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ADME–Tox in drug discovery: integration of experimental and computational technologies

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Over the past ten years, *in vitro* experimental tools to characterize ADME–Tox profiles of compounds have been applied in early stages of the drug discovery process to increase the success rate of discovery programmes and to progress better candidates into drug development. Application of *in silico* ADME–Tox models has further enhanced discovery support, enabling virtual screening of compounds and thus, application of ADME–Tox at every stage of the discovery process. Ultimately, effective and efficient ADME–Tox support of discovery will depend on a complementary and synergistic use of experimental and *in silico* ADME–Tox.

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▼ Drug discovery and development is a lengthy and costly process, taking an average of 15 years and US$880M to generate a successful medicine [1]. The process is characterized by high attrition rate; up to 76% between target and IND [2] and ~90% by the end of clinical trials [3]. This is at least partly due to inadequate ADME–Tox properties [3,4].

A rational approach to increase the efficiency and reduce the cost of pharmaceutical R&D is to reduce the attrition rate in the costly downstream stages by increasing the attrition rate in the less costly, earlier stages of the process – a 'fail early, fail cheap' strategy that has been widely accepted in the pharmaceutical industry [5–10]. The major approach towards this involves moving ADME–Tox evaluations into early discovery stages, such as lead identification or optimization (Figure 1), to be conducted in parallel with activity and selectivity assays. This enables all properties to be optimized simultaneously, thus resulting in cost and time savings. To meet the new challenges of large compound number and shortened cycle time that are characteristic of early discovery, *in vitro* experimental ADME–Tox assays have undergone significant modifications. In addition, *in silico* models using computational technology have emerged to further expand the ADME–Tox tools. This review provides an overview of the new approaches that have evolved since the mid-1990s, focussing on the integrated and synergistic applications of experimental and computational ADME–Tox tools.

Experimental ADME–Tox tools in drug discovery

The goal of *in vitro* ADME–Tox characterization is to provide, with reasonable accuracy, a preliminary prediction of the *in vivo* behavior of a compound to assess its potential to become a drug. Many factors combine to determine this, therefore, a variety of experimental assays have been developed to characterize each aspect of the processes (Table 1). The tools involved include physicochemical methods and biological assays using subcellular fractions, primary cell culture, immortalized cell lines, tissues and whole organs. Two major challenges faced by these assays in the discovery phase are higher throughput and shorter time for data turnaround, both of which are a result of the need to rapidly screen a large number of compounds. To meet these challenges, new formats of *in vitro* ADME–Tox assays serving early discovery have been developed from traditional assays through protocol simplification and making use of technology advancement.

Protocol simplification

Traditional ADME–Tox assays were designed as detailed experimental approaches to characterize a process by its underlying mechanisms.

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For example, characterization of the metabolism or transport of a compound involve investigation at multiple concentrations and time points [11,12] with a throughput of a few compounds per week. This format is not sufficient to enable the evaluation of hundreds or thousands of compounds quickly, as is necessary in early discovery and, furthermore, the evaluation of compounds in early discovery is not likely to require the details and depth of data provided by such traditional approaches. However, these approaches might still be useful as secondary assays during lead optimization or preclinical development (Table 1).

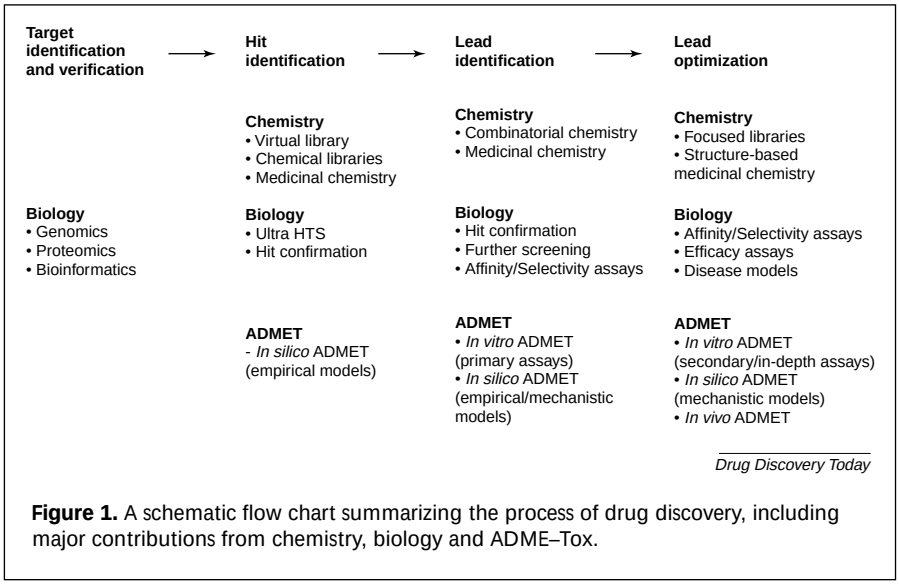

Figure 1. A schematic flow chart summarizing the process of drug discovery, including major contributions from chemistry, biology and ADME–Tox.

These shortfalls necessitated the development of simplified primary assays that provide surrogate measures of the parameters characterizing the processes but that are still of sufficient validity to be used in decision-making on the progression of compounds. Typical examples include the measurement of metabolic stability or permeability of a compound at a single concentration and single time point [13] or the evaluation of the CYP450 inhibition potential of a compound at a single concentration, in place of determining the IC_{50} or inhibition constant value (K_i) [14,15].

Technology advancements

Protocol simplification and a widespread use of microtiter plate formats have made it possible for many assays to be automated, using robotic systems. This has significantly increased the throughput of sample generation and pushed the bottleneck of *in vitro* ADME–Tox assays from sample generation to sample analysis. The new bottleneck is being addressed with new technological advancements in analytical methods, such as LC–MS(–MS), that comprise shortened run time, higher capacity injectors and the combination of multiple injection heads with one MS–MS detector [16,17]. Alternative detection methods that simultaneously read the entire microtiter plate is another approach that has enhanced throughput and shortened turnaround time. Such methods include fluorescent readouts for the CYP450 inhibition screening assay [18,19] and turbidimetry or nephelometry in solubility determinations [20,21].

In summary, recent advances in *in vitro* ADME–Tox technologies have enhanced throughput to an unprecedented level [22]. Further improvements through process optimization will provide an even greater opportunity for generating data to support the integrated use of experimental and *in silico* ADME–Tox tools in drug discovery (discussed later).

The emergence of *in silico* ADME–Tox tools

Despite the increased throughput provided by the simplified *in vitro* ADME–Tox assays, throughput capacity remains low in comparison with that of HTS activity assays or combinatorial chemistry, consequently limiting the application of these assays to only a fraction of compounds evaluated in discovery. The need for increased ADME–Tox throughput to fully meet the demands of discovery has led to renewed and increasing interests in computational or *in silico* models that make predictions based on chemical structures alone (reviewed in [23–31] and selectively summarized in Table 1).

As shown in Table 1, a wide variety of *in silico* ADME–Tox models are available, with different levels of complexities and throughputs. The extent of computation, both to calculate the required compound descriptors used in the model and to actually fit the model to the observed data, is the primary factor that limits the throughput of these models. This computation need is dependent on the complexity of the model and this is determined by the particular process being modeled. For example, a model of bioavailability is more complicated than a model of any of the underlying processes, such as solubility and dissolution rate, passage through lipophilic membranes and associated efflux by transporters or metabolism in the gut wall and/or in the liver. The simpler models can subsequently be integrated, if needed, into a more complex model to provide a complete picture of the overall process. This approach will keep the modeling of underlying

Table 1. Selected experimental and predictive ADME–T tools in support of drug discovery

	Experimental [5–10,21,22,45]		Computational [12,23–31,46–49]
	Primary assays (throughput >100 compounds per week)	**Secondary assays (throughput <100 compounds per week)**	
Absorption [50–55]			
Physicochemical methods	IAM, PAMPA, solubility	LogD7.4, pKa, pH-dependent solubility	Empirical models on ClogP, MlogP, solubility models (in H_2O or with little DMSO)
Human intestinal absorption (HIA)	Unidirectional cell permeability (Caco-2, MDCK, MDCK-II, HT29)	Bidirectional cell permeability, Intestinal tissue, intestinal perfusion, pharmacokinetic studies using animals	Empirical models on Caco-2 passive permeability, HIA
Metabolism [56]			
Metabolic stability	Human or rodents liver microsomes or S9 fraction	Hepatocytes (human or animals), expressed enzymes	Regioselectivity models for CYP 3A4, 2C9 and 2D6, empirical models on liver microsomal half life, metabolite prediction (METEOR, COMPACT, META, MetabolExpert)
Drug–drug interaction [58]	CYP inhibition screening using fluorescent substrates, single point or IC50 determination	*Ki* determination of CYP inhibition using drug probes and LC–MS(–MS)	Empirical models for CYP enzyme inhibition
Distribution			
Blood–brain barrier (BBB) penetration [58,59]	PAMPA using BBB specific lipids [61]	Brain capillary endothelial cells, brain perfusion, brain/blood ratio in animals	Empirical models on BBB penetration models [61]
Efflux [63]	Calcein uptake, Pgp-ATPase assay	Bi-directional transport with inhibitors using parent or transfected cell lines, brush border membrane vesicles, bi-directional intestinal perfusion with appropriate inhibitors, knock-out rodents	Mechanistic model for Pgp substrate
Protein binding [63]	Human serum albumin (HSA) binding	Plasma protein binding	Empirical models on ClogP, LogHSA
Toxicity [64,65]			
Basic cytotoxicity	Mitochondrial inhibition (MTT or alamar blue) or LDH using cell lines (e.g. 3T3, HepG2)	Using metabolism-competent cells, such as hepatocytes from human or rodents	COMPACT, TOPKAT, DEREK
Genotoxicity	SOS/*umu* test [66]	AMES assay	TOPKAT, DEREK

Abbreviations: IAM, immobilized artificial membrane; PAMPA, parallel artificial membrane permeability assay.

processes simple and less demanding, in terms of computational power.

The currently available computational ADME–Tox models can be classified into two main categories – empirical and mechanistic [30]. Empirical models use statistical tools to explore the relationships, either linear or nonlinear, between certain structural descriptors and observed parameters of a particular ADME–Tox property [32–35]. Their relative simplicity has fostered application in almost all ADME–Tox properties and they account for a majority of the currently available models (Table 1). Such models require minimal computational power and, as a result, have high throughputs of up to thousands or millions of molecules per hour, – sufficient to meet even the most demanding needs of ADME–Tox support for discovery. Conversely, mechanistic models, use quantum mechanics

methods to calculate atomic interactions between small molecules and macromolecules, for example, enzymes or transporter proteins, that are involved in a certain ADME–Tox process [36–39]. They require 3D structures of ligands and macromolecules and thus, require more computational power in comparison to the empirical models. This results in a relatively lower throughput, varying from tens to a few hundred compounds per hour. Although this throughput is still more than that of the highest capacity of *in vitro* ADME–Tox assays, further improvements in algorithms and computational power will enhance the use of these models.

The major limitation to widespread use of *in silico* ADME–Tox methods is their predictability, which currently varies between 60% and 90%. There are several reasons for this limitation. The first depends on the type of model. The predictability of empirical models is generally limited to the chemistry space that is covered by the compounds in the training set or those fairly close to them. Mechanistic models, however, are more predictive on an expanded chemistry space because they are based on atomic and molecular interactions between the ligand and the corresponding macromolecule. In both cases, the use of a more diverse set of chemical molecules in model development will ensure a better predictability and wider applicability. Second, the perceived lack of predictability is partly due to the comparisons drawn between the predictions and the *in vivo* behavior of compounds. This is challenging because of the complicated nature of *in vivo* ADME–Tox processes, caused by multiple underlying mechanisms, some of which can not be represented adequately in the models. More appropriately, comparisons of predictions should be made to results of *in vitro* ADME–Tox assays that tend to focus on specific mechanistic processes and are thus, less complicated than *in vivo* profiles. Another significant advantage of this approach is that results from *in vitro* assays are more readily available and easier to generate, making model validation and refinement more feasible. Finally, the predictability of both model types depends on large and good quality datasets being used in their development and refinement [40]. Therefore, efforts should be made to generate datasets with balanced quality and diversity to enhance predictability.

In silico approaches have undergone significant improvement in terms of the variety of models available and the predictability of ADME–Tox properties. However, the predictability of *in silico* ADME–Tox needs further improvement and the integrated use of *in silico* and *in vitro* ADME–Tox tools, as proposed in the following section, is one approach that could facilitate this process.

The integration of *in vitro* and *in silico* ADME–Tox tools

Both experimental and computational ADME–Tox have been used in support of the discovery process and each has its own unique advantages and uses but also distinct limitations. The adoption and use of experimental ADME–Tox is almost universal across all companies, whereas the application of *in silico* ADME–Tox is less widespread and the extent to which the results obtained from it are used in decision-making is unclear. As discussed previously and in more detail elsewhere [23–31], *in silico* ADME–Tox approaches are coming of age and their wider use and application is perhaps justified. The optimal approach for ADME–Tox support of discovery will be one that uses both *in vitro* and *in silico* ADME–Tox in a complementary way and ensures that ADME–Tox is used and considered at almost every stage of the discovery process, from hit identification to lead optimization (Figure 1). This overall approach will be designed in a way that takes advantage of the strengths of the processes and also helps to overcome or improve their limitations. Such a system is depicted in Figure 2 and described below.

Hit identification stage

As shown in Figure 2, the application of ADME–Tox starts at the 'hit identification' stage, where the objective is to identify compounds that demonstrate initial feasibility to interact with a target. This process has evolved from testing a few chemical compounds to screening the entire collection of a company's library (which might run into millions of compounds) with (ultra) high throughput biological assays (Figure 1). Even with the increased throughput of *in vitro* ADME–Tox assays, they still do not have the capacity to screen such a large collection. This demand for throughput makes *in silico* ADME–Tox particularly suitable for application at this stage. The enhanced throughput provided, particularly by the empirical models, enables evaluation of a large number of compounds. In addition, they require only structures of compounds to make predictions, and can therefore also be used for screening virtual compound libraries [41–43].

The primary goal of the application of *in silico* ADME–Tox models at this stage is to identify compounds or series with the least acceptable drug-like properties to eliminate them from consideration. The risks of this approach are the potential for false positives and false negatives. False positives are easier to resolve because they will be subjected to other tests further into the process that will eliminate them. False negatives are potentially more contentious because of the risk of missing the next 'blockbuster' drug. However, owing to the large numbers of compounds involved (up to millions), the law of probability

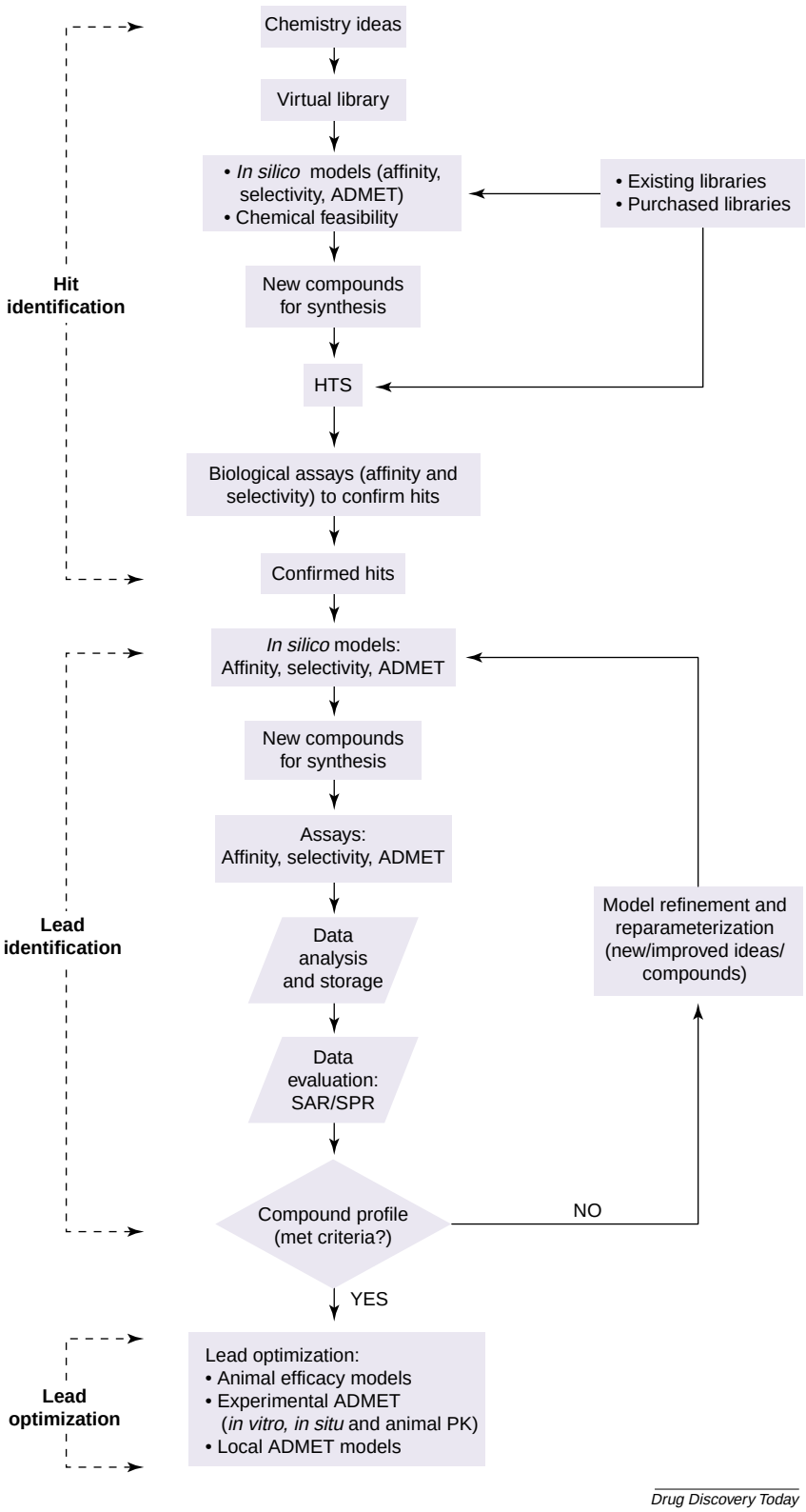

Figure 2. Proposed workflow for a drug discovery project, showing the integrated use of computational and experimental technologies in the assessment and selection of compounds.

ensures that many compounds with potentially good characteristics will make it through. Another goal of ADME–Tox application here is to identify potential weaknesses and liabilities in the selected series to highlight the issues that will form the focus of improvement/optimization efforts. The use and success of this approach significantly relies on the scope of available compounds from which choices can be made; the higher the number of compounds, the less concern there will be about false negatives.

Compound libraries usually possess broad chemical diversity, therefore, the *in silico* models that are needed to screen these libraries are inevitably 'global' models, that are designed to cover a broad scope of chemistry space. This is achieved by training the model with compounds from numerous chemotypes. Many global models are commercially available and are suitable for use at this stage, particularly for companies without the required in-house resource or experience for model development. The level of predictability should be adequate for the goal at this stage, given the chemical diversity and number of compounds involved. Furthermore, model refinement (discussed in the following section) should provide a way to continuously improve the predictability of the global models.

Lead identification stage

Following identification and confirmation of hits, the selected series now enter the 'lead identification' stage. Characterized by a significantly reduced number of chemical series and compounds, depending on the 'hit rate' from the primary screening, the objective here is to identify a small number of chemical series with the activity, selectivity and drug-like properties required in potential candidates. This stage involves an iterative use of both experimental and *in silico* ADME–Tox

technologies (Figure 2). Although empirical models could continue to identify weaknesses or liabilities in compounds and series, the application of *in silico* ADME–Tox should focus on predictions of chemical modifications that will improve the ADME–Tox properties of compounds to be produced in the next round of synthesis. For this purpose, mechanistic models are particularly appropriate because of their enhanced ability to make predictions based on atomic and molecular interactions between these compounds and macromolecules. Also, the focus is now shifting to specific chemicals or series, rather than large numbers of compounds and thus, the throughput capacity of mechanistic models should be sufficient. With the use of *in silico* models, the overall number of compounds to be synthesized and experimentally tested is reduced to better suit the capacity of subsequent assays.

Application of *in vitro* assays is focused on measurement of the ADME–Tox properties of the newly synthesized compounds. The primary goal of such measurements is to assess if the compounds have the desired properties to become a lead. Those compounds that meet the required criteria can be advanced to the next stage of evaluation along the discovery path. The experimental data can also be used in the assessment of predictability of *in silico* models. This will confirm those predictions or reveal differences between predictions and observed properties. The results would provide valuable input, particularly in areas of predictive failures, for the refinement of *in silico* ADME–Tox models. In other words, the positive and negative results can be used to redefine the model to help it become more predictive. The refined models can then be used to predict the next set of compounds to be synthesized by building on this round of predictions, synthesis and experimental measurements. This then, becomes an iterative process [22], which eventually leads to lead optimization. In addition to adjusting the parameters of global models, the experimental data can also be used for building 'local' ADME–Tox models that are specific to a single chemical series. Local models are particularly useful for optimizing a potential lead series in the later stage of lead identification or early in lead optimization.

Lead optimization

The lead optimization stage is characterized by improving pharmacological and pharmaceutical properties in a single or a small number of chemical series. Similar to the process of lead identification, the optimization of ADME–Tox properties consists of an iterative workflow – starting from *in silico* prediction, to chemical synthesis, to experimental testing and confirmation, and to model refinement. Here, the major tools for *in silico* ADME–Tox are mechanistic models or empirical local models built with newly generated data, whereas, on the experimental side, secondary assays and *in vivo* tests in animals are frequently used. In summary, the integrated approach makes the best use of experimental and *in silico* ADME–Tox and enables them to complement each other well. The earlier use of *in silico* ADME–Tox on large numbers of compounds and to predict improved candidates serves as a filter for a more manageable number of compounds entering *in vitro* ADME–Tox testings. The availability of *in vitro* results on sets of compounds predicted to have better properties provides valuable data to assess predictability of *in silico* ADME–Tox models and, more importantly, data which can be used to adjust the parameters and refine the models in the continuous improvement of predictability.

Practical considerations

Commercial versus in-house models

A variety of *in silico* models for ADME–Tox are commercially available and have been reviewed recently by Waterbeemd and Gifford [27]. Commercial models tend to cover a broad chemical diversity and are usually used as global models for series selection. Many companies can not afford the extensive investment and chemical resource for an *ab initio* building of global models, therefore, the readiness for immediate use of a commercial package is an attractive option. The major drawback with commercial models is that they might not be directly applicable to the chemical series of interest if a project is narrowly focused.

Another potential limitation exists in cases where the modification of parameters and refinement of the models is not possible. By contrast, in-house models have advantages in both of these situations. Furthermore, local ADME–Tox models, being series-specific, are better built using in-house data. In answer to this, commercial vendors could provide opportunities for users to make the best use of their products by enabling customers to modify parameters for their own internal use or by providing such services themselves. In addition, the protocols and conditions for generating experimental data need to be transferred to customers for generation of follow-up data on a consistent platform.

Modeling-compatible in vitro assays

In the integrated process, *in vitro* ADME–Tox has two major roles – project support and model improvement. Experimental data are used not only to determine the ADME–Tox features for each compound but also for refining existing global models and for building local models, if necessary. These two roles are potentially conflicting: sufficient throughput is required for project support and higher quality data are required for modeling, thus, a balance between data quantity and quality is required. The data

generated by certain simplified experimental ADME–Tox assays might not be usable for this purpose. For example, single-point metabolic stability or CYP450 inhibition data are of limited value in developing robust models. Likewise, unidirectional (apical-to-basolateral) permeability results are not appropriate in the building of a passive diffusion model because the method does not distinguish passive diffusion from efflux and other active transport processes. However, it should be noted that these data might be suitable at some stages of the discovery process in decision-making on compound progression.

An alternative and feasible approach is to modify the *in vitro* ADME–Tox assays towards more modeling-compatible formats that can provide high quality data for model development, while maintaining an adequate throughput for project support. Examples of such modeling-compatible assays include: i) generation of IC_{50} data for CYP450 enzyme inhibition assays, which are better than single-point data but not as resource-demanding as the generation of the ideal K_i values ; ii) generation of a rate constant of metabolism, rather than a single-point percentage remaining, to aid development of a metabolism rate model; and iii) generation of bi-directional permeability data that provide information on transport mechanisms. Although these assay formats will have lower throughputs than their simpler counterparts, the use of *in silico* ADME–Tox for compound selection should reduce the number of compounds that would otherwise need to be tested experimentally. The experimental data being produced by these methods can be used for better support of projects and can serve as a sufficient data pool for model refinement and local model building.

Resource issues

The modeling-compatible *in vitro* assays should not necessarily require more resources. Although the number of samples for each tested compound will increase, the use of *in silico* models should reduce the number of compounds that need to be tested. In addition, the afore-mentioned progresses in automation and higher-throughput bioanalytical techniques will further secure a reasonable throughput while the data quality is enhanced. Finally, process optimization, as described in the following section, will significantly contribute to a smoother workflow and increased efficiency.

Process optimization

The integrated use of *in silico* and *in vitro* ADME–Tox, particularly the model refinement process, which relies on newly generated experimental data, imposes an unprecedented demand for faster data turnaround. Currently, the ADME–Tox process in drug discovery, from compound submission for screening to the final data release to the project team consists of multiple steps and multiple assays. Optimization of this process can be achieved by using informatics to integrate and manage the components, ensuring an almost seamless operation, which in turn, results in a significantly shortened cycle time [44]. Figure 3 demonstrates an example of the optimized process.

The workflow starts with an on-line submission of requests to a central repository, where plate reformatting and replication are conducted simultaneously for ADME–Tox and other pharmacological assays by a robotic system. The data from all the parallel assays are uploaded into a database that automatically triggers an e-mail notification of data release to the project team and managers. This parallel processing means that data becomes available at about the same time, thus, enabling an 'across-the-board' evaluation of the suitability of the compounds tested. Using this optimized process, the delay in a typical sequential process is reduced and the turnaround time can be as short as five business days. It also provides a system to maintain data and information integrity by reducing manual intervention. Furthermore, an electronic plate or compound-tracking can be achieved when barcode labeling and scanning are performed on all plates throughout the process. This enables the identification of any delay during the plate flow and provides guidance for further improvements. Various aspects of this integration are already in use at most companies and complete integration can be achieved with minimal additional effort. Also, the recent emergence of commercial software, such as the Galileo™ *in vitro* LIMS (http://www.innaphase.com/products_galileo.html), for improved management of *in vitro* assay data, is a valuable development.

Standardization of in vitro assays

The process of *in silico* ADME–Tox model refinement requires that all *in vitro* ADME–Tox data are generated under consistent conditions. Therefore, standardization of assay conditions, such as cell line, assay protocol, enzyme and substrate concentrations, temperature and time points, is of paramount importance to a successful modeling effort. Given the variability associated with biological data (between, and even within, laboratories), it is essential to acquire data using consistent assay formats, methods and set-ups. This will ensure the comparability between initial dataset for model development and the follow-up datasets for adjustment of model parameters, refinement and optimization. In a time of global, multi-site companies, standardized protocols are a necessity: they should be used across all sites of a company to ensure full use of the larger

datasets available. It will also ensure a more uniform assessment of projects, which is particularly useful because most projects originate from screening the same libraries. The challenge for commercial developers of *in silico* models is that they will be required to provide the assay protocols that were used in initial data generation to clients so that subsequent data for adjustment of model parameters can be generated consistently. This standardization, although challenging, is crucial to the success of experimental and computational ADME–Tox integration.

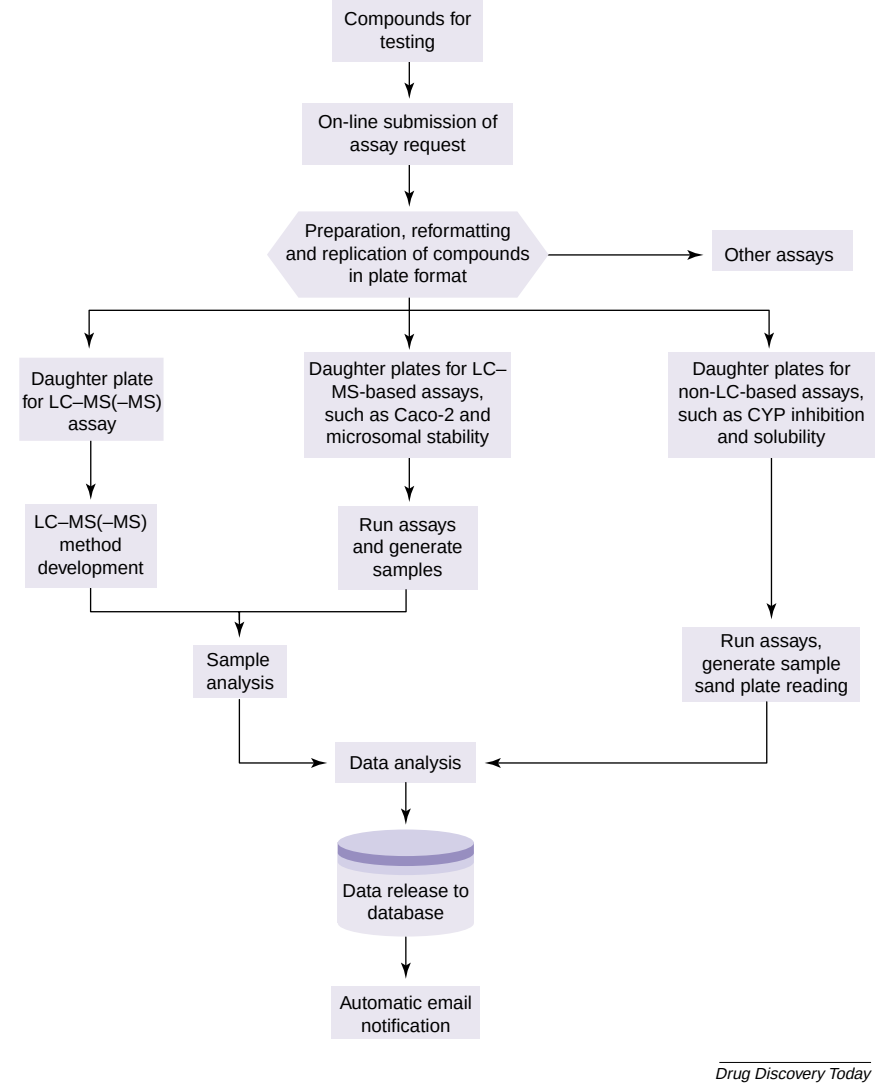

Figure 3. Process optimization of *in vitro* ADME–Tox assays. Key components include centralized plate replication, simultaneous assay running, LC–MS(–MS) method development in parallel with sample generation, electronic data flow and plate tracking, contributing to a shorter data turnaround time.

Conclusions and prospects

The initial goal of introducing ADME–Tox in discovery was to increase the success rate of drug development projects by addressing ADME–Tox issues, one of the major causes of project failure, at the early stages of discovery and at a relatively low cost. This effort has been ongoing over the past few years. The application of experimental ADME–Tox tools is now almost universal and has progressed with significant improvements in throughput and turnaround time that have been achieved through assay simplification, automation and employment of advanced new technologies. The use of *in silico* ADME–Tox tools has shown a steady progress because of its potential to provide a much higher throughput than experimental ADME–Tox and because of recent improvements in predictability. Indeed, tools are now available to enable the application of ADME–Tox at every stage of the discovery process, including the decision on which compounds or series to make and which compounds meet the required criteria for progression to the next stage.

The application of ADME–Tox in discovery has advanced towards its goal but there are still challenges to a full realization of its potential. Although the benefits of experimental ADME–Tox in decision-making are well established, its limited throughput does not enable every compound to be evaluated. By contrast, *in silico* ADME–Tox can provide a much higher throughput but a perceived lack of predictability has limited its use; there is less reliance on the output from this method to make decisions. The way forward, to a full integration of ADME–Tox in discovery, therefore, seems to depend on the ability to apply the two approaches in a complementary and synergistic manner. The throughput limitations of *in vitro* ADME–Tox assays can be overcome by a judicious use of *in silico* ADME–Tox at earlier stages involving evaluation and selection from a large number of compounds. Furthermore, *in silico* models can be used to predict which compounds to synthesize, based on confirmed hits and structural modifications that would be necessary to obtain more drug-like compounds. This use of *in silico* ADME–Tox serves as a filter to select a manageable number of compounds for

testing by *in vitro* ADME–Tox assays at later stages. The results from the assays are used for project evaluation, *in silico* prediction confirmation and as a source of high quality data for *in silico* ADME–Tox model refinement. This concept is mutually beneficial and ultimately forms the foundation of an iterative process to produce the candidate compounds with the desired characteristics. The whole process can be better managed through an informatics system for improved efficiency and better data or information integrity.

Advances in other areas are necessary for successful implementation of such an integrated approach. Improved algorithms and an increase in computing power are needed to increase the quality and throughput of *in silico* ADME–Tox models, thus enabling the application of more predictive, although computation-intensive, mechanistic models to larger numbers of compounds and, perhaps, at earlier stages of discovery. In addition, there is an increasing need for reconfigurating *in vitro* ADME–Tox assays from the current simplified versions into formats that will generate data more suitable for model development and refinement with the following advantages: i) the balanced data quality and throughput will satisfy the needs for both modeling and project support; ii) mechanism-specific assays will facilitate data interpretation and modeling and; iii) assay standardization will generate follow-up data for adjustment of model parameters. Like the process optimization involved in the application of experimental ADME–Tox in drug discovery, the integration of experimental and *in silico* ADME–Tox into discovery will require further adjustments to ensure a system that enables optimal realization of mutual benefits. Through these improvements, ADME–Tox will be integrated into and used at every stage of the discovery process, thus, achieving its goal – a more efficient and productive drug discovery process.

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