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Quantitative systems toxicology: modelling to mechanistically understand and predict drug safety

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Abstract

Reliable prediction and prevention of adverse drug reactions (ADRs) remains a key challenge in the development of new medicines. Advanced mathematical and computational modelling approaches, which incorporate cutting-edge mechanistic understanding of ADRs in concert with systematically collected data addressing knowledge gaps, are integral components of model-informed drug discovery and development (MID3). These approaches provide a precise, quantitative framework for predicting and mitigating safety risks in the earliest phases of drug development. Here, we highlight recent developments in the burgeoning field of quantitative systems toxicology (QST), including insights into the current state-of-the-art, as well as outcomes from the Innovative Medicines Initiative (IMI) 2 TransQST project. QST models that describe the disruption of cardiovascular, gastrointestinal, hepatic and renal physiological functions following drug exposure are presented, along with recommendations for their application in drug discovery and development.

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Introduction

Safety is a major cause of attrition in drug discovery and development^{1,2} and is, thus, a key consideration when making decisions at the major transition points in the drug pipeline (Box 1). Historically, data from testing drug candidates in animal models have been crucial in many of these decisions. Recently, however, new approach methodologies (NAMs) such as complex in vitro models and computational modelling and simulation have gained attention as an alternative to testing in animals^{3,4}. These approaches support the 3Rs principles – replacement, reduction and refinement – for animal use in scientific experiments⁵ and are also well-aligned with the current vision from the US Food and Drug Administration (FDA) to phase out animal testing in the coming years⁶.

Mechanistic systems modelling and simulation approaches in general have proven invaluable for drug discovery and development through the integration of knowledge and data into mathematical models, predicting clinical outcomes and enabling the simulation of ‘what-if’ scenarios^{7,8}. Although they have different aims and applications, three related systems-modelling disciplines applicable for studying drug safety and toxicity – systems biology, systems pharmacology and systems toxicology – use computational and mathematical modelling to analyse complex biological systems.

Systems biology models aim to describe the complex interactions between biological components at various scales, from molecular to organismal levels, to understand how these relationships give rise to the function and emergent behaviour of organisms⁹. Quantitative systems pharmacology (QSP) builds on systems biology to study how drugs interact with biological systems and to provide a mechanistic understanding of drug action and efficacy^{10,11}. Similarly, quantitative systems toxicology (QST), in the context of its use in pharmaceutical discovery and development, is defined as an approach to quantitatively and mechanistically understand and predict the toxic effects of a therapeutic agent on an organism through the integration of computational and experimental methods^{10,11}. Although QSP and QST are underpinned by similar methodologies to model complex biological interactions, QST specializes in understanding and predicting drug-induced adverse effects on biological systems.

QST models have the potential to increase confidence in the translational safety assessment of candidate molecules and to inform project progression decisions on the path towards regulatory submissions^{12,13}. Such models can describe on-target–on-pathway toxicity (that is, exaggerated pharmacology), on-target–off-pathway toxicity, and off-target toxicities dependent on their scope and data availability¹⁰, thereby enhancing the precision of safety evaluations and increasing confidence in nonclinical safety data packages for drug candidates. An internal analysis at AstraZeneca has found that compounds with lower confidence in their preclinical safety profiles had a higher rate of termination for safety reasons in clinical trials than those with higher confidence².

A number of academic–industry joint initiatives in recent years have focused on the development of QST models, many of which are discussed in this manuscript, including the Innovative Medicines Initiative (IMI) 2 TransQST project, which ran from 2017 to 2022 with the goal of developing QST models for liver, kidney, cardiovascular and gastrointestinal–immune systems¹² (Box 2). Nevertheless, challenges remain regarding gaps in data and knowledge for model development and validation, the availability and accuracy of physiological organ-specific models, the mechanistic understanding of nonclinical and clinical adverse drug reactions (ADRs), and the translational

relevance needed for effective safety decision-making when progressing drug candidates into clinical testing at transition point 2 (TP2, see Box 1). It is also important to note that there is no ‘one-size-fits-all’ QST model, and safety decisions are often not binary (safe versus unsafe). Instead, they are made on a case-by-case basis, considering the identified safety concern and whether the anticipated incidence and severity of ADRs are acceptable in the context of the indication (for example, oncology compared with obesity) and current standard of care. It should also be acknowledged that, although beyond the scope of this manuscript, QST models have applications beyond the pharmaceutical industry, including environmental safety, agrochemicals and cosmetics.

In this Perspective, after briefly presenting the fundamentals of QST models and approaches to building them, we describe several QST models covering cardiovascular, gastrointestinal, liver and kidney toxicity, focusing on their construction and envisioned applications. We also discuss opportunities and challenges in applying QST models to support safety decisions and provide recommendations for effectively building and applying QST models in drug safety assessment. Although we mainly focus on published QST models for small molecules, QST approaches are becoming increasingly important for evaluating the safety of other modalities, such as biologics and cell therapies (see Box 3).

Quantitative systems toxicology: a primer Introduction to quantitative models and their relationship to QST models

Quantitative modelling approaches include empirical, data-driven models and mechanistic, knowledge-driven models. Both of these model types are used in the context of drug discovery and development, including those that predict and elucidate drug effects on biological systems and the effects of systems on the drug (for example, metabolism and elimination)¹⁴. These approaches can also be combined, incorporating both empirical data and mechanistic insights to enhance predictive power. Definitions and comparisons of these modelling terms are shown in Table 1.

Examples of empirical, data-driven models include pharmacokinetic and toxicokinetic (PK and TK) and pharmacodynamic and toxicodynamic (PD and TD) models, along with their combinations, pharmacokinetic–pharmacodynamic (PK–PD) and toxicokinetic–toxicodynamic (TK–TD) models. These models link the kinetics of compound exposure to the dynamic pharmacological–toxicological response via empirical mathematical descriptions without necessarily representing mechanistic processes such as organ pathobiology. Examples of such models include concentration–effect models based on half-maximal inhibitory concentration (IC₅₀) values¹⁵ and time-dependent models that describe the temporal profile of the drug concentration and associated dynamic changes in response¹⁶. Empirical models effectively support drug development and guide decision-making by highlighting correlations and forecasting pharmacological and toxicological responses within the context of observed data. Thus, their development relies heavily on data availability, and their predictive power is limited outside the specific empirical frame for which they were developed.

As a result, the field has advanced towards mechanistic models that provide greater predictive power by leveraging existing biological understanding complemented by specific data to address uncertainties. Examples of mechanistic models include physiologically based pharmacokinetic (PBPK) models, as a subset of physiologically based kinetic

Box 1 | Transition points in safety testing and development of QST models

As drug candidates progress from early discovery through nonclinical studies and clinical trials towards approval, they pass through important transition points (TPs, see the figure) at which the complexity of safety test(s) increases and different safety decisions are addressed. Differences in these safety decisions and the key questions addressed at each TP dictate the type of models required as part of an integrated quantitative systems toxicology (QST) strategy.

TP1: Will the molecule be safe enough in nonclinical tests? Early in discovery, multiple molecules are screened for safety using *in silico* and *in vitro* methods, and subsequently, they may be tested in nonclinical animal models. Insufficient nonclinical safety in short-term nonclinical animal studies may preclude advancing a candidate molecule to the required good laboratory practice (GLP) nonclinical studies that should demonstrate sufficient safety for an investigational new drug (IND) application for clinical testing.

An established application of QST modelling at this TP is in the translation of *in vitro* screening data for cardiac ion channels (for example, hERG, Nav1.5). Inhibition data for molecules against these channels are routinely generated as part of off-target safety pharmacology screening^{123,124} because inhibition of these channels can affect cardiomyocyte action potential, resulting in arrhythmias such as Torsades de Pointes. Mechanistic QST models simulating the function of multiple ion channels are used to estimate the potential of arrhythmia based on inhibition data for screened molecules^{43,114}. Through application of these models, safety pharmacologists can contextualize *in vitro* hazards, integrating data for multiple ion channels and assessing risk at clinically relevant concentrations.

TP2: Will nonclinical safety findings translate to humans?

GLP nonclinical safety studies establish the dosing parameters allowed for safety testing in humans under an IND. Nonclinical animal data may over-predict risk (too many false positives), thereby preventing human testing, or may be found to have under-predicted adverse effects in humans (too many false negatives) when human safety findings occur that were not identified in nonclinical studies.

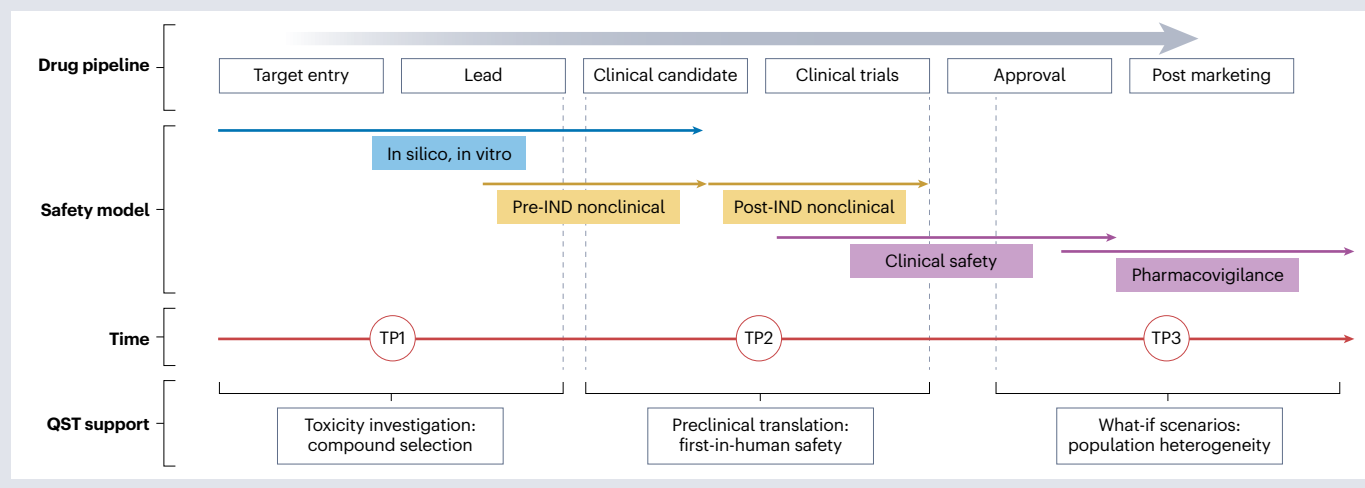
Although clinical testing is generally safe, serious adverse events not anticipated by nonclinical studies can occur¹²⁵. Thus,

safety predictions at TP2 focus on extrapolation from one species (nonclinical) to another (human). QST models for target tissues that encompass species-specific physiological parameters and integrate species-specific pharmacokinetics can inform the translation of toxicity findings from nonclinical species to humans. For example, QST modelling and simulation has been used to translate the nonclinical incidence of diarrhoea to human by accounting for species differences in pharmacokinetics and turnover of the gastrointestinal epithelia^{30,64}.

When unanticipated human safety findings occur in clinical testing, further nonclinical studies may be initiated to elucidate underlying mechanisms and identify mitigation strategies, such as modified dosing regimens. An example is the unexpected observation of changes of hepatotoxicity biomarkers in the clinic (for example, levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)). Here, modelling and simulation with the DILIsym QST platform has been used to investigate clinical findings. Integrating clinical data and additional *in vitro* data to support modelling (for example, mitochondrial toxicity assays), DILIsym has been used to understand the relative contribution of specific mechanisms of drug-induced liver injury (DILI) to observed changes in liver biomarkers⁸⁴. Coupled with PBPK modelling, this can be used to simulate alternative dosing regimens that may mitigate the DILI liability. Although the retrospective application of QST modelling may not be able to prevent termination of a candidate molecule, the results can inform subsequent projects and increase the probability of success.

TP3: Will testing in controlled clinical trials predict safety in the general population?

If the safety and efficacy data from clinical trials support marketing approval, the new drug will become available to a wider and more diverse population of patients. Establishing confidence in safety predictions can be considered a problem of sample size and population heterogeneity resulting from genotype or phenotype, disease state, age, sex, ethnicity and environmental factors. QST models such as those described for TP2 can be parameterized to be representative of different patient groups. Where physiological differences in parameters associated with pharmacokinetics or drug effect are established, ‘what-if’ simulations may be run in virtual populations informing



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translational safety between patient groups and identifying at-risk individuals¹¹⁴.

Recognizing the stepwise nature of safety testing and that safety decisions differ at each transition point is critical to establishing an

integrated QST strategy to address the unique safety questions at each step.

Figure adapted with permission from ref. 12, American Chemical Society.

(PBK) models^{17–19} (Table 1), QSP models and QST models. On the basis of a granular understanding of drug absorption, distribution, metabolism and excretion (ADME), as well as compound physicochemical properties, PBPK models predict human tissue exposures resulting from different dosing regimens (that is, dose, route, frequency and duration). QSP models, primarily used for understanding drug efficacy, incorporate target modulation in the context of human disease, based on physicochemical drug properties and known molecular and biological systems dynamics. Analogously, QST models are used to explore safety hazards and risks, and incorporate on-target and off-target modulation in the context of relevant patient populations based on physicochemical drug properties and the understanding of tissue or organ dynamics at relevant scales. The differences between PK–PD, PBPK and QSP models have been previously described in Bradshaw et al.²⁰.

By integrating PBPK and QSP or QST approaches, exposure and target modulation can be modelled before clinical testing, helping to select efficacious and safe dosing regimens and test clinical hypotheses. The resulting modelling framework can be further refined as additional preclinical – and eventually clinical – data become available, and it may also be repurposed for other investigational drugs.

QST models and their specific challenges

Although PBPK and QSP models are well-established in pharmaceutical R&D, mechanistic models used in the context of drug safety – that is, QST models – have fewer established precedents and face specific challenges. These include uncertainty about whether the toxicity is an on-target-mediated or off-target-mediated effect. Indeed, there is often a poor mechanistic understanding of the link between compound–target interactions and clinical adverse outcomes, unlike QSP models, which typically focus on well-understood on-target effects. Toxicities may emerge in organs other than those wherein the therapeutic target is present, especially when both on-target and off-target properties of a clinical candidate are poorly characterized. Mechanistic modelling of the complex pathobiology underpinning ADRs is, therefore, challenging without adequate experimental biomarkers to identify toxicity mechanisms or quantitatively inform key events contributing to toxicity initiation and progression. Conventional safety studies are not routinely designed to reveal such detailed mechanistic information, and often only measure safety end points at the end of the study period. Such study designs provide limited quantitative data suitable for modelling, and so they may need to be adjusted to include measurements of tissue or organ response over time, helping to identify the dynamic behaviour of injury and repair processes to inform QST modelling.

Ideally, QST models would be able to predict toxicity at any stage of drug development, both in scenarios wherein a subgroup of patients is predicted to be affected and, more broadly, in large populations. Modelling the diversity of specific patient subpopulations requires careful consideration of the underlying heterogeneity of the general population and its relation to specific mechanisms of toxicity. Central

challenges faced in QST include addressing sources of uncertainty and understanding population diversity to achieve high sensitivity and specificity in predicting toxicity.

Key considerations and recommendations when building QST models

QST models are typically designed to recapitulate the dynamics of essential organ physiology, the response to drug perturbations, and the recovery of homeostasis for reversible toxicities. The biology represented by QST models can span multiple scales of organization (for example, molecule, cell, tissue, organ and system), and outputs will include clinical end points informing on safety. Given the unique biology of each organ, it is crucial to quantitatively understand the molecular, biochemical and physiological processes that regulate organ functionality and how their perturbation leads to specific clinical outcomes. For instance, models that represent electrophysiological functions in cardiomyocytes are used for predicting the risk of QTc prolongation and Torsades de Pointes (a potentially lethal form of arrhythmia), whereas models that capture cell cycle and differentiation dynamics in the intestinal epithelium are used for understanding dose-limiting gastrointestinal toxicities associated with cancer therapy.

Many systems biology models describe physiological cell and tissue functions, and these have been applied to elucidate biology and mechanisms of diseases^{21,22}. Such systems models can serve as an ideal starting point for developing QST models. Repurposing existing models is an efficient use of current knowledge, but it relies on the robust reporting and reproducibility of published systems models²³. Although a comprehensive review of basal physiological models for all four organ systems is beyond the scope of this Perspective, readers are referred to existing literature²⁰.

In practice, safety decisions consider key questions arising from mechanistic understanding of the target or compound, as well as empirical observations. Questions may arise from theoretical safety hazards identified during target safety reviews²⁴ or subsequently from findings in preclinical studies, when toxicities are off-target and unexpected. For example, a gastrointestinal liability may be anticipated when modulating intended or unintended pharmacological targets involved in cell cycle regulation, which can affect the rapid proliferation of intestinal epithelial cells. Gastrointestinal safety concerns may also arise at later stages of development based on preclinical findings for which the underlying mechanism of toxicity may not be identified. Relevant preclinical findings can include data from *in vitro* experiments such as impaired intestinal organoid proliferation, or μ -opioid receptor binding – which has been linked to nausea and vomiting – or from *in vivo* studies demonstrating weight loss, diarrhoea or histopathological changes in the intestinal tissue of preclinical species.

The application of QST modelling and simulation to answer key safety questions will depend on model availability, ability to establish credibility within the intended context of use and access to the necessary data. Data requirements tend to increase in cases of off-target

toxicities, particularly when the underlying mechanisms of toxicity are not fully understood and warrant further investigation. The types of decisions that the model could inform include target or compound prioritization, the design of in vivo toxicology studies, first-in-human dose selection, risk mitigation strategies, clinical trial design and patient inclusion criteria. Modelling may also support decisions to halt compound development. QST models can additionally provide deeper mechanistic insight into the processes underlying toxicity^{25–27}.

Taking these considerations together, we recommend the following stepwise approach when starting to develop a QST model:

1. Establish a solid understanding of the essential processes underpinning normal biology and pathobiology leading to adverse outcomes – this knowledge is the foundation of a mechanistic QST model.
2. Clearly define the question of interest and the context of use of the model: at what stage(s) of drug discovery and development will the model be applied, and what decisions will the model output inform? This clarity will help in formulating an appropriate evaluation strategy for the QST model before application to the pharmaceutical compound of interest. Guidance currently under development by The International Council for Harmonisation (ICH) in the form of the proposed ICH M15 document for model-informed drug development provides a helpful structure to document these key model details and strategies²⁸.
3. Identify and clearly define data requirements to implement and apply the model to a novel compound in development. In addition, clearly outline a model evaluation plan and ensure that required input and output data are available to support this activity.

QST model development

Building a QST modelling framework for a system typically includes three main components (Fig. 1):

- A quantification of the systemic and tissue exposure of the active agent over time at the point or scale of activity: This may involve PBPK modelling^{17–19} (Table 1). Coupling of a comprehensive

kinetic model of the active agent to the organ physiology model is required when the affected organ has an impact on the ADME characteristics of the agent.

- A hypothesis for the toxicity mechanism, which may be distinct from the mechanism of therapeutic effect²⁹. This may include the disruption of transcription or protein signalling networks, metabolic pathways, electrical or mechanical processes, cellular events such as mitosis or apoptosis, and so on.
- A digital representation of the normal physiological functions of the organ or tissue, including the primary processes susceptible to drug-induced perturbation, mechanisms underlying the reversibility of adverse drug effects if relevant, and features that enable clinical monitorability (biomarkers or clinical end points): These need to be represented within the model and rationally connected by processes spanning relevant hierarchies of biological complexity.

During model development, uncertainties and knowledge gaps may be circumvented through the integration of diverse data sources into the model. Data sources include *in silico*, *in vitro*, *in vivo* and clinical data sets and should be systematically structured and accompanied by comprehensive metadata to facilitate reuse across different approaches and applications.

Model parameters anchor the theoretical modelling framework to observable phenomena, and the identification of their values often serves as a principal means of resolving uncertainties. QST model parameters enable a quantitative understanding of system homeostasis and drug-induced responses. Parameters can be broadly categorized as either system parameters, which describe the physiological and biochemical properties of the biological system, or drug-specific parameters, which define the pharmacokinetic and toxicological characteristics of the compound and its specific interactions with the system. For QST model applications to pharmaceutical compounds, the system parameters will in many cases remain fixed, and only the drug-specific parameters need to be adjusted for the specific compound of interest. An exception

Box 2 | Data management for QST modelling: the TransQST approach

The implementation and validation of quantitative systems toxicology (QST) models requires diverse nonclinical and clinical data and review and, where appropriate, repurposing of existing models. This requirement is best served by adherence to the principles of findability, accessibility, interoperability and reusability (FAIR) across the multidisciplinary scientific community driving QST¹¹. FAIR data principles will also be critical in leveraging emerging artificial intelligence or machine learning tools as part of MID3.

Rather than creating new, custom data resources, the recent Innovative Medicines Initiative Translational Quantitative Systems Toxicology Consortium (TransQST) adapted the infrastructure and expanded the content of well-established benchmark data resources such as ChEMBL¹²⁶ and ExpressionAtlas¹²⁷ (see 'Related links'). New technical capabilities were developed to facilitate the capture of new data types and terminologies, and these were used within these resources. New data were curated and dynamically integrated into the modelling process via these established data repositories.

For example, drug label black-box warnings, toxicity classes of withdrawn drugs, and adverse side effects of other compounds were curated in ChEMBL. Liver and kidney toxicogenomic and healthy control expression data sets were curated in ExpressionAtlas. To support dynamic data integration, reusable open KNIME workflows¹²⁸ that actively retrieve information from multiple repositories such as DrugBank¹²⁹, IUPHAR¹³⁰, PharmGKB¹³¹ and Therapeutic Target Database¹³² (see 'Related links') were developed to build drug target–protein profiles from millions of data points.

The use of previously established and widely adopted resources for data curation ensures project sustainability and impact, while minimizing the data management burden. QST models and software tools developed by the TransQST consortium using these integrated safety–toxicity data sources were also published and made accessible to the scientific community via established repositories such as BioModels (see Table 2 and Supplementary information).

Box 3 | Quantitative systems toxicology for emerging modalities

In the past three decades, therapeutic modalities beyond traditional small-molecule drugs have emerged. These are enabling the modulation of disease mechanisms previously considered challenging, if not impossible, to target ('undruggable')¹³³.

Newer modalities include recombinant enzymes and monoclonal antibodies, antibody–drug conjugates, nucleic acids, peptides, cell-based therapies and targeted protein degraders^{134–137}. As the development of these new therapeutic modalities accelerates, there is an increasing need for more reliable, human-relevant toxicology models to optimize their safety. QST could also be applied in this respect.

One notable example of a computational model for evaluating the safety of a new treatment modality, designed to extend the ability to simulate drug-induced liver injury (DILI) beyond the small molecules covered by DILIsym (previously described), is BIOLOGXsym, developed by Simulations Plus in collaboration with the University of Pittsburgh Drug Discovery Institute (UPDDI). BIOLOGXsym predicts biologics-induced liver injury (BILI)¹³⁸. The BIOLOGXsym platform includes representation of relevant liver biochemistry and the mechanistic effects of biologics on liver pathophysiology, and it was

developed to be used in conjunction with clinically relevant data from a human biomimetic liver microphysiological system — vascularized Liver Acinus MicroPhysiology System (vLAMPS) — a next-generation organ-on-chip system allowing for comparison of toxicity in liver cells collected from healthy donors versus those collected from donors with liver illness¹³⁹.

In proof-of-concept simulations, BIOLOGXsym effectively predicted clinically observed hepatotoxicity for two biologic drugs, cimaglermin alfa^{140,141} and tocilizumab, a humanized monoclonal antibody targeting the IL-6 receptor that is approved for various autoimmune and inflammatory diseases and associated with modest alanine aminotransferase elevations¹⁴². With further development of the platform, it is anticipated that BIOLOGXsym will become suitable for use in supporting drug development projects including vaccines, blood and blood components, allergenics, somatic cells, tissues, recombinant therapeutic proteins, and, potentially, gene therapy vectors and beyond.

Table 3 presents some key references for areas of potential QST model utility in exploring toxicity questions associated with emerging modalities.

arises in heterogeneous or disease populations wherein system parameters must reflect perturbed or disease states in addition to, or instead of, healthy physiology. In such cases, system parameters are adjusted to accurately represent the relevant pathological or divergent conditions.

Evaluation and refinement of QST models

Following the development of a QST model, model verification ensures that the model is implemented correctly according to its specifications. Verification does not require that the model accurately represents the real-world system it aims to mimic; rather, it ensures that the model adheres to the logical structure and assumptions originally outlined. This involves checking that the model is free from errors in its code, equations and algorithms. The process confirms that the computational components function as intended without any bugs and that the mathematical operations correctly translate the underlying theoretical framework.

Model validation, by contrast, assesses how well the model represents the real-world system it is intended to simulate. It focuses on establishing the credibility and relevance of the model by comparing its output to empirical data. This step involves demonstrating that the model accurately replicates a range of observed biological phenomena across various conditions, ensuring reliable prediction of relevant biological end points (for example, injury biomarkers in serum) and is 'fit for purpose'. Validation requires iterative interaction with experimental data, parameter adjustments and potentially recalibration or re-evaluation of model structure and hypotheses to enhance predictive accuracy.

In summary, verification is about 'building the model right', ensuring technical accuracy in its construction, whereas validation is about 'building the right model', ensuring that it accurately reflects biological reality. Both processes are essential for developing robust and reliable models for model-informed drug discovery and development (MID3).

Importantly, QST models undergo continuous refinement through iterative test–refine–test cycles, utilizing independent data and emerging knowledge on toxicity mechanisms and organ physiology (Fig. 1). Such ongoing refinement often arises when repurposing the models within the pipeline for new investigational drugs. The development and validation of the gastrointestinal QST model from Gall et al.³⁰ is illustrated in Fig. 2 as an example.

Verified versions of validated models should be systematically managed and distributed with documentation to ensure reproducibility and facilitate continuous development. Resources such as FAERS³¹, SIDER³², BioStudies³³ and Open Targets³⁴ can support these aspects. The BioModels database²¹ offers a selection of physiologically relevant mechanistic models, promoting accessibility and reproducibility (Box 2).

Organ-based QST models

Many published QST models have demonstrated proof of concept or have been applied in research contexts, whereas broader acceptance and routine implementation are still evolving, reflecting the burgeoning nature of the field. Validation of QST models for their context of use is essential, as well as a clear definition of the applicability domain for each of the models.

In this section, we discuss established QST models for the cardiovascular system, gastrointestinal system, liver and kidney, detailing their context of use in drug development, current status, and further verification or validation required for application in drug development projects (Table 2). Among these examples are some well-established tools that inform *in vitro* to *in vivo* extrapolation (for example, cardiac electrophysiology modelling) or elucidate mechanisms of unexpected clinical observations (such as drug-induced liver injury).

Cardiovascular system

Cardiotoxicity is a critical concern in drug development^{2,35,36}. In recent years, numerous innovative approaches have emerged for investigating

drug-induced cardiotoxicity, with mathematical models for predicting drug-induced cardiotoxicity now widely used in the pharmaceutical industry¹¹. Aligned with NAM-based safety assessment, many of these approaches focus on human physiology rather than animal models, incorporating specific cardiac diseases or conditions.

In contrast to the QST models for gastrointestinal, liver and kidney toxicities, which are often largely centred around the modelling of functional cell turnover in the organs (for example, gastrointestinal epithelial cells, hepatocytes and renal epithelial cells, respectively), QST models for the heart attempt to describe the dysregulation of physiology for the whole integrated cardiovascular system. This is best exemplified by human-based modelling and simulation for the prediction of pro-arrhythmic adverse outcomes³⁷. This is in line with the objectives of the cardiac safety paradigm proposed by the Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative^{38,39}, to use a new mechanistic, model-informed approach to predict the risk of drug-induced Torsades de Pointes in the clinic.

CiPA incorporates detailed mathematical models of cardiac ion currents and their perturbation by drugs that may alter cardiac currents based on in vitro or structure activity model data indicating an interaction with one or more cardiac ion channels. By simulating these interactions, researchers can predict the potential effects of drugs on cardiac action potentials. In one such approach, an accuracy of 89% was demonstrated in predicting the pro-arrhythmic effect of a drug in patients using electrophysiological simulations of populations of virtual human cardiomyocytes^{40,41}.

The profile of the virtual cardiomyocyte models can be optimized to mimic population variability, including disease phenotypes more prone to arrhythmias, thereby maximizing the efficiency and accuracy of the approach. Although cardiac action potential models have primarily assumed steady-state operating drug concentrations (usually represented by multiples of the putative effective therapeutic maximal concentration), the use of PBPK modelling with more physiological models of cardiac tissue distribution has been explored and published as part of the TransQST project and can be applied to predict tissue concentration as an input for cardiac electrophysiology models⁴². Also within TransQST, a tissue-based model based on the rabbit Purkinje fibre assay was applied to develop a novel cardiac QST model that allows simulation of the cardiac action potential

and that could quantitatively reproduce 80% of recordings from the experimental assay⁴³.

Beyond pro-arrhythmia, predicting drug-induced adverse effects on cardiac contractility poses an additional challenge in drug development, and this was also a focus of the TransQST project. Unwanted changes in the mechanical function of the heart can be detrimental, especially in patients at risk owing to pathogenic variants in genes encoding proteins involved in the constitution of cardiac sarcomeres, such as *MYH7* and *MYBPC3*⁴⁴. Ineffective cardiac contractility causes a decrease in cardiac output that could lead to myocardial infarction and, ultimately, heart failure. Recent studies have shown how modelling and simulation of human electromechanical function could replicate experimental recordings of drug-induced effects in human cardiomyocytes^{45,46} and the effect of myocardial infarction on electromechanical function from ionic to whole-organ function^{47,48}. Human electromechanical modelling and simulation was also able to predict and explain drug effects in human cardiac electrophysiology and contractility in the context of specific gene variants⁴⁹.

Cardiac electrophysiology QST models, integrating high-throughput ion channel screening data^{43,50,51}, are now routinely applied as screening tools in drug discovery, and the resulting predictions inform cardiac safety assessment. Application of these models is possible in the early stages of lead identification and optimization.

In accordance with the definition of QST (Table 1), the outcome of these modelling and simulation studies is not simply the prediction of the cardiovascular safety end points for compounds but also an explanation of the mechanisms underpinning cardiotoxic effects and the identification of the specific profiles of the patients that may experience these effects. These are the bases of important emerging concepts such as the ‘digital twin’ for predictive modelling in cardiology⁵² and in silico trials for virtual testing of new therapies^{50,53,54}. An example is provided in Fig. 3.

Another focus of the TransQST project was the characterization of haemodynamic drug effects – encompassing changes in blood pressure, heart rate and cardiac contractility – in preclinical in vivo models, an important step in the assessment of cardiovascular safety of investigational compounds that may affect one or more of these parameters⁵⁵. Preclinical haemodynamic studies seek to identify any haemodynamic effects of compounds and characterize the underlying

Table 1 | Definitions of modelling approaches and their context of use

	Dose/exposure	Efficacy	Toxicity
Empirical modelling	Pharmacokinetics (PK) and toxicokinetics (TK) Empirical kinetic models capture the change of drug concentration over time without necessitating mechanistic insight Models are parameterized through fitting to data with PK representative of efficacious therapeutic concentrations and TK of supratherapeutic doses	Pharmacodynamic (PD) (PK)PD models link compound exposures to the change of physiological or therapeutic end points without necessitating mechanistic insight	Toxicodynamic (TD) (TK)TD models link compound exposures to the change of pathological or safety end points without necessitating mechanistic insight
Systems biology modelling	Physiologically based kinetics (PBK)^a PBK models integrate physiology of ADME processes (absorption, distribution, metabolism, elimination) and compound properties for a mechanistic prediction of systemic and tissue exposure	Quantitative systems pharmacology (QSP) QSP models ideally integrate compound exposure with knowledge of molecular mechanisms related to efficacy, to better understand target-mediated effects (that is, on-target)	Quantitative systems toxicology (QST) QST models ideally integrate compound exposure with knowledge of molecular mechanisms related to safety, to better understand adverse effects (that is, on-target–off-tissue, off-target)

^aPBK modelling is an umbrella term encompassing physiologically based pharmacokinetic modelling (PBPK) and physiologically based toxicokinetic modelling (PBTK). Individual approaches can be informative when applied as stand-alone models. More commonly, approaches within the domains of dose/exposure, efficacy and toxicity are combined to address specific questions, for example, combining PK and PD modelling (that is, PK–PD modelling) to predict efficacy end points or combining PBK and QST modelling to predict safety end points.

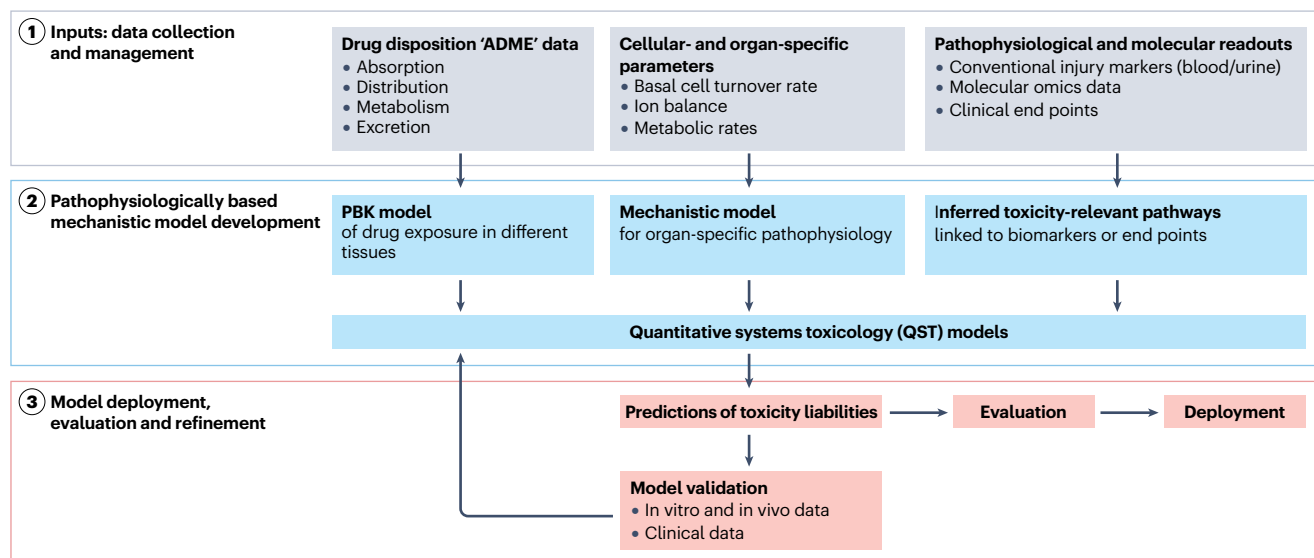


Fig. 1 | Construction and validation of quantitative systems toxicology models. The development of quantitative systems toxicology (QST) models requires the integration of several types of data (1) and modelling techniques (2), which typically include drug pharmacokinetics, a cell-specific and organ-specific physiology model, mechanism(s) of toxicity and toxicodynamic biomarker(s). Cellular systems and physiology models can be expanded to include additional potential mechanisms of toxicity through the inclusion of molecular network models. In recent years, there has been a growing interest in utilizing the knowledge captured within large and complex omics data, particularly

toxicogenomics data, which are now more frequently gathered to investigate molecular responses to drugs as part of a QST approach to serve as a surrogate for cellular and tissue responses. QST models ideally combine tissue-level exposure information, organ physiology, molecular network models and quantitative measurements of molecular and functional changes occurring across multiple levels of biological organization. Iterative cycles of ‘predict–learn–confirm’ (3) are used to refine and validate the accuracy of predictions of toxicity liabilities from nonclinical data and available clinical data. PBK, physiologically based kinetics. Adapted with permission from ref. 12, American Chemical Society.

mechanisms, typically using a dose-escalation design in preclinical species such as rats and dogs. In TransQST, models were expanded to disentangle processes relevant to the dysregulation of haemodynamics, such as heart rate, vascular resistance and contractility^{56–58}. These models have been applied for several compounds²⁵. A relevant property of haemodynamic QST models, as with QST models presented previously, is their ability to distinguish between biological system (species)-specific parameters and drug-specific parameters, enabling inter-species scaling from preclinical species to humans²⁶. The use of haemodynamic models may guide experimental design of preclinical studies, avoiding more complex measurements of cardiac output and optimizing the number of animals and study duration²⁷.

Further details of models for understanding cardiotoxicity developed or expanded as part of the TransQST project are included in Supplementary Table 1.

Gastrointestinal system

The toxic effects of antiproliferative drugs on the gastrointestinal epithelium, leading to the disruption of the epithelial barrier, mucosal inflammation and diarrhoea, can be life-threatening and require careful monitoring and treatment in affected patients with cancer^{59–62}. This adverse effect is often identified only during clinical development and frequently necessitates dose reduction or treatment interruption, potentially compromising overall efficacy and patient outcomes⁶³.

Assessment of the gastrointestinal risk associated with proposed therapeutic regimens before first-in-human studies is, therefore, a common and important safety question for investigational oncology

drugs, but one for which a quantitatively reliable answer is generally lacking. A previous translational modelling work has demonstrated the possibility of predicting clinical gastrointestinal adverse events associated with cancer treatments from preclinical data by modelling drug-induced disruption of epithelial cell dynamics⁶⁴. However, there is currently a limited body of published works focused specifically on intestinal toxicity modelling.

An important objective of the TransQST project was to advance and expand this area of research. The consortium focused on developing gastrointestinal QST modelling strategies to address critical safety questions at TP2, as outlined in Box 1 – for example, supporting first-in-human studies by predicting the risk of clinical diarrhoea. Figure 2 outlines the model-building process that served as a proof of concept, focusing on 5-fluorouracil (5-FU)-induced intestinal toxicity³⁰.

The central model hypothesis was that intestinal toxicity and diarrhoea induced by antiproliferative agents result from epithelial ulceration and mucosal inflammation, triggered by the disruption of proliferation in intestinal crypts, as previously reported⁶⁵. Model development, therefore, focused on two main components (see Fig. 2): a 5-FU PBPK model and a dynamic model describing epithelial homeostasis and its disruption by 5-FU, together with associated biomarkers and end points. The 5-FU PBPK model encompasses the generation of the main 5-FU active metabolites and recapitulates the temporal profiles of their free concentrations in both plasma and epithelium. The epithelial model describes the proliferation and differentiation of stem and progenitor cells into mature epithelial cells that migrate to the villus and are ultimately shed into the lumen. Epithelial homeostasis is

maintained by a precise balance between proliferation, differentiation and shedding, which is restored following perturbations such as drug challenges. The toxicity mechanisms of active 5-FU metabolites involve inducing cell cycle arrest and apoptosis in proliferating crypt cells, leading to epithelial injury and a reduction in cell numbers within the crypt and villus.

System model parameters governing epithelial dynamics were sourced from previously published research in humans, whereas the identification of 5-FU-specific parameter values was based on preclinical experiments performed in mice. Although PBPK models are typically parameterized using only *in vitro* data, here, the concentrations of 5-FU and its metabolites measured in mice were used to quantify 5-FU metabolism and the formation of its key active metabolites. Parameter values specific to the mechanism of toxicity were determined from cell counts in the crypt and villus of mice and, together with recorded diarrhoeal events, enabled the establishment of a quantitative relationship between epithelial injury and the incidence of diarrhoea. Other model elements include citrulline kinetics – a plasma biomarker synthesized by enterocytes and decreased with injury⁶⁶ – and relevant gene expression in epithelial cells, which may be of interest for broader research or additional safety assessments, but are not relevant to the specific question posed here.

In deployment (Fig. 2), model predictions of diarrhoea incidence across a range of 5-FU dosing regimens demonstrated strong concordance with clinical data. Similarly, when the model was applied to predict

doxorubicin-induced clinical diarrhoea – after re-parameterizing the drug-specific toxicity parameters with additional data from mice treated with doxorubicin – it again demonstrated a high degree of accuracy in predicting clinical diarrhoea incidence³⁰. These results demonstrate the utility of this strategy in forecasting the risk of clinical diarrhoea associated with 5-FU and doxorubicin, two broad-spectrum cytotoxic agents, and, therefore, in addressing the above defined safety question relevant to TP2 (Box 1).

The application of the same modelling strategy to assess the gastrointestinal safety of gefitinib, a molecularly targeted agent, however, failed to recapitulate the clinical incidence of gefitinib-induced diarrhoea. This is owing to a lack of detectable epithelial toxicity in mice, even at exposures far exceeding those that elicit diarrhoea in patients. As a result, although the model structure remained appropriate, its parameterization captured the relative resistance of the mouse intestine rather than the human epithelial response³⁰.

This highlights that the success of this type of model depends on the suitability of the preclinical experimental platform to precisely quantify toxicity, and it is heavily dependent on cross-species differences in drug toxicity and/or organ physiology. The data gap may ultimately be mitigated by the increased application of human-relevant NAMs, including human-derived intestinal organoids and microphysiological systems (MPS), into preclinical safety assessments. The development of mechanistically refined modelling strategies with decreased data dependencies that explicitly address species-specific toxicology and physiology will further mitigate these limitations⁶⁷.

In this regard, agent-based modelling offers a powerful computational framework for constructing advanced mechanistic models by enabling the implementation of the individual behaviour of the so-called agents, such as molecules or cells, interacting across scales of biological organization to give rise to the collective response of the system, tissue, organ or organism⁶⁸. Agent-based modelling requires extensive biological knowledge and increased computational resources and skills⁶⁸. In turn, these models enable full integration of complex phenomena with high granularity at multiple time and spatial scales, and they result in superior predictive performance with reduced data dependency. The intestinal crypt is particularly amenable to developing agent-based models, with a wealth of single-cell and signalling pathway information⁶⁹. Several agent-based models have been proposed to describe the complexity and dynamic nature of single cells in the epithelium^{70–72}.

In a recently published agent-based model, also developed within the scope of the TransQST project, each cell within the intestinal crypt was explicitly represented, incorporating intracellular protein networks governing cell cycle progression and DNA or RNA synthesis⁷³, which are mechanisms frequently targeted by cancer treatments^{74,75}. Moreover, cells in the model interact mechanically in the three-dimensional geometry of the crypt, and they communicate through multiple signalling pathways, such as Wnt, Notch, BMP, RNF43 and ZNRF3, and Hippo–YAP, which modulate cell differentiation and fate, response to the mechanical environment, and feedback mechanisms, ultimately forming a representation of the self-organizing intestinal epithelium. This model recapitulates recent findings relating to the importance of Lgr5⁺ stem cells in the maintenance of crypt homeostasis⁷⁶ and cell retrograde motion, reported using intravital microscopy⁷⁷. It also describes the epithelial dynamics observed during 5-FU-induced injury and recovery⁷³.

Each implemented process in this model can be mechanistically perturbed to simulate a variety of drug effects and diseases across

1 Data requirements

- Preclinical data on 5-FU ADME
- Preclinical data on 5-FU/metabolite-induced epithelial toxicity, and response of biomarkers and endpoints
- Qualitative and quantitative data on human epithelial cell dynamics

2 Model development

- 5-FU epithelial metabolism and exposure (PBK) model
- Model describing epithelial dynamics with cell arrest and apoptosis induced by 5-FU metabolites, dynamics of linked biomarkers, tissue disruption, recovery and associated diarrhoea risk

3 Deployment, evaluation and refinement

- Predictions of clinical epithelial injury and diarrhoea risk at relevant dosing regimes
- Comparison with independent clinical observations, if available
- Model refinement with emergent preclinical and clinical data

Fig. 2 | Example implementation of a gastrointestinal toxicity model. The steps to develop the gastrointestinal quantitative systems toxicology (QST) model from Gall et al.³⁰ to predict the risk of clinical intestinal toxicity induced by 5-fluorouracil (5-FU) included the following: (1) collection of preclinical data on 5-FU absorption, distribution, metabolism and elimination (ADME) processes, mechanisms of toxicity of active metabolites, and human data on epithelial dynamics and composition; (2) model development incorporating a 5-FU physiologically based kinetics (PBK) component to describe epithelial metabolite exposure, and a dynamic model of epithelial homeostasis, disruption by 5-FU, and recovery, integrating relevant biomarkers and clinical end points; (3) application of the model to predict clinical epithelial injury and diarrhoea risk, with iterative validation and refinement driven by ongoing comparison with clinical observations and continuous incorporation of new preclinical and clinical data. By accurately predicting diarrhoea caused by 5-FU, and after re-parameterization with additional *in vivo* data by doxorubicin, the model underscores the utility of QST models in translating preclinical toxicity into clinically relevant adverse events.

Table 2 | Examples of QST models, their contexts of use and availability

Example model	Envisaged initiator for modelling	Application or decision type	Technology readiness and additional steps for use to inform drug discovery or development	Availability and form of model deployment	Refs and links
Virtual assay — contractility model (via the TransQST consortium)	Routine screening	Compound prioritization, early detection of contractility issues	Further validation needed; can expand the context of use beyond ion channel effects	Software package with a user interface available via Oxford University Innovation licensing	114 https://www.cs.ox.ac.uk/ccs/virtual-assay/
GI ODE-based QST model (via the TransQST consortium)	Early target safety flag or in vitro/in vivo findings	Translation of preclinical toxicity into clinical risk of diarrhoea occurrences Dose and schedule optimization to minimize risk of GI toxicity in animals and humans	Further validation and testing to understand the applicability domain needed	Model available in BioModels and model code provided in publication	30 https://www.ebi.ac.uk/biomodels/MODEL2212120003
DILIsym	Early target safety flag or in vitro/in vivo findings including clinical adverse events	Insight into the mechanisms of toxicity and predictions of liver injury markers for dose optimization and compound prioritization	Technology is established, applied in research and included in submissions to regulators	Software package with user interface available via Simulations Plus license	82,85,115 https://www.simulations-plus.com/software/dilisyms/
Renal QST model	Early target safety flag or in vitro/in vivo findings	Understand time course progression of renal toxicity risk in animal studies and apply model to predict safe dose	Clinical translation of animal findings to humans; validation with clinical data and model use in the potential applicability domain are still to be explored	Model descriptions available in the literature	107

GI, gastrointestinal; ODE, ordinary differential equation; QST, quantitative systems toxicology.

scales of biological organization, enabling clinical predictions with reduced data requirements. For instance, the model was used to simulate how inhibition of the activity of CDK1, a cell cycle-related protein, disturbs the cell cycle protein network and cell proliferation alongside impacts on epithelial organization. Importantly, this was achieved without requiring additional input data⁷³. These results underscore the potential of advanced mechanistic modelling to improve the precision of safety assessments and to explore underlying toxicity mechanisms.

Further details of models of gastrointestinal toxicity developed or expanded as part of the TransQST project are included in Supplementary Table 2.

Liver

The liver is a primary target organ of toxicity for many drugs owing to its role in drug metabolism and clearance. Drug-induced liver injury (DILI) is a leading cause of drug attrition and market withdrawal^{78,79}, making the early prediction of DILI a critical challenge in drug development⁸⁰. One of the most advanced tools for in silico prediction and investigation of drug-induced liver toxicity is DILIsym, developed through the DILI-sim initiative, a precompetitive public–private partnership^{81,82}.

Toxicity mechanisms covered by DILIsym include oxidative stress, mitochondrial respiration interference and bile acid accumulation, which together account for a large proportion of hepatocellular injuries⁸². DILIsym models these subcellular mechanisms alongside subsequent hepatocyte death and regeneration with a system of ordinary differential equations and algebraic expressions⁸³.

Drug and metabolite exposures driving toxicity are described by a PBPK model, with hepatic injury feeding back on hepatic drug clearance. Specific toxicity mechanisms are parameterized based on the potency of both parent drug and major metabolites determined by

in vitro assays. Cellular mechanisms for hepatocellular toxicity are linked to hepatocyte death, regeneration and the time-dependent release of liver injury biomarkers into serum, such as alanine aminotransferase (ALT). The ultimate aim of the model is to predict clinical liver injury using preclinical in vitro data as model inputs.

DILIsym can be considered the current state-of-the-art for predicting DILI and has the ability to simulate and predict the occurrence of liver injury in mouse, rat, dog and human populations. It has been used to investigate the clinical risk and underlying mechanisms of hepatotoxicity for several drugs reported to cause DILI in clinical trials or post-licensing^{84–87}. For example, the calcitonin gene-related peptide (CGRP) receptor antagonist telcagepant failed during late-stage clinical trials owing to DILI. By coupling in vitro mechanistic data – indicating inhibition of the mitochondrial electron transport chain and bile acid transporters – with clinical pharmacokinetic simulations, DILIsym predicted DILI with clinical dosing regimens of telcagepant, but not with ubrogepant, rimegepant or atogepant, which are now approved CGRP receptor antagonists⁸⁵. DILIsym has also been used to investigate the hepatotoxicity of the vasopressin V2 receptor antagonist tolvaptan in patients with autosomal-dominant polycystic kidney disease^{84,86} and to explain why preclinical species failed to reflect the DILI seen in an early clinical trial of the novel epilepsy therapy PF-0489516239.

In recent years, we have seen increased use of molecular omics data to understand and predict toxicity liabilities in clinical toxicology⁸⁸. In the public domain, researchers at Aachen University have developed an approach termed PBPK-based in vivo contextualization of in vitro toxicity data, or ‘PICD’. This approach uses public gene expression data from primary human and rat hepatocytes as in vitro toxicity data sources. PBPK simulations then identify in vivo drug doses associated with an interstitial area under the curve in the liver equal to the in vitro exposure^{89–91}.

Within the TransQST project, there were also multiple different efforts to advance DILI prediction, including some approaches using omics data:

- Proof-of-concept integration of PBPK models with genome-scale metabolic networks and gene regulation networks for exploring questions related to stress on hepatocyte metabolism⁹²
- Modelling of hepatic steatosis with valproic acid⁹³
- Development of a prototype model for liver injury or regeneration demonstrating the ability of modelling to link gene expression to liver injury outcomes⁹⁴
- Development of a reactive metabolite model with paracetamol and differential Nrf2-related gene expression to explore species differences in response between rat and mouse⁹⁵
- Development of a logic-based modelling framework for exploring stress response pathways based on time-resolved proteomic data in the liver⁹⁶

For more details of these models, see Supplementary Table 3. Other examples of QST models being developed to provide information on the liver safety of emerging modalities such as recombinant proteins and monoclonal antibodies are provided in Table 3 and Box 3.

Kidney

The kidney has an important role in drug disposition and excretion, making it a common target tissue for drug-induced injury⁹⁷. Several computational modelling approaches have been developed to understand and predict potential drug-induced kidney injury (DIKI) at an early stage to prevent drug attrition in the latter phases of drug development⁹⁸.

PBPK-based modelling and simulation have been applied widely in pharmaceutical companies for dose optimization in patients with renal impairment and to predict drug–drug interactions (DDIs) that might affect drug efficacy and patient safety⁹⁹. Renal DDIs can be mediated through competitive inhibition of tubule-expressed membrane transporter proteins. Such DDIs may be exacerbated by drugs affecting electrochemical gradients along tubules that modulate the activity and directionality of transporter proteins. In addition to competitive inhibition of renal secretion of co-administered drugs, other drugs may also competitively inhibit the active secretion of endogenous compounds, such as creatinine^{100–102}. PBPK modelling has also been applied to the prediction of drug-induced crystal nephropathy¹⁰³, which may result

from the precipitation of a drug in the kidney or from the crystallization of endogenous compounds, such as uric acid.

Although target-tissue exposure is an important component in predicting drug safety, the modelling of downstream mechanisms is vital in understanding the monitorability and reversibility of safety liabilities, elucidating species differences and informing translation to the clinic. Cisplatin is a well-studied nephrotoxicant, inducing injury via multiple, interrelated mechanisms including DNA damage, oxidative and endoplasmic reticulum stress, apoptosis, inflammation and vascular injury¹⁰⁴. It has been used as an exemplar compound for the development of computational models ranging from TK–TD approaches to more mechanistic QST models building on published models of kidney physiology.

Fukushima et al. implemented a TK–TD model with which they were able to simulate different single-dose regimens of cisplatin in rats (intravenous infusion versus bolus) and corresponding changes in a biomarker of renal function, serum creatinine¹⁰⁵. Simulations using this model indicated that changes in serum creatinine were driven not only by the maximum systemic concentration of cisplatin but also by the duration of exposure above a threshold concentration.

Although informative, this TK–TD approach does not provide mechanistic insight, has limited capacity for cross-species translation, and does not couple nephrotoxicity to tissue exposure – a fundamental consideration in modelling cisplatin-induced kidney injury. Measurement of tissue exposure also helps in differentiating the nephrotoxic liabilities of cisplatin from other platinum-based antitumour agents such as carboplatin. Cisplatin has been shown to be a substrate for transporter proteins expressed in the basolateral membranes of proximal-tubule epithelial cells (for example, OCT2 and CTR1), whereas carboplatin is only a substrate for CTR1, limiting renal exposure and its adverse effects on the kidney¹⁰⁶.

A kidney QST model developed by Gebremichael et al. can be considered to be the current state-of-the-art¹⁰⁷. This model couples drug pharmacokinetics, an existing systems physiology model of renal and basic cardiovascular function, sub-models of proximal tubular epithelial cell (PTEC) injury, and a set of biomarkers of renal function and injury¹⁰⁷. As with the model of Fukushima et al., Gebremichael and colleagues connected simulated systemic PK of single-dose cisplatin (that is, drug concentration in the plasma) to the downstream occurrence of kidney toxicity. This model was also applied to simulate the renal toxicity of gentamicin in rats at different concentrations with a

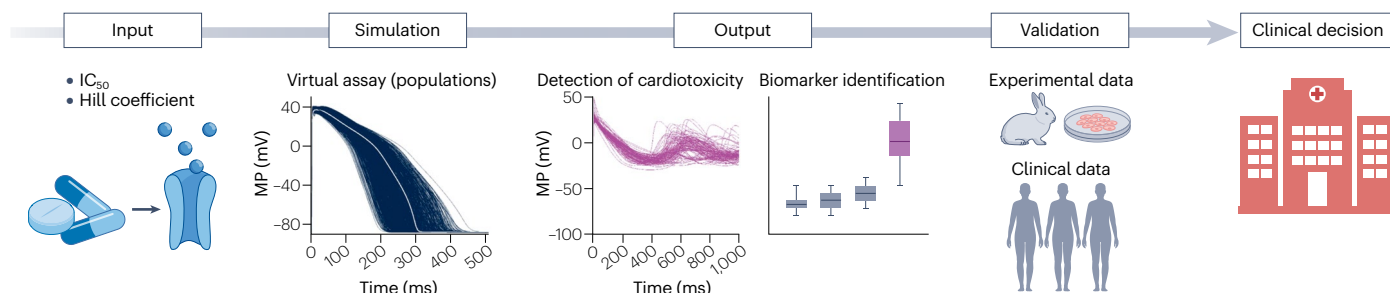


Fig. 3 | Overview of the virtual assay platform for in silico drug trials to predict drug-induced pro-arrhythmic cardiotoxicity. In vitro data on drug activity against ion channels are generally used as inputs⁵⁰. The Virtual Assay simulation software allows the use of both built-in or newly generated populations (that is, disease phenotypes) of virtual human cardiac cell models for the detection of potential cardiotoxicity and the identification of biomarkers

of drug action across the entire population. Potential cardiotoxicity is informed by the simulations combined with experimental data from in vivo and in vitro models. The findings can be further validated using pre-existing clinical trial data in public repositories, if available. IC_{50} , half-maximal inhibitory concentration; MP, membrane potential.

reasonable accuracy, demonstrating applicability of such a QST model across different compounds.

However, it should be noted that although this model mechanistically accounts for the downstream consequences of PTEC injury in terms of renal function, PTEC injury itself is modelled as a simple cell-death turnover model independent of the mechanisms of cellular injury. Nonetheless, neither of the published models accounts for the impact of reduced renal function on the pharmacokinetics of subsequent doses¹⁰⁸, and thus, both have limitations when applied to repeat-dose scenarios.

Simulations Plus (see 'Related links') are also developing a tool analogous to DILIsym called RENAsym for predicting DIKI¹⁰⁹. Models of DIKI are currently less widely applied across the pharmaceutical industry, although there is interest in more model development in this area¹¹.

During the TransQST project, efforts to advance DIKI prediction focused on the development of a prototype QST model with cisplatin as the exemplar compound using newly generated preclinical data in rats¹¹⁰. The model was able to predict the severity grading of lesions on renal proximal tubules, and associated serum and urinary injury biomarkers, with potential integration of transcriptomic data, applications to other compounds such as carboplatin, and species translation to humans. Further details are provided in Supplementary Table 4.

Outlook for QST modelling

The application of QST models to inform decision-making in the pharmaceutical industry remains largely an experimental, as-needed approach. For QST to be implemented effectively as a standard tool in pharmaceutical safety assessment, further refinements are required.

One key issue is the definition of the 'end-user' and establishment of a communication plan to stakeholders. The following questions should be clarified: will model code be executed by QST scientists or by subject-matter experts such as safety pharmacologists through a customized user interface? How will simulation outputs be visualized, communicated and reported? How to make a QST model available is also important to consider and may be related to its context of use. Software with a user interface – as opposed to a set of computational scripts that require programming expertise to operate – may affect both usability and the nature of the end-user. For example, a user interface could enable a safety pharmacologist to interact directly with haemodynamic or contractility models to test their hypotheses and use this alongside their own expert interpretation of experimental data^{27,57}. This level of interaction and application has less tendency to occur if a computational scientist is needed to generate and provide model predictions for each scenario of interest to the safety pharmacologist. Conversely, a closed software tool such as DILIsym⁸¹ may not allow a computational scientist to readily make modifications to the model to maximize the utility and relevance to a specific application of interest for end-users, and engagement with the model providers would be needed, limiting utility in early compound evaluation.

A major obstacle encountered in QST model validation is the availability of appropriate data. Mechanisms for easily sharing and accessing such data accelerate progress (see Box 2). Ensuring model sustainability with robust data storage and accessibility aligned with FAIR principles¹¹¹ supports model development, evaluation of predictive performance, and validation within the context of use.

Model reproducibility is also fundamental, and although this is true generally across modelling fields, it is especially important when models are used to inform safety decisions in drug development programmes. A roadmap for assessing and improving the

Table 3 | Potential uses of QST models in assessing toxicity risks of emerging modalities

Modality	Modelled toxicity	QST utility	Refs.
AAV gene therapy	Hepatotoxicity, immune response	Dose selection, immune modulation	116
ADCs	Payload-induced hepatotoxicity	Linker or payload design, PK-toxicity correlation	117,118
CAR-T cell therapy	CRS, neurotoxicity (ICANS)	Personalized dosing, IL-6 blockade strategies	119
Bispecific antibodies	CRS, off-tumour T cell activation	Cytokine engagement tuning	120,121
mRNA therapeutics	Innate immunity, inflammation	LNP optimization, sequence safety	122

AAV, adeno-associated viruses; ADCs, antibody–drug conjugates; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; IL-6, interleukin-6; LNP, lipid nanoparticle; PK, pharmacokinetics; QST, quantitative systems toxicology.

reproducibility of systems biology models was recently published²³, and the recommendations it set out can help to address this issue.

In any scenario, the active advocacy of expert application scientists and senior management for the use of modelling and simulation within a company is essential for successfully embedding QST models. Establishing effective, multidisciplinary teams that leverage modelling approaches to inform projects and generate the necessary supporting data in a timely fashion is critical. For such multidisciplinary teams to succeed, effective communication between individuals who work in different disciplines is needed, with a clear and shared understanding of the safety question of interest, the availability and applicability of models in the specific context of use, what assumptions are being made in the models, and what input data are required. Where a foundational model already exists – such as a published QST model – this can accelerate implementation for a specific project. Industry–academic collaborations such as the recent TransQST consortium (Box 2 and Supplementary information) exemplify how such a multidisciplinary collaboration can be assembled and how it can be instrumental in model development and validation.

A sponsor, such as a pharmaceutical company, deals not only with uncertainty around the safety of candidate drugs but also with uncertainty around how regulatory agencies will perceive the overall risk–benefit profile of a candidate – both during regulatory review and through post-marketing surveillance. Consequently, sponsors (particularly large ones) may adopt a conservative approach to applying novel tools such as QST models in assessing drug safety. To mitigate the risk that conservatism impedes the application of QST models, sponsors and regulators should ideally engage in early and continuous dialogue to reach alignment on model-informed strategies. This contrasts with the more routine use of QSP models, which have demonstrated utility in supporting efficacy assessments in clinical trials and in guiding the design of studies or selection of patient populations wherein mispredictions generally do not result in additional risks for the safety of patients.

The considerations above are not exhaustive, but they capture some of the key lessons from pharmaceutical industry and public–private consortia as QST continues to evolve^{12,112}. QST researchers have the opportunity to learn from other disciplines under the MID3 umbrella to address some of the challenges involved²⁰. For example, mechanistic PBPK

modelling and simulation is well-established, exemplifying the iterative ‘predict–learn–confirm’ cycle through its application in nonclinical-to-clinical translation, human dose prediction, evaluation of dosing route and regimens, prediction in diverse patient populations, DDI predictions and virtual bioequivalence studies. Best practices in the application of PBPK modelling, including establishing scaling factors for in vitro to in vivo extrapolation-based modelling, the development of software tools for drug metabolism and pharmacokinetics (such as [GastroPlus](#), [Simcyp](#) and [PKSim](#); see ‘Related links’), and defined areas of application across an increasing number of modalities beyond small-molecule drugs, have contributed to the successful use and continued development of PBPK. Notably, PBPK models are also often essential components of QST models, in which they are used to predict drug concentrations in target organs wherein safety concerns are being evaluated.

QST holds the potential to drive a transformation in the evaluation of drug safety through the same kind of iterative development cycle as PBPK modelling. The continued advancement of QST approaches will require multidisciplinary collaboration across industry and academia, as well as coordinated integration with other MID3 disciplines such as PBPK and QSP, and will almost certainly draw on emerging artificial intelligence tools. As QST establishes itself as a core component of MID3, it will further progress the 3Rs principles (replace, reduce, refine) in the use of animals in pharmaceutical development – particularly in light of recent changes to FDA requirements^{6,113} – and ultimately become a pillar of drug development in the twenty-first century.

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Competing interests

C.P. and L.G. are employees and shareholders of AstraZeneca. K.A.B., C.P.F. and E.I.R. are employees of GSK. F.S., M.M. and P.T. are employees and shareholders, and R.J.B. is a retired employee and shareholder of Sanofi. D.C., S.T. and L.L. are employees and shareholders of AbbVie. M.K. is an employee of Orion Corporation. L.L. is a shareholder of Johnson & Johnson. J.S.-R. reports in the past 3 years funding from GSK and Pfizer and fees or honoraria from Trave Therapeutics, Stadapharm, Astex, Owkin, Pfizer, Grunenthal, Tempus and Moderna. F.V. is an employee of Servier. J.A.W. is an employee of Vertex Pharmaceuticals. D.J.L. is an employee of Eli Lilly and Company. All other authors declared no competing interests for this work.

Additional information

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