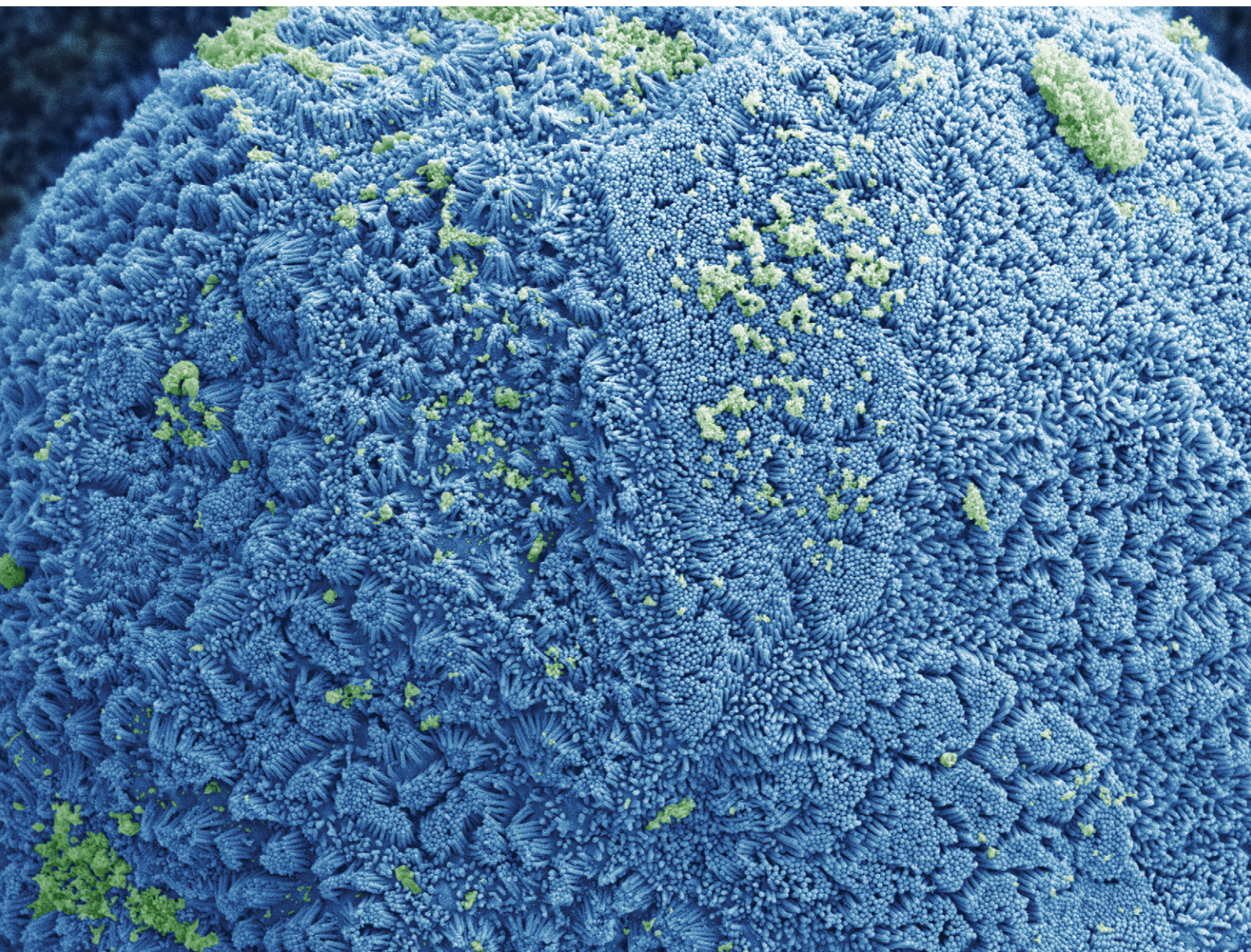


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CRITICAL REVIEW

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Developing *in vitro* assays to transform gastrointestinal safety assessment: potential for microphysiological systems



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Developing *in vitro* assays to transform gastrointestinal safety assessment: potential for microphysiological systems†

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Drug-induced gastrointestinal toxicities (DI-GITs) are among the most common adverse events in clinical trials. High prevalence of DI-GIT has persisted among new drugs due in part to the lack of robust experimental tools to allow early detection or to guide optimization of safer molecules. Developing *in vitro* assays for the leading GI toxicities (nausea, vomiting, diarrhoea, constipation, and abdominal pain) will likely involve recapitulating complex physiological properties that require contributions from diverse cell/tissue types including epithelial, immune, microbiome, nerve, and muscle. While this stipulation may be beyond traditional 2D monocultures of intestinal cell lines, emerging 3D GI microtissues capture interactions between diverse cell and tissue types. These interactions give rise to microphysiologies fundamental to gut biology. For GI microtissues, organoid technology was the breakthrough that introduced intestinal stem cells with the capability of differentiating into each of the epithelial cell types and that self-organize into a multi-cellular tissue proxy with villus- and crypt-like domains. Recently, GI microtissues generated using miniaturized devices with microfluidic flow and cyclic peristaltic strain were shown to induce Caco2 cells to spontaneously differentiate into each of the principle intestinal epithelial cell types. Second generation models comprised of epithelial organoids or microtissues co-cultured with non-epithelial cell types can successfully reproduce cross-‘tissue’ functional interactions broadening the potential of these models to accurately study drug-induced toxicities. A new paradigm in which *in vitro* assays become an early part of GI safety assessment could be realized if microphysiological systems (MPS) are developed in alignment with drug-discovery needs. Herein, approaches for assessing GI toxicity of pharmaceuticals are reviewed and gaps are compared with capabilities of emerging GI microtissues (e.g., organoids, organ-on-a-chip, transwell systems) in order to provide perspective on the assay features needed for MPS models to be adopted for DI-GIT assessment.

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Introduction

Drug-induced gastrointestinal toxicities (DI-GITs) are the most common category of adverse events (AEs) both during clinical trials¹ and after drug approval.² When incidence of drug-induced AEs is broken down by individual symptoms, five GI AEs (nausea, vomiting, constipation, diarrhoea, and abdominal pain) rank among the 12 most frequent.¹ These AEs occur across all drug modalities and span all therapeutic areas.³ However, the consequences of GIT often differ from those in other organ systems with respect to timing of discovery, medical response, impact on drug development, and effects on patients. Specifically, GIT risks are frequently discovered late in development during clinical trials. Since GITs are generally non-life-threatening, the medical response tends to focus on dose reduction and/or palliative co-therapies (e.g., anti-diarrheal).^{4,5} Whilst such clinical management strategies avoid drug attrition,



symptomatic relief is transient, and the underlying issues are not addressed. Hence, the existing safety assessment paradigm results in (i) drugs advancing into the clinic with GI liabilities, (ii) GI-related limitations on dosing/efficacy persisting throughout the drug life cycle, and (iii) patients bearing the burden of reduced quality of life, reduced drug compliance, and compromised efficacy.⁴

In an ideal scenario, DI-GIT would be identified early with assays suitable for screening out the liability during preclinical drug discovery. One recent review evaluated toxicity-related drug attrition by target organs during the preclinical and clinical phases. GI toxicity was the least likely cause of failure in the preclinical phase despite DI-GITs being the most common AEs in the clinical phase.⁵ One notable limitation on GI preclinical testing cascades is the lack of *in vitro* assays suitable for routine screening. New *in vitro* assays that can predict DI-GITs will therefore be positioned for significant impact. The demand will likely extend beyond a single assay; ultimately a suite of *in vitro* assays may be utilized such that each of the major GI AEs is supported with a frontline screening assay for hazard detection along with multiple investigative assays for mechanistic follow-up.

For many GI AEs, microphysiological systems appear uniquely suited to fill the void in *in vitro* assays. MPS are

biomimetic devices recapitulating natural physiology of human or animal tissues in a microenvironment that induces *in situ* cell phenotypes (e.g., architecture, polarity, genetic expression, differentiation, response to mechanical factors such as stretch and perfusion, etc.). For the purposes of this manuscript, MPS are also defined as going beyond traditional 2D sandwich culture and could include several of the following design aspects: a multi-cellular environment within a biopolymer or tissue-derived matrix, a 3D structure that replicates essential anatomical architecture, mechanical factors such as stretch, shear force or perfusion, incorporate primary or stem cell-derived cells that express appropriate cell type-specific phenotypic markers and functional capabilities, and/or inclusion of immune system components. In contrast to traditional 2D assays, which are mostly comprised of monocultures of intestinal cell lines, 3D microtissues recapitulate the interactions among diverse cell and tissue types. Complex interactions between intestinal epithelium, immune cells, microbiome, nerves, and muscles are emerging as the essential biology underlying most DI-GITs. It follows that an array of diverse GI MPS models may be needed, each capturing a microphysiological interaction between the minimal set of tissues needed to emulate one particular GI AE in order to transform predictive accuracy for DI-GIT. To guide

Table 1 Safety assessment priorities for GI MPS models in drug discovery

Priority risks to be assessed	
Pan-therapeutic area GI AEs	Top 5 most common DI-GI AEs: nausea, vomiting, constipation, diarrhea, and abdominal pain, all functional toxicities
Oncology GI AEs	The leading DI-GI AE in oncology is mucositis. Chemotherapeutics and targeted oncotherapeutics disrupt epithelial renewal by on-target cytostatic and cytotoxic mechanisms leading to compromised barrier function, infection, and diarrhea
Priority microphysiologies to be replicated	
Enterocyte-microbiome-immune interaction	Disturbances in the homeostatic interaction between epithelial renewal and maintenance of tight barrier function, bacterial penetration, and immune surveillance is fundamental to GI disease and toxicity. Replicating this microphysiology may transform <i>in vivo</i> predictivity of <i>in vitro</i> models
Enterochromaffin-neuronal interaction	Enterochromaffin epithelial cells are proposed to act as epithelial chemosensors, transducing information via serotonergic synapses with enteric neurons. This microphysiology may yield breakthroughs enabling <i>in vitro</i> models to predict nausea, vomiting, or abdominal pain
Priority capabilities to be included	
Functional endpoints	Functional endpoints measuring mechanistically aligned microphysiologies are needed rather than cytotoxic endpoints which has been insufficient to predict organ-specific toxicity ⁶
Moderate throughput for mechanistic studies	Moderate throughput assays (e.g., 96-well) are needed for investigative studies aiming to elucidate mechanisms by pharmacological characterization
Genetic manipulation	Capacity to manipulate gene expression is needed for mechanistic toxicity studies. Genetic inhibition needed to distinguish on- vs. off-target mechanisms of toxicity
Cross-species comparison	Comparing toxicity responses across species used for <i>in vivo</i> safety assessment (rat, dog, non-human primate (NHP), human) is needed for translating preclinical animal study toxicities into clinical risk
Exposure control	To match oral drugs, access to apical surface is needed to mimic the selectively high lumen exposure; drug washout capacity is needed to match pulsatile exposure
Response kinetics for PK-PD modeling	The ability to mimic clinical drug exposure profiles and measure the resulting drug responses (i.e., pharmacokinetic/pharmacodynamic, PK/PD) is needed. In particular, matching sampling frequency/duration with clinical dose schedules and oral-like exposure (apical dosing with washout) is needed to inform PK-PD mathematical modeling to optimize clinical dose schedules
Cellular kinetics for mechanistic modeling	Mechanistic kinetic endpoints need to be measured over a time scale that is clinically matched to drug exposure duration (washout) and schedule (weekly)
High-throughput for frontline screening	384-Well throughput is desired to enable routine frontline screening during chemistry optimization to assess and minimize DI-GIT risk



the design of GI-MPS assays to meet safety assessment needs, this review details a drug discovery perspective on assay features of high priority (Table 1). The topics discussed include: screening assays for early detection, investigative models for mechanistic elucidation, tool compounds for assessing predictive performance, gaps and opportunities emerging with epithelial-only GI microtissues, specific microphysiologies that need to be emulated to align with top GI AEs, kinetic data to support mathematical modelling, and guidance on assay endpoint selection.

Frontline assays for GIT risk detection

Currently, there are no *in vitro* screening assays suitable for routine GIT risk assessment early in drug discovery. Rather, preclinical DI-GIT risk detection depends on testing in animal models. The performance record of the industry's *in vivo*-focused cascade has been quantitatively assessed in two surveys of multinational pharmaceutical companies. Retrospective evaluation of compounds with clinical GIT (100% prevalence) revealed that non-rodent studies had 83% clinical agreement whereas rodent studies had 46% agreement.⁷ Conversely, selecting compounds with preclinical GIT and prospectively assessing the diagnostic value of different species revealed a proportionate reduction in uncertainty of 87%, 28%, and 15%, NHP, dog, and rat respectively.⁸ Both studies confirm that the current *in vivo* testing cascade is highly dependent on data from higher-order species and delivers suitable predictive accuracy if non-human primate is included. However, due to both ethical and cost considerations, testing in non-rodent species is often appropriately limited to a few potential compounds that are evaluated in a species that may not necessarily be well-suited for human GI functionality. Thus, the standard preclinical *in vivo* safety assessment package supports detecting GIT just prior to entering clinical trials but inherently lacks the capacity to select/design drug molecules with improved safety.

Frontline *in vitro* assays are a high priority need. Concordance with clinical events for a given GI AE is the primary criterion used to determine whether an assay is worthy of implementation. For DI-GIT, routine clinical assessment includes the range of signs and symptoms, duration, and severity scored based on a 5-grade system provided by the National Cancer Institute Common Toxicity Criteria (NCI CTC). GI toxicities are determined to be drug-related if the AE (i) coincides with drug treatment, (ii) resolves with removal, and (iii) is exacerbated by re-challenge. Since most GI toxicities resolve with drug removal, tissue biopsies are difficult to procure from the site of injury and thus for GI AEs discovered in clinical studies, histopathology is most likely not available to provide mechanistic insight, hence the toxicities are therefore defined functionally. The clinical data that is routinely and publicly available to assess the predictive performance of an *in vitro* assay consists of functional symptoms, frequency of occurrence, and total drug

exposure in plasma (*i.e.*, drug bound to serum proteins plus free/unbound drug).

Qualification of an *in vitro* assay with evidence establishing the predictive accuracy for a particular clinical AE⁹ is paramount for a new MPS assay to gain adoption in drug discovery. The confirmation of performance requires well-characterized positive and negative reference compounds to determine the suitability of a model for use in exploring a specific question. For broader toxicological applications, the compound set used should include diverse chemistry and cover multiple modalities (*e.g.*, small molecules, biologics, nucleic acids, *etc.*), if possible. Alternatively, a set of compounds could be used to qualify an assay for narrow usage within a chemical series. To provide sets of drugs for evaluating GI MPS model predictive performance, clinical data were mined for marketed drugs known to cause nausea, vomiting, or constipation. Approved drugs with high or low incidence were selected and the total plasma exposures associated with the clinical findings were identified (Tables 2–4; for references see ESI† Tables S1, S2, and S3, respectively). A similar set of compounds for diarrhoea (Table 5, see references in ref. 10) was tested in an *in vitro* human GI microtissue and revealed that barrier function measured by transepithelial electrical resistance (TEER) predicted clinical

Table 2 Assay qualification compound set for nausea

Drug name	Nausea drug incidence	Nausea placebo incidence	Clinical $C_{\max}$ (total, M)
Carboplatin	89%	Not determined	8.5×10^{-5}
Oxycodone	67%	13%	4.9×10^{-8}
Pomalidomide	36%	Not determined	2.7×10^{-7}
Cabozantinib	47%	21%	1.1×10^{-6}
Bortezomib	64%	Not determined	1.3×10^{-6}
Raltitrexed	58%	Not determined	2.4×10^{-6}
Tapentadol	49%	13%	2.5×10^{-7}
Bosutinib	46%	Not determined	3.8×10^{-7}
Ixabepilone	42%	Not determined	5.0×10^{-7}
Decitabine	42%	16%	3.2×10^{-7}
Pemetrexed	39%	Not determined	2.5×10^{-4}
Lubiprostone	31%	5%	2.6×10^{-11}
Varenicline	30%	10%	1.9×10^{-8}
Ritonavir	30%	8%	1.6×10^{-5}
Mycophenolic acid	29%	Not determined	8.3×10^{-5}
Docetaxel	25%	Not determined	2.7×10^{-6}
Tramadol (ER)	23%	8%	1.3×10^{-6}
Duloxetine	22%	9%	1.3×10^{-7}
Pentostatin	22%	Not determined	1.3×10^{-6}
Dofetilide	5%	4%	8.6×10^{-9}
Febuxostat	1%	1%	8.7×10^{-6}
Amlodipine	3%	2%	1.0×10^{-8}
Verapamil	3%	Not determined	9.9×10^{-8}
Maprotiline	2%	Not determined	2.3×10^{-7}
Fesoterodine	1%	1%	8.4×10^{-9}
Furosemide	1%	Not determined	6.7×10^{-6}
Nadolol	0.5%	Not determined	4.3×10^{-7}
Simvastatin	0.4%	0.6%	1.1×10^{-7}
Finasteride	0.1%	0%	2.2×10^{-7}
Haloperidol	0%	0%	2.7×10^{-8}
Valsartan	2%	2%	6.8×10^{-6}
Prednisone	1%	0%	5.9×10^{-8}
Oxazepam	'Rare'	Not determined	1.6×10^{-6}



Table 3 Assay qualification compound set for vomiting

Drug name	Vomiting drug incidence	Vomiting placebo incidence	Clinical $C_{\max}$ (total, M)
Carboplatin	82%	Not determined	8.5×10^{-5}
Oxycodone	42%	3%	4.9×10^{-8}
Raltitrexed	37%	Not determined	2.4×10^{-6}
Bosutinib	37%	Not determined	3.8×10^{-7}
Bortezomib	36%	Not determined	1.3×10^{-6}
Tapentadol	32%	3%	2.5×10^{-7}
Ixabepilone	29%	Not determined	5.0×10^{-7}
Cinacalcet	27%	15%	1.1×10^{-7}
Cabozantinib	26%	2%	1.1×10^{-6}
Decitabine	25%	9%	3.2×10^{-7}
Pemetrexed	25%	Not determined	2.5×10^{-4}
Mycophenolic acid	23%	Not determined	8.3×10^{-5}
Docetaxel	19%	Not determined	2.7×10^{-6}
Ritonavir	17%	4%	1.6×10^{-5}
Varenicline	5%	2%	1.9×10^{-8}
Duloxetine	5%	4%	1.3×10^{-7}
Lubiprostone	5%	1%	2.6×10^{-11}
Amlodipine	1%	Not determined	1.0×10^{-8}
Furosemide	1%	Not determined	6.7×10^{-6}
Nadolol	0.5%	Not determined	4.3×10^{-7}
Simvastatin	0.2%	0%	1.1×10^{-7}
Prednisone	0%	0%	5.9×10^{-8}
Verapamil	Not noted	Not determined	9.9×10^{-8}
Valsartan	<1%	Not determined	6.8×10^{-6}
Maprotiline	'Rarely'	Not determined	2.3×10^{-7}
Haloperidol	0%	0%	2.7×10^{-8}
Guaifenesin	Not noted	Not determined	1.1×10^{-5}
Oxazepam	Not noted	Not determined	1.6×10^{-6}
Dofetilide	Not noted	Not determined	8.6×10^{-9}
Fesoterodine	0%	0%	8.4×10^{-9}
Febuxostat	<1%	Not determined	8.7×10^{-6}

diarrhoea with 83% accuracy¹⁰ which would be suitable for drug screening.¹¹ We propose these four well-annotated compound sets as standards to be used to benchmark predictive accuracy of future MPS assays. Drug concentrations tested in the *in vitro* systems should achieve multiples of the clinical plasma $C_{\max}$ when feasible, as is often done for liver and other *in vitro* systems such as for the potassium channel hERG,¹² to closely replicate local concentrations found under clinical use and in toxicology studies and to compare results across assays more effectively.

Investigative assays to elucidate GIT mechanisms

Current preclinical approaches to elucidate mechanisms of DI-GIT are generally dependent on *in vivo* or *ex vivo* models. A foundational component of mechanistic GI toxicological studies includes testing in animal models to characterize the histopathology associated with the functional symptoms. Follow-up studies rely on a suite of models specialized for three functionalities: motility, secretion, or nausea/emesis (Table 6 and reviewed in ref. 4). Motility is evaluated with organ bath, charcoal meal test, or fecal pellet counts. Newer approaches include the wireless SmartPillTM and Bravo capsule as well as imaging transit of nonabsorbable markers using radiography or ultrasonography. Wireless capsules can measure pH, thus offering a less invasive measure of gastric secretion compared to traditional monitoring using cannula inserted in the stomach, intestine, or bile duct. Emesis is assessed by counting instances of vomiting in ferrets or dogs while nausea can be

Table 4 Assay qualification compound set for constipation

Drug name	Constipation incidence	Constipation placebo	Clinical $C_{\max}$ (total, M)
Clomipramine	47%	11%	2.9×10^{-7}
Bortezomib	43%	Not determined	1.3×10^{-6}
Pomalidomide	36%	Not determined	2.7×10^{-7}
Decitabine	35%	14%	3.2×10^{-7}
Alosetron	29%	6%	1.7×10^{-8}
Cabozantinib	28%	6%	1.1×10^{-6}
Clozapine	25%	Not determined	1.3×10^{-6}
Hydromorphone	23%	Not determined	1.7×10^{-8}
Tramadol (ER)	17%	4%	1.3×10^{-6}
Oxycodone	15%	1%	5.0×10^{-8}
Ondansetron	14%	6%	1.7×10^{-7}
Duloxetine	11%	3%	1.3×10^{-7}
Morphine	11%	Not determined	8.4×10^{-8}
Cabergoline	10%	0%	1.1×10^{-10}
Voriconazole	<2%	Not determined	8.6×10^{-6}
Amlodipine	≤ 1 but $>0.1\%$	Not determined	1.0×10^{-8}
Nadolol	0.5%	Not determined	4.3×10^{-7}
Lubiprostone	1%	1%	2.6×10^{-11}
Simvastatin	0.3%	0.4%	1.1×10^{-7}
Zolmitriptan	<0.1%	Not determined	3.4×10^{-8}
Dofetilide	Not noted	Not determined	8.6×10^{-8}
Prednisone	0%	0%	5.9×10^{-8}
Fomepizole	Not noted	Not determined	2.1×10^{-4}
Trimethoprim	Not noted	Not determined	8.9×10^{-6}
Oxazepam	Not noted	Not determined	1.6×10^{-6}
Ritonavir	0.2%	0.4%	1.6×10^{-5}
Finasteride	0.1%	0%	2.2×10^{-7}

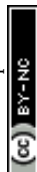


Table 5 Assay qualification compound set for diarrhoea

Drug name	Diarrhoea drug incidence	Diarrhoea placebo incidence	Clinical $C_{\max}$ (total, M)
Afatinib	96%	Not determined	7.8×10^{-8}
Colchicine	77%	14%	1.7×10^{-8}
Idarubicin	73%	Not determined	8.8×10^{-8}
Tacrolimus	72%	Not determined	8.3×10^{-8}
Imatinib	60%	Not determined	3.2×10^{-6}
Capecitabine	55%	Not determined	9.7×10^{-6}
Axitinib	54%	Not determined	5.6×10^{-8}
Bortezomib	51%	Not determined	1.3×10^{-6}
Prostacyclin	50%	Not determined	9.9×10^{-10}
Crizotinib	49%	Not determined	2.2×10^{-7}
Sorafenib	43%	13%	6.2×10^{-6}
Docetaxel	42%	Not determined	3.7×10^{-6}
Diacerein	41%	8%	1.3×10^{-5}
Quinidine	40%	Not determined	1.2×10^{-5}
Miglustat	89%	Not determined	6.1×10^{-6}
Metformin HCl	53%	12%	8.9×10^{-6}
Stavudine	50%	Not determined	2.4×10^{-6}
Mycophenolate mofetil	48%	Not determined	5.7×10^{-5}
Acetaminophen	1%		1.4×10^{-4}
Verapamil	2%		9.9×10^{-8}
Dofetilide	3%		8.6×10^{-9}
Amiodarone	<2%		4.7×10^{-6}
Fondaparinux	<3%		8.4×10^{-7}
Alfuzosin	Not noted		3.2×10^{-8}
Triamcinolone	Not noted		2.7×10^{-8}
Ranolazine	Not noted		1.4×10^{-5}
Fomepizole	Not noted		2.1×10^{-4}
Haloperidol	Not noted		2.7×10^{-8}
Isoprenaline	Not noted		2.7×10^{-9}
Amlodipine	Not noted		1.0×10^{-8}
Dexamethasone	Not noted		1.6×10^{-7}
Finasteride	Not noted		2.2×10^{-7}
Nadolol	Not noted		4.3×10^{-7}
Nifedipine	Not noted		4.6×10^{-7}
Methoxsalen	Not noted		1.5×10^{-6}
Flecainide	Not noted		9.3×10^{-7}
Maprotiline	Not noted		2.3×10^{-7}
Dexmedetomidine	Not noted		4.7×10^{-9}
Furosemide	Not noted		6.7×10^{-6}

assessed in rodents by quantifying pica behaviour, *e.g.*, measuring consumption of non-nutritive material.¹³ Permeability and ion transport can be quantified with *ex vivo* intestinal tissues in Ussing chambers.¹⁴ Precision-cut intestinal slices are an alternative approach to defined MPS approaches.^{15,16} These *in vivo* and *ex vivo* tools are primarily descriptive in nature thereby leaving a barrier to elucidating

mechanisms. In pharmaceutical companies, mechanistic studies need to confirm pharmacological mechanism often through genetic manipulation to determine target involvement. Critical endpoints include assessing barrier fidelity, immune surveillance, and epithelial cell renewal.

New GI MPS models capable of supporting mechanistic investigative toxicology is a need that rivals frontline screening assays for impact, but such models must be developed to meet different standards. Although the diversity of investigative questions/applications varies, the critical question to address is whether a DI-GIT is mediated by intended or unintended mechanisms. This distinction is critical for devising next steps in dealing with a GIT, whether to modify the structure of the drug molecule (to mitigate off-target activity) or abandon the drug target or modify dose schedule (to address on-target toxicity). Moderate throughput, more flexibility, and deep access to molecular events in the model are needed to support these types of investigative studies. Specifically, *in vitro* assays need to enable (i) controlled drug exposure levels and duration, (ii) testing of drugs with differing potency, selectivity, chemical structure, (iii) accurate reflection of *in vivo* molecular functionality of the target and (iv) genetic manipulation of target expression. In addition, kinetic data are valued for informing mathematical modelling used to discover novel mechanistic-based clinical mitigation strategies (see below: Enabling PK-PD and mechanistic mathematical modelling). Compared to the limitations of mechanistic animal models described above, MPS assays with meaningful biology for supporting investigative pharmacology and/or genetic manipulation have the potential to transform studies elucidating mechanisms of GIT.

One reoccurring investigative concern of critical importance for GITs is the need to resolve *in vivo* safety findings that differ between preclinical species and determine how these differences translate into human risk. Drug development regulatory guidance documents for conducting human clinical trials require safety assessment in one rodent and one non-rodent species. The most common species, rat and dog, have morphological and/or functional features of their intestinal tracts that complicate clinical translation. Rat intestines have a large cecum and almost half of the stomach surface area is a non-glandular region similar to the human esophagus, but such a region is not found in human stomachs. Rats are relatively resistant to diarrhoea and are incapable of vomiting. Since dogs are carnivores, their stomach acid has a lower pH and the overall length of the GI tract is shorter than in humans. Likely related to these morphologic and functional differences, dogs are more prone to diarrhoea and emesis.¹⁷ Having a GI MPS that can be used with multiple species (*e.g.*, rat/dog/human) would be exceptionally valuable for assessing translational safety risk. If drug-induced toxicity is observed in a preclinical species, MPS from that same species would be used to determine if a suitable toxicity signal can be



Table 6 Methods used to assess pharmaceuticals gastrointestinal safety

GI drug AEs	<i>In vivo</i> models	<i>Ex vivo/In vitro</i> models	Microtissue models
• Nausea • Vomiting	• Histopathology of GI • Pica, consumption of kaolin (rat) • Emesis, retching count (ferret/dog)	• Not currently available	• Not currently available
• Diarrhea • Constipation	• Histopathology of GI • Fecal pellet count (rat) • Meal motility tests with phenol red or charcoal (rat) • Capsules (SmartPill™ & Bravo) • Motility imaging (radiography, ultrasonography)	• Ussing chamber • GI segment in organ bath, assessment of smooth muscle function	• Organoid swelling (secretory diarrhea) • Transwell μ-tissue TEER (diarrhea)
• Abdominal pain	• Histopathology of GI	• Not currently available	• Not currently available

replicated with drug treatment using the *in vitro* assay. This positive response provides confidence in the ability to interpret the results from human MPS for predicting the effect in humans. However, without positive *in vitro* – *in vivo* concordance in the preclinical MPS, interpreting a negative response in a human MPS (should it occur) is speculative at best.¹⁸ Assays with validated species translation and clinical predictivity will help reduce reliance on testing in animals.

Microtissues-current limitations, critical features, opportunities for MPS

Complex intestinal organoids and next-generation models are likely to be the first *in vitro* assays implemented for routine GIT screening. Since organoid technology has been aptly reviewed elsewhere,^{19,20} comments here are focused on their applications and emerging features relevant to developing assays for drug-induced GIT. For existing organoid models, this review highlights (i) the currently available data on assay predictive performance and (ii) the limitations for drug screening. For next-generation models, this review highlights (iii) the breakthrough in interactions between diverse cell types, and (iv) the emerging differentiation cues available to induce the more specialized microphysiologies aligned with GI AEs.

Perhaps the most straightforward and direct application is using GI organoids to screen for drug-induced cytotoxicity and/or reduced cell growth (see caveat on this endpoint in: Selecting assay endpoints for DI-GIT). Although concordance has been seen within small sets of 3 to 4 oncology drugs,^{21,22} studies using larger, diverse compound sets such as in Tables 2–5 will be required to determine which *in vitro* endpoints yield sufficient predictivity for particular clinical GI AEs. Independent of those findings, there will be broad demand to extend organoid technology with second generation platforms tailored for DI-GIT applications.

The capacity to control drug exposure is essential for most drug discovery applications but is limited with current organoid technology. Organoids grow embedded in hydrogel which complicates the ability to control/determine the free

drug concentration at the organoid exterior. Control of exposure in the luminal space of traditional (apical-in) organoids is limited by the spherical 3D configuration combined with the tendency of epithelial cells to form tight-junctions which may effectively seal these cysts to penetration by some drugs. For orally administered drugs, exposure in the intestinal lumen can be particularly high. *In vitro* assays that replicate selectively high exposure at the apical surface of enterocytes will be needed. In addition, organoids shed senescent enterocytes into the organoid interior as part of the normal life cycle. The resulting cellular debris accumulates in the organoid core (vs. being cleared *via* the GI tract *in vivo*). Consequently, organoids are typically physically fragmented and passaged on a weekly basis to remove the cellular debris. This limits the time course for a drug treatment experiment and complicates studies where longer-term exposure is required, *e.g.* to compare drug dosing schedules. The recently developed reversed polarity (apical-out) organoids are an important advancement that addresses some of these challenges.²³ Replating organoid-derived cultures into transwells or microdevices may be required to allow dual exposure control at both apical and basal surfaces (*e.g.*, ref. 24). Techniques for automated microinjection of organoids with drugs and microbes may be valuable particularly for processes involving highly oxygen sensitive anaerobic microbiota.^{25,26} Alternatively, 2-dimensional culture platforms yield microtissues with access to both apical and basal surfaces.^{10,27,28}

The breakthrough feature of GI microtissues may be delivering complex epithelial cultures that capture interactions between diverse intestinal cell types. Previous *in vitro* models based on monocultures of intestinal cell lines (*e.g.*, Caco2) were successfully used as frontline assays for intestinal drug absorption/permeability but were limited as a routine screen for intestinal toxicity. The minimal unit necessary to recapitulate GI epithelial biology/toxicity will vary based on mechanism of action but at a broad level will require major epithelial cell type functions including: stem cell renewal, Paneth cell support, transient amplifying cell proliferation, goblet cell secretion, and enterocyte barrier function. Rare intestinal cell types may be important contributors particularly for replicating drug pharmacology



that is reflected in these cells, since expression of unique combinations of G-protein coupled receptors has recently been used to distinguish previously unknown intestinal cell types.^{29,30} Beyond replicating the presence of primary and rare epithelial cell types, organoids capture the interactions between diverse cell-types. Organoids self-organize into crypt and villus-like domains which is spatial evidence of coordinated interactions between diverse cell-types – the definition of a microphysiology. The interactions between these diverse cellular types are the foundation of GI microphysiologies and may be tailored by carefully combining differentiation cues and specifically designed microdevices to induce biology relevant for GIT.

The cues that direct microtissue differentiation/specialization are being discovered at a rapid pace and merit brief review here. In the original organoid manuscript,³¹ the combination of cues included defined soluble growth factors (Wnt/ β -catenin signalling pathway agonist R-spondin 1, epidermal growth factor, Noggin) and extracellular matrix. Although less appreciated, non-spherical GI microtissues have been developed by combining stimuli consistent with a microtiter plate format such as transwell culturing, air-liquid interface, and undefined growth factors from a feeder layer of mesenchymal cells.^{32–35} In the future, the growth factor cues may be switched from proteins to small molecules in order to improve consistency, reduce cost, and refine pharmacology. To date, small molecule inhibitors of GSK3 (CHIR-99021) and the BMP type I receptor (LDN-193189) have been shown to maintain intestinal stem cells.³⁶ Novel small molecule factors may be discovered using platforms like the micraft array which allows high-throughput screening of factors that replicate stem cell niche conditions.³⁷ In addition to growth factor/chemical support, 3D structure and 3D gradients can provide important differentiation cues. Collagen scaffolds microengineered to replicate the 3D crypt-villus-like enfolding promoted crypt compartmentalization of stem cells and lineage differentiation along the villus. 3D gradients of growth factors further promoted specialization zones including both crypt-to-villus gradients of growth factors³⁸ and villus-to-crypt gradients of the bacterial metabolites, *e.g.*, butyrate.³⁹ The power of gradients has been harnessed in 2D cultures with microholes distributed in a planar array. Above the microholes, where concentrations of growth factors were the highest, stem/proliferative zones formed and as these cells migrated radially, they became more differentiated.⁴⁰ Finally, physical and mechanical factors add another means for inducing differentiation. Subjecting Caco2 cells to fluid flow (shear stress 0.02 dyne per cm²) induced a 6-fold increase in cell height yielding a columnar shape similar to healthy human intestinal epithelium.⁴¹ Adding cyclic peristaltic strain has minimal effect on cellular architecture but increases changes in paracellular permeability and aminopeptidase activity.⁴¹ Models with both microfluidic flow and cyclic strain, induced Caco2 and primary human intestinal cells to differentiate into diverse nature epithelial cells (absorptive, goblet,

enteroendocrine and Paneth) that assemble with villus-like morphology.^{42,43} Taken together, the success of growth factors, 3D structure, chemical gradients, and physical/mechanical factors in stimulating epithelial morphogenesis affords GI MPS model developers a diverse toolbox to induce specialized GI microtissues (reviewed in ref. 35 and 44). Hopefully, innovative combinations of these factors can be discovered that coax microtissues into emulating microphysiologies aligned with each GI AE.

A priority microphysiology to replicate: enterocyte/immune/microbiome interaction

Intestinal microbes, enterocytes, and immune cells engage in a complex interaction that are unique to symbiotic gut biology and is a high priority for replication in MPS models. The commensal microbiome is essential for digestion of dietary fibers, proteins, and peptides, yet presents a constant threat of infection and disease pathology.⁴⁵ Enterocytes form a barrier to prevent microbial invasion, while still allowing nutrient absorption. Immune cells detect and respond to harmful microbial penetration yet are not chronically activated by commensal microbes. Disturbances in the homeostatic balance between enterocyte/immune/microbiome tissues underpin many GI diseases and toxicities. This interactive nature of GI diseases is evident in inflammatory bowel diseases (IBD) such as food allergy and celiac disease, which have intestinal epithelial barrier dysfunction as a primary contributing factor. Hence, IBD are often termed leaky gut diseases (reviewed in ref. 46 and 47). Conversely, immune cell responses can alter enterocyte barrier function and disease. Release of cytokines TNF- α and IL-13 contributes to barrier dysfunction in inflammatory diseases such as Crohn's and ulcerative colitis. Inhibiting TNF- α dramatically reduces gut inflammation and largely restores the gut barrier in Crohn's disease (reviewed in ref. 48 and 49). Disturbances in the enterocyte/immune/microbiome interaction can also be central to toxicological responses. For example, approximately 50% of all patients treated with chemotherapy will suffer from treatment-related gastrointestinal mucositis. The pathogenesis of mucositis involves changes in intestinal epithelial renewal, barrier function, and immune response (reviewed in ref. 50). Immune checkpoint inhibitors can induce immune-related GI adverse events, with 10–30% frequency being reported in clinical trials and covering a wide spectrum of symptoms (reviewed in ref. 51). Finally, intestinal microbiota can contribute to drug metabolism and formation of reactive metabolites that can adversely affect both efficacy and toxicity.⁵²

Achieving the goal of including components of the microbiome in MPS models poses a significant technical hurdle and offers a powerful tool to drive GI microtissue specialization. Since most gut bacteria are obligate or



facultative anaerobes, co-culturing with oxygen-requiring enterocytes requires specialized microdevices. Technical feasibility has been demonstrated with several *in vitro* platforms using Caco2-derived epithelial cells and anaerobic gut bacteria (reviewed in ref. 53). Selecting the appropriate constituents in these microdevices present challenges for both the epithelia and microbiota. The epithelial barrier will likely need to be comprised of more cell types than enterocytes forming monolayer with tight junctions. Goblet cells will be needed to secrete the protective mucus layer (50–450 μm thick in the small intestine) that physically separates microbes from the epithelium. Tuft cells may be needed to support the defence against protozoa and helminth infections (reviewed in ref. 44). Selecting microbiome components (bacterial strains, viruses, and eukaryotes) will need to be defined at a granular level to reduce risk of experimental inconsistency across systems and between laboratories. However, an opportunity to study patient-specific microbiomes could replicate patient/microbiome-specific GI disease and toxicity. At the *in vivo* level, germ-free animals lacking commensal bacteria exhibit thinner villi, shallower crypts, and reduced cellular proliferation.⁵⁴ Reductionist *in vitro* studies reveal that microbial metabolites, like short-chain fatty acids butyrate and acetate, enhance epithelial tight junction protein expression and barrier function *in vitro*.^{55,56} There is broad evidence that exposure to biodiverse microbiota correlates with improved immune function and immune-reduced disease,^{57–59} leading to the hypothesis that diverse microbial exposure is necessary to educate and shape host immunity.^{60–63} Thus, for models able to surmount these technical difficulties, adding a microbiome component is expected to deliver a powerful cue for enterocyte and immune cell development.

Recent GI MPS microdevices have integrated microbiome/enterocyte/immune microphysiology.^{64,65} The mechanical cues of cyclic peristaltic strain and microfluidic flow were used in an MPS model to coax Caco2 cells to differentiate into diverse epithelial cells that assemble with villi-like morphology. This model was then inoculated with a microbiome and unlike prior co-cultures where bacteria overgrowth prevented sustained co-culture, bacteria in this MPS microtissue reached a steady state that was maintained for more than a week. Cessation of the cyclic peristaltic-like stretch triggered bacterial overgrowth, which is consistent with post-surgical complication of bacterial overgrowth observed in some patients that lose ileal peristaltic tone.⁶⁴ Further experiments revealed that co-culturing pathogenic bacteria in the microtissues induced villus atrophy, which could be prevented with probiotic and antibiotic treatments. The addition of immune cells derived from peripheral blood (PBMCs) along with lipopolysaccharide endotoxins, stimulated epithelial cells to produce proinflammatory cytokines (IL-8, IL-6, IL-1 β , and TNF- α) sufficient to induce villus injury and compromise intestinal barrier function. This breakthrough MPS model replicating enterocyte/immune/microbiome microphysiology *in vitro* has profound potential for clinical translation. Opportunities for

future iterations of improved GI-MPS models include finding novel methods that expand the mucosal immune features by including resident immune cells, capturing innate and adaptive immune responses, and replicating antigen sampling and activation functions.

Epithelial-neuronal microphysiology – modelling nausea, vomiting, abdominal pain, and constipation

Integrating sensory epithelial cells and signal transducing enteric neurons is a priority for developing *in vitro* models of the most common DI-GITs: nausea, vomiting, abdominal pain, and constipation. Enterochromaffin (EC) cells act as epithelial chemosensors, transducing information *via* serotonergic synapses with enteric neurons. Alterations in EC cell-released serotonin have been implicated in nausea, vomiting, GI dysmotility, visceral hypersensitivity disorders, and inappetence.^{66,67} Serotonin receptor 5-HT₃ targeted therapeutics are used to treat chemotherapy-induced emesis, chronic nausea, and visceral pain disorders. EC cells in organoids, when co-cultured with neurons, form functional 5HT₃R synapses capable of sensing and transducing noxious stimuli from distinct sources including ingested chemicals, commensal organisms, and endogenous regulatory pathways.⁶⁸

The enteric nervous system (ENS) of the small intestine and colon has autonomous reflex pathways controlling intestinal contractile activity, local blood flow and transmucosal movement of fluids⁶⁹ and therefore compounds that disrupt ENS function likely elicit diarrhoea or constipation depending on the affected pathway. Emerging *in vitro* models have paired stem cells from human intestinal organoids with neural crest cells differentiated into neurons. When these cells are implanted *in vivo* they form neuroglial structures similar to a myenteric and submucosal plexus.⁷⁰ Implanting these co-cultures *in vivo* is an emerging approach but offers potential for building epithelial-neuronal models. To support development of *in vitro* models capable of assessing drugs for the risk of constipation, we have generated a constipation validation test set in (Table 4) which includes drugs with primary effects on neurons and smooth muscle.

Enabling PK–PD and mechanistic mathematical modelling

In instances where GITs are mediated by on-target mechanisms, such as for many oncotherapeutics, it is illogical to expect that optimizing chemistry and reducing off-target pharmacology will mitigate the liability. In such cases, an alternative strategy is to optimize the clinical dose and schedule to maximize therapeutic margin. Historically, effective and tolerated treatment schedules have been identified by extensively testing different schedules in clinical trials, an approach that is empirical and costly. Recent work has demonstrated that dosing schedules can be rationally



designed using mathematical models derived by relating drug exposure pharmacokinetics to efficacy and toxicity pharmacodynamics. For example, PK-PD modelling of capecitabine GIT in animal and clinical studies was used to predict a clinical dose and schedule with improved efficacy and reduced on-target toxicity.⁷¹ Application of modelling is often limited by access to robust kinetic data. Hence MPS models able to deliver kinetic data to inform modelling have the potential to assist in clinical management of DI-GIT.

PK-PD models are empirical and can resolve the relationship between concentration and effect over time. The iterative rounds of data collection required for model predictions to be tested and refined are often lacking and have limited implementation of this approach. In early clinical trials focused on safety, patient numbers and sampling frequency are limited, while switching to preclinical animal studies introduces cross-species differences. Furthermore, determination of GIT in animal studies depends on terminal histological evaluation, which limits sampling frequency and is an endpoint that can be an 'indirect' reflection of toxicity, since the tissue damage is several steps downstream from the primary toxicity target effect. By contrast, human derived MPS models offer a variety of endpoints to quantify response dynamics, with automated high frequency sampling and live real-time observation to feed mathematical modelling. For example, TEER data from GI microtissues were used to model drug-induced intestinal barrier damage and recovery dynamics.¹⁰ For the particular drug investigated, modelling revealed complex epithelial responses comprising damage, recovery, and sensitization. However, due to the slow recovery kinetics, all predicted dosing schedules include extended drug holidays, that are too long to maintain the target inhibition needed for efficacy.¹⁰ Although a viable clinical schedule could not be ascertained as achieved with capecitabine,⁷¹ both examples demonstrate the important value of utilizing PK-PD models in GIT clinical plans.

In contrast to PK-PD models, mechanistic mathematical modelling accounts for the underlying biology and provides a causality between inputs and outputs. Mechanistic models can be expanded to include multiple compounds affecting the same underlying physiology processes. Models have been developed to describe the temporal^{72,73} or spatiotemporal cell dynamics⁷⁴⁻⁷⁷ in the GI epithelium and to elucidate epithelial organization and renewal. Emerging multiscale approaches⁷⁸ utilize a comprehensive understanding of the main biological processes to enable the prediction of toxicity and recovery at multiple temporal and spatial (e.g., cells, organ, organism) scales. As an example, development of intestinal tumorigenesis has been modelled based on Wnt-activating mutations.⁷⁹ MPS platforms capable of providing data with enough resolution to parametrize these models and/or test hypotheses will be applicable for developing mechanistic models of DI-GIT affecting epithelial renewal. Mechanistic-inspired modelling has been applied to DI-GIT and successfully predicted disruption of epithelial proliferation and recovery following oncology

therapies.⁸⁰ Parameters fitted included normal rates of doubling, arrest, death, and shedding for each epithelial cell-type. For those MPS platforms capable of providing these data, this mechanistic model should be applicable for DI-GIT affecting epithelial renewal.

Selecting assay endpoints for DI-GIT

Experience with *in vitro* toxicity screening across diverse organ systems suggests several general principles for selecting endpoints for DI-GIT detection in MPS. Cytotoxicity-related endpoints often fail to accurately predict organ-specific toxicities even when the cytotoxicity is assessed in organ-specific cultures (i.e., hepatic, cardiac, or kidney-derived).^{6,81} Cytotoxicity endpoints do add to interpretations of other endpoints but may not be a useful measure alone. Second, Valentin and colleagues observed that most GI AEs are functional toxicities thereby indicating that functional screening assays should be prioritized.⁴ Third, strategies for managing GIT in the clinic have exploited dose scheduling, thereby suggesting that kinetic data should be generated, if possible, to inform mathematical modelling and guide discovery of optimized clinical schedules (discussed in Enabling PK-PD and mechanistic mathematical modelling). Selecting assay endpoints that measure GI-specific functions in a non-destructive, re-readable manner will likely enhance predictivity and expand utility.

Examples of MPS suitable endpoints that capture GI-specific functions and their relationships to drug toxicity include the following:

(i) Intestinal barrier function is a core role of the GI epithelia and its disruption is associated with drug-induced diarrhoea and inflammatory bowel diseases. Robust barrier data have been achieved with GI microtissues using TEER in transwells¹⁰ and microfluidic devices.⁶⁴ Another electrically-based method, impedance, delivers robust barrier data but requires electrodes on solid surfaces.⁸² TEER and impedance measurements are non-destructive, label-free and can be repeated to generate kinetic data for modelling. Measuring barrier changes by dye diffusion eliminates the need for electrodes but lacks the exquisite sensitivity of electrical measures⁸³ and is an endpoint assay.

(ii) Immune function is central to GI homeostasis, disease and DI-GIT, thus quantifying immune changes resulting as a primary drug response or secondary to barrier disruption and microbial-related stimulation will be fundamental. Inflammatory responses have been quantified in MPS models by measuring proinflammatory cytokines released into the culture media from GI microtissues cultured in microfluidic devices.^{64,65}

(iii) EC cell chemosensory signalling is a GI function of particular interest due to linkage with nausea, vomiting and visceral pain. Endpoints possibly as simple as measuring serotonin release also warrant exploration.

(iv) Continual renewal of epithelial cells is a core GI physiological process encompassing the maintenance of



quiescent reserve stem cells within the intestinal crypts through to enterocyte senescence at the tip of villi. Endpoints that quantify diverse epithelial cell types, ideally with high-content capacity to distinguish cell types over time will allow assessment of cell type-specific drug toxicity and adaptive population shifts. A prototypic example for this application would be the drug-induced differentiation of proliferative cells into goblet cells by Notch/ γ -secretase inhibitors.⁸⁴

(v) Mucus secreted by goblet cells is an essential frontline protective barrier for the GI epithelium. Causal linkage between changes in mucus secretion and GIT have not been widely reported however it is not routinely examined.⁸⁵ Exploratory assessment of mucus secretion in an MPS model suggests this endpoint has potential for drug toxicity testing.⁶⁵

(vi) Finally, secretory diarrhoea can occur as a consequence of disrupted electrolyte homeostasis. Although not a common mechanism for drug-induced diarrhoea, it can be quantified by measuring drug-induced GI organoid swelling as exemplified by an assay designed to detect modulators of CFTR mutations to treat cystic fibrosis.⁸⁶

Preclinical biomarkers of GIT are endpoints that may prove valuable for some MPS models. Potential biomarkers include L-citrulline, which is associated with reduced enterocyte mass, calprotectin, increases of which are associated with IBD, and miR-194, which has been shown to correlate well with histopathology and may detect transient GI damage that could be repaired. Other biomarkers used *in vivo* that could be useful *in vitro* include diamine oxidase for small intestinal mucosal lesions, gastrin for injury to the duodenum and gastric antrum, pepsinogen for inflammation of the gastric mucosa, and I-FABP for cell damage of enterocytes. An advantage of many MPS models is that the material used is transparent or sufficiently translucent to allow imaging of the cells, providing the opportunity to correlate biochemical biomarkers with cell morphology.

Novel biomarkers for GIT are a high priority need and could be revealed by MPS models that include endpoints for unbiased biomarker discovery. While the existing biomarkers of GIT have shown fit-for-purpose as pre-clinical tools, they suffer from significant inter-species variability and lack demonstrable clinical translation. For many clinicians, the best biomarker of GIT is still diarrhoea (or bloody diarrhoea indicating colitis) (reviewed in ref. 87). As an example of the expected impact, the cleaved ectodomain of KIM1 has emerged as a circulating biomarker of renal proximal tubule injury and is widely used to facilitate the early diagnosis of renal toxicity/disease. For GIT, MPS *in vitro* models may be sufficiently reductionist to enable elucidation of novel biomarkers. Once MPS models are established for a particular GIT, multi-omics screening for biomarkers should be considered.

Assay qualification and context of use

While assay qualification is an important step in the eventual use of an MPS as a predictive tool in drug discovery, for MPS assays to guide clinical studies (such as an *in vitro* biomarker

or an MPS-driven mathematical model) the standards are higher, and guidance set by the NIH and FDA related to biomarkers and clinical assessments should be appreciated.⁸⁸ Qualification is driven from clearly defining the context of use (CoU) which is “a statement that fully and clearly describes the way the medical product development tool is to be used and the medical product development-related purpose of the use”.⁸⁸ This includes both the precise statement of the purpose and the conditions upon which the assay is to be evaluated. In the case of a GI MPS model, the CoU would describe the segment of the GI tract being modelled, along with the toxicity/disease of interest, the expected outcomes, and how the data will be used. As an example, ‘the small intestine MPS can be used to assess toxicity of drugs during exposures of up to two weeks. Epithelial barrier function and biomarkers such as L-citrulline and diamine oxidase can be assessed to demonstrate toxicity. Data can be used to discriminate between drug candidates or illustrate mechanisms of toxicity’. When working with a CoU statement, the proposed use guides the qualification studies and the data obtained can feed back to modify the CoU statement.

The boundaries of utility within the CoU framework can be defined by characterization and comparison of the *in vitro* model to *in vivo* responses and GI functions being modelled. Characterization can range from gene and protein expression, morphology, cell function, and pharmacologic and toxicologic responses. The layering of characterization efforts with effects from well characterized molecules is the foundation of sound model development and ultimately can be used as a rationale to expand CoU (see above: Frontline assays for GIT risk detection). Examples include a colon cancer line, LS180, which was qualified in a narrow CoU approach on mycophenolic acid (MPA) induced GI injury. The authors demonstrated key MPA metabolism genes and consistent cytotoxicity during line passaging.⁸⁹ A second example is a transwell human GI microtissue model where TEER IC₁₅ values were used to predict drug-induced diarrhoea. Across a set of 30 compounds, human microtissue TEER data were a better predictor of diarrhoea than data derived from Caco2 cells.¹⁰ This study is a *de facto* blueprint for model development and establishment of a CoU for a screen to identify drug-induced diarrhoea.

Challenges for GI MPS models specific to assessing DI-GIT

In addition to the challenges of recapitulating normal GI physiology (reviewed in ref. 19 and 90), MPS models for DI-GIT screening will encounter several application-specific challenges. The next major advance requires supplementing epithelial microtissues with additional tissue types. Among the leading GITs, the diverse mechanisms are proposed to include contributions from immune cells, microbiome, nerve, smooth muscle, and vasculature. Some of these tissue functions require complex organization such as enteric



neural networks, circular *versus* longitudinal muscle layers, or circulating *versus* resident immune cell concentrated into discrete domains (gut-associated lymphoid tissue) and in the extreme case, some instances of nausea and vomiting that are mediated by the CNS. A central challenge for MPS model development will be assembling the minimal set of necessary tissues with appropriate 3D structure to recapitulate the targeted toxicity.

Two additional challenges unique to assessing DI-GIT arise in use-specific context. GI MPS can be used in drug discovery in either a proactive mode (to predict GI toxicity in advance of *in vivo* testing) or reactive mode (responding to a preclinical *in vivo* or clinical finding). The prerequisite to deploy an assay for proactive screening is establishing accuracy of clinical translation. Concordance requires testing drugs *in vitro* at concentrations that reflect the toxic concentration. A key challenge for this will require knowledge of site of action and local concentration. It is difficult to determine if toxicity is a response to acute high concentration of drug that occurs in the intestinal lumen after oral dosing or reflects secondary systemic exposures. Intraluminal drug exposures can be several orders of magnitude higher than plasma,⁹¹ which makes it difficult to know whether toxicity is an on-target response or is due to modulation of lower affinity off-targets at these high concentrations.

After a toxicity has been identified, the next goal is typically to identify an underlying mechanism for toxicity and then determine if chemical modifications of the lead compound can improve tolerability. However, the mechanisms and pathogenesis underlying DI-GITs are often unknown. Although diarrhoea has been considered the best indicator for GIT, it gives little to no information on the underlying mechanism or the target cells for toxicity.⁸⁷ Thus, a challenge for intestinal MPS models will be to reveal mechanistic insights of DI-GIT with histology, cell type analysis, or similar high-content tissue-level data. Mechanistic insight at the molecular level is equally important as this enables preclinical to clinical translation assessment. Elucidating causal linkage between target pharmacology and toxicity is a reoccurring need requiring molecular approaches such as gene knockout.

GI microtissues typically require a source of intestinal stem cells. Fetal-derived stem cells carry ethical concerns that severely restrict their use within pharmaceutical companies. Sourcing stem cells from biopsied adult intestinal tissue is a minimal requirement. Assays capable of utilizing induced pluripotent stem cells (iPSC)-derived stem cells will be important for comparing healthy and diseased patient populations without the need for biopsy.⁷⁰

Conclusions

From a drug discovery perspective, there is high demand for *in vitro* systems that can accurately detect drug-induced GITs. Given the complexity of cell types, their anatomic distribution and biochemical mechanisms that regulate GI homeostasis

and function, approaches based on simple cell lines in 2D assays will remain limited. New strategies are needed. Fortunately, the ability to generate multi-cell type intestinal microtissues using either primary stem cells or iPSCs are providing greater opportunities for success in the near-term. Incubating these physiologically-relevant microtissues in transwell, organoid or bioprinted scaffolds with additional tissue-relevant parameters such as pulsatile stretch, fluid flow, and an anaerobic microbial luminal environment may enable predictive *in vitro* systems for drug-induced GI toxicity.

As with any assay, the balance between complexity and utility is dependent on CoU. For example, moderate throughput screens for a pharmacological endpoint require testing at multiple drug concentrations and replicate samples to generate quantitative values for structure–activity relations and therapeutic index insight. Assays of this type tend to minimize biocomplexity, utilize an economy of scale and platform endpoints to enable cost-effective testing. In contrast, assays designed to provide mechanistic understanding of toxicity or disease typically require increased complexity and cell types combined with bespoke endpoints where low throughput is acceptable. By definition, MPS models attempt to recapitulate tissue biology rather than single cell-type biology and therefore begin at the more complex end of the assay spectrum. However, they need not be restricted to low throughput environments since many of the detection endpoints described in detail (see Selecting assay endpoints for DI-GIT) are compatible with higher throughput screening platforms. Indeed, microtiter plate-based 3D microtissues have been developed^{83,92} demonstrating that moderate throughput MPS screening is possible. Testing clinically-defined positive and negative control compounds for particular GITs (such as those described in: Frontline assays for GIT risk detection) in an MPS will help establish their suitability for use as a predictive screen for human DI-GIT and to investigate underlying mechanisms of action.

Remarkable progress has been made in the past decade in the development of complex GI microtissues and MPS with the goal of emulating normal intestinal features/function. These efforts provide confidence that we are making headway towards the goal of using *in vitro* systems in preclinical drug discovery to identify and mitigate DI-GITs. The rate of progress will depend, in part, on continued investment in MPS technologies, intestinal cell biology research and identification/application of endpoints and biomarkers of drug-induced GI dysfunction.

Conflicts of interest

There are no conflicts of interest to declare. All authors are/were current employees of their respective institutions with substantive contributions to the manuscript in the form of conceptualization, analysis, and writing (original drafting, reviewing, and editing).



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References

- 1 C. Federer, M. Yoo and A. C. Tan, *Assay Drug Dev. Technol.*, 2016, **14**, 557–566.
- 2 C. Tamaki, T. Nagayama, M. Hashiba, M. Fujiyoshi, M. Hizue, H. Kodaira, M. Nishida, K. Suzuki, Y. Takashima, Y. Ogino, D. Yasugi, Y. Yoneta, S. Hisada, T. Ohkura and K. Nakamura, *J. Toxicol. Sci.*, 2013, **38**, 581–598.
- 3 J. Luo and R. A. Cisler, *Cancer Inf.*, 2016, **15**, 211–217.
- 4 A. Al-Saffar, A. Nogueira da Costa, A. Delaunoy, D. J. Leishman, L. Marks, M. L. Rosseels and J. P. Valentin, *Handb. Exp. Pharmacol.*, 2015, **229**, 291–321.
- 5 D. Cook, D. Brown, R. Alexander, R. March, P. Morgan, G. Satterthwaite and M. N. Pangalos, *Nat. Rev. Drug Discovery*, 2014, **13**, 419–431.
- 6 Z. Lin and Y. Will, *Toxicol. Sci.*, 2012, **126**, 114–127.
- 7 H. Olson, G. Betton, D. Robinson, K. Thomas, A. Monro, G. Kolaja, P. Lilly, J. Sanders, G. Sipes, W. Bracken, M. Dorato, K. Van Deun, P. Smith, B. Berger and A. Heller, *Regul. Toxicol. Pharmacol.*, 2000, **32**, 56–67.
- 8 T. M. Monticello, T. W. Jones, D. M. Dambach, D. M. Potter, M. W. Bolt, M. Liu, D. A. Keller, T. K. Hart and V. J. Kadambi, *Toxicol. Appl. Pharmacol.*, 2017, **334**, 100–109.
- 9 J. A. Wagner, *Annu. Rev. Pharmacol. Toxicol.*, 2008, **48**, 631–651.
- 10 M. F. Peters, T. Landry, C. Pin, K. Maratea, C. Dick, M. P. Wagoner, A. L. Choy, H. Barthlow, D. Snow, Z. Stevens, A. Armento, C. W. Scott and S. Ayehunie, *Toxicol. Sci.*, 2019, **168**, 3–17.
- 11 E. Genschow, H. Spielmann, G. Scholz, A. Seiler, N. Brown, A. Piersma, M. Brady, N. Clemann, H. Huuskonen, F. Paillard, S. Bremer and K. Becker, *ATLA, Altern. Lab. Anim.*, 2002, **30**, 151–176.
- 12 W. S. Redfern, L. Carlsson, A. S. Davis, W. G. Lynch, I. MacKenzie, S. Palethorpe, P. K. Siegl, I. Strang, A. T. Sullivan, R. Wallis, A. J. Camm and T. G. Hammond, *Cardiovasc. Res.*, 2003, **58**, 32–45.
- 13 C. Grant, L. Ewart, D. Muthas, D. Deavall, S. A. Smith, G. Clack and P. Newham, *Toxicol. Appl. Pharmacol.*, 2016, **296**, 10–18.
- 14 L. L. Clarke, *Am. J. Physiol.*, 2009, **296**, G1151–G1166.
- 15 I. A. de Graaf, P. Olinga, M. H. de Jager, M. T. Merema, R. de Kanter, E. G. van de Kerkhof and G. M. Groothuis, *Nat. Protoc.*, 2010, **5**, 1540–1551.
- 16 M. Li, I. A. de Graaf and G. M. Groothuis, *Expert Opin. Drug Metab. Toxicol.*, 2016, **12**, 175–190.
- 17 A. M. Holmes, J. A. Rudd, F. D. Tattersall, Q. Aziz and P. L. Andrews, *Br. J. Pharmacol.*, 2009, **157**, 865–880.
- 18 T. R. Van Vleet, M. J. Liguori, J. J. Lynch, 3rd, M. Rao and S. Warder, *SLAS Discovery*, 2019, **24**, 1–24.
- 19 S. E. Blutt, J. R. Broughman, W. Zou, X. L. Zeng, U. C. Karandikar, J. In, N. C. Zachos, O. Kovbasnjuk, M. Donowitz and M. K. Estes, *Exp. Biol. Med.*, 2017, **242**, 1633–1642.
- 20 S. Rahmani, N. M. Breyner, H. M. Su, E. F. Verdu and T. F. Didar, *Biomaterials*, 2019, **194**, 195–214.
- 21 T. Grabinger, L. Luks, F. Kostadinova, C. Zimmerlin, J. P. Medema, M. Leist and T. Brunner, *Cell Death Dis.*, 2014, **5**, e1228.
- 22 S. Hoyle, A. M. Bonavita, A. Murdoch, M. Brown and C. Booth, presented in part at the European Organisation for Research and Treatment of Cancer, 2016.
- 23 J. Y. Co, M. Margalef-Catala, X. Li, A. T. Mah, C. J. Kuo, D. M. Monack and M. R. Amieva, *Cell Rep.*, 2019, **26**, 2509–2520, e2504.
- 24 Y. Wang, R. Kim, S. J. Hwang, J. Dutton, C. E. Sims and N. L. Allbritton, *Anal. Chem.*, 2018, **90**, 11523–11530.
- 25 S. Bartfeld, T. Bayram, M. van de Wetering, M. Huch, H. Begthel, P. Kujala, R. Vries, P. J. Peters and H. Clevers, *Gastroenterology*, 2015, **148**, 126–136, e126.
- 26 I. A. Williamson, J. W. Arnold, L. A. Samsa, L. Gaynor, M. DiSalvo, J. L. Cocchiario, I. Carroll, M. A. Azcarate-Peril, J. F. Rawls, N. L. Allbritton and S. T. Magness, *Cell. Mol. Gastroenterol. Hepatol.*, 2018, **6**, 301–319.
- 27 Y. Wang, I. L. Chiang, T. E. Ohara, S. Fujii, J. Cheng, B. D. Muegge, A. Ver Heul, N. D. Han, Q. Lu, S. Xiong, F. Chen, C. W. Lai, H. Janova, R. Wu, C. E. Whitehurst, K. L. VanDussen, T. C. Liu, J. I. Gordon, L. D. Sibley and T. S. Stappenbeck, *Cell*, 2019, **179**, 1144–1159, e1115.
- 28 Y. Wang, M. DiSalvo, D. B. Gunasekara, J. Dutton, A. Proctor, M. S. Lebhar, I. A. Williamson, J. Speer, R. L. Howard, N. M. Smiddy, S. J. Bultman, C. E. Sims, S. T. Magness and N. L. Allbritton, *Cell. Mol. Gastroenterol. Hepatol.*, 2017, **4**, 165–182, e167.
- 29 D. Grun, A. Lyubimova, L. Kester, K. Wiebrands, O. Basak, N. Sasaki, H. Clevers and A. van Oudenaarden, *Nature*, 2015, **525**, 251–255.
- 30 A. L. Haber, M. Biton, N. Rogel, R. H. Herbst, K. Shekhar, C. Smillie, G. Burgin, T. M. Delorey, M. R. Howitt, Y. Katz, I. Tirosh, S. Beyaz, D. Dionne, M. Zhang, R. Raychowdhury,



- W. S. Garrett, O. Rozenblatt-Rosen, H. N. Shi, O. Yilmaz, R. J. Xavier and A. Regev, *Nature*, 2017, **551**, 333–339.
- 31 T. Sato, R. G. Vries, H. J. Snippert, M. van de Wetering, N. Barker, D. E. Stange, J. H. van Es, A. Abo, P. Kujala, P. J. Peters and H. Clevers, *Nature*, 2009, **459**, 262–265.
 - 32 F. Boccellato, S. Woelffling, A. Imai-Matsushima, G. Sanchez, C. Goosmann, M. Schmid, H. Berger, P. Morey, C. Denecke, J. Ordemann and T. F. Meyer, *Gut*, 2019, **68**, 400–413.
 - 33 A. Ootani, X. Li, E. Sangiorgi, Q. T. Ho, H. Ueno, S. Toda, H. Sugihara, K. Fujimoto, I. L. Weissman, M. R. Capecchi and C. J. Kuo, *Nat. Med.*, 2009, **15**, 701–706.
 - 34 X. Wang, Y. Yamamoto, L. H. Wilson, T. Zhang, B. E. Howitt, M. A. Farrow, F. Kern, G. Ning, Y. Hong, C. C. Khor, B. Chevalier, D. Bertrand, L. Wu, N. Nagarajan, F. A. Sylvester, J. S. Hyams, T. Devers, R. Bronson, D. B. Lacy, K. Y. Ho, C. P. Crum, F. McKeon and W. Xian, *Nature*, 2015, **522**, 173–178.
 - 35 Y. Wang, R. Kim, S. S. Hinman, B. Zwarycz, S. T. Magness and N. L. Allbritton, *Cell. Mol. Gastroenterol. Hepatol.*, 2018, **5**, 440–453, e441.
 - 36 Y. Li, Y. Liu, B. Liu, J. Wang, S. Wei, Z. Qi, S. Wang, W. Fu and Y. G. Chen, *Cell Discovery*, 2018, **4**, 49.
 - 37 A. D. Gracz, I. A. Williamson, K. C. Roche, M. J. Johnston, F. Wang, Y. Wang, P. J. Attayek, J. Balowski, X. F. Liu, R. J. Laurenza, L. T. Gaynor, C. E. Sims, J. A. Galanko, L. Li, N. L. Allbritton and S. T. Magness, *Nat. Cell Biol.*, 2015, **17**, 340–349.
 - 38 Y. Wang, D. B. Gunasekara, M. I. Reed, M. DiSalvo, S. J. Bultman, C. E. Sims, S. T. Magness and N. L. Allbritton, *Biomaterials*, 2017, **128**, 44–55.
 - 39 Y. Wang, R. Kim, D. B. Gunasekara, M. I. Reed, M. DiSalvo, D. L. Nguyen, S. J. Bultman, C. E. Sims, S. T. Magness and N. L. Allbritton, *Cell. Mol. Gastroenterol. Hepatol.*, 2018, **5**, 113–130.
 - 40 R. Kim, Y. Wang, S. J. Hwang, P. J. Attayek, N. M. Smiddy, M. I. Reed, C. E. Sims and N. L. Allbritton, *Lab Chip*, 2018, **18**, 2202–2213.
 - 41 H. J. Kim, D. Huh, G. Hamilton and D. E. Ingber, *Lab Chip*, 2012, **12**, 2165–2174.
 - 42 M. Kasendra, A. Tovaglieri, A. Sontheimer-Phelps, S. Jalili-Firoozinezhad, A. Bein, A. Chalkiadaki, W. Scholl, C. Zhang, H. Rickner, C. A. Richmond, H. Li, D. T. Breault and D. E. Ingber, *Sci. Rep.*, 2018, **8**, 2871.
 - 43 H. J. Kim and D. E. Ingber, *Integr. Biol.*, 2013, **5**, 1130–1140.
 - 44 J. S. Dutton, S. S. Hinman, R. Kim, Y. Wang and N. L. Allbritton, *Trends Biotechnol.*, 2019, **37**, 744–760.
 - 45 A. Koh, F. De Vadder, P. Kovatcheva-Datchary and F. Backhed, *Cell*, 2016, **165**, 1332–1345.
 - 46 K. R. Groschwitz and S. P. Hogan, *J. Allergy Clin. Immunol.*, 2009, **124**, 3–20, quiz 21–22.
 - 47 A. Michielan and R. D'Inca, *Mediators Inflammation*, 2015, **2015**, 628157.
 - 48 A. Buckley and J. R. Turner, *Cold Spring Harbor Perspect. Biol.*, 2018, **10**, a029314.
 - 49 P. Suenart, V. Bulteel, L. Lemmens, M. Noman, B. Geypens, G. Van Assche, K. Geboes, J. L. Ceuppens and P. Rutgeerts, *Am. J. Gastroenterol.*, 2002, **97**, 2000–2004.
 - 50 Y. Toucheffeu, E. Montassier, K. Nieman, T. Gastinne, G. Potel, S. B. des Varannes, F. Le Vacon and M. F. de La Cochetiere, *Aliment. Pharmacol. Ther.*, 2014, **40**, 409–421.
 - 51 M. A. Samaan, P. Pavlidis, S. Papa, N. Powell and P. M. Irving, *Nat. Rev. Gastroenterol. Hepatol.*, 2018, **15**, 222–234.
 - 52 H. Li, J. He and W. Jia, *Expert Opin. Drug Metab. Toxicol.*, 2016, **12**, 31–40.
 - 53 J. Z. H. von Martels, M. Sadaghian Sadabad, A. R. Bourgonje, T. Blokzijl, G. Dijkstra, K. N. Faber and H. J. M. Harmsen, *Anaerobe*, 2017, **44**, 3–12.
 - 54 A. Parker, M. A. E. Lawson, L. Vaux and C. Pin, *Environ. Microbiol.*, 2018, **20**, 2337–2353.
 - 55 C. Y. Hsieh, T. Osaka, E. Moriyama, Y. Date, J. Kikuchi and S. Tsuneda, *Physiol. Rep.*, 2015, **3**(3), e12327.
 - 56 H. B. Wang, P. Y. Wang, X. Wang, Y. L. Wan and Y. C. Liu, *Dig. Dis. Sci.*, 2012, **57**, 3126–3135.
 - 57 T. Haahtela, S. Holgate, R. Pawankar, C. A. Akdis, S. Benjaponpitak, L. Caraballo, J. Demain, J. Portnoy, L. von Hertzen and W. A. O. S. C. O. C. Change and Biodiversity, *World Allergy Organ. J.*, 2013, **6**, 3.
 - 58 I. Hanski, L. von Hertzen, N. Fyhrquist, K. Koskinen, K. Torppa, T. Laatikainen, P. Karisola, P. Auvinen, L. Paulin, M. J. Makela, E. Vartiainen, T. U. Kosunen, H. Alenius and T. Haahtela, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 8334–8339.
 - 59 B. N. Lambrecht and H. Hammad, *Nat. Immunol.*, 2017, **18**, 1076–1083.
 - 60 L. V. Hooper, D. R. Littman and A. J. Macpherson, *Science*, 2012, **336**, 1268–1273.
 - 61 C. L. Maynard, C. O. Elson, R. D. Hatton and C. T. Weaver, *Nature*, 2012, **489**, 231–241.
 - 62 L. W. Peterson and D. Artis, *Nat. Rev. Immunol.*, 2014, **14**, 141–153.
 - 63 H. Sokol, B. Pigneur, L. Watterlot, O. Lakhdari, L. G. Bermudez-Humaran, J. J. Gratadoux, S. Blugeon, C. Bridonneau, J. P. Furet, G. Corthier, C. Grangette, N. Vasquez, P. Pochart, G. Trugnan, G. Thomas, H. M. Blottiere, J. Dore, P. Marteau, P. Seksik and P. Langella, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 16731–16736.
 - 64 H. J. Kim, H. Li, J. J. Collins and D. E. Ingber, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, E7–E15.
 - 65 W. Shin and H. J. Kim, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E10539–E10547.
 - 66 M. D. Gershon, *Curr. Opin. Endocrinol., Diabetes Obes.*, 2013, **20**, 14–21.
 - 67 G. M. Mawe and J. M. Hoffman, *Nat. Rev. Gastroenterol. Hepatol.*, 2013, **10**, 473–486.
 - 68 N. W. Bellono, J. R. Bayrer, D. B. Leitch, J. Castro, C. Zhang, T. A. O'Donnell, S. M. Brierley, H. A. Ingraham and D. Julius, *Cell*, 2017, **170**, 185–198, e116.
 - 69 J. B. Furness, *Nat. Rev. Gastroenterol. Hepatol.*, 2012, **9**, 286–294.
 - 70 M. J. Workman, M. M. Mahe, S. Trisno, H. M. Poling, C. L. Watson, N. Sundaram, C. F. Chang, J. Schiesser, P. Aubert, E. G. Stanley, A. G. Elefanty, Y. Miyaoka, M. A.



- Mandegar, B. R. Conklin, M. Neunlist, S. A. Brugmann, M. A. Helmuth and J. M. Wells, *Nat. Med.*, 2017, **23**, 49–59.
- 71 K. A. Cadoo, D. Gajria, E. Suh, S. Patil, M. Theodoulou, L. Norton, C. A. Hudis and T. A. Traina, *npj Breast Cancer*, 2016, **2**, 16006.
- 72 M. D. Johnston, C. M. Edwards, W. F. Bodmer, P. K. Maini and S. J. Chapman, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 4008–4013.
- 73 A. Parker, L. Vaux, A. M. Patterson, A. Modasia, D. Muraro, A. G. Fletcher, H. M. Byrne, P. K. Maini, A. J. M. Watson and C. Pin, *Cell Death Dis.*, 2019, **10**, 108.
- 74 P. Buske, J. Galle, N. Barker, G. Aust, H. Clevers and M. Loeffler, *PLoS Comput. Biol.*, 2011, **7**, e1001045.
- 75 F. A. Meineke, C. S. Potten and M. Loeffler, *Cell Proliferation*, 2001, **34**, 253–266.
- 76 G. R. Mirams, C. J. Arthurs, M. O. Bernabeu, R. Bordas, J. Cooper, A. Corrias, Y. Davit, S. J. Dunn, A. G. Fletcher, D. G. Harvey, M. E. Marsh, J. M. Osborne, P. Pathmanathan, J. Pitt-Francis, J. Southern, N. Zenzemi and D. J. Gavaghan, *PLoS Comput. Biol.*, 2013, **9**, e1002970.
- 77 C. Pin, A. J. Watson and S. R. Carding, *PLoS One*, 2012, **7**, e37115.
- 78 D. Groen, J. Borgdorff, C. Bona-Casas, J. Hetherington, R. W. Nash, S. J. Zasada, I. Saverchenko, M. Mamonski, K. Kurowski, M. O. Bernabeu, A. G. Hoekstra and P. V. Coveney, *Interface Focus*, 2013, **3**, 20120087.
- 79 T. Thalheim, P. Buske, J. Przybilla, K. Rother, M. Loeffler and J. Galle, *J. R. Soc., Interface*, 2016, **13**, 20160218.
- 80 H. Shankaran, A. Cronin, J. Barnes, P. Sharma, J. Tolsma, P. Jasper and J. T. Mettetal, *CPT: Pharmacometrics Syst. Pharmacol.*, 2018, **7**, 26–33.
- 81 R. Clothier, M. J. Gomez-Lechon, A. Kinsner-Ovaskainen, A. Kopp-Schneider, J. E. O'Connor, P. Prieto and S. Stanzel, *Toxicol. In Vitro*, 2013, **27**, 1347–1356.
- 82 R. J. Haines, R. S. Beard, Jr., R. A. Eitner, L. Chen and M. H. Wu, *PLoS One*, 2016, **11**, e0154351.
- 83 S. J. Trietsch, E. Naumovska, D. Kurek, M. C. Setyawati, M. K. Vormann, K. J. Wilschut, H. L. Lanz, A. Nicolas, C. P. Ng, J. Joore, S. Kustermann, A. Roth, T. Hankemeier, A. Moisan and P. Vulto, *Nat. Commun.*, 2017, **8**, 262.
- 84 J. H. van Es, M. E. van Gijn, O. Riccio, M. van den Born, M. Vooijs, H. Begthel, M. Cozijnsen, S. Robine, D. J. Winton, F. Radtke and H. Clevers, *Nature*, 2005, **435**, 959–963.
- 85 H. Yamamoto, K. Ishihara, Y. Takeda, W. Koizumi and T. Ichikawa, *BioMed Res. Int.*, 2013, **2013**, 276186.
- 86 J. F. Dekkers, C. L. Wiegerinck, H. R. de Jonge, I. Bronsveld, H. M. Janssens, K. M. de Winter-de Groot, A. M. Brandsma, N. W. de Jong, M. J. Bijvelds, B. J. Scholte, E. E. Nieuwenhuis, S. van den Brink, H. Clevers, C. K. van der Ent, S. Middendorp and J. M. Beekman, *Nat. Med.*, 2013, **19**, 939–945.
- 87 D. F. Carr, S. Ayehunie, A. Davies, C. A. Duckworth, S. French, N. Hall, S. Hussain, H. R. Mellor, A. Norris, B. K. Park, A. Penrose, D. M. Pritchard, C. S. Probert, S. Ramaiah, C. Sadler, M. Schmitt, A. Shaw, J. E. Sidaway, R. G. Vries, M. Wagoner and M. Pirmohamed, *Pharmacol. Ther.*, 2017, **172**, 181–194.
- 88 F.-N. B. W. Group, *BEST (Biomarkers, EndpointS, and other Tools) Resource Silver Spring (MD)*, 2016.
- 89 S. Heischmann and U. Christians, *Xenobiotica*, 2018, **48**, 433–441.
- 90 H. Yu, N. M. Hasan, J. G. In, M. K. Estes, O. Kovbasnjuk, N. C. Zachos and M. Donowitz, *Annu. Rev. Physiol.*, 2017, **79**, 291–312.
- 91 R. Cristofolletti, N. Patel and J. B. Dressman, *J. Pharm. Sci.*, 2016, **105**, 2712–2722.
- 92 D. T. T. Phan, X. Wang, B. M. Craver, A. Sobrino, D. Zhao, J. C. Chen, L. Y. N. Lee, S. C. George, A. P. Lee and C. C. W. Hughes, *Lab Chip*, 2017, **17**, 511–520.

