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Predictive in vitro toxicology screening to guide chemical design in drug discovery

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Abstract

The safety profile of a novel drug candidate is highly determinant of its chance of success to be launched for treatment of patients. The integration of predictive toxicology in the drug discovery process is essential to identify and mitigate potential safety issues in a timely manner and contributes to improved productivity in pharmaceutical R&D, especially for small molecules, which is the focus of this review. A range of predictive in vitro toxicology assays has been established, which we use as part of AstraZeneca's proactive discovery safety screening strategy, with the aim to influence chemical design and obtain candidate drugs with the right safety profile. Here, we discuss recent progress in some of the main areas of toxicity, such as cardiotoxicity, hepatotoxicity, CNS toxicity, genetic toxicity, and nephrotoxicity, and also considerations around assay validation, predictivity, and throughput, which are relevant to secure actual impact on drug design. Finally, we share our perspectives on future opportunities and innovation.

Addresses

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Discovery safety, Drug discovery, Predictive toxicology, Investigative toxicology, Drug safety.

Introduction

Drug discovery and development is a long, costly process and, despite an uplift in the number of newly approved drugs in recent years, also relatively inefficient because only a small proportion of novel candidate drugs finally reach the market [1–3]. The main reasons for attrition are lack of efficacy and unfavorable safety [4,5]. In 2014, a systematic longitudinal analysis of the AstraZeneca small-molecule drug project portfolio demonstrated that safety/toxicity was causative of most failures in preclinical stages and phase I and also led to project closures in phase 2 comparable with industry averages [5,6]. This exemplified the need for establishing and implementing novel predictive safety assays [5]. A similar need emerged in the cosmetics industry, because of the ban on animal testing in 2013. These insights have undoubtedly accelerated the transformation of traditional toxicology, in which animal models constituted the main tools, into an innovative and advanced science encompassing molecular and cellular systems and computational biology [7–9]. Here, we discuss the application of predictive in vitro toxicology assays to the discovery of novel drug candidates with the right safety profile.

Integrated safety screening strategies

To reduce the number of expensive failures in late development, safety testing is nowadays performed in earlier stages of drug development in addition to the actual pivotal toxicology studies that enable progression into the clinic. We have argued that simply frontloading toxicology studies are not sufficient. In addition, it is important that the safety discipline is properly integrated in the drug discovery process, such that the toxicologist is an equal partner to the other relevant disciplines [10,11]. The purpose is to influence the pursued therapeutic concepts and ensure timely mitigation and also to proactively design safety into the molecules, similar to other drug properties, rather than to retrospectively try to modify an otherwise optimal molecule that is found to be toxic [10,11]. This has led to the development and implementation of various in silico and in vitro methods in the drug discovery process [12–16]. Early signs of consequent success emerged recently: The implementation of the 'Right Safety' concept from project inception, one of the pillars of AstraZeneca's 5R framework to increase R&D productivity, has reduced safety-related attrition [2,5].

Generic vs customized screening

Historical analysis of safety-related causes for termination of drug development programs or market withdrawals has been informative about what safety signals are not readily detected or responded to. Clearly, cardiovascular, liver, and central nervous

system (CNS) toxicities are the most common and impactful safety concerns [5,17]. Gastrointestinal and constitutional adverse reactions are common but can, depending on their severity and the tolerability in the disease indication, often be managed in the clinic [2,17–20]. Then, there are clinical toxicities that are impactful but do not occur as frequently (e.g. respiratory toxicity) or are very rare because they are adequately detected preclinically (e.g. genetic toxicity and carcinogenicity) [5,17]. It is relevant to note that the nature of the safety concerns that occur can depend on the target organ or route of administration [5,20]. Examples include effects on the respiratory system, which occur more frequently with drugs aimed to treat respiratory diseases, and on the CNS, which are more common for drugs against CNS disorders [5]. This shows that not only drug mechanisms but also drug distribution can be essential for a drug's safety profile: Drugs targeting respiratory disorders are frequently administered by inhalation, and those targeting CNS disorders are typically designed to enter the brain. It also exemplifies the need for patient-centric risk assessment, that is, to take characteristics of the target patient population into consideration, such as increased sensitivity due to the disease pathophysiology, comorbidities, and comedications.

As a result, in vitro safety screening strategies in drug discovery projects are truly dynamic paradigms. They will include a set of generic assays that are used across projects to identify and mitigate the most important risk factors versus common target organs and clearly undesirable effects (e.g. genetic toxicity) and assess general secondary pharmacology profiles [13,14,21,22]. They will typically also include customized assays to deal with more project- or target-specific safety questions.

Assay throughput and predictivity

The aim to impact chemical design early during small-molecule drug discovery imposes several demands on the in vitro assays that are used. In our experience, it is important that assays are compatible with the daily practice of drug discovery decisions, for example, that they can be executed in a miniaturized format with short turnaround times. This secures adequate throughput to test a large number of compounds across a wide concentration range in a routine screening setup. That is essential to obtain sufficient information to generate structure–activity relationships (SAR) to timely influence the chemical design cycles and thus steer chemical design toward optimized safety properties. It also ensures that compounds can be tested in low-volume assays, which is helpful because the quantity of available compound material is typically relatively low, in particular when hit series are characterized during early lead generation.

Perhaps most important is that the predictivity of the assays is well established. It needs to be clear what the assay results mean in term of risk assessment for clinical safety or, at least, for later toxicology studies, otherwise data may not be factored into decision-making. This is neither trivial nor easy but certainly possible. Ideally there would be a clear link between an IC50 value from an in vitro screening assay endpoint and a clinical exposure at which a particular safety outcome occurs. For example, drugs that inhibit the equilibrative nucleoside transporter 1 can, by means of accumulation of extracellular adenosine, cause dyspnea and bronchospasm. A recent analysis showed that the in vitro potency data of an equilibrative nucleoside transporter 1 assay could be directly applied to semiquantitatively assess the risk of dyspnea/bronchospasm in a relevant patient population [23]. Similarly, for inhibition of vascular endothelial growth factor receptor 2, which is linked to the incidence of hypertension, it has been shown that drugs require a margin of >10-fold between the IC50 in a cellular vascular endothelial growth factor receptor 2 assay and the unbound plasma concentration to exert minimal risk of hypertension [24]. Establishing the predictivity of novel predictive toxicology assays relies on the availability of an appropriate set of validation compounds that are well annotated for the clinical safety endpoint that is to be predicted and that represent a pharmaceutically relevant chemical space [25]. It is also relevant to note that the test concentration range for validation compounds needs to be related to the therapeutically relevant exposure in humans. Optimal predictivity, in particular for multiparametric cellular assays that predict organ toxicity, is typically obtained within a therapeutic index (e.g. the concentration at which an endpoint in the assay is perturbed relative to the clinical Cmax) range of 30–200, depending on the target organ, cell system, and assay readouts [25–28]. This indicates in vitro test concentrations need to exceed the therapeutic clinical exposure.

The predictivity of an assay relies on its sensitivity and specificity, and these may conflict. Optimizing the assay for high sensitivity, the ability to identify compounds with toxic potential, can result in low specificity, when true negatives are identified as toxic. To obtain optimal predictivity of an assay, it is important to establish appropriate cutoffs to classify test compounds as toxic or nontoxic [27,29]. In early discovery, high specificity is important to avoid rejection or needless optimization of potentially safe compounds. High sensitivity is important to identify and mitigate risk factors early and to reduce and replace the need for downstream in vivo studies, although suboptimal sensitivity can be complemented by more complex assays that are used in later stages of drug discovery. It is important to recognize that such assay cutoffs are applied to create a binary outcome (toxic vs nontoxic), whereas more quantitative (probabilistic) assessment would often be more

appropriate. Ultimately, exposure margins will often guide risk assessment. In the next sections, we will discuss several target organ areas for which predictive assays have been developed and integrated in discovery safety screening paradigms.

Cardiotoxicity

Cardiovascular toxicity has been a main cause of preclinical and clinical safety issues across the industry [5,17,20,30,31]. Generic secondary pharmacology assay panels therefore encompass many targets with a link to cardiovascular safety [21,22]. Historically, the majority of cardiac events have involved prolongation of QT interval or arrhythmias [31]. Detecting inhibitors of the human ether-a-go-go-related gene (hERG)—encoded potassium channel, which delay the ventricular action potential repolarization, and performing QT studies has therefore been emphasized in the current regulatory guidelines, and several analyses have shown consistent high concordance between preclinical hERG and QT assessment with clinical outcomes [20,32—34]. The current strategy has however been criticized for low specificity and for not directly assessing ventricular proarrhythmia or Torsade de Pointes (TdP) [35,36]. The Comprehensive In vitro Proarrhythmia Assay (CiPA) initiative aims to establish better predictions of proarrhythmia risk, by integrating the experimental results on several key cardiac ion channels, using in silico analysis, and measuring action potentials in cardiomyocytes [36—38]. The advancement of stem cell—derived cardiomyocytes has enabled the development of in vitro assays in human cells, assessing cardiomyocyte contractility, Ca^{2+} transients, field potential, or impedance, and these assays have the throughput, predictivity, and reproducibility to serve as routine screening assays in discovery projects [39—43]. Reduced contractility can also result from structural cardiotoxicity, and new multiparameter high-throughput screening assays to assess that have also been established to provide early insight into the mechanisms of cardiotoxicity [44—46]. More complex systems, integrating stem cell—derived cardiomyocytes into 3D structures such as engineered hearts [47,48] or into microtissue cocultures [48,49], have been developed more recently to improve structural maturation. These are currently particularly useful for mechanistic investigation but could, with increased throughput, also fit early discovery safety screening.

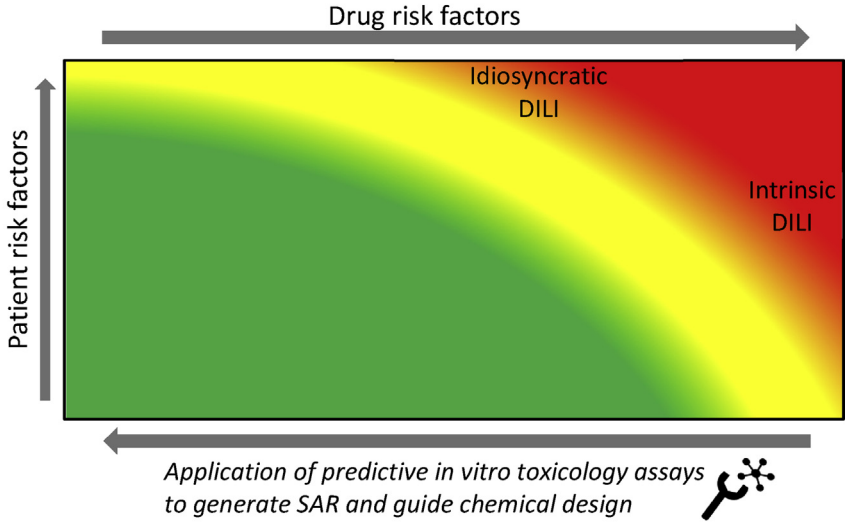
Hepatotoxicity

Along with cardiovascular toxicity, drug-induced liver injury (DILI) has been a major cause for candidate drug attrition and marketed drug withdrawal [20]. Traditionally, DILI has been subdivided into two classes. Intrinsic DILI is acute, is dose-dependent, has a high incidence, and can often be attributed to drug-related risk factors with known causal mechanisms. Idiosyncratic DILI is less strictly dose-dependent, occurs as isolated events, often after weeks or months of treatment, and is typically attributed to the complex interplay of drug-related and individual patient-related risk factors (Figure 1A).

In general, in vitro two-dimensional (2D) cell systems and standard preclinical models are suitable to identify molecules carrying well-known drug-related DILI risk factors [50,51]. As an example, compounds that exert mitochondrial toxicity, such as by inhibition of complexes in the respiratory electron transport chain and thereby reducing ATP production, are very frequently associated with liver toxicity, which highlights the need to assess this risk early [52]. Although assay endpoints, such as mitochondrial toxicity, can be downstream of a variety of off-targets, it is our experience that if sufficient effort is made, it is often possible to generate understanding of SAR to optimize chemical series and mitigate the risk timely (Figure 1B). In vitro screening assays assessing mitochondrial toxicity, cytotoxicity, bile acid homeostasis, and oxidative stress are stable, are cost-effective, and have adequate throughput to enable optimization campaigns [27,51,53]. Such assays are often based on HepG2 cells and, in particular if readouts are assessed in combination, identify roughly 50% of DILI-positive compounds while maintaining very high specificity (>90%) [27]. In addition, screening cascades will often include in vitro screening for potential inhibition of the human bile salt transport pump, Multidrug resistance-associated protein transporters (MRP) transporter proteins, or multidrug resistance protein 3 (MDR3), although it is still debated how relevant each of these assays is to predict for DILI [51,53—57]. In later stages of drug discovery, more refined cellular systems with a more accurate phenotype can be used to further increase predictivity. Primary hepatocytes (from human and other species), tissue slices, and three-dimensional (3D) microtissue systems such as liver spheroids better reflect liver biology in terms of cell metabolism, transporter expression, and/or tissue architecture and can enable long-term culturing and interspecies comparison [53,56,58,59]. Still, current conventional models are insufficient to assess the complex interplay of drug-related risk factors and individual patient-related risk factors [60,61]. For example, the adaptive immune system plays a role in a proportion of idiosyncratic DILI, which is related to specific human leukocyte alleles (HLA) [51,53,62]. The next generation of hepatotoxicity models will have to incorporate a multitude of such factors. Emerging microphysiological liver systems are promising to fill that gap in current screening paradigms. Although these models currently are too resource-intensive to offer routine screening opportunities, they do enable incorporation of coculturing and appropriate liver tissue architecture and also patient-centric parameters such as HLA type [63,64].

Figure 1

a DILI risk assessment matrix



b Project example

	AZ 1	AZ 2	AZ 3	AZ 4	AZ 5	AZ 6	AZ 7	AZ 8	AZ 9	AZ 10
Cytotoxicity assay gluc (IC_{50})	No Tox	No Tox	No Tox	No Tox	200	No Tox	No Tox	No Tox	150	46
Cytotoxicity assay gal (IC_{50})	No Tox	No Tox	No Tox	No Tox	No Tox	220	200	17	14	1.9
Ratio gluc/gal	NA	NA	NA	NA	<1	>1.1	>1.3	>15	11	24
OCR (IC_{50})	No Tox	No Tox	No Tox	No Tox	No Tox	70	53	15	16	1.4

Steering away from drug-induced liver injury (DILI) risk. **(a)** Depicted is a DILI risk assessment matrix from low (green) to high (red) risk, describing the complex interplay between drug risk factors (e.g. cytotoxicity, mitochondrial toxicity, reactive metabolites, oxidative stress and transporter protein inhibition) and patient risk factors (e.g. age, gender, genetics, comedications, health status and lifestyle). The established in vitro and in vivo models are generally good at identifying intrinsic DILI drug risk factors and can impact design. An understanding of, and consequently tools to identify and quantify, patient risk factors to stratify patients into well-defined risk groups are beginning to emerge (e.g. HLA type). Ultimately, the aim is to develop a quantitative risk assessment matrix that integrates drug and patient risk factors. **(b)** Data from three in vitro assays are depicted for ten compounds (AZ1–AZ10) from a chemical series in a drug discovery project: (i) cytotoxicity of HepG2 cells cultured in medium with high-glucose (gluc), (ii) cytotoxicity of HepG2 cells cultured in medium with galactose (gal), and (iii) oxygen consumption rate (OCR) in HepG2 cells. A high gluc/gal ratio indicates cytotoxicity is mediated by mitochondrial toxicity (as cells are more dependent on mitochondrial oxidative phosphorylation when grown on galactose [52]), which can be confirmed with the OCR assay. By linking structural features of the chemical series to the data, it is possible to influence chemical design, steer away from mitochondrial toxicity, and thus ensure delivery of candidate drugs with an optimized safety profile. IC50 values are indicated in μM. Not toxic: no observed toxicity up to 250 μM. NA: not applicable.

CNS toxicity

Direct or indirect nervous system toxicities such as headache, nausea, dizziness, fatigue/somnolence, or pain are some of the most common adverse events in clinical phase I trials [19,20,65]. CNS effects, such as seizures, can have severe impact on preclinical and clinical drug development [5,17]. Milder symptoms such as nausea can often be managed for severe disease indications but may still drive differentiation from other treatments. Most preclinical models to assess CNS effects rely on animal models (e.g. behavioral changes in the Irwin test) and are therefore less suited for screening in early discovery. The in vitro screening tools include assessment of binding or functional activity on ion channel and receptor panels with a well-established link to seizure liability, which is typically conducted as part of generic secondary pharmacology screening, and electrophysiology in brain slices [66,67]. Primary rodent CNS culture assays, containing relevant cell types but lacking the structural component, represent a more high-throughput option which readily detect seizure liability; however, it is not yet well established when the cultures reach maturity and correct receptor/channel expression [68]. More novel high-content screening approaches that, for example, assess neurite outgrowth patterns or the use of induced pluripotent stem cell-derived (iPS)-derived cultures may develop into relevant high-throughput screening tools for certain types of neurotoxicity, but further validation may be required to warrant adoption of such assays as part of standard screening paradigms [68,69]. An obvious strategy to mitigate CNS-related effects is to influence the distribution of the drug by proactive chemical design of physicochemical properties, as opposed to designing blood–brain barrier penetrance for centrally acting drugs [70].

Genetic toxicity

Because DNA damage is associated with carcinogenesis, there are clear guidelines and well-established testing batteries of in silico, in vitro, and in vivo assessments to predict the outcome of the long-term carcinogenicity studies and ensure human safety [71]. These encompass the bacterial mutagenicity Ames test and assays to assess genotoxic potential in mammalian cells, such as the mouse lymphoma assay, in vitro micronucleus, or chromosomal aberration assay [71]. In the pharmaceutically relevant chemical space, the mouse lymphoma assay and Ames test have a minimum specificity of 85% and 97%, respectively [72], which indicates a positive result in these assays is often regarded as indication that further leads optimization is required, unless thorough follow-up in vivo work can demonstrate irrelevance to man.

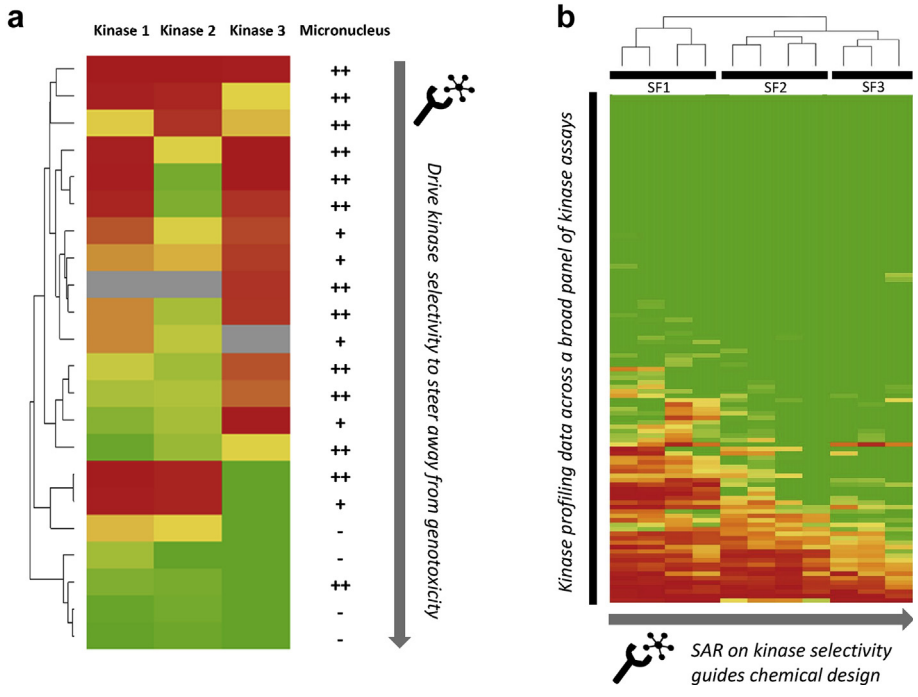
Several novel multiplex assays to identify genotoxic compounds, based on flow cytometry or high-content imaging, have been developed in recent years, and because these are compatible with plate-based assay formats, they are suitable for discovery safety screening [29,73–76]. The advantage of obtaining multiple endpoints in novel genotoxicity screens, such as the ToxTracker, Multiflow™ DNA Damage, or MEGA-screen assays [75–77], is that one can simultaneously analyze cytotoxicity and micronucleus induction as well as gain insight into whether a clastogenic or aneugenic mechanism is at play. Combining genetic toxicity data with secondary pharmacology panel data, across a set of compounds within a chemical series, can enable identification of causative off-target promiscuity, and this insight can subsequently be applied to influence chemical design (Figure 2A).

Other recent advancements in the field include assessment of broader carcinogenic potential using nongenotoxic endpoints related to cell signaling or gene expression [77–79]. Such novel approaches should also result in a better understanding of carcinogenic mechanisms of action.

Nephrotoxicity

Although drug-induced kidney injury (DIKI) may occur less frequently than cardiac toxicity or DILI, it does represent a substantial safety burden to the

Figure 2



Kinase profiling to influence chemical design. (a) A chemical series in a kinase drug discovery project had an intrinsic micronucleus issue for which the SAR was not understood. Because inhibition of off-target kinases can induce micronucleus formation [102], compounds were profiled against a broad panel of kinase assays. Hierarchical clustering of 22 compounds from the series, based on data for three kinases in the panel (red: >80% inhibition, green: <20% inhibition, gray: no data), separated micronucleus negative (–) from almost all positive (++ strong positive, + borderline positive) compounds. SARs on the biochemical kinase assays could subsequently be applied to guide chemical design, drive optimization of the general kinase selectivity, and thus steer away from micronucleus risk. **(b)** Kinase profiling data across 125 kinase assays, depicted here by % inhibition (red: 75–100%, amber: 50–75%, yellow: 25–50%, green: 0–25%), were applied for hierarchical clustering of compounds within one chemical series in a discovery project. Using this approach, three distinct structural features (SF1-3) were identified, which differentiated on selectivity. We currently apply this approach to larger compound sets in kinase drug discovery programs to drive optimization toward a more selective profile [89]. SAR, structure–activity relationship.

development of novel drugs. Up to 10% of historical safety-related drug development failures were due to DIKI, and it also affects clinical application of currently used drugs [5,80]. Discovery safety screening for DIKI has lagged behind the DILI field, but in recent years, significant progress has been reported. Compared with standard cell lines, the use of more advanced cell systems, including various proximal tubular epithelial cell models and kidney organoids, allows replication of relevant physiological features such as expression of relevant transporters or metabolizing enzymes [28,81–86]. We recently established a novel nephrotoxicity assay that, aided by machine learning, exploits the assessment of a combination of subtle precytotoxic cell health parameters, including actin cytoskeleton, nuclear density, and mitochondrial function, in a conditionally immortalized proximal tubular epithelial cell line [28]. By combining four assay parameters and compound exposure, the assay identifies 75% of proximal tubular toxicants (66% of overall nephrotoxicants) without producing false positives. Owing to the high predictivity and simple setup, such an assay presents an attractive opportunity for incorporation in standard screening approaches in early discovery.

Tailored approaches

In addition to assays addressing risk factors around common target organs, drug discovery safety screening plans typically include a set of assays that are tailored to the target organ, target class, modality, indication, or specific safety concerns around the intended pharmacological target based on a target safety assessment [11,87]. For example, candidates that are intended for inhaled administration may cause toxicity in the respiratory tract, which is typically discovered in repeat dosing animal studies. Recent progress includes the finding that epithelial barrier integrity in a 3D human lung cell model based on bronchial epithelial cells cultured at air–liquid interface is predictive of in vivo respiratory toxicity [88]. Such in vitro models allow for earlier risk identification and an opportunity for mitigation.

Early safety strategies are also tailored to the target class, such as kinase inhibitors. Type I and II kinase inhibitors will generally have poor selectivity because they target the structurally conserved ATP pocket. Even though modern approaches in kinase drug discovery include a focus on kinase selectivity, such compounds will generally hit off targets as exposure increases in safety studies [89]. It is thus essential to integrate specific kinase assays, considering close homologs and undesired off-targets, in early screening strategies, to drive optimization (Figure 2B). Identification of allosteric inhibitors may overcome this, and although this is not the most straightforward lead identification strategy for kinase projects, it may contribute to long-term success of the project.

While many discovery safety screening paradigms are based on historical analysis of small molecules, they need to be readily adapted for other modalities. Emerging insights into novel modalities need to be integrated, such as specific proinflammatory effects or thrombocytopenia for antisense oligonucleotides [90].

An alternative to applying predictive in vitro toxicology assays as screening tools to design candidate drugs with the right safety profile is to influence biodistribution. For example, proactive targeting strategies using Triantennary N-acetyl galactosamine (GalNAc3) or glucagon-like peptide 1 conjugation can be exploited to specifically direct antisense oligonucleotides to the liver or pancreas, respectively [90–92]. In addition, pursuing an inhaled route of administration, which usually results in lower systemic exposure compared with, for example, an oral route, may lead to a larger Therapeutic Index (TI) for peripheral safety issues, other than local in the lung [93].

Future perspectives

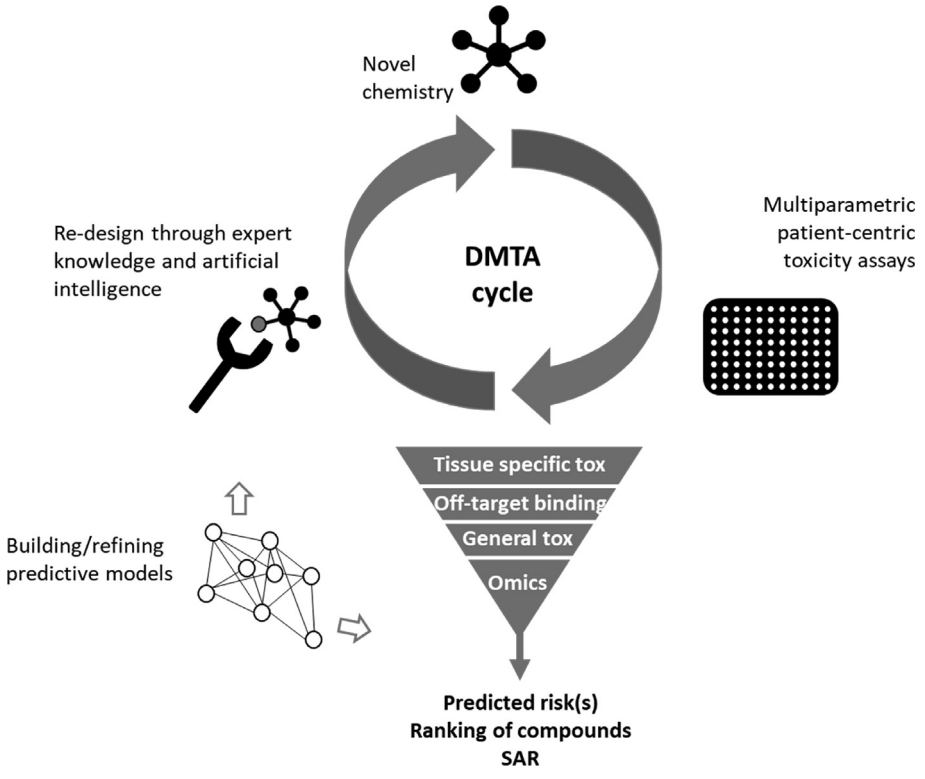
As the field of applied predictive toxicology is advancing rapidly, it is relevant to consider the next steps of innovation that will contribute to the discovery of candidate drugs with the right safety. We believe this will be driven by a combination of (i) an evolving need for drug candidates with an even further refined and optimized safety profile and (ii) an increased ability to integrate the generation and analysis of large data sets in decision-making.

With an increasing number of innovative medicines on the market, it can be expected that the safety profile will become an increasingly relevant parameter for differentiation of novel drugs. As new medicines obtain therapeutic benefit, even in difficult areas such as oncology, and consequent disease modification allows patients to recover or at least live longer, superior safety will be essential for sustained quality of life of patients, to ensure compliance, as well as for viable value propositions to payers. It will require discovery safety screening paradigms to be further optimized, potentially by covering target organs beyond those discussed previously, but certainly by further enhancing predictivity by integrating physiologically relevant models early, such as microphysiological systems which are based on human cells, recapitulate relevant structural and functional organ characteristics, and can be coupled to create multiorgan systems in vitro [64]. However, to use more complex models in early discovery screening paradigms, further technical advancements are required to increase assay throughput and reduce resources required to employ them. Furthermore, there is a need for more patient centricity because disease characteristics and interindividual variability can impact the susceptibility to a certain toxicity. For example, a long-acting

muscarinic antagonist bronchodilator was discontinued when clinical studies on patients with chronic obstructive pulmonary disease (COPD) revealed an initial drop in forced expiratory volume and adverse respiratory events, something that was not observed in healthy volunteers, and which was hypothesized to relate to an increased susceptibility/sensitivity of the inflamed tissue in COPD lungs [94]. In addition, rosiglitazone, previously a widely prescribed treatment for diabetes, was associated with increased risk of myocardial infarction and withdrawn from the European market, but still, subsequent meta-analysis on the link to cardiotoxicity has produced varying results [95–99]. This exemplifies that patients carry unique risk factors and that a personalized medicine approach is warranted in which candidate drug–related risk factors are matched to patient-related risk factors to assess their risk benefit profile. The use of human primary cells and induced pluripotent stem cells enables adapting assays to include genome-specific phenotypes of the patient population for identification of responders or risk groups. In vitro models with specific genotypes or disease contexts are therefore promising opportunities to increase patient centricity. However, owing to the complex interplay of many factors, including genetics, disease context, lifestyle, comedications, patient history, etc., that impact drug safety, it is relevant to recognize that the design of truly personalized drug safety for the individual patient is not yet addressed by current innovation in discovery safety screening.

As we get access to more and more complex big data, enabled by rapid development of laboratory technologies (e.g. high-content imaging, sequencing of genomes and transcriptomes, proteomics, metabolomics, etc), and witness digitalization of in vivo data and human health records, the challenge for data-driven decision-making in drug discovery is shifting from data generation to data interpretation. Artificial intelligence and machine learning will be essential to be able to structure, handle, and interpret the data and to drive novel hypothesis generation based on the resulting

Figure 3



Drug safety design-make-test-analyze (DMTA) cycle 2.0. Novel chemistry is iteratively tested in a battery of high-throughput multiparametric toxicity assays of varying complexity to assess general or tissue-specific toxicity, multiomics, and off-target binding. Patient centricity, by introducing disease models, context, or patient genotypes, adds value to predictions. Pattern recognition algorithms and predictive modeling, enabled by artificial intelligence, identify inherent risks and generate structure–activity relationship (SAR) to aid redesign or facilitate ranking of compounds based on multidimensional information. Building and continuous refining of these predictive models by machine learning and big data sharing will facilitate increasingly accurate risk assessment and faster design of candidate drugs with the right safety. In practice, this will be integrated in a multidisciplinary DMTA cycle, including all relevant drug properties.

knowledge. One of the current key challenges for the development of predictive machine learning models is the access to reliable, structured, standardized data, which can be used as training sets. The eTOX consortium, a data-sharing project, initiated the generation of a curated database by collecting preclinical toxicological data from academic groups and pharmaceutical companies to develop in silico tools to predict drug toxicity [100]. This field is currently very dynamic and will undoubtedly impact the way we discover novel drugs [101]. Discovery safety screening paradigms can be expected to evolve from the current approach (in which single assays are applied for single drug–related risk factors) toward the application of patient-centric (primary cells or genetically modified), high-throughput cell and microtissue panels with multiple endpoints (imaging and various -omics platforms) simultaneously, followed by machine learning algorithm–based analysis to identify a broad range of safety concerns (Figure 3). Such a multiparametric approach would consider potential correlations and synergies between the endpoints to make more comprehensive in vitro predictions in an integrated discovery safety strategy.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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