**TOWARDS better models and MECHANISTIC biomarkers for drug-induced gastrointestinal injury**

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**ABSTRACT**

Adverse drug reactions affecting the gastrointestinal (GI) tract are a serious burden on patients, healthcare providers and the pharmaceutical industry. GI toxicity encompasses a range of pathologies in different parts of the GI tract. However, to date no specific mechanistic diagnostic / prognostic biomarkers or translatable pre-clinical models of GI toxicity exist. This review will cover the current knowledge of GI ADRs, existing biomarkers and models with potential application for toxicity screening/ monitoring. We focus on the current gaps in our knowledge, the potential opportunities and recommend that a systematic approach is needed to identify mechanism-based GI biomarkers with potential for clinical translation.

**KEYWORDS**

Gastrointestinal

Toxicity

Adverse drug reaction

Biomarker

*In vitro*

*In vivo*

**ABBREVIATIONS**

|  |  |
| --- | --- |
| ADR | Adverse Drug Reaction |
| COX | Cyclooxygenase |
| DILI | Drug-induced liver injury |
| DMTA | Design-make-test-analyse |
| GI | Gastrointestinal |
| iPSC | Induced pluripotent stem cell |
| NSAID | Non-steroidal anti-inflammatory drug |
| VOC | Volatile organic compound |

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1. **INTRODUCTION**

Adverse drug reactions (ADR) involving the gastrointestinal (GI) tract are a significant and frequent problem, creating a major burden to patients, as well as healthcare providers and the pharmaceutical industry ([Pusztaszeri, et al., 2007](#_ENREF_113); [Redfern, et al., 2010](#_ENREF_114)). Drug toxicity to the GI tract covers a multitude of pathologies which reflects the complex physiological, histological and microbiome heterogeneity within this system. Upper gastrointestinal injuries, such as acute gastric erosions, reactive gastritis and peptic ulceration, caused by nonsteroidal anti-inflammatory drugs account for the commonest cause of ADRs in the UK with ~12,000 hospital admissions and 2,000 deaths per annum ([Blower, et al., 1997](#_ENREF_17)). Lower GI toxicity (often manifested by clinical symptoms such as diarrhea, constipation and abdominal cramps), is a major dose-limiting safety concern for several classes of compounds ([Pusztaszeri, et al., 2007](#_ENREF_113)) including cytotoxic chemotherapeutic agents, targeted cancer therapies such as kinase inhibitors and immune checkpoint inhibitors. The incidence of chemotherapy-induced diarrhea has been reported to be as high as 50–80% of treated patients ([Benson, et al., 2004](#_ENREF_14)) with rates of severe or life-threatening diarrhea up to 30% with some regimens ([Stein, et al., 2010](#_ENREF_137)). A recently approved selective, oral phosphatidylinositol 3-kinase delta inhibitor (idelalisib), for the treatment of several types of leukemia and lymphoma, is associated with severe GI toxicity 6 and contains a black box warning in the US prescribing information for fatal and/or serious and severe diarrhea or colitis. Furthermore, T cell activation with systemically-administered immune checkpoint inhibitors of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-1 (PD-1), for use in melanoma and other solid tumours, has been shown to result in GI adverse events including diarrhea and colitis and in rare cases, bowel perforation([Villadolid & Amin, 2015](#_ENREF_147)). However, if identified early, the GI-related adverse events can be reversible, or clinically manageable.

Diarrhea is also common in patients receiving oral small molecule tyrosine kinase inhibitors, such as erlotinib, lapatinib and sorafenib with an occurrence of between 30-60% for all grades of diarrhea ([Stein, et al., 2010](#_ENREF_137))and is dose-dependent, although it is unknown whether the effects are associated predominantly with luminal or systemic exposure.

Diarrhea is thus a major cause of treatment discontinuation and decreased drug efficacy and is likely to affect the pharmacokinetics of oral dosage regimens. Despite the importance of drug-induced GI toxicity, there are substantial gaps in our knowledge of the mechanisms and pathogenesis. Given the novelty of targets which are being pursued particularly in cancer, we need to be cognisant of the possibility of novel mechanisms as has recently been shown for dasatinib, where decreasing immune tolerance against intestinal microflora ([Eskazan, et al., 2014](#_ENREF_35)) or an autoimmune etiology ([Villadolid & Amin, 2015](#_ENREF_147)) has been implicated.

Pre-clinical safety assessment of new medicines does provide some degree of prediction of human toxicities, albeit that this varies with site of toxicity (e.g. prediction of liver and skin toxicity is worse than that for haematological, cardiovascular and GI toxicity) and the class of compounds being evaluated. Furthermore, a combination of rodent and non-rodent models (usually dog and non-human primate) is better than rodent models only ([Olson, et al., 2000](#_ENREF_99)). The availability of robust mechanism-based biomarkers and better pre-clinical (*in vitro* and *in vivo*) models would certainly help in translation to clinical applications. Mechanistic biomarkers are markers embedded in the pathogenesis of the toxicity and can therefore be considered more informative and more accurately reflect the underlying pathology. By contrast, monitorable biomarkers are usually by-products and often surrogate markers of the pathophysiological process. Mechanistic biomarkers, however, are considered more challenging to develop into validated clinically utilisable tools for safety monitoring.

This review discusses approaches to developing better *in vitro* models and mechanistic biomarkers for gastrointestinal injury and seeks to identify areas where collaborative efforts should be focused from the perspective of all stakeholders (pharmaceutical and biotechnology companies, contract research organizations, regulatory agencies and academia). Principally, the following specific points and themes are addressed:

* What pathologies come under the banner of GI toxicities and what do we understand about mechanisms in different parts of the GI tract and determinants of severity?
* What areas of GI toxicity (upper/middle/lower GI tract) are particular problems in drug development for the industry?
* What lessons can be learned from industry case-studies and existing paradigms of GI toxicity to inform ongoing research and development?
* Do we have validated *in vitro*/*in vivo* models to identify mechanisms based on pathologies?
* What *in vitro*/*in vivo* tools need to be developed for further understanding of mechanisms and translation?
* How does the pharmaceutical industry foresee using these tools for decision making?

1. **CLINICAL PROBLEM AND DISEASE BURDEN**

The term “gastrointestinal toxicity” can be considered to encompass a great many pathologies affecting a number of different tissues and organs which constitute the GI tract. GI toxicity can manifest in a number of ways including: nausea/ vomiting, intestinal inflammation, ulceration/perforation, altered fecal output and abdominal discomfort/pain. These symptoms do not necessarily imply toxicity involving a specific organ/region of the GI tract and are non-specific, arising as a result of a number of other non-drug-related conditions. The route of administration can have a bearing on the risk profile of drugs associated with GI toxicity and indeed many pathologies can be directly attributable to oral administration (e.g aspirin) and consequent direct GI exposure. However, it should be pointed out that there are a plethora of examples of i.v. administered drugs that cause GI toxicity though systemic exposure (e.g. chemotherapeutics such as 5-fluorouracil ([C. S. Lee, et al., 2014](#_ENREF_74))).

**Upper GI Tract**

Given its function to rapidly transit ingested substance into the stomach, exposure of the esophagus to drugs is often only momentary. Thus, toxicity to the esophagus only occurs when the passage of drugs is interrupted and a toxic substance is left in contact with the mucosa long enough to induce damage. This is often referred to as “pill oesophagitis” and can sometimes also result in ulcer formation. Predisposing factors include impaired swallowing, insufficient water when taking tablets or a patient lying down after taking their medication. Symptoms include heartburn, chest pain, dysphagia and odynophagia. There are a significant number of drugs which are known to cause this localized topical toxicity ([Petersen & Jaspersen, 2003](#_ENREF_103)) (summarized in Table 1). It is thought that oesophageal injury arises from caustic coatings, direct medication injury and poor oesophageal clearance of pills leading to acute inflammation. Further damage occurs when the toxic contents of a drug pill/capsule remain in the oesophagus long enough to produce mucosal lesions ([Jaspersen, 2000](#_ENREF_59)).

Due to the slower transit of food and drugs through the stomach, ingested compounds can remain in situ for several hours and this theoretically makes the stomach particularly vulnerable to drug toxicity. However, the highly efficient mucosal protective mechanism of the stomach means that most potential toxins are able to pass safely through without issue.

There are, however, a small number of drugs which elicit toxicity in the stomach (Table 1), the most notable of which are non-steroidal anti-inflammatory drugs (NSAIDs) which can cause a significant incidence of peptic ulcers and, in the most serious cases, bleeding and even perforation. In the case of NSAIDs, toxicity is thought to occur via a two-fold mechanism:

1. cyclooxygenase 1(COX-1) inhibition results in disruption of the mucosal epithelium; and
2. a localized topical toxic effect on the epithelium.

NSAID-induced ulceration is a considerable problem with an incidence estimated at 0.3-2.5% of users ([Goldstein, et al., 2000](#_ENREF_45); [Singh, 2000](#_ENREF_129)).

**Lower GI Tract**

It is often difficult to establish drug-induced injury to the small intestine because symptoms are often mild and non-specific. Indeed, until the advent of capsule endoscopy, the ileum and jejunum remained largely inaccessible to examination. This has led to suggestions that the incidence of small intestine toxicity may have previously been under-estimated though there are a number of drugs that are known to cause small intestinal toxicity (Table 1).

Indeed, symptomatically, it is often impossible to distinguish toxicity of the small intestine from that occurring in the large intestine. Lower GI toxicity can broadly be divided into two distinct categories:

* Acute toxicity - this can be predictable, on-target and dose-related, as observed with many chemotherapeutic drugs.
* Chronic toxicity - this can manifest as drug-induced colitis or small intestinal ulceration and is often the result of long-term drug exposure (e.g. aspirin).

Particularly in the acute setting, patients will typically present with a symptom, most frequently diarrhea, the causal drug will be withdrawn and gut motility will be non-specifically slowed by administration of loperamide. Often no further investigation is undertaken since the available invasive procedures (such as colonoscopy) would be considered inappropriate in patients who may already be considerably unwell. Thus, investigation as to the specific site and mechanism of toxicity is often overlooked. Non-invasive clinical tools which were able to identify the exact site of toxicity (small or large intestine) or even the specific mechanism would prove a powerful means by which clinicians could assess toxicity and help make better informed decisions about treatment regimens.

**Clinical Assessment of GI Toxicity**

In the non-acute setting, a patient will typically be referred to a gastroenterology specialist complaining of diarrhea and GI drug toxicity may form part of the physician’s differential diagnosis. Tests would be requested to rule other possible causes such as infection, inflammation and cancer. These will include routine blood tests such as full blood count, urea and electrolytes, liver function tests, C - reactive protein, as well as stool culture and fecal calprotectin concentration.

In order to assess the extent of gastrointestinal toxicity and exclude other possible causes of symptoms, a clinician will commonly utilise one of three invasive procedures: oesophago-gastroduodenoscopy (esophagus, stomach and proximal duodenum); capsule enteroscopy (small intestine); or colonoscopy (large intestine). Additionally, cross sectional imaging may be arranged (e.g. CT scan).

Currently there are a very small number of clinical biomarkers utilized for the assessment of GI pathophysiology. Fecal haemoglobin is often used when GI disease/ injury is suspected and considered to be a good “rule-out” marker ([Mowat, et al., 2016](#_ENREF_93)) but perhaps the most widely utilized example is fecal calprotectin (or often lactoferrin in the US). Calprotectin is commonly used to evaluate inflammation in inflammatory bowel diseases (IBDs) (including colitis and Crohn’s disease) to which it demonstrates a strong correlation ([D'Haens, et al., 2012](#_ENREF_30)). Though clearly an excellent general marker of GI inflammation, it does not demonstrate the specificity pertaining to site/mechanism of damage/insult that would be required for a robust, translatable biomarker of GI toxicity. In this regard, it may be good to draw parallels with renal injury, where biomarkers affecting the different parts of the nephron have been identified and undergone validation, at least in animal models ([Ozer, et al., 2010](#_ENREF_100)).

From a clinical/regulatory perspective better mechanism-based biomarkers of GI toxicity would allow implementation of better, more rigorous, patient monitoring and thus application of enhanced risk management strategies to alleviate GI toxicity risk with both new and existing medicines.

**Incidence, burden and regulatory perspective**

GI ADRs represent a significant burden to both patients and healthcare providers. A study in the US suggested that, of an estimated 701,547 emergency department visits attributed to ADRs in 2004-2005, 99,914 (14%) were due to gastrointestinal toxicity([Budnitz, et al., 2006](#_ENREF_20)). Indeed, mortality associated with GI toxicity, though low in incidence, can be an issue. A study of 18,820 admissions to a UK hospital identified 17 deaths (0.1%) due to gastrointestinal injury attributable to NSAID use ([Pirmohamed, et al., 2004](#_ENREF_109)). An emerging GI safety issue of considerable incidence is found with new cancer biologic immunotherapies (immune checkpoint inhibitors). For example, ipilimumab used in the treatment of metastatic melanoma, has been shown to cause severe GI toxicity (grade 3-4 colitis) in 8-12% patients([Larkin, et al., 2015](#_ENREF_73); [Robert, Schachter, et al., 2015](#_ENREF_117)).

From the perspective of the public bodies responsible for the licensing and regulation of therapeutics, it is clear that GI toxicity constitutes a significant volume of safety issues spontaneously reported each year. In an analysis of 26,000 spontaneous ADR reports by patients and health care professionals to the UK Yellow card scheme over a 2 year period from October 2005, it was found that 32.3% of patient reports, and 19.7% of health-care professional reports, were of gastrointestinal disorders ([Avery, et al., 2011](#_ENREF_7)). These numbers serve to illustrate the potential benefits (health and economic) that could be gained from the development and application of sensitive and specific clinical tests for GI toxicity.

In a study of 1015 healthy volunteers recruited to phase 1 trials ([Sibille, et al., 1998](#_ENREF_128)), a total of 43 severe adverse reactions were reported of which 10 (23%) were attributable to GI symptoms. Analysis has also suggested that GI toxicity constitutes 67% of all ADRs in phase III trials ([Redfern, et al., 2010](#_ENREF_114)). These figures suggest that there is still a considerable unmet need within the pharmaceutical industry, for more sensitive and specific predictive clinical and pre-clinical models and biomarkers of GI toxicity.

1. **DETECTING GI TOXICITY IN DRUG DISCOVERY/ EARLY DEVELOPMENT**

**Pre-clinical**

The high incidence of drug-induced GI toxicity in the clinic is exacerbated by the paucity of translatable tools available in the drug discovery setting. The lack of reliable and translatable *in vitro* models and non-invasive biomarkers of GI toxicity causes a reliance on histopathology for its detection. While histopathology may be a sensitive biomarker, it is a resource-intensive endpoint, requires a large sample size, and it occurs relatively late in the drug discovery process and is thus not conducive for the early detection or screening of drug-induced injury.

A key issue pre-clinically is the clear difference between species with regards to sensitivity to detect GI toxicity. Dogs, for example are highly sensitive to emesis and diarrhea while rats do not vomit and are considered to be more resistant to diarrhea. Since dog studies are likely to be carried out later than rat, this can hinder early detection of these particular toxicities. Bridging preclinical findings to human, it is likely that some GI toxicities translate more consistently than others which could presents a real problem for human risk assessment in drug discovery.

Early detection of toxicity is critical for enabling the selection of safer small molecule and, to a lesser extent, protein, therapeutics. If detected in the lead generation or lead optimization stages of drug discovery, then a bioassay for the detection of GI toxicity can be included in the design-make-test-analyse (DMTA) cycle, enabling the active selection of safer molecules. The cardiac repolarization potassium channel hERG provides an example of how including predictive screening tools in the DMTA cycle can significantly reduce the incidence of toxicity ([Cook, et al., 2014](#_ENREF_28)). Unfortunately, often little is known about the molecular underpinnings of GI toxicity, making such prospective biochemical screening in drug discovery difficult. While there are a number of cellular *in vitro* assays currently used to investigate mechanisms of GI toxicity, there are no published accounts of these assays being used as predictive screening tools in the drug discovery setting (Table 4). This is likely due to a combination of the challenges inherent in the complexities of intestinal biology and the necessity for high levels of validation required to establish the accuracy and the domain of applicability for each assay([Judson, et al., 2013](#_ENREF_61)). The scale of investment needed to appropriately validate each *in vitro* toxicity model for the drug-discovery setting is daunting. A major consideration regarding the degree of validation required to apply to pre-clinical screens in early drug discovery is how the data produced is likely to be utilised and what decisions are made based on it. Abandoning a chemical series because of perceived *in vitro* GI toxicity is a very conservative approach but screens could be used to rank compounds or series for subsequent progression so as to minimise the potential impact of a false positive observation of toxicity.

It is hoped that the development of *in vitro* toxicity prediction tools will benefit from the efforts of public-private partnerships, like those for models of drug-induced liver injury (DILI) (the IMI-related Mechanism-based Integrated systems for the Prediction of DILI: MIP-DILI ([Kenna, 2012](#_ENREF_65))) and drug-induced cardiotoxicity (HESI Cardiac Safety Technical Committee([Pierson, et al., 2013](#_ENREF_105))). Efforts by public-private partnerships to validate *in vitro* models of GI toxicity would potentially be an important first step in enabling its detection earlier in the drug discovery DMTA cycle, leading to the selection of safer molecules for clinical development.

**Clinical**

In addition to the significant clinical burden and post marketing safety concerns, GI toxicity also represents a significant source of development compound attrition for the pharmaceutical industry. Despite low levels of compound failures in the pre-clinical phase (3%)([Redfern, et al., 2010](#_ENREF_114)), gastrointestinal toxicity contributed to 9% of project closures in the clinic ([Cook, et al., 2014](#_ENREF_28)), reinforcing the poor performance of pre-clinical GI toxicity prediction approaches.

1. **Biomarkers**

**Pre-clinical GI toxicity biomarkers**

A number of pre-clinical biomarkers of GI tract pathophysiology currently exist (Table 2) and indeed a number of these have been proposed as putative pre-clinical GI toxicity biomarkers ([John-Baptiste, et al., 2012](#_ENREF_60)). Existing biomarkers of GI toxicity however lack specificity, sensitivity, and technical validity and have a lack of demonstrable clinical translation as well as having significant inter-species variability as highlighted by case study 1 GI biomarker (s) that anchors to histopathology, has temporal biomarker performance characteristics and human translation potential will significantly mitigate human GI safety risks especially with compounds that have low exposure safety margins (relative to exposures of GI pathology and highest human exposures).

**Current clinical biomarkers for assessment of GI toxicity**

For many clinicians, the best biomarker of GI toxicity is still diarrhea (or bloody diarrhea indicating colitis). Indeed, in some instances, such as with tyrosine kinase inhibitors, it is even considered a good indicator of drug efficacy ([Shah, et al., 2013](#_ENREF_127)). However, as a biomarker, diarrhea gives little indication as to the specific mechanism or target cells of toxicity.

It is clear that the clinician currently lacks, at his/her disposal, adequate mechanistic biomarkers of GI toxicity which are sufficiently sensitive or specific. The advent of tissue/cell-specific biomarkers of GI toxicity could greatly benefit patients by potentially more accurately pinpointing the site of injury and removing the need for invasive procedures.

In the context of gastrointestinal toxicity, an effective clinically utilisable biomarker needs to fulfil at least one of the following functions:

1. *Prediction*. Identifying patients at risk of GI toxicity prior to treatment. The treatment can then be modified or individualized. Preventative strategies can also be put in place for patients where an alternative therapeutic regimen is not available.
2. *Diagnosis***.** Determining the presence, severity and specific location of toxicity.
3. *Prognosis*.Assessing the efficacy of interventions used to treat underlying toxicity

Existing *in vivo* biomarkers with potential applications for GI toxicity essentially fall in to three categories: a) blood (serum/plasma), b) fecal and c) orally administered probes (measured in urine/breath). In terms of the pathophysiology that they represent, they are broadly markers of three key areas: a) epithelial mass/ health; b) inflammation and c) permeability. A host of biomarkers (including RNA [miRNA], protein and metabolite based platforms), for which a relationship between levels and the extent of one or more of these areas, have been identified (Table 2).

In order to enable future translation of GI toxicity biomarkers from “bench to bedside”, it is critical that there is clear mechanistic understanding of the cell type(s) and site of the GI tract affected, and for the resulting tissue pathophysiology to be correlated with the biomarker.. Though many of the biomarkers noted in Table 2 can be viewed as discriminatory for broad physiological processes attributable to the pathogenesis, they lack specificity for site of toxicity or indeed the specific cell type that has been injured.

**Genetic markers of Gastrointestinal ADRs**

In addition to dynamic biomarkers, genetic factors which predispose individuals to susceptibility to GI toxicity warrant further research. Pharmacogenetics has the potential to identify predictive and prognostic genetic markers of adverse drug reactions. Examples, where pre-emptive genetic testing is now mandatory prior to prescribing include *HLA-B\*57:01* for abacavir hypersensitivity ([Mallal, et al., 2008](#_ENREF_84)), *HLA-B\*15:02* for carbamazepine hypersensitivity ([Chung, et al., 2004](#_ENREF_25)), and thiopurine methyl transferase *(TPMT)* for azathioprine-induced bone marrow suppression ([Relling, et al., 2013](#_ENREF_115)). Despite the plethora of gastrointestinal pathologies attributable to therapeutics and, in many examples (e.g. NSAIDs), their apparent high incidence, there have been relatively few genetic association studies undertaken to date (Table 3). Indeed, only a very few examples are considered clinically important (e.g. the *UGT1A1\*28* variant and the GI toxicity caused by irinotecan, a colorectal cancer treatment ([Iyer, et al., 2002](#_ENREF_58))).

Thus, pharmacogenetics still represents an area of great potential for predictive markers of GI toxicity. However, in order to achieve the goal of clinically utilisable genetic markers, studies will need to focus on standardizing phenotype definitions, as with other ADRs ([Alfirevic, et al., 2014](#_ENREF_4); [Behr, et al., 2012](#_ENREF_13); [Pirmohamed, Aithal, et al., 2011](#_ENREF_107); [Pirmohamed, Friedmann, et al., 2011](#_ENREF_108)) so as to allow independent replication of associations, a key requisite for clinical translation of pharmacogenetic markers.

**Potential areas for novel biomarker development**

As well as the more traditional sources and approaches of biomarkers (including miRNA efforts) for gastrointestinal markers of pathophysiology (Table 3), there is growing weight of evidence to suggest that metabolomics data from “novel” sample sources may provide a wealth of potential markers of GI disease including GI toxicity. One such source is volatile organic compounds (VOCs). These are the “chemical signatures” emitted by fecal and breath samples which have the potential to act as proxies for different GI disease states ([Probert, et al., 2009](#_ENREF_111)). Fecal VOC profiles have been demonstrated to discriminate between two inflammatory bowel disorders with differing GI localisation: ulcerative colitis and Crohn’s disease ([Ahmed, et al., 2013](#_ENREF_2)). Thus the possibility that VOCs can actually provide information as to the site of pathogenesis (and toxicity) is very real. A non-invasive metabolomic marker which could provide accurate information about the site of toxicity would potentially be a very powerful diagnostic/ prognostic clinical tool, but of course this would need to be tested in carefully designed clinical studies

With the advent of cheaper, more sensitive DNA sequencing technologies and the completion of the human microbiome project ([Human Microbiome Project, 2012](#_ENREF_55)), a great deal of focus has been directed towards understanding the composition of the human microbiome and its role in health and disease. Indeed, a change in the gut microbiota composition could be seen both as a putative marker of GI pathogenesis, as has suggested in Crohn’s disease ([Gevers, et al., 2014](#_ENREF_43)), or even part of the etiology. The disposition of a number of drugs has been shown to be altered as a consequence in changes to the microbiota including the anti-viral sorivudine, and lovastatin which is used to treat hypercholesterolemia ([Klaassen & Cui, 2015](#_ENREF_72)). It is plausible that altered disposition of a drug may lead to either GI or systemic toxicity, or alternatively, a change in the microbiome may be intimately connected with the pathogenesis of the toxicity, as has been suggested for colitis associated with CTLA4 inhibitors. ([Villadolid & Amin, 2015](#_ENREF_147)) Thus, future investigations need to consider how and when the human microbiome may yield biomarkers of GI toxicity which are predictive as well diagnostic/prognostic. Such biomarkers of microbiome compositional change could potentially be derived from direct next generation sequencing data but also metabolomic proxies such as fecal bile acids ([Duboc, et al., 2012](#_ENREF_33)),,

Presently there is a lack of both pre-clinical and clinical biomarkers which are patho-physiologically relevant and mechanism-based. This is, in part, due to a lack of availability of robust , *in vitro* and *in vivo* models which can demonstrate translations between *in vivo* and *in vitro* pre-clinically and subsequently bridge to the GI toxicity observed clinically.

1. **Model systems**

***In vivo* models**

Typically, the first-line compound toxicity screening for lead identification and clinical candidate validation in the pre-clinical phase of drug discovery will involve animal studies. In rodent models, the simplest assessment of GI toxicity is the fecal pellet method ([Marks, et al., 2013](#_ENREF_86)) which measures changes in the number, appearance and weight of pellets in response to a test compound. The limitation to this method is that while it is indicative of overall GI toxicity it gives little indication of site of injury. Gastric emptying and or intestinal motility assessment can be used additionally to indicate site of injury (gastric or intestinal). This is done by administering (after test compound) meals containing markers of liquid (phenol red) or solid transport (charcoal/ barium sulphate). Levels of marker in the tissue can then be assessed *ex vivo* to determine drug modulation of gastric/ intestinal motility. Though such models of GI toxicity are effective, their utility as translatable indicators of human GI toxicity is limited. Rodent models have been estimated to be only predictive of 50% of clinical drug toxicities and still only 70% when taking into account non-rodent animal models([Olson, et al., 2000](#_ENREF_99)).

In larger pre-clinical species, emerging “pill” imaging and measurement techniques may allow for more accurate and site-determining assessment of GI toxicity. Indeed, the utility of “capsule endoscopy” technologies has already been demonstrated for determining GI tract pH and gastric residence ([Mahar, et al., 2012](#_ENREF_83)) and GI motility ([Marks, et al., 2013](#_ENREF_86)). The use of gastrointestinal mucosal visualisation methodologies ([Liu, et al., 2013](#_ENREF_81); [Oliva, et al., 2012](#_ENREF_98)) can also provide a significant amount of information related to tissue injury. These technologies may allow for more detailed and accurate clinical or pre-clinical assessment of tissue specificity of GI toxicity without the need for more invasive procedures in the future.

**In vitro models**

If a pre-clinical safety signal is detected *in vivo* then GI toxicity may subsequently be investigated mechanistically in relevant *in vitro* models. However, generally no pre-clinical *in vitro* predictive GI toxicity screening is carried out. As case study 2 highlights, there are also significant issues with regards to pre-clinical to clinical species translatability of existing *in vitro* models of GI toxicity. In the case study example, a difference in sensitivity to Brd4 inhibitors between species *in vitro* was potentially over-predicting human toxicity. Several *in vitro* models of the gastrointestinal tract which have however been well validated and have the potential to be developed as predictive toxicology screening tools (Table 4). Since the pathobiology of drug induced intestinal damage is complex and involves the interplay of multiple intricate pathways including molecular and cellular events ([Sultani, et al., 2012](#_ENREF_138)), a multi-parametric approach may be needed to identify relevant biomarkers of GI toxicity.

Existing *in vitro* gastrointestinal models largely focus on predicting oral drug absorption ([Antunes, et al., 2013](#_ENREF_5); [Z. Huang, et al., 2014](#_ENREF_54); [N. Li, et al., 2013](#_ENREF_79)), understanding intestinal secretion, digestion([Sung, et al., 2011](#_ENREF_140)), and intestinal permeability; studying Helicobacter pylori pathogenesis in the stomach ([Bartfeld, et al., 2014](#_ENREF_12); [Fiorentino, et al., 2013](#_ENREF_40); [Zhang, et al., 2002](#_ENREF_158)) and furthering developmental understanding ([Feng, et al., 2013](#_ENREF_38); [B. M. Kim, et al., 2005](#_ENREF_68); [McCracken, et al., 2014](#_ENREF_90)). Very few GI models have the primary focus of toxicity. However, models are rarely created on an *ad hoc* basis and are often adapted to suit requirements; therefore modifications of the large range of current models gives scope for the development of accurate toxicity biomimetics. In addition, models have been developed to mimic GI disease such as inflammatory bowel disease ([Leonard, et al., 2010](#_ENREF_77)). It may therefore be possible to partially predict any differential toxicity responses between patients with GI diseases and healthy patients. It is important to bear in mind that the models described (Table 4) are not mutually exclusive; in fact the most translatable future biomimetics are likely to be a conglomerate of several of these systems.

Typically *in vitro* screening tools for toxicity have often been in the form of either immortalized cell lines which are phenotypically similar to the targeted tissue in question or alternatively isolated primary cells. However, over recent years there has been significant growth in the application of *in vitro* organoid models in gastroenterological research. The term organoid, in the context of the GI tract, has been applied to a plethora of “mini-organ models” (Table 4). However it is important to note these have differences in the cells from which they are been derived including: a) isolated GI cells or induced pluripotent stem cells (iPSCs), b) cells isolated from different locations in the GI tract, and c) isolated cells from healthy or diseased tissue. As such, the many examples of GI organoids described in the literature have very varied phenotypes and this is something which will require significant consideration when selecting suitable models for application to pre-clinical toxicity screening.

**Future toxicity screening models**

A key issue for future *in vitro* GI toxicity screening models will be the incorporation of population-based screening into pre-clinical evaluation. At present many *in vitro* models are derived from a single cell source/individual. High-throughput, population-based *in vitro* screening models utilizing induced pluripotent stem cells, ([Paull, et al., 2015](#_ENREF_102)) differentiated into intestinal organoids, enteroids, or organotypic tissue models models, which could be applied, pre-clinically, for detection of GI safety signals which may not become apparent until phase 3 or post-marketing could be investigated. In turn, the need for large collections of population derived organoids will require a greater commitment to bio-banking efforts in order to supply such samples at a significant scale for pre-clinical assays such as the biobank currently developed from the Lgr5 organoids ([van de Wetering, et al., 2015](#_ENREF_145)).

A large proportion of cell models of the GI tract, as detailed in Table 4, are derived from epithelial cells and whilst these are a key target of drug toxicity, it is also important to consider that other cell types within the GI tract may either be targets of compounds or play a key role in the pathogenesis of drug toxicity. Likewise, when considering application of an *in vitro* GI model, it is important to consider its relevance given the intended route of clinical exposure (e.g. oral, IV) and the associated impact of metabolic toxification or de-toxification (e.g. gut and/or hepatic). In the same way that future GI toxicity biomarkers need to be sensitive/ specific for different organ systems and cells, *in vitro* models screens will be needed that are specific to a multitude of different cell types through which GI toxicity might be elicited. Availability of such models is vital for translational studies of drug-host cell interactions that are capable of predicting human responses.

Recent advances in “Human-on-a-chip” technology allow pulsatile flow of physiological-range fluids to mimic the effects of chronic dosing ([I. Maschmeyer, et al., 2015](#_ENREF_88)). Perhaps more significant in this technology is the ability to cover the chip’s microfluidic channels in endothelial cells to model the tissue vasculature. This has great potential in applications of screening for endothelial cell mediated toxicity and ultimately ADRs which is currently over-looked.

Another factor over-looked within current *in vitro* models of GI tract pathogenesis is the role of the innate immune system. Indeed, a significant number of GI toxicities exhibit an inflammatory component. However at present there are no robust *in vitro* systems utilized to model these particular ADRs. Co-culture models utilizing target organ cells with isolated immune cells have been developed for other ADRs, for example hepatotoxicity ([Rose, et al., 2016](#_ENREF_118)). Application of such methodologies to GI toxicity could offer an opportunity to model those ADRs and open up the possibility to understand better the aetiology and pathogenesis of immune mediated toxicities.

Altered gut motility is a significant symptom of many of the drug toxicities which have been observed in patients over the years. Therefore, it stands to reason that toxic insult to cells which mediate vagus nerve regulation of GI tract motility might play a key role in many of the drug-induced pathogeneses. Though culture of vagus cell-types is entirely possible, incorporation into more complex co-culture models may prove technically demanding.

There are several other GI regulatory cell types which could be putative targets of GI drug toxicity (Figure 2) and so *in vitro* models incorporating them need to be considered if mechanistic understanding of GI toxicities is to be developed within a systematic framework. Additional cell types include but are not limited to smooth muscle cells, fibroblasts, immune cells, enteroendocrine cells and neuronal cells (both within the GI tract and peripherally)

In order to identify the specific mechanisms by which a given drug causes a GI ADR, we need to understand firstly which cells are the target of the toxic insult and what is the most appropriate model for the purpose of a) future screening out of said toxicity and b) understanding the pathogenesis. Secondly, we need to understand how toxicity targeted to a particular cell type can infer altered functionality of other cell types, which can then elicit the toxicity phenotype.

1. **CONCLUSIONS AND RECOMMENDATIONS**

Currently the most widely accepted biomarker of GI toxicity is simply altered gastric transit (diarrhea/constipation). Whilst, in many instances, it is indicative of toxicity, little information can be gleaned as to the specific site or mechanism of toxicity. For clinical decision making, this may be sufficient indication of toxicity but for pre-clinical and clinical drug development, better, more sensitive and specific mechanistic biomarkers are essential.

The field of gastrointestinal toxicity could learn significant lessons from other organ drug toxicity research. Renal ([Bonventre, et al., 2010](#_ENREF_18)) and hepatic ([J. I. Clarke, et al., 2016](#_ENREF_26)) toxicity act as paradigms whereby systematic approaches have been applied to identify novel mechanism-based biomarkers which are utilisable as sensitive and robust pre-clinical markers of organ toxicity.

However unlike the liver and kidney, the GI tract is not one organ but a coordination of several organ systems, each with its own unique morphology and constituent cell types. As such, the identification, characterization and implementation of new, effective and utilisable pre-clinical models and biomarkers of GI toxicity require a systematic approach. Understanding the unique mechanisms of different drug induced GI pathologies using a combination of approaches and biomarkers will be needed to develop better clinically-utilisable quantitative predictive models and mechanism-based biomarkers to improve the problem of drug-induced GI toxicity during both drug development and clinical use.

Efforts to address the following issues are encouraged and supported:

1. Given that the GI tract is a heterogeneous organ and is subject to a range of different diseases and pathologies (and treatment modalities) it is essential that a more systematic evaluation (involving industry, academia and regulators, in collaboration) is undertaken to identify and characterize (and qualify) panels of GI biomarkers for their context of use, according to specific regions of the GI tract, and related to specific pathologies.  In this respect, it may well be worth applying the lessons learned (over many years) from biomarker identification studies carried out in different nephron segments affected by various nephrotoxicants ([Bonventre, et al., 2010](#_ENREF_18)).  The GI tract is currently lagging significantly behind the liver and kidney in terms of injury biomarkers and their relationships to certain pathologies (and anatomical region of pathology).
2. Significant recent advances have been made in efforts to develop *in vitro* models which can evaluate and predict the GI response to injurious agents.  There is a need however to develop multiple co-culture models (to include epithelial cell, fibroblast and immune cell compartments) and to provide open access to standardized protocols as currently there is enormous variability and a lack of reproducibility of data from *in vitro* models.  It is expected that these models will play a major role in helping to identify novel and predictive biomarkers of cellular injury.
3. Currently little is known about the molecular mechanisms which contribute to drug-induced GI- toxicity in man and, as a result, treatment of the adverse reactions (e.g. diarrhea) is the only means of ensuring continuation of therapy, albeit with agents (e.g. loperamide for diarrhea) which are relatively non-specific and possibly inappropriate (potential for colitis with loperamide). A better understanding of the mechanism of toxicity (by industry and clinicians) may improve the design (and safety) of the therapeutic agent and/or facilitate a more specific approach to alleviation of adverse effects leading to improved dose intensity that may help to improve clinical outcomes for patients.
4. There are currently significant gaps in understanding of translation from preclinical models and in bridging to man and patients which need to be addressed urgently. GI toxicity studies in rodents may be misleading due to the lack of an emetic reflex and the relative resistance of mice and rats to drug-induced diarrhea. A more translatable effect may only be observed from studies carried out in other species such as dogs, which are typically conducted later (and at greater expense) in the drug screening cascade. It is therefore important that interpretation of test results, and therefore applicability to the human setting, takes into account the limitations of the animal species used, and is correlated with other test systems.
5. Greater understanding of the roles of the innate and adaptive immune system and the intestinal microbiota in drug-induced gastrointestinal injury is needed.  These can be modelled to some extent *in vitro* and there have been some preliminary efforts by various groups studying the role of inflammatory cells and cytokines in this setting.
6. Pre-competitive data sharing of targets which are associated with toxicity offers the potential to avoid costly drug discovery and development efforts by the pharmaceutical industry.  Publication of identified target-associated GI toxicities allows opportunities for a reduction in animal usage and can minimise time and cost spent by the scientific community on futile drug discovery efforts.

**CONFLICTS OF INTEREST**

Some authors of this paper are employed in the pharmaceutical industry or serve as consultants to the pharmaceutical industry. However, the subjects presented in the paper do not advocate or support purchase of any of the products offered by the respective organisations.

**INDUSTRY CASE STUDY 1: *In vivo*/ *in vitro* biomarker translation ( Peptidase Inhibitor)**

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Intestinal toxicity in beagle dogs treated with a reversible and selective peptidase inhibitor.

Dose- and exposure-dependent decrease in plasma citrulline seen in dogs after repeated oral administration of drug correlated very well with small intestinal histopathological findings (crypt necrosis, villus atrophy, enterocyte loss).

Based on physiological range of plasma citrulline a clear cut-off value for intestinal mucosal toxicity was established (4µg/mL), however a clear cut-off could not be established in initial studies in rat.

Results demonstrate that plasma citrulline is a potential translational safety biomarker for small intestinal toxicity in dogs.

As a biomarker of small-intestinal toxicity, plasma citrulline appears to have some potential in non-clinical safety studies. However, further work is required to determine its applicability in humans as well as other pre-clinical species. The application of citrulline to *in vitro* models of intestinal disease/toxicity also requires further exploration.

**INDUSTRY CASE STUDY 2: *in vitro* model species translation (Brd4 Inhibitor)**

Brd4 is an epigenetic ‘reader’ and drives the transcriptional elongation of a suite of protoncogenes, including cMyc. Brd4 is activated or amplified in a wide range of tumour types and inhibitors have shown a broad preclinical efficacy profile.

Critical questions for the drug discovery project were to identify potential dose-limiting toxicities, understand whether these toxicities were inherent to the target and whether these toxicities would limit exploration of the pharmacology of Brd4 inhibition in patients.

*In vivo* daily oral dosing of rats with a novel Brd4 inhibitor at pharmacologically relevant exposures, resulted in decreased food intake, and rapid weight loss. Histopathological findings were consistent with Myc inhibition including intestinal villous atrophy, with the duodenum appearing especially sensitive region of the GI tract. However, further investigations into the mechanism of intestinal villous atrophy revealed that although Brd4 inhibitor did block Myc mRNA there was no corresponding decrease in proliferation. The cause for the intestinal villous atrophy was found to be loss of fast cycling duodenal stem cells.

*In vitro* toxicity of Brd4 inhibitors in a “mini-gut” small intestine model ([Sato, et al., 2011](#_ENREF_120); [Sato, et al., 2009](#_ENREF_121)) correlated with potency against a tumour cell-line, suggesting that the intestinal toxicity is driven by Brd4 activity.

Mice used in xenograft studies tolerated higher AUC exposures of the Brd4 inhibitor than rats. Where humans sit on this scale is unknown. Understanding different species sensitivities was critical for the project to move forward into the clinic. Using the ‘mini-gut’ system human intestinal cells were shown to be least sensitive species tested: human<mouse<rat<dog.

The use of in vitro models, in this example, demonstrates the potential to model in vivo toxicological observations, and compare species sensitivities. However, the ability to translate these *in vitro* model endpoints to preclinical and clinical endpoints, and therefore predict symptoms such as diarrhea is still a significant gap.

**Figure1.** Existing biomarkers and *in vitro* models of gastrointestinal pathophysiology and exemplar GI ADR causal drugs.



**Table 1.** Examples of drugs/ biologics associated with gastrointestinal toxicity.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Drug/ Biologic** | **Indication** | **Affected GI Tissue** | | | | **Phenotype(s)** | **Ref** |
| **Esophagus** | **Stomach** | **Small Intestine** | **Large Intestine** |
| 5-Fluorouracil | Colorectal, breast, stomach, pancreatic cancer | X |  |  | x | Stomatitis, diarrhea, nausea, | ([Porta, et al., 1994](#_ENREF_110); [Tebbutt, et al., 2003](#_ENREF_141)) |
| Alendronate | Osteoporosis | x | x |  |  | Upper gastrointestinal ulceration | ([Graham & Malaty, 1999](#_ENREF_47); [Graham, et al., 1997](#_ENREF_48)) |
| Amoxicillin-clavulanic acid | Bacterial infection |  |  | x | x | Diarrhea | ([Gillies, et al., 2015](#_ENREF_44)) |
| Ascorbic acid | Vitamin C deficiency | x |  |  |  | Nausea, abdominal pain, diarrhea, | ([Hathcock, 1997](#_ENREF_50)) |
| Aspirin | Analgesia, anticoagulant | x | x | x |  | Oesophagitis, Gastroduodenal ulceration/perforation/bleeding, small intestinal enteropathy | ([Fortun & Hawkey, 2005](#_ENREF_41); [Jaspersen, 2000](#_ENREF_59); [Sostres, et al., 2010](#_ENREF_133)) |
| Bevacizumab | Renal, lung, colon, ovarian, cervical cancer | x | x | x | x | Stomatitis, oral mucositis, diarrhea, , | ([Elting, et al., 2013](#_ENREF_34)) |
| Ciprofloxacin | Bacterial infections, Anthrax |  |  | x | x | Diarrhea | ([Yildirim, 2015](#_ENREF_155)) |
| Clopidogrel | Anticoagulant, stroke prevention | x | x |  |  | Dyspepsia, constipation, peptic/ gastro duodenal ulceration, gastric haemorrhage | ([Harker, et al., 1999](#_ENREF_49)) |
| Clozapine | Schizophrenia |  |  | X | x | Gastrointestinal hypomotility, ischaemic colitis, gastrointestinal necrosis | ([Palmer, et al., 2008](#_ENREF_101); [Peyriere, et al., 2009](#_ENREF_104)) |
| Dabigatran | Anticoagulant, stroke prevention | x | x |  |  | Dyspepsia, gastrointestinal bleeding, | ([Bloom, et al., 2014](#_ENREF_16); [Hoffman & Galle, 2013](#_ENREF_51)) |
| Irinotecan | Colorectal cancer |  |  | x | x | Late diarrhea | ([Stein, et al., 2010](#_ENREF_137)) |
| Nivolumab | Advanced melanoma; non-small cell lung cancer |  |  | x | x | Nausea/ vomiting, diarrhea, constipation | ([Robert, Long, et al., 2015](#_ENREF_116)) |
| *NSAIDs e.g.:*  Celecoxib  Diclofenac  Ibuprofen  Indomethacin  Naproxen  Sulindac | Analgesia, osteoarthritis, rheumatoid arthritis |  | X | X |  | Gastroduodenal ulceration, perforation, bleeding | ([Sostres, et al., 2010](#_ENREF_133)) |
| *Proton Pump Inhibitors e.g.:*  Esomeprazole  Lansoprazole  Omeprazole  Pantoprazole  Rabeprazole | Gastroduodenal ulcers, gastroesophageal reflux |  | x | X |  | Hypergastrinaemia, enterochromaffin-like (ECL) cell hyperplasia, diarrhea | ([Lundell, et al., 2015](#_ENREF_82)) |
| Quinidine | Heart arrhythmias | x |  |  |  | Pill oesophagitis | ([Jaspersen, 2000](#_ENREF_59)) |
| Serotonin reuptake inhibitors | Depression, anxiety, OCD |  | x |  |  | Upper gastrointestinal bleeding in combination with aspirin or NSAIDs | ([Lewis, et al., 2008](#_ENREF_78)) |
| *Tyrosine kinase inhibitors e.g.:*  Bosutinib  Erlotinib  Imatinib | Chronic myeloid leukaemia  Pancreatic, non-small cell lung cancer  Chronic myeloid leukaemia, GI stromal tumours |  | x  x  x | x  x  x | x  x  x | Diarrhea nausea/vomiting abdominal pain  Diarrhea, stomatitis  Diarrhea, loss of appetite/anorexia | ([Kantarjian, et al., 2014](#_ENREF_63))  ([Abdel-Rahman & Fouad, 2015](#_ENREF_1))  ([Sodergren, et al., 2014](#_ENREF_132)) |

**Table 2.** Existing *in vivo* biomarkers of gastrointestinal injury.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Biomarker** | **Tissue Specificity** | **Marker of** | **Species** | **Ref** |
| **Blood (Serum/Plasma)** | Gastrin-17 | Esophagus | Epithelial mass/health | Human | ([Sipponen, et al., 2005](#_ENREF_130)) |
| Gastrin | Gastric Antrum/ Duodenum | Ulceration | Rat/Dog | ([Garcia-Sancho, et al., 2005](#_ENREF_42); [Sun, et al., 2002](#_ENREF_139)) |
| Eosinophilic cationic protein (ECP) | Gastric Mucosa | Inflammation | Human | ([Aydemir, et al., 2004](#_ENREF_8)) |
| Pepsinogen | Gastric mucosa | Epithelial mass/health | Human | ([Y. K. Huang, et al., 2015](#_ENREF_53)) |
| Prohepcidin | Gastric Mucosa | Epithelial cell mass/health | Rat/Human | ([H. K. Kim, et al., 2013](#_ENREF_70); [Schwarz, et al., 2012](#_ENREF_125)) |
| Trefoil Factor 3 (TFF3) | Gastric mucosa | Epithelial mass/health | Human | ([Aikou, et al., 2011](#_ENREF_3)) |
| Vitamin B12 | Gastric Mucosa | Malabsorption | Human | ([Schenk, et al., 1999](#_ENREF_122)) |
| Ghrelin | Stomach/ Small Intestine | Motility, gastric acid secretion, gastric emptying | Human | ([Muller, et al., 2015](#_ENREF_94)) |
| Diamine oxidase (DAO) | Small intestine | Epithelial mass/health | Rat/Human | ([John-Baptiste, et al., 2012](#_ENREF_60); [Miyoshi, et al., 2015](#_ENREF_91)) |
| Citrulline | Small Intestine | Epithelial mass/health | Rat /Dog/Human | ([Crenn, et al., 2008](#_ENREF_29); [Dossin, et al., 2011](#_ENREF_32); [John-Baptiste, et al., 2012](#_ENREF_60)) |
| CD64 | Small/Large intestine | Inflammation | Human | ([Tillinger, et al., 2009](#_ENREF_142)) |
| C-reactive protein | Small/Large intestine | Inflammation | Human | ([Ki, et al., 2009](#_ENREF_67)) |
|  | | | | | |
| **Fecal** | miR-194 | Small intestine | Epithelial damage | Rat | ([John-Baptiste, et al., 2012](#_ENREF_60)) |
| Calprotectin | Small/Large Intestine | Neutrophil infiltration/inflammation | Human | ([Burri & Beglinger, 2014](#_ENREF_21)) |
| Lactoferrin | Small/Large Intestine | Inflammation | Human | ([Walker, et al., 2007](#_ENREF_148)) |
| Bile acids | Small/ Large Intestine | Dysbiosis of gut flora | Human | ([Duboc, et al., 2012](#_ENREF_33)) |
|  | | | | | |
| **Orally Administered Probes** | 51Cr-EDTA (urine) | Gastroduodenal/Small intestine | Permeability | Rat | ([Yanez, et al., 2003](#_ENREF_154)) |
| 13C Sucrose (Breath) | Small Intestine | Permeability | Rat/Human | ([J. M. Clarke, et al., 2006](#_ENREF_27); [Wardill, et al., 2013](#_ENREF_151)) |

**Table 3**. Genetic variants associated with risk of drug-induced gastrointestinal injury.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Drug** | **Indication** | **GI Toxicity Phenotype** | **Gene** | **Associated variant/allele** | **PGx Dosing Guidelines** | **Ref** |
| **Irinotecan** | Advanced colorectal cancer | Diarrhea | *UGT1A1* | \*28 | Yes | ([Etienne-Grimaldi, et al., 2015](#_ENREF_36)) |
| **5-Fluorouracil** | Various cancers | Diarrhea | *DPYD* | \*2A/\*13/rs67376798 | Yes | ([Caudle, et al., 2013](#_ENREF_24)) |
| **Methotrexate** | Rheumatoid arthritis, psoriasis, Crohn’s disease | Nausea, diarrhea, dyspepsia, stomatitis | *GGH* | c.-401CC vs. c.-401CT/TT | No | ([Dervieux, et al., 2006](#_ENREF_31)) |
| *MTHFR* | c.1298AC/CC vs. c.1298AA  rs1801131 | No |
| *ATIC* | c.347GG vs. c.347CC/CG | No |
| *MS* | c.2756AA vs. c.25756AG/GG | No |
| *MTRR* | c.66GG vs. c.66AA/AG | No |
| **Cyclophosphamide** | Various cancers | Moderate/ severe diarrhea and nausea/vomitus | *GSTP1* | \*I/V and \*V/V  rs1695 | No | ([Zhong, et al., 2006](#_ENREF_159)) |
| **Mixed NSAIDs** | Inflammation/ Analgesia | Gastric ulceration | *CYP2C8* | \*3 | No | ([Blanco, et al., 2008](#_ENREF_15))  ([Pilotto, et al., 2007](#_ENREF_106))  ([Musumba, et al., 2013](#_ENREF_95)) |
| *CYP2C9* | \*2 and \*3 |
| *CYP2C19* | \*17 |
| **Leflunamide** | Rheumatoid Arthritis | Diarrhea, nausea/vomitus, abdominal pain and weight loss | *CYP1A2* | \*1F CC | No | ([Grabar, et al., 2008](#_ENREF_46)) |

**Table 4.** Existing *in vitro* upper and lower gastrointestinal models with potential application for toxicity studies and screening. \*Species for cell lines taken from those available from American Tissue Culture Collection (ATCC).

|  |  |  |  |
| --- | --- | --- | --- |
| **UPPER GI** | | | |
| **Model Type** | **Species\*** | **Cells and Methods Utilized** | **Examples of Applications** |
| **Cancer cell lines** | Human  Mice  Rat  Goat | * True oesophageal cell lines: CP-B, CP-C and CP-D. * Falsified oesophageal adenocarcinoma cell lines derived from other tissues: SEG-1, BIC-1 and SK-GT-5 ([Souza, et al., 2002](#_ENREF_134)), ([Zeevaart, et al., 2009](#_ENREF_157)) * Gastric cancer cell lines: AGS, MKN45, MKN28 and SNU-1 ([Zhang, et al., 2002](#_ENREF_158)), ([Xu, et al., 2016](#_ENREF_152)) | * Exploring pathogenesis and treatment of gastric ulcers * Investigating oncogenic pathways in gastric carcinomas * Characterizing Barrett’s esophagus |
| **Non-tumour-derived immortalized cell lines** | Human  Cow | * Gastric epithelium-derived cells: JOK-1, GES-1 ([Okayama, et al., 2000](#_ENREF_97); [Zhang, et al., 2002](#_ENREF_158)) * Esophagus epithelium-derived cells: Het-1A and CP, respectively | * As above |
| **Primary gastric or oesophageal cells** | Human  Mice | * Normal or cancerous cells are isolated from patient samples or animal models ([Bartfeld, et al., 2014](#_ENREF_12); [Cao, et al., 2002](#_ENREF_22); [Lemieux, et al., 2011](#_ENREF_76); [Marchetti, et al., 2003](#_ENREF_85); [Sato, et al., 2011](#_ENREF_120)) | * Evaluating gastric cancer progression and treatment * Investigating pathogenesis of Barrett’s esophagus |
| **Transwell models** | Human  Mice  Dog | * Gastric cell lines, such as NCI-N87 ([Fiorentino, et al., 2013](#_ENREF_40); [Lemieux, et al., 2011](#_ENREF_76)) * Primary gastric epithelial cells ([B. M. Kim, et al., 2005](#_ENREF_68)) | * Studying gastric development and physiology * Determining drug permeability * Epithelial integrity studies |
| **3D culture models** | Human | * 3D nanofibre scaffolds ([Y.-j. Kim, et al., 2009](#_ENREF_71); [Sîrbu-Boeţi, et al., 2009](#_ENREF_131)) | * Predicting cytotoxicity of chemotherapeutic drugs |
| **Gastric organoids** | Human  Mice | Oesophageal organoids:   * Human epithelial tissue from patients with Barrett’s esophagus ([Sato, et al., 2011](#_ENREF_120))   Gastric organoids:   * Rodent embryonic stem cell line ([Noguchi, et al., 2015](#_ENREF_96)) * Rodent, or human ([Bartfeld, et al., 2014](#_ENREF_12)) gastric D44/Lgr5+ stem cells ([Barker, et al., 2010](#_ENREF_11); [Feng, et al., 2013](#_ENREF_38); [Schumacher, et al., 2015](#_ENREF_124))([Barker, et al., 2010](#_ENREF_11); [Feng, et al., 2013](#_ENREF_38); [Schumacher, et al., 2015](#_ENREF_124))([Barker, et al., 2010](#_ENREF_11); [Feng, et al., 2013](#_ENREF_38); [Schumacher, et al., 2015](#_ENREF_124))([Barker, et al., 2010](#_ENREF_11); [Feng, et al., 2013](#_ENREF_38); [Schumacher, et al., 2015](#_ENREF_123)) * Rodent gastric troy+ stem cells ([Stange, et al., 2013](#_ENREF_136)) * Human induced-pluripotent stem cells ([McCracken, et al., 2014](#_ENREF_90)) * Rodent ([Schumacher, et al., 2015](#_ENREF_124)) or human ([Schlaermann, et al., 2014](#_ENREF_123)) gastric glands * Rodent gastric tissue ([X. Li, et al., 2014](#_ENREF_80)) | * Studying normal development and tumour progression * Modelling diseases (e.g Ménétrier disease and Barrett’s esophagus), and infection (e.g. *H.pylori*). * Determining signalling pathways involved in proliferation |
|  | | | |
| **LOWER GI** | | | |
| **Model Type** | **Species\*** | **Cells and Methods Utilized** | **Examples of Applications** |
| **Cancer cell lines** | Human  Rat  Mouse | * Large intestinal cell lines: HT29, HCT116, SW480/SW620 and Caco-2 * Small intestinal cell lines: limited number available * Caco-2, the most commonly used cell line, is used to model both the small and large intestines. ([Carrasco-Pozo, et al., 2013](#_ENREF_23); [Samak, et al., 2011](#_ENREF_119); [Seth, 2004](#_ENREF_126)) | * Monolayer culture for simple studies ([Carrasco-Pozo, et al., 2013](#_ENREF_23); [Samak, et al., 2011](#_ENREF_119); [Seth, 2004](#_ENREF_126)) * Cells incorporated into transwell studies, 3D culture models, organoids and organs-on-a-chip. |
| **Non-tumour-derived immortalized cell lines** | Human  Rat | * Small intestinal cells: IEC-16/18 and FHs 74 Int. * Large intestinal cells: very few available. | * As above ([Fan, et al., 2014](#_ENREF_37)), ([Hong, et al., 2014](#_ENREF_52)) |
| **Primary intestinal epithelial cells** | Human  Mouse | * Human ([Ayehunie, et al., 2013](#_ENREF_9); [Ayehunie, et al., 2014](#_ENREF_10)) ([Kauffman, et al., 2013](#_ENREF_64)) or mouse ([Booth, et al., 1995](#_ENREF_19); [Moon, et al., 2013](#_ENREF_92)) colonic epithelial cells from biopsy tissues | * As above |
| **Transwell models** | Human  Rat | The following have been utilized in trans-well models:   * Intestinal cell lines (Caco-2 ([Carrasco-Pozo, et al., 2013](#_ENREF_23); [Tong, et al., 2014](#_ENREF_143))and IEC-6 ([Fan, et al., 2014](#_ENREF_37))), * Human primary intestinal epithelial cells([Kauffman, et al., 2013](#_ENREF_64)) * Human induced pluripotent stem cell (iPSC)-derived intestinal cells ([Kauffman, et al., 2013](#_ENREF_64)) * Co-culture methods ([Antunes, et al., 2013](#_ENREF_5); [Hyung Choi, et al., 2004](#_ENREF_56); [N. Li, et al., 2013](#_ENREF_79); [Pusch, et al., 2011](#_ENREF_112)) | * Determining mechanism and likelihood of GI toxicity by measuring monolayer permeability ([Hyung Choi, et al., 2004](#_ENREF_56)) * Predicting drug absorption ([Antunes, et al., 2013](#_ENREF_5); [Artursson, et al., 2001](#_ENREF_6); [N. Li, et al., 2013](#_ENREF_79); [Moon, et al., 2013](#_ENREF_92); [Pusch, et al., 2011](#_ENREF_112)) |
| **3D culture models** | Human  Rat | * 3D scaffolds produced by 3D printing ([M. Lee, et al., 2005](#_ENREF_75)) or moulding ([Sung, et al., 2011](#_ENREF_140); [Wang, et al., 2009](#_ENREF_150)) * Membranes with crypt-like topography([Wang, et al., 2010](#_ENREF_149)) * Hollow fiber bioreactors ([Yu, et al., 2014](#_ENREF_156)) * Co-culture methods ([Ayehunie, et al., 2013](#_ENREF_9); [Ayehunie, et al., 2014](#_ENREF_10); [N. Li, et al., 2013](#_ENREF_79)) | * Evaluating absorption by incorporation within trans-well model ([N. Li, et al., 2013](#_ENREF_79)) |
| **Intestinal organoids** | Human  Mouse  Pig | * Rodent ([Yamada, et al., 2002](#_ENREF_153)) or human ([Finkbeiner, et al., 2015](#_ENREF_39)) embryonic stem cell lines * Rodent ([Moon, et al., 2013](#_ENREF_92); [Sato, et al., 2009](#_ENREF_121)) human([Sato, et al., 2011](#_ENREF_120)) and pig ([Khalil, et al., 2016](#_ENREF_66)) intestinal stem cells * Rodent ([Ueda, et al., 2010](#_ENREF_144)) or human ([Ayehunie, et al., 2013](#_ENREF_9)), ([Spence, et al., 2011](#_ENREF_135)) iPSCs * Rodent ([Sato, et al., 2009](#_ENREF_121)) or human ([Jung, et al., 2011](#_ENREF_62)) whole crypt preparations | * Determining absorption, metabolism and efficacy of chemotherapeutic agents ([Imura, et al., 2010](#_ENREF_57)) |
| **‘Organs/Humans-on-a-chip’ micro-devices** | Human  Rat | Different cell types/tissue slices have been used including:   * Caco-2 cells ([Imura, et al., 2010](#_ENREF_57); [H. J. Kim, et al., 2012](#_ENREF_69)) * Human primary intestinal epithelial cells ([Ilka Maschmeyer, Tobias Hasenberg, et al., 2015](#_ENREF_87); [Ilka Maschmeyer, Alexandra K. Lorenz, et al., 2015](#_ENREF_89)) * Rat intestinal slices ([van Midwoud, et al., 2010](#_ENREF_146)) | * Determining PK parameters (absorption, bio-activation and metabolism) * Ascertain cytotoxicity of chemotherapeutics |

**Figure 2.** A schematic representation highlighting key cell types of lower GI tract physiology which may be putative targets of drug toxicity**.** 

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