care measures to reduce their risk of developing severe GI toxicity. This would not only improve clinical outcomes and long-term prognosis, but also improve patient quality of life whilst on otherwise life-saving chemotherapy treatment. This critical review has provided evidence to suggest TIR domain innate immune signalling pathway SNPs are suitable candidate predictors for severe GI toxicity risk following 5-FU-based chemotherapy.

However, further investigation is required to thoroughly understand the effect of these TIR domain innate immune signalling pathway SNPs on the mechanisms underlying the development of GI toxicity. To allow the 'bench to beside' translation of TIR domain innate immune signalling pathway SNPs as clinical predictors for severe GI toxicity risk, it is critical to not only associate, but also identify mechanisms by which these SNPs influence the development of severe GI toxicity in addition to understanding the exact functional impact of the SNPs themselves.

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Compliance with ethical standards

Conflict of interest Samantha Korver declares she has no conflict of interest. Rachel Gibson is a consultant for Kaleido Biosciences, Mundipharma and Onyx Pharmaceuticals, and has received research funding from Onyx Pharmaceuticals and AstraZeneca. Joanne Bowen has received research funding from AstraZeneca, Helsinn, Pfizer and Puma Biotechnology Inc. Janet Coller declares she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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