

# De-Risking Drug Discovery Programmes Early with ADMET

Katya Tsaioun<sup>1</sup> and Steven A. Kates<sup>2</sup>

<sup>1</sup>*Apredica, a Cyprotex Company, Watertown, MA*

<sup>2</sup>*Ischemix LLC, Maynard, MA*

*USA*

## 1. Introduction

Drug attrition that occurs in late clinical development or during post-marketing is a serious economic problem in the pharmaceutical industry (1). The cost for drug approvals is approaching \$1 billion USD, and the cost of advancing a compound to Phase 1 trials can reach up to \$100 million USD according to the Tufts Center for the Study of Drug Development, Tufts University School of Medicine (2). The study also estimates a \$37,000 USD direct out-of-pocket cost for each day a drug is in the development stage and opportunity costs of \$1.1 million USD in lost revenue (2). Given these huge expenditures, substantial savings can accrue from early recognition of problems that would demonstrate a compound's potential to succeed in development (3).

The costs associated with withdrawing a drug from the market are even greater. For example, terfenadine is both a potent hERG cardiac channel ligand and is metabolized by the liver enzyme Cyp3A4. Terfenadine was frequently co-administered with Cyp3A4 inhibitors ketoconazole or erythromycin (4). The consequent overload resulted in increases in plasma terfenadine to levels that caused cardiac toxicity (5) resulting in the drug to be withdrawn from the market (6) at an estimated cost of \$6 billion USD. Another example is the broad-spectrum antibiotic trovafloxacin, which was introduced in 1997 and soon became Pfizer's top seller. The drug was metabolically activated *in vivo* and formed a highly reactive metabolite causing severe drug-induced hepatotoxicity (7). Trovafloxacin was black labeled in 1998 (8) costing Pfizer \$8.5 billion USD in lawsuits (9). With the new ability to measure hERG and other important ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) parameters early in the discovery and development process, such liabilities are now recognized earlier allowing for safer analogs to be advanced to more expensive formal preclinical and clinical stages.

The purpose of preclinical ADMET also referred to as early DMPK (drug metabolism and pharmacokinetics) is to reduce the risk similar to above and avoid spending scarce resources on weak lead candidates and programs. This allows drug-development resources to be focused on fewer, but more-likely-to-succeed drug candidates. In 1993, 40 % of drugs failed in clinical trials because of pharmacokinetic (PK) and bioavailability problems (10). Since then, major technological advances have occurred in molecular biology and screening to allow major aspects of ADMET to be assessed earlier during the lead-optimization stage. By

the late 1990s the pharmaceutical industry recognized the value of early ADMET assessment and began routinely employing it with noticeable results. ADME and DMPK problems decreased from 40% to 11% (4). Presently, a lack of efficacy and human toxicity are the primary reasons for failure (11).

The terms “drugability” and “druglikeness” were first described by Dr. Christopher Lipinski, who proposed “Lipinski’s Rule of 5” due to the frequent appearance of a number “5” in the rules (12). The Rule of 5 has come to be a compass for the drug discovery industry (13). It stipulates that small-molecule drug candidates should possess:

- a molecular weight less than 500 g/mol
- a partition coefficient (logP – a measure of hydrophobicity) less than 5
- no more than 5 hydrogen bond donors
- no more than 10 hydrogen bond acceptors

A compound with fewer than three of these properties is unlikely to become a successful orally bioavailable drug. There are exceptions to Lipinski’s Rule of 5 that have become marketed drugs, such as those taken up by active transport mechanisms, natural compounds, oligonucleotides and proteins.

The drug discovery industry is experiencing dramatic structural change and is no longer just the domain of traditional large pharmaceutical companies. Now venture-capital-funded startups, governments, venture philanthropy and other non-profit and academic organizations are important participants in the search for new drug targets, pathways, and molecules. These organizations frequently form partnerships, sharing resources, capabilities, risks and rewards of drug discovery. Thus, it is becoming increasingly important to ensure that investors, donors, and taxpayers’ money is efficiently used so that new safe drugs for unmet medical needs may be delivered to the public. ADMET profiling has been proven to remove poor drug candidates from development and accelerate the discovery process.

Although lack of efficacy and unexpected toxicity are the major causes of drug failure in clinical trials, a prime determinant is the ability of a drug to penetrate biological barriers such as cell membranes, intestinal walls, or the blood brain barrier (BBB). For drugs that target the Central Nervous System (CNS) such as stroke, *in vitro* efficacy combined with the inability to penetrate the BBB typically result in poor *in vivo* efficacy in patients. The delivery of systemically administered drugs to the brain of mammals is limited by the BBB as it effectively isolates the brain from the blood because of the presence of tight junctions connecting the endothelial cells of the brain vessels. In addition, specific metabolizing enzymes and efflux pumps such as P-glycoprotein (P-gp) and the multi-drug-resistance protein (MRP), located within the endothelial cells of the BBB, actively pump exogenous molecules out of the brain (14, 15). This is one of the reasons for CNS drugs having a notoriously high failure rate (16). In recent years, 9% of compounds that entered Phase 1 survived to launch and only 3-5% of CNS drugs were commercialized (16). Greater than 50% of this attrition resulted from failure to demonstrate efficacy in Phase 2 studies. Over the last decade, Phase 2 failures have increased by 15%. Compounds with demonstrated efficacy against a target *in vitro* and in animal models frequently proved to be ineffective in humans. Many of these failures occur due to the inability to reach the CNS targets such as in stroke due to lack of BBB permeability. For drugs targeted to reduce damage from a stroke, the delivery method, BBB permeability, and drug metabolism and clearance can provide life or death to a patient if the drug is not delivered to the target tissue in its active form in a matter of hours from the event.

Due to the extraordinary cost of drug development, it is highly desirable to have effective, cost-efficient and high-throughput tools to measure BBB permeability before proceeding to expensive and time consuming animal BBB permeability studies or human clinical trials. With *in vitro* tools available, promising drug candidates with ineffective BBB penetration may be improved by removing structural components that mediate interaction(s) with efflux proteins, and/or lowering binding to brain tissue at earlier stages of development to increase intrinsic permeability (17).

The development of drugs targeting CNS requires precise knowledge of the drug's brain penetration. Ideally, this information would be obtained as early as possible to focus resources on compounds most likely to reach the target organ. The physical transport and metabolic composition of the BBB is highly complex. Numerous *in vitro* models have been designed to study kinetic parameters in the CNS, including non-cerebral peripheral endothelial cell lines, immortalized rat brain endothelial cells, primary cultured bovine, porcine or rat brain capillary endothelial cells and co-cultures of primary brain capillary cells with astrocytes (18, 19, 20). *In vitro* BBB models must be carefully assessed for their capacity to reflect accurately the passage of drugs into the CNS *in vivo*.

Alternatively, several *in vivo* techniques have been used to estimate BBB passage of drugs directly in laboratory animals. *In vivo* transport across the BBB was first studied in the 1960s using the early indicator diffusion method (IDM) of Crone (21). Other *in vivo* techniques were later proposed including brain uptake index (BUI) measurement (22), *in situ* brain perfusion method (23, 24), autoradiography and intracerebral microdialysis (25). Unfortunately these methods have limitations including sophisticated equipment, technical expertise, mathematical modeling, species differences, invasiveness, and low throughput and render them unsuitable for use during early stages of drug discovery and development. Hence, *in vitro* and *in vivo* models remain mere approximations of the complex BBB and their relevance to human pharmacology must be carefully considered. The most appropriate method to conduct controlled experiments is to cross-compare the BBB passage of a series of compounds evaluated with both *in vitro* and *in vivo* models. This enables cross-correlations of pharmacokinetic data and the assessment of the predictive power of *in vitro* and *in vivo* tests.

## 2. The evolving science of ADMET

Regulatory authorities have relied upon *in vivo* testing to predict the behavior of new molecules in the human body since the 1950s. Bioavailability, tissue distribution, pharmacokinetics, metabolism, and toxicity are assessed typically in one rodent and one non-rodent species prior to administering a drug to a human to evaluate safety in a clinical trial (Phase 1). Biodistribution is assessed using radioactively labeled compounds later in development because it is expensive both in terms of synthesizing sufficient amounts of radioactively labeled compound and for performing the animal experiments (22).

Pharmacodynamic (PD) effectiveness of test compounds is typically assessed initially through *in vitro* models such as receptor binding, followed by confirmation through *in vivo* efficacy models in mice or rats. The predictive ability depends on the therapeutic area and the animal model. Infectious disease models are considered to have the best predictive ability, whereas CNS and oncology animal models are generally the least predictive of human efficacy. Understanding the PK/PD relationship is crucial in determining the mechanism of action and metabolic stability of the molecule which can explain and support

efficacy results. *In vivo* pharmacokinetic (PK) studies in a variety of animal models are routinely used for lead optimization to assess drug metabolism and absorption. There are significant differences in absorption and metabolism among species from animal studies, which may cause conflicting predictions of degradation pathways of new chemical entities (NCEs).

Toxicity and safety studies are performed in models that are relevant to the NCE's mode of action and therapeutic area. *In vivo* toxicity models are required for IND (Investigational New Drug Application) to the US Food and Drug Administration, but have substantial predictive weaknesses. In a retrospective study of 150 compounds from twelve large pharmaceutical companies, the combined animal toxicity study of rodents and non-rodents accurately predicted only 50% of the human hepatotoxicity. This poor level of accuracy in animal toxicity studies caused large numbers of compounds to be removed from development without proceeding into clinical trials with the potential of demonstrating safety in human subjects (26). The other ~50% whose toxicity could not be predicted was attributed to "idiosyncratic human hepatotoxicity that cannot be detected by conventional animal toxicity studies". Although it is widely recognized that mechanisms for toxicity are frequently quite different between species, animal testing remains the "gold standard" for required regulatory and historical data reasons. The US FDA and other regulatory agencies are in the process of evaluating alternatives to animal testing, with the aim of developing models that are truly predictive of human mechanisms of toxicity, and limiting *in vivo* toxicology testing.

### 3. The ADMET feedback loop

As discussed above, historically ADMET studies were focused on *in vivo* assays. These are time- and resource-intensive, and generally low throughput assays resulting in their implementation later in the development process, when more resources are released to study the few molecules that have advanced to this stage. With the advent of *in vitro* high-throughput screening, molecular biology and miniaturization technologies in the 1990s, early ADMET assays were developed to predict *in vivo* animal and human results, at a level of speed and cost-effectiveness appropriate for the discovery stage. This produced a major advance in the science of ADMET and has created a new paradigm that drug discovery programs follow in advancing compounds from hit to lead, from lead to advanced lead, and on to nominated clinical candidates. Now, early in the discovery phase, using human enzymes and human-origin cells, drug discovery programs are able to obtain highly actionable information about the drug-likeness of new molecules, the potential to reach target organ, and early indications of known human mechanisms of toxicities. ADMET assessment of varying complexity is currently routinely performed on compounds that have shown *in vitro* efficacy and in conjunction with or just prior to demonstrating early proof of principle *in vivo*.

The application of early ADMET is unique to each drug discovery program. The development path from discovery to IND is not straightforward and is dependent on the therapeutic area, route of administration, chemical series, and other parameters. Correspondingly, the importance of the various ADMET assays is based upon the specifics of the drug discovery program. ADMET assays can also be categorized into those that are routine and those reserved for more advanced profiling. This division is also a function of cost effectiveness and the need for the specific information. For instance, data regarding

induction of human liver enzymes and transporters are not relevant during the hit-to-lead phase and is normally obtained for fewer more advanced candidates.

In some cases the FDA requests data from *in vitro* ADMET assays. For example, *in vitro* drug-drug interaction (DDI) studies may now be conducted under the guidance from FDA dated September 2006. The guidance document precisely outlines methods to conduct CYP-450 inhibition and induction and P-gp interaction studies (27). This package is now typically included in an IND submission.

How should a discovery team employ early ADMET? The answer is not simple and formulaic – it is a process. It is useful to start from the ultimate goal and work backwards towards discovery. The drug discovery and development team should first define the target product profile (TPP), which includes indication, intended patient population, route of administration, acceptable toxicities, and ultimately will define the drug label. The TPP invariably will evolve during the life of the project, but having major parameters of TPP established initially maintains a collaboration and focus between disciplines such as biology and chemistry, discovery and development, pre-clinical and clinical groups. Once the TPP is identified, then major design elements of the Phase 2 and 3 clinical trials can be outlined leading to questions about the tolerability, toxicity and safety of the molecule. These parameters will then define the GLP toxicity studies in animals, which will guide the team to the discovery and preclinical development data to be addressed in an early ADMET program.

How is this information implemented in the discovery phase? If a compound has high target receptor binding and biological activity in cells and in relevant *in vivo* animal models, what are the chances of it becoming a successful drug? A molecule needs to cross many barriers to reach its biological target. In order to obtain this goal, a molecule must be in solution and thus the first step is typically to assess the solubility of a compound. A solubility screen provides information about the NCE's solubility in fluids compatible with administration to humans. Chemical and metabolic stability is a further extension of the intrinsic properties of a molecule. Chemical stability in buffers, simulated gastric and intestinal fluids, and metabolic stability in plasma, hepatocytes or liver microsomes of different species can be measured to predict the rate of decay of a compound in the different environments encountered in the human body.

The second step is to define the absorption properties and the bioavailability of a molecule. Measurement of permeability across Caco-2 cell monolayers is a good predictor of human oral bioavailability. For CNS drugs, assessment of BBB penetration would be performed at this stage and is usually a key component of lead optimization campaigns. Passive BBB permeability may be assessed using BBB-PAMPA assays whereas potential for active uptake or efflux may be determined using *in vivo* models or cell lines naturally expressing endogenous human intestinal or BBB transporters (such as CaCO-2 cell line) or cell lines overexpressing specific transporters (such as MDCK-MDR1).

Measurement of binding to plasma proteins indicates the degree of availability of the free compound in the blood circulation. This is critical as only unbound drugs are able to reach the target and exert their pharmacologic effects. Metabolism and drug-drug interaction issues are discovered by screening for inhibition of cytochrome P450 liver enzymes (CYP450). All these assays allow chemists and biologists to obtain actionable information and provide a link between structure-activity (SAR) and structure-properties (SPR) relationships that drive decisions on selection of chemical series and molecules.

The next step is the involvement of drug-drug interactions and is required for advanced lead optimization. The effect of drug transporters on permeability and the effect of drugs on transporter activity can be measured in Caco-2, MDCK-MDR1 or other models. P-gp interactions are particularly important for CNS drugs due to high expression of these efflux transporters in the human BBB. Early knowledge about these interactions is instrumental to the medicinal chemistry strategy and helps drive lead optimization.

The effect of a compound on CYP-450 metabolism can be identified by determining the 50% inhibitory concentration ( $IC_{50}$ ) for each CYP-450. These relationships between the NCE and metabolizing enzymes need to be evaluated in the context of the human effective dose and maximum effective plasma concentrations. These human data are not normally available at early stages of discovery, but could be extrapolated from animal PK/PD results for compounds in more advanced stages of development. It is important to understand these transporter and CYP-450 relationships for the following.

1. The compound may affect the effective plasma concentrations of other concomitantly administered drugs if metabolized by the same CYPs (i.e., terfenadine).
2. If the parent drug is a CYP inducer, it may increase the clearance rate of concomitantly administered drugs which are metabolized by these CYPs. This may result in a decrease in these drugs' effective plasma concentrations, thus decreasing their pharmacologic effect.
3. Metabolites formed *via* CYP metabolism may be responsible for undesirable side effects such as organ toxicity.
4. The metabolite of a compound may actually be responsible for compound's efficacy, and not the parent compound. The metabolite may even have a better efficacy, safety, and pharmacokinetics profile than its parent. As a result, metabolism can be exploited to produce a better drug which will impact the medicinal chemistry strategy.
5. The identification of drug-metabolizing enzymes involved in the major metabolic pathways of a compound assists to predict the probable drug-drug interactions in humans. This information also may be used to design human clinical trials to detect unnecessary drug-drug interaction.

ADMET is a tool that supports overall program goals. Similar to the Rule of 5 that requires only 3 of the 4 conditions to be met, seldom will negative results from a single ADMET assay terminate a compound's development or the overall program. The results are more likely to alter the medicinal chemistry direction.

After assessing compounds in a few simple mechanistic systems such as plasma and liver microsomal stability screens in relevant species, lead optimization phase is started that includes assays which identify potential liabilities. Finally, at the stage of advanced lead optimization and development, more-complex systems are used to more thoroughly understand a compound's metabolic fate and absorption mechanism to drive efficient development. As ADMET roadblocks are discovered, the cycle is repeated until a clear path is found (Figure 1).

#### 4. Impact of ADMET

Early ADMET provides the data necessary for selecting preclinical candidates by providing crucial information to medicinal chemists and accelerates the timelines for IND and subsequently NDA submission which translates to lengthier commercialization under

patent protection and greater profits. For investors, this is a major parameter. For philanthropic organizations and from standpoint of public policy, it means increasing the time of clinical benefit to the public. Data compiled by the Tufts Center for Drug Discovery have identified that for a typical, moderately successful proprietary drug (\$350 million USD annual sales) each day's delay equates to \$1.1 million USD in lost patent protected revenues that provide the return on investment needed to fund drug discovery (3). Further, shorter discovery and development timelines provide faster liquidity events for venture capital and angel investors. As drug discovery requires a longer commercialization than any other form of product development, its slowness to produce returns is a major impediment for obtaining investment. Accelerating drug discovery and development should attract more investment in drug discovery research.

## ADMET Feedback Loop

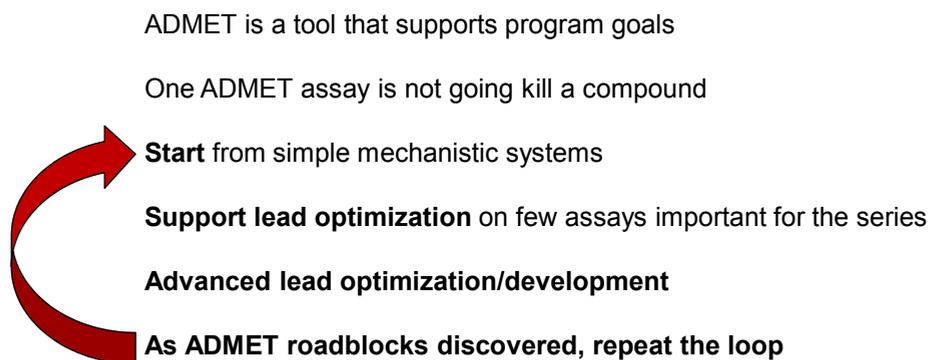


Fig. 1. ADMET Feedback Loop.

ADMET technologies remain an active area of research. There are many challenges in accurately measuring BBB penetration which may be one of the reasons for poor human efficacy of CNS drug candidates. Another challenge is detection of all mechanisms of human idiosyncratic toxicity. These mechanisms cause the most expensive, harmful, and disheartening form of drug attrition - post-commercialization toxicity. Many idiosyncratic drug reactions are due to formation of short-lived reactive metabolites that bind covalently to cell proteins (28). The extent to which a compound will generate these metabolites can now be detected before a compound is administered to humans signifying progress. Other mechanisms of human toxicity can be observed early in discovery and are briefly described in the following section.

### 5. New ADMET tools

Penetrating the BBB is a challenge particular to CNS drug discovery. Another obstacle caused by BBB permeability is that many drugs not intended as CNS therapeutics cause neurotoxicity. Artificial membrane permeability assays (PAMPA and BBB-PAMPA) offer a

cost-effective and high-throughput method of screening for passively absorbed compounds but do not predict active transport in or out of the brain.

### 5.1 *In Vitro* model of human adult Blood-Brain Barrier

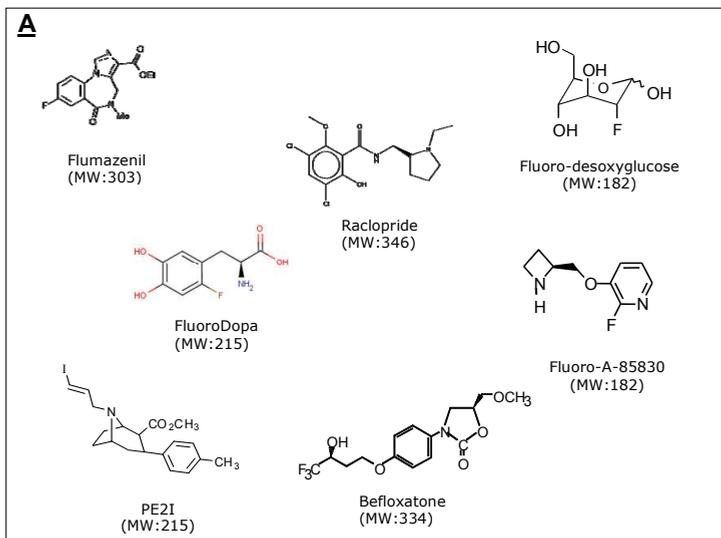
Many new drugs designed for CNS may show exceptional therapeutic promise due to their high potency at the target site, but lack general efficacy when administered systemically. In many cases, the problem is due to lack of penetration of the BBB and this has become a major problem that has impeded the discovery and development of active CNS drugs. CEA Technologies previously reported the development of a new co-culture-based model of human BBB able to predict passive and active transport of molecules into the CNS (29). This new model consists of primary cultures of human brain capillary endothelial cells co-cultured with primary human glial cells (18, 29). The advantages of this system include:

- i. made of human primary culture cells
- ii. avoids species, age and inter-individual differences since the two cell types are removed from the same person
- iii. expresses functional efflux transporters such as P-gp, MRP-1, 4,5 and BCRP.

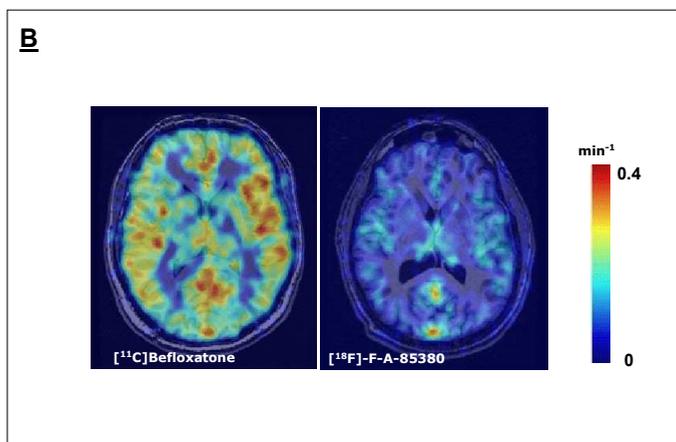
This model has potential for assessment of permeability of drug and specific transport mechanisms, which is not possible in PAMPA or other cell models due to incomplete expression of active transporters.

One important step in development of any *in vitro* model is to cross-correlate *in vitro* and *in vivo* data in order to validate experimental models and to assess the predictive power of the techniques (30). The human BBB model was validated against a “gold standard” *in vivo* model and has shown an excellent *in vitro-in vivo* correlation (29, 31). In this carefully designed *in vivo-in vitro* correlation study the authors reported the evaluation of the BBB permeabilities for a series of compounds studied correlatively *in vitro* using a human BBB model and *in vivo* with quantitative PET imaging (29). Six clinical PET tracers with different molecular size ranges (Figure 2) and degree of BBB penetration were used including [<sup>18</sup>F]-FDOPA and [<sup>18</sup>F]-FDG, ligands of amino acid and glucose transporters, respectively. The findings demonstrate that the *in vitro* co-culture model of human BBB has important features of the BBB *in vivo* including low paracellular permeability, well developed tight junctions, functional expression of important known efflux transporters and is suitable for discriminating between CNS and non-CNS compounds. To further demonstrate the relevance of the *in vitro* human system, drug permeation into the human brain was evaluated using PET imaging in parallel to the assessment of drug permeability across the *in vitro* model of the human BBB. *In vivo* plasma - brain exchange parameters used for comparison were determined previously in humans by PET using a kinetic analysis of the radiotracer binding. 2-[<sup>18</sup>F]Fluoro-A-85380 and [<sup>11</sup>C]-raclopride show absent or low cerebral uptake with the distribution volume under 0.6. [<sup>11</sup>C]-Flumazenil, [<sup>11</sup>C]-befloxatone, [<sup>18</sup>F]-FDOPA and [<sup>18</sup>F]-FDG show a cerebral uptake with the distribution volume above 0.6. The *in vitro* human BBB model discriminates compounds similar to *in vivo* human brain PET imaging analysis. This data illustrates the close relationship between *in vitro* and *in vivo* pharmacokinetic data ( $r^2$  0.90,  $p < 0.001$ ) (Figure 2). Past *in vivo-in vitro* studies often did not have good correlations for substances transported into or out of the brain *via* active transport. Presumably this is due to experiments being performed either with models that did not have adequate expression of active human transporters (such as PAMPA or MDCK cells) or using too high concentrations of compounds *in vitro*, which are known to saturate

the transporters. Using the radioactive labeled probes and the small amounts of compounds avoids these issues.

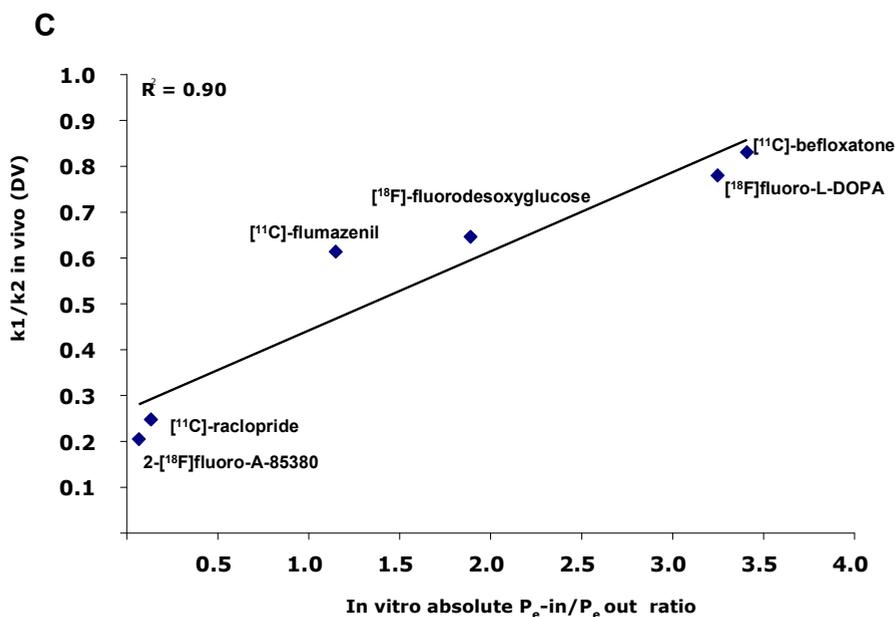


A. Chemical structures of radioligands investigated and used clinically.



B. Typical imaging data. Co-registered PET-MRI images representing the  $k_1$  obtained in human after intravenous injection of  $^{11}\text{C}$ -Befloxatone (left) and  $^{18}\text{F}$ -F-A-85380 (right). The PET images representing the  $k_1$  are as follows: PET image obtained at 1 min post injection (mean value between 30 sec and 90 sec) is considered as independent to the receptor binding. This image (in Bq/mL) is corrected from the vascular fraction ( $F_v$  in Bq/mL, considered as 4% of the total blood concentration at 1 min) and divided by the arterial plasma input function (AUC<sub>0-1</sub> min of the plasma concentration, in Bq\*min/mL). The resulting parametric image, expressed in min<sup>-1</sup>, represent an index of the  $k_1$  parameter of the radiotracer.

Fig. 2. *In vitro-in vivo* drug transport correlation.



C. *In Vivo* Distribution Volume (DV) as function of the in vitro  $P_{e-out}/P_{e-in}$  ratio (Q). Regression line was calculated, and correlation was estimated by the two tailed non parametric Spearman test. [ $^{11}\text{C}$ ]PE2I radioligand was not plotted in the figure since the *in vivo*  $K_1/k_2$  parameter in human is not available.

Fig. 2. *In vitro-in vivo* drug transport correlation (continued).

In conclusion, this *in vitro* human BBB model offers great potential for both being developed into a reproducible screen for passive BBB permeability and determining active transport mechanisms. Due to its high-throughput potential, the model may provide testing large numbers of compounds of pharmaceutical importance for CNS diseases. Validation work is in progress in which activity of transporters that are important in CNS BBB are being assessed in a functional assay and compared between CaC0-2 and hBBB models (31).

## 5.2 Mechanisms of human toxicity

Idiosyncratic hepatotoxicity or drug-induced liver injury (DILI) occurs in only one out of about 10,000 patients and is usually statistically impossible to discover during clinical trials. In spite of its name which means “rare event with undefined mechanism”, some mechanisms have now been defined including mitochondrial toxicity and the formation of reactive metabolites. Another mechanism of human toxicity that is not limited to the liver, but may also affect lung, spleen, and heart tissues is phospholipidosis.

### 5.2.1 Mitochondrial toxicity

Mitochondrial toxicity is increasingly implicated in drug-induced idiosyncratic toxicity. Many of the drugs that have been withdrawn from the market due to organ toxicity have been found to be mitochondrial toxicants (32). Mitochondrial toxicants injure mitochondria by

inhibiting respiratory complexes of the electron chain, inhibiting or uncoupling oxidative phosphorylation, inducing mitochondrial oxidative stress, or inhibiting DNA replication, transcription, or translation (33).

Toxicity testing of drug candidates is usually performed in immortalized cell lines that have been adapted for rapid growth in a reduced-oxygen atmosphere. Their metabolism is often anaerobic by glycolysis despite having functional mitochondria and an adequate oxygen supply. Alternatively, normal cells generate ATP for energy consumption aerobically by mitochondrial oxidative phosphorylation. The anaerobic metabolism of transformed cell lines is less sensitive to mitochondrial toxicants causing systematically underreporting in toxicity testing (33, 34). To address this issue, HepG2 and NIH/3T3 cells can be grown in media in which glucose is replaced by galactose (32). The change in sugar results in the metabolism of the cell to possess a respiratory substrate that is both more similar to normal cells and sensitive to mitochondrial toxicants without reducing sensitivity to non-mitochondrial toxicants (Figure 3).

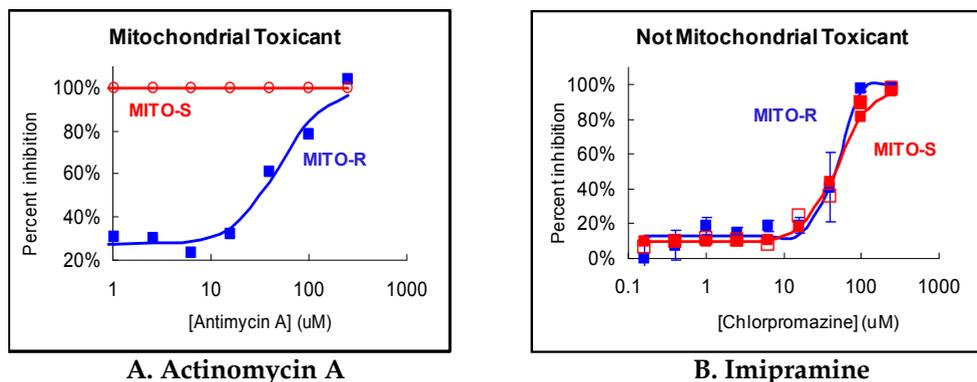


Fig. 3. Effect of Antimycin A, a compound known to be toxic to mitochondria (A) and Imipramine (B) on parent HepG2 cells (Mito-R - blue) and a HepG2 cell line that has been developed to become sensitive to mitochondrial toxicants (Mito-S - red).

### 5.2.2 Reactive metabolites formation

Another property of compounds that can cause idiosyncratic toxicity is their ability to form reactive intermediates (35). Formation of short-lived reactive metabolites is known to be the mechanism of toxicity of some compounds such as acetaminophen (36). The formation of reactive metabolites can be identified by incubating test compounds with liver microsomes and adding glutathione to trap the reactive intermediates which are then identified by LC/MS/MS (Figure 4). Conversion of more than 10% of the test agent to reactive intermediates indicates that the compound may be implicated in idiosyncratic toxicity.

### 5.2.3 Phospholipidosis

Phospholipidosis is a lysosomal storage disorder and can be caused by drugs that are cationic amphiphiles (37). The disorder is considered to be mild and often can self-resolve. However, drugs that cause phospholipidosis can also produce organ damage, and thus this disorder is a concern to the regulatory agencies (37). A cell-based assay for phospholipidosis

has been developed (38) which involves accumulation of a fluorescent phospholipid resulting in an increase of fluorescence in the lysosomes of cells that have been treated with drugs that cause phospholipidosis (Figure 5). If phospholipidosis is absent, the phospholipid is degraded and fluorescence does not increase. Increases in fluorescence are normalized to cell numbers since many of these drugs are also cytotoxic (Figure 5).

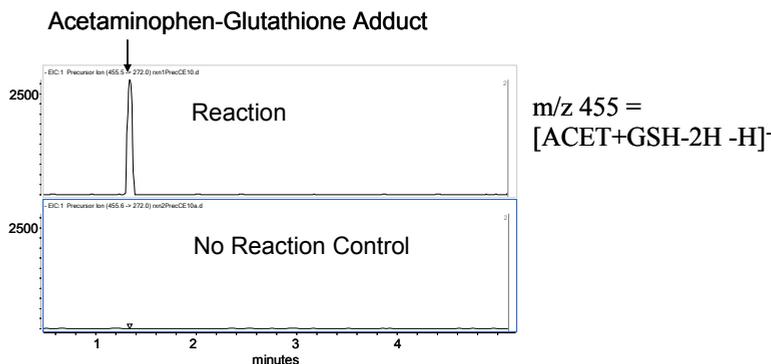


Fig. 4. Formation of Reactive Metabolites of Acetaminophen. Acetaminophen was incubated with microsomes and glutathione in the presence and absence of NADPH. An adduct of glutathione with acetaminophen was formed in the presence of NADPH. When NADPH was absent (No Reaction Control), no adduct was formed.

#### Phospholipidosis of Compounds in HepG2 Cells

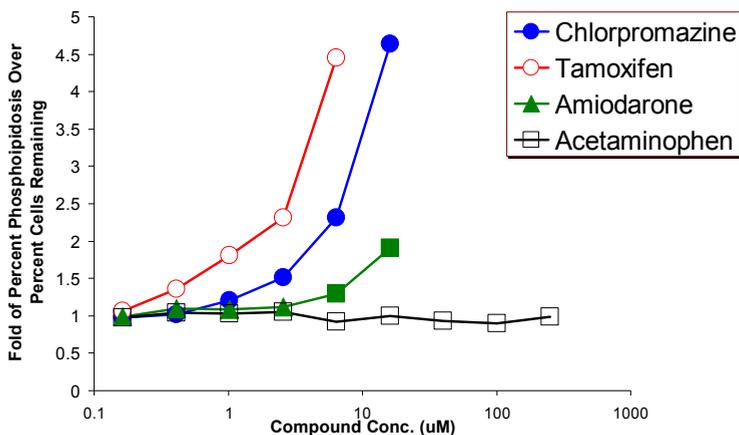


Fig. 5. Drug-induced phospholipidosis (PLD) is determined by measuring the accumulation of a fluorescent phospholipid in cells treated with increasing drug concentrations. Fluorescence is measured and normalized to cell number. Fluorescence is increased in cells treated with compounds that are known to cause PLD (chlorpromazine, tamoxifen, amiodarone), but it is not increased in cells treated with a compound that is known not to cause PLD (acetaminophen).

#### 5.2.4 High-content toxicology: The present and future of predictive toxicology

Drug safety is a major concern for the pharmaceutical industry with greater than 30% of drug candidates failing in clinical trials as a result of toxicity (3, 10). Furthermore, there are numerous examples of drugs which have been withdrawn from the market or given black box warnings as a result of side effects not identified in clinical trials. Developing and commercializing a drug is a large financial commitment and failure at this stage can be catastrophic for a company. To address this problem, there has been a significant drive to incorporate toxicity assessment at much earlier stages in the drug discovery and development process.

It is well recognized that animal models are often not reflective of human toxicity. This is corroborated by a large percentage of drugs fail in the clinic through toxicity despite having progressed through costly preclinical animal studies. Human hepatotoxicity, as well as hypersensitivity and cutaneous reactions, are particularly difficult to identify during regulatory-based animal studies. Only 50% of drugs found to be hepatotoxic in clinical studies showed concordance with animal toxicity results (39, 40). In addition, there are profound ethical issues associated with the widespread use of animals for this purpose. Initiatives such as ECVAM, ICCVAM and NC3Rs are currently addressing this problem by identifying alternatives to animal safety testing. The cosmetics industry is at the forefront and starting in 2013 there is an anticipated total EU ban of the sale of cosmetics tested on animals.

The introduction of more relevant and sophisticated *in vitro* human systems is essential to overcome these issues and will enable higher throughput screening assays to be implemented earlier and more cost effectively. The widespread use of *in vitro* methods has to some extent been hampered by their relatively poor predictive capability as traditionally only single markers of toxicity have been investigated. However, in many cases, drug toxicity is a highly complex process which can manifest itself *via* multiple different mechanisms. Predictions of toxicity can only be improved by investigating a broad panel of markers and their relationship to each other.

### 6. The power of high content screening

Technologies such as high content screening (HCS) have transformed cell biology and enabled subtle changes in multiple cellular processes to be tracked within the same cell population and well. The technique uses either fluorescently labeled antibodies or dyes to stain specific areas of the cell which have critical roles in cell health or the maintenance of cellular function. The impact of concentration-dependent and time-dependent drug exposure on these cellular processes can be investigated and related to specific toxicological or efficacious responses. The ability to analyze multiple end points simultaneously, yet selectively, is a major advantage and as well as being more sensitive, allows greater predictivity and an improved mechanistic understanding over traditional single endpoint measurements (41).

The power of HCS in toxicity assessment was illustrated in two key papers authored by scientists at Pfizer (41, 42) where a panel of 6 to 8 key toxicity markers were identified and used to predict human hepatotoxicity. The articles highlight the improved predictive power of HCS over existing conventional *in vitro* toxicity assays and over traditional preclinical animal tests. HCS technology is now routinely used for *in vitro* toxicity assessment in large pharmaceutical companies.

## 7. CellCiphr™ – bridging the gap between *in vitro* and *in vivo*

It is critical to link the *in vitro* HCS information to animal or human pathology and establish a relationship to the *in vivo* data. The patterns observed in the key toxicity markers are often characteristic of specific mechanisms of known pathology.

The CellCiphr™ system utilizes the powerful technique of HCS and combines this with a novel classifier system which is underpinned by a large database of information for drugs with known toxicological profiles. Using this system the toxicological profiles generated by HCS can be compared with known drugs for which animal or clinical data are available. Specific changes in cellular function observed for particular mechanisms of toxicity can then be recognized and used to predict for unknown NCEs.

It is well recognized that toxicological events can be organ specific, time-dependent and concentration-dependent. The CellCiphr™ system investigates three different panels which represent general cytotoxicity (using HepG2 cells) or organ specific toxicity (using primary rat hepatocytes as a model for hepatotoxicity or H9c2 cardiomyocytes as a model for cardiotoxicity). The panels have been validated for the most relevant parameters for each particular cell type. Depending on the panel, these include the following cell health parameters: cell cycle arrest, nuclear size, oxidative stress, stress kinase activation, DNA damage response, DNA fragmentation, mitochondrial potential and mass, mitosis marker, cytoskeletal disruption, apoptosis, steatosis, phospholipidosis, ROS generation, hypertrophy and general cell loss. To assess early and late stage toxic responses, CellCiphr™ investigates exposure at 3 different time points. Dose dependent effects are investigated by exposing the cells to 10 different concentrations of the compound.

Data are represented as AC<sub>50</sub> (concentration at which average response is 50% of control activity) for each cell health parameter, and the collection of AC<sub>50</sub> values over the entire cell feature set comprises the response profile. Proprietary visual and quantitative data mining tools including CellCiphr™ Classifiers, correlation analysis and cluster analysis are used to analyze the profiles (Figure 6). Using the CellCiphr™ approach, there are a number of different ways by which the data can be interpreted.

1. Similarity profile plots can identify potential mechanisms of actions by correlating unknown test compound response with known control compounds where the mechanisms of action are already known.
2. The relative toxicity of compounds in a series can be predicted by the CellCiphr™ Classifier and used to rank compounds for prioritization of the most promising candidates.
3. The most potent or earliest cellular response can be extracted from the data which may highlight the optimal endpoint(s) for designing higher throughput systems to investigate SAR within a series.

Detailed mechanistic data can be generated for specific compounds. In the case of nimesulide which has been withdrawn from the market for severe hepatotoxicity, the CellCiphr™ Hepatotoxicity Profiling Panel scored this drug as the most toxic of the nonsteroidal anti-inflammatory drugs (NSAIDs). The toxicity was associated with a specific mechanistic profile characterized by an early oxidative stress response captured as a decreased mitochondrial membrane potential after 1 hour of exposure. This insult drives the development of an apoptotic response at subsequent time points measured in the release of cytochrome C from the mitochondria and activation of the DNA damage response. Finally, prolonged exposure to nimesulide is marked by the accumulation of lipids in lysosomes and other vesicles (Figure 7). The early effect on cell loss may also indicate a necrotic response in addition to apoptosis.

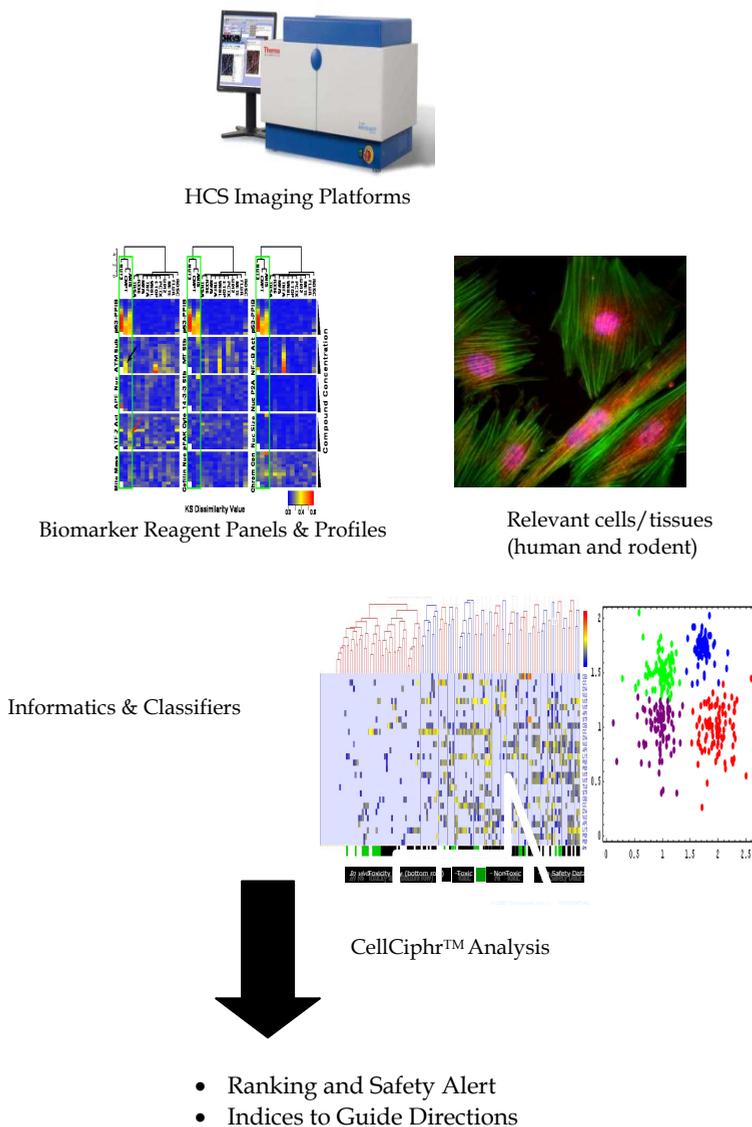


Fig. 6. The CellCiphr™ system uses HCS imaging platforms to identify specific patterns of biomarker response following exposure to multiple concentrations of compound at multiple time points. Proprietary visual and quantitative data mining tools have been developed to analyse the profiles and compare unknown compound response against known *in vivo* pathology.

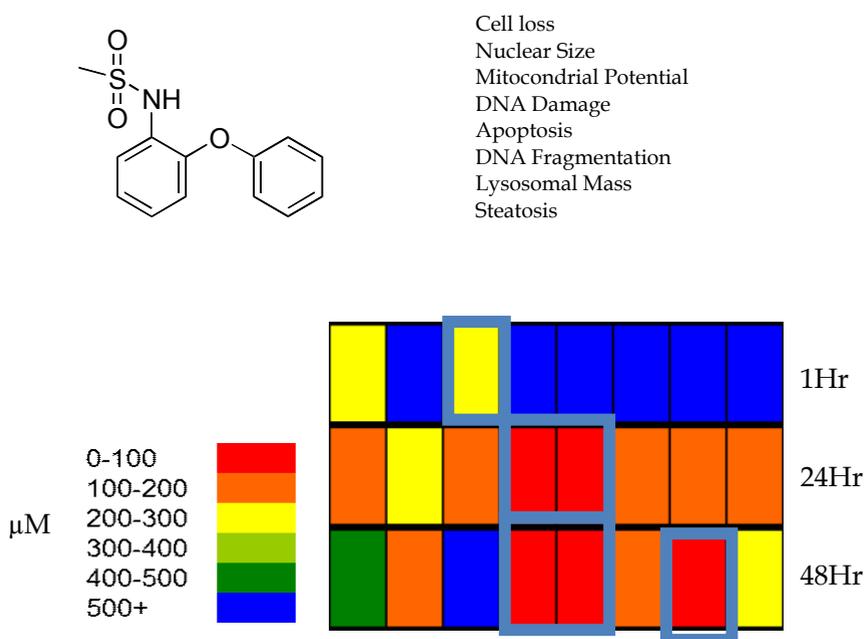


Fig. 7. Nimesulide, which has been withdrawn from the market in several countries over concerns of severe hepatotoxicity, was scored as the most toxic of the NSAIDs by the CellCiphr™ Hepatotoxicity Profiling Panel. The toxicity is associated with a specific mechanistic profile at sub-lethal doses.

### 7.1 Future strategies

In summary, CellCiphr™ is shown here as an example of a novel approach which identifies time dependent, sub-lethal effects on cell health and function. The system illustrates significant improvements over existing single end point assays and has the ability to predict mechanistic outcomes by correlating with known compound profiles and pathology. By expanding the CellCiphr™ database, improving bioinformatics platform and increasing the number of panels to cover new organ specific cells, it will continue to improve the reliability of the classification. Toxicological response is influenced by many factors including dose administration (including tissue exposure levels), time of exposure and/or accumulation in specific cells. Many of these factors are influenced by the pharmacokinetics of the drug administered and its effect on absorption, distribution, metabolism and excretion. Considering ADME data in conjunction with multiparametric measurements of *in vitro* toxicology is likely to be an important consideration in the future direction of predictive toxicology. Incorporation of human PK parameters prediction in models such as CellCiphr to ensure that cytotoxicity is relevant to projected tissue exposure is actively being pursued.

## 8. Genotoxicity

Genotoxicity of drugs is an important concern to the regulatory authorities. The FDA recommends a number of *in vitro* and *in vivo* tests to measure the mutagenic potential of

chemical compounds, including the Ames test in *Salmonella typhimurium* (43). GreenScreen GC, a new, high-throughput assay that links the regulation of the human GADD45a gene to the production of Green Fluorescent Protein (GFP) has become available. The assay relies on the DNA damage-induced up-regulation of the RAD54 gene in yeast measured using a promoter-GFP fusion reporter (44). The test is more specific and sensitive for genotoxicity than those currently recommended by the FDA such as the Ames and mouse lymphoma tests.

## 9. Current challenges and future directions

A large amount of progress in the field of ADMET profiling has occurred in the last 15 years. This progress has decreased the proportion of drug candidates failing in clinical trials for ADME reasons providing optimism in an otherwise declining productivity in drug discovery. The principal barrier now is the toxicity portion of ADMET. The prediction of human-specific toxicology must be improved.

Cell-based assays using established cell lines and co-cultures have been used to determine toxicity to various organs, but many of these cell lines have lost some of the physiological activities present in normal cells. HepG2 cells, for instance, have greatly reduced levels of metabolic enzymes. Primary human hepatocytes can be used but are expensive, suffer from high donor-to-donor variability, and maintain their characteristics for only a short time. Three-dimensional models have been developed for cell-based therapies including micropatterned co-cultures of human liver cells that maintain the phenotypic functions of the human liver for several weeks (45). This development should provide more accurate information about toxicity when used in ADMET screening and could be extended to other organ-specific cells leading to integrated tissue models in the "human on a chip" (46). The potential of stem cells to differentiate into cell lines of many different lineages may be exploited to develop human and animal stem-cell-derived systems for major organ systems (47).

High content screening (HCS) has been used for early cytotoxicity measurement since 2006 and provides great optimism (41). This method has been optimized for hepatocytes and is more predictive of hepatotoxicity than other currently available methods and in the future could be applied to cells of other organs.

Molecular profiling is another alternative and is defined as any combination or individual application of mRNA expression, proteomic, toxicogenomic, or metabolomic measurements that characterize the state of a tissue (48). This approach has been applied in an attempt to develop profiles or signatures of certain toxicities. Molecular profiles, in conjunction with agents that specifically perturb cellular systems, have been used to identify patterns of changes in gene expression and other parameters at sub-toxic drug concentrations that might be predictive of hepatotoxicity including idiosyncratic hepatotoxicity (49). In the future, larger data sets, high-throughput gene disruptions, and more-diverse profiling data will lead to more-detailed knowledge of disease pathways and will facilitate in target selection and the construction of detailed models of cellular systems for use in ADMET screening to identify toxic compounds early in the discovery process. The combination of *in silico*, *in vitro*, and *in vivo* methods and models into multiple content data bases, data mining, and predictive modeling algorithms, visualization tools, and high-throughput data-analysis solutions can be integrated to predict systems' ADMET properties. Such models are starting to be built and should be widely available within 10 years (50). The use of these tools will lead to a greater understanding of the interactions of drugs with their targets and predict their toxicities.

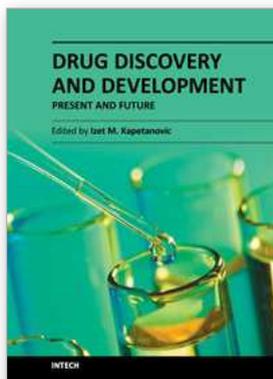
To conclude, the future should provide a decrease in late-stage development failures and withdrawals of marketed drugs, faster timelines from discovery to market, and reduced development costs through the reduction of late-stage failures.

## 10. References

- [1] Kaitin KI. (2008). Obstacles and opportunities in new drug development. *Clinical Pharmacology & Therapeutics* 83, 210-212.
- [2] DiMasi, J.A., Hansen, R.W. & Grabowski, H.G. (2003). The price of innovation: new estimates of drug development costs. *Journal of Health Economics* 22, 151-185.
- [3] Kola, I. & Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nature Reviews Drug Discovery* 3,711-715.
- [4] Honig, P.K., Woosley, R.L., Zamani, K., Conner, D.P. & Cantilena, L.R., Jr. (1992). Changes in the pharmacokinetics and electrocardiographic pharmacodynamics of terfenadine with concomitant administration of erythromycin. *Clinical Pharmacology & Therapeutics* 52, 231-238.
- [5] Honig, P.K., Wortham, D.C., Zamani, K., Conner, D.P., Mullin, J.C. & Cantilena, L.R. (1993). Terfenadine-ketoconazole interaction. Pharmacokinetic and electrocardiographic consequences. *Journal of the American Medical Association* 269, 1513-1518.
- [6] US Food and Drug Administration. (1998). Seldane and generic terfenadine withdrawn from market. FDA Talk Paper T98-10. Available at <http://www.fda.gov/bbs/topics/answers/ans00853.html>.
- [7] Ball, P., Mandell, L., Niki, Y. & Tillotson, G. (1999). Comparative tolerability of the newer fluoroquinolone antibacterials. *Drug Safety* 21, 407-421.
- [8] US Food and Drug Administration. Public Health Advisory. Trovan (trovafloxacin/alatrofloxacin mesylate). June 9, 1999. Available at <http://www.fda.gov/cder/news/trovan>.
- [9] Stephens, J. (May 7, 2006). "Panel Faults Pfizer in '96 Clinical Trial In Nigeria". *The Washington Post*: p. A01. Available at <http://www.washingtonpost.com/wp-dyn/content/article/2006/05/06/AR2006050601338.html>.
- [10] Kubinyi, H. (2003). Drug research: myths, hype and reality. *Nature Reviews Drug Discovery* 2, 665-668.
- [11] Schuster, D., Laggner, C. & Langer, T. (2005). Why drugs fail - a study on side effects in new chemical entities. *Current Pharmaceutical Design* 11, 3545-3559.
- [12] Lipinski, C.A., Lombardo F., Dominy, B.W. & Feeney, P.J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 23, 3-25.
- [13] Lipinski, C.A. (2000). Drug-like properties and the causes of poor solubility and poor permeability. *Journal of Pharmacological and Toxicological Methods* 44, 235-249.
- [14] Schinkel, A.H., Smit, J.J., van Tellingen, O., Beijnen J.H., Wagenaar, E., van Deemter, L., Mol, C.A., van der Valk, M.A., Robanus-Maandag, E.C., te Riele H.P., Berns, A.J.M., & Borst, P. (1994). Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 77(4), 491-502.
- [15] Schinkel, R.S. & Minn, A. (1999). Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain. *Cell Mol Biol* 45, 15-23.
- [16] Hurko, O. & Ryan, J.L. (2005). Translational research in central nervous system drug discovery. *NeuroRx* 2(4), 671-82.
- [17] Liu, X. & Chen, C. (2005). Strategies to optimize brain penetration in drug discovery. *Curr Opin Drug Discov Devel* 8(4), 505-12.

- [18] Megard, I., Garrigues, A., Orlowski, S., Jorajuria, S., Clayette, P., Ezan, E., Mabondzo, A. (2002). A co-culture-based model of human blood-brain barrier: application to active transport of indinavir and in vivo-in vitro correlation. *Brain Res* 927(2), 153-67.
- [19] Begley, D.J., Lechardeur, D., Chen, Z.D., Rollinson, C., Bardoul, M., Roux, F., Scherman, D., Abbott, N.J. (1996). Functional expression of P-glycoprotein in an immortalised cell line of rat brain endothelial cells, RBE4. *J Neurochem* 67, 988-95.
- [20] Deli, M.A., Abraham, C.S., Kataoka, Y., Niwa, M. (2005). Permeability studies on *in vitro* blood-brain barrier models: physiology, pathology and pharmacology. *Cell Mol Neurobiol* 25, 59-120.
- [21] Crone, C. (1963). The Permeability of Capillaries in Various Organs as Determined by Use of the 'Indicator Diffusion' Method. *Acta Physiol Scand* 58, 292-305.
- [22] Oldendorf, W. H. (1970). Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res* 24(2), 372-6.
- [23] Takasato, Y., Rapoport, S.I. & Smith, Q.R. (1984). An *in situ* brain perfusion technique to study cerebrovascular transport in the rat. *Am J Physiol* 247(3 Pt 2), H484-93.
- [24] Kakee, A., Terasaki, T., Sugiyama, Y. (1996). Brain efflux index as a novel method of analyzing efflux transport at the blood-brain barrier. *J Pharmacol Exp Ther* 277(3), 1550-9.
- [25] Elmquist, W.F. & Sawchuk, R.J. (1997). Application of microdialysis in pharmacokinetic studies. *Pharm Res* 14(3), 267-88.
- [26] Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Van Deun, K., Smith, Berger P.B., & Heller, A. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology* 32, 56-67.
- [27] US Food and Drug Administration Website. Guidance for Industry, Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling September, 2006 Available at: <http://www.fda.gov/CDER/guidance/6695dft>.
- [28] Uetrecht, J. (2008). Idiosyncratic drug reactions: past, present, and future. *Chemical Research in Toxicology* 21, 84-92.
- [29] Jossierand, V., Pélerin, H., de Bruin, B., Jego, B., Kuhnast, B., Hinnen, F., Ducongé, F., Boisgard, R., Beuvon, F., Chassoux, F., Daumas-Duport, C., Ezan, E., Dollé, F., Mabondzo, A., Tavitian, B. (2006). Evaluation of drug penetration into the brain: a double study by *in vivo* imaging with positron emission tomography and using an *in vitro* model of the human blood-brain barrier. *J Pharmacol Exp Ther* Jan 316(1), 79-86.
- [30] Pardridge, W.M., Triguero, D., Yang, J. & Cancilla, P.A. (1990). Comparison of *in vitro* and *in vivo* models of drug transcytosis through the blood-brain barrier. *J Pharmacol Exp Ther* 253(2), 884-91.
- [31] Jacewicz, M., Guyot, A.-C., Annand, R., Gilbert, J., Mabondzo, A., Tsaïoun, K. Contribution of Transporters to Permeability Across Cell Monolayers. Comparison of Three Models. Apredica, CEA. American Association of Pharmaceutical Scientists, poster presentation, Atlanta, GA, November 2008
- [32] Dykens, J.A. & Will, Y. (2007). The significance of mitochondrial testing in drug development. *Drug Discovery Today* 12,777-785.
- [33] Rodríguez-Enríquez, S., Juárez, O., Rodríguez-Zavala, J.S., & Moreno-Sánchez, R. (2001). Multisite control of the Crabtree effect in ascites hepatoma cells. *European Journal of Biochemistry* 268, 2512-2519.
- [34] Marroquin, L.D., Hynes, J., Dykens, J.A., Jamieson, J.D. & Will, Y. (2007). Circumventing the Crabtree effect: Replacing media glucose with galactose

- increases susceptibility of HepG2 cells to mitochondrial toxicants. *Toxicological Sciences* 97, 539-547.
- [35] Williams, D.P. & Park, B.K. (2003) Idiosyncratic toxicity: the role of toxicophores and bioactivation. *Drug Discovery Today* 8, 1044-1050.
- [36] Liu Z.X. & Kaplowitz, N. (2006). Role of innate immunity in acetaminophen-induced hepatotoxicity. *Expert Opinion on Drug Metabolism & Toxicology* 2, 493-503.
- [37] Ademuyiwa, O., Agarwal, R., Chandra, R., and Behari, J.R. (2009). Lead-induced phospholipidosis and cholesterologenesis in rat tissues. *Chemical & Biological Interactions* 179, 314-320.
- [38] Natalie, M., Margino, S., Erik, H., Annelieke, P., Geert, V. & Philippe, V. (2009). A 96-well flow cytometric screening assay for detecting in vitro phospholipidosis-induction in the drug discovery phase. *Toxicology In Vitro* 23, 217-226.
- [39] Olson, H., Betton, G., Stritar, J. & Robinson, D. (1998). The predictivity of the toxicity of pharmaceuticals in humans from animal data - an interim assessment. *Toxicol Lett* 102-103, 535-538.
- [40] Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Doratoi, M., Van Deun, K., Smith, P., Berger, B. & Heller, A. (2000) Concordance of Toxicity of Pharmaceuticals in Humans and Animals. *Regul Toxicol Pharmacol* 32, 56-67.
- [41] O'Brien, P.J., Irwin, W., Diaz, D., Howard-Cofield, E., Krejsa, C.M., Slaughter, M.R., Gao, B., Kaludercic, N., Angeline, A., Bernardi, P., Brain, P. & Hougham, C. (2006). High concordance of drug-induced human hepatotoxicity with in vitro cytotoxicity measured in a novel cell-based model using high content screening. *Arch Toxicol* 80, 580-604.
- [42] Xu, J.J., Henstock, P.V., Dunn, M.C., Smith, A.R., Chabot, J.R. & de Graaf, D. (2008). Cellular Imaging Predictions of Clinical Drug-Induced Liver Injury. *Toxicol Sci* 105(1), 97-105.
- [43] US Food and Drug Administration Guidance for Industry. S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. Available at: <http://www.fda.gov/CDER/guidance/1856fnl>.
- [44] Hastwell, P.W., Chai, L.-L., Roberts, K.J., Webster T.W., Harvey J.S., Rees R.W., & Walmsley, R.M. (2006). High-specificity and high-sensitivity genotoxicity assessment in a human cell line: Validation of the GreenScreen HC GADD45a-GFP genotoxicity assay. *Mutation Research* 607, 160-175.
- [45] Khetani, S.R. & Bhatia, S.N. (2008). Microscale culture of human liver cells for drug development. *Nature Biotechnology* 26, 120-126.
- [46] Viravaidya, K. & Shuler, M.L. (2004). Incorporation of 3T3-L1 cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies. *Biotechnology Progress* 20, 590-597.
- [47] Yang, L., Soonpaa, M.H., Adler, E.D., Roepke, T.K., Kattman, S.J., Kennedy, M., Henckaerts, E., Bonham, K., Abbott, G.W., Linden, R.M., Field, L.J. & Keller, G.M. (2008). Human cardiovascular progenitor cells develop from a KDR<sup>+</sup> embryonic-stem-cell-derived population. *Nature* 453, 524-529.
- [48] Stoughton, R.B. & Friend, S.H. (2005). How molecular profiling could revolutionize drug discovery. *Nature Reviews Drug Discovery* 4, 345-350.
- [49] Kaplowitz, N. (2005). Idiosyncratic drug hepatotoxicity. *Nature Reviews Drug Discovery* 4, 489-499.
- [50] Ekins, S., Nikolsky, Y. & Nikolskaya, T. (2005). Techniques: application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends in Pharmacological Sciences* 26, 202-209.



## **Drug Discovery and Development - Present and Future**

Edited by Dr. Izet Kapetanović

ISBN 978-953-307-615-7

Hard cover, 528 pages

**Publisher** InTech

**Published online** 16, December, 2011

**Published in print edition** December, 2011

Drug discovery and development process aims to make available medications that are safe and effective in improving the length and quality of life and relieving pain and suffering. However, the process is very complex, time consuming, resource intensive, requiring multi-disciplinary expertise and innovative approaches. There is a growing urgency to identify and develop more effective, efficient, and expedient ways to bring safe and effective products to the market. The drug discovery and development process relies on the utilization of relevant and robust tools, methods, models, and validated biomarkers that are predictive of clinical effects in terms of diagnosis, prevention, therapy, and prognosis. There is a growing emphasis on translational research, a bidirectional bench to the bedside approach, in an effort to improve the process efficiency and the need for further innovations. The authors in the book discuss the current and evolving state of drug discovery and development.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Katya Tsaïoun and Steven A. Kates (2011). De-Risking Drug Discovery Programmes Early with ADMET, Drug Discovery and Development - Present and Future, Dr. Izet Kapetanović (Ed.), ISBN: 978-953-307-615-7, InTech, Available from: <http://www.intechopen.com/books/drug-discovery-and-development-present-and-future/de-risking-drug-discovery-programmes-early-with-admet>

# **INTECH**

open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.