Minireview

Drug discovery and development: Biomarkers of neurotoxicity and neurodegeneration

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Impact statement

Attrition in drug discovery and development remains a major challenge. Safety/toxicity is the most prevalent reason for failure with cardiovascular and CNS toxicities predominating. Non-invasive biomarkers of neurotoxicity would provide significant advantage by allowing earlier prediction of likely neurotoxicity in preclinical studies as well as facilitating clinical trials of new therapies for neurodegenerative conditions such as Parkinson's disease (PD) and multiple sclerosis (MS).

Abstract

The discovery and development of new drugs are vital if we are to improve and expand treatment options available to improve outcomes for patients. Overall, therapeutic strategies fall into two broad categories: small molecules and biologics, although more recently there has been a growth in novel platforms such as miRNAs and oligonucleotides. On average, the development of a small molecule drug takes around 12 years and costs around $50m. Despite this huge investment of time and money, attrition remains a major challenge and very few molecules actually make it through to the market. Here, we look at reasons for attrition in the small molecule field with a focus on neurotoxicology and efforts being made to improve success via the development of imaging and fluidic biomarkers. We also look at learnings from other models of CNS damage and degeneration such as Parkinson's disease, traumatic brain injury, and multiple sclerosis since these may offer the opportunity to improve tools available to nonclinical toxicologists in the early detection of potential neurotoxicity. Reciprocally, learnings from studies of animal neurotoxicity may offer better ways to potentially monitor patients during clinical development of new drugs for neurodegeneration.

Keywords: Biomarkers, central nervous system, drug development, drug safety, neurodegeneration, toxicology

Drug discovery and development: An overview

Small molecule drug discovery begins with the selection of a target based on linkage of the target with disease, target expression across tissue and species and likely 'drugability' of the target (Figure 1). Also important at this stage is a target safety assessment (TSA) to characterize the potential for unwanted side effects of target inhibition or activation.[1,2] Once a target is selected, then the search for chemistry that can interact with the target begins usually via high-throughput screening (HTS) of chemical collections containing millions of molecules. Options are narrowed down via exploration of the impact of chemical modification both on potency/selectivity and also on other key parameters such as solubility/partitioning. This is the optimum time to run early safety screens such as genetic toxicology and the potential to form reactive metabolites, since any liabilities can be designed out in an iterative design-make-test cycle. This also applies to removing liability associated with hERG (human Ether-à-go-go-Related Gene) since inhibition of potassium currents through this ion channel is linked to a potentially fatal cardiac arrhythmia called Torsades de Pointes. Ultimately, one molecule is selected to go forward for GLP toxicology testing. This is an expensive phase of drug development, costing on average $7m/molecule[3] and is pivotal in generating the data to ensure the safety of patients and volunteers is protected in the first time in human (FTIH) clinical trials.
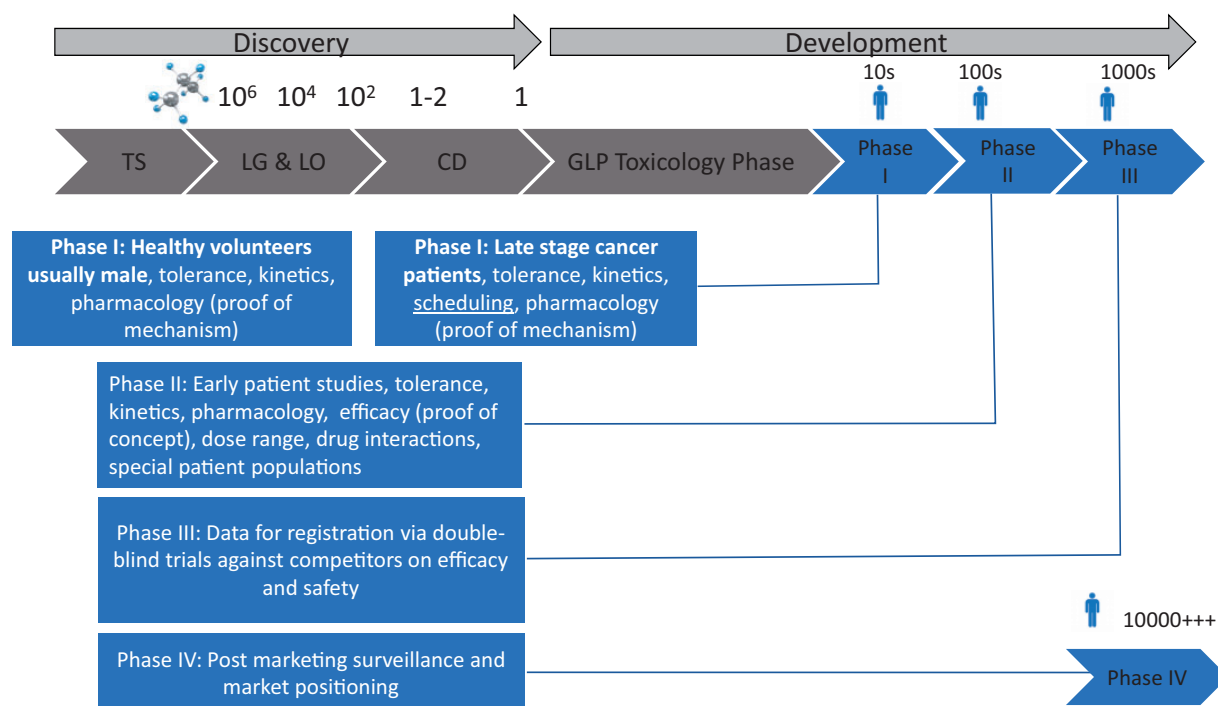
Figure 1. The drug discovery and development paradigm for small molecules. Target selection (TS) is followed by lead generation (LG) and lead optimization (LO). One or two candidate drugs (CDs) are selected to begin more extensive *in vitro* and *in vivo* testing, providing the data to select one molecule to go forward for good laboratory practice (GLP) toxicology testing in support of first time in human (FTIH) clinical trials. (A color version of this figure is available in the online journal.)

The majority of FTIH clinical trials are conducted in healthy male volunteers and are aimed at establishing tolerance, kinetics, pharmacology (proof of mechanism) and offer the opportunity to detect early signals of potential efficacy. Small doses that would not be expected to cause any adverse outcome are used. For drugs aimed at treating life-threatening conditions such as cancer, phase I is conducted in late stage cancer patients where scheduling is also studied alongside tolerance, kinetics, pharmacology and potential early signals of efficacy. For most cancer drugs, even low doses are likely to be associated with toxicities; hence, it would be unacceptable to expose healthy volunteers.

Phase II takes place in groups of usually 100–500 patients and builds on earlier data on tolerance, kinetics and pharmacology to gain data on efficacy (proof of concept), dose range, and drug interactions. Phase III typically studies thousands of patients and is aimed at generating the data for registration via double-blind trials against current standard of care looking at detailed measurements of efficacy and safety in a broader population.

Navigating the regulatory framework

The typical pattern of drug discovery and development is supported by a regulatory framework that aims to standardize the data sets needed to support each transition to ensure maximum efficiency while ensuring patient and volunteer safety is protected. Briefly, specific International Council for Harmonisation (ICH) guidelines[4] specify the different areas of testing to be undertaken to create the 'FTIH package' that is normally required for Phase 1

clinical trials, wherever they occur in the world (US, Europe, Japan, China, South America, India, etc.) (Figure 2).

In general toxicology, the FTIH decision is supported by two species toxicology testing, usually in the rat and dog. A maximum tolerated dose (MTD) is established followed by a period (7–14 days) of repeat dosing to ensure the proposed MTD can be sustained over the usual one-month period of testing (Figure 2). A low dose is then chosen that is a likely no effect level and a mid-dose is chosen to give a dose response. In Europe, the start of the nonrodent studies is usually slightly staggered in case of any unexpected issues.

In safety and secondary pharmacology, unwanted effects of the compounds are studied in a growing panel of likely unwanted targets (secondary pharmacology), starting with around 20 and building to >300 receptors, kinases, ion channels and others as the compound approaches FTIH. At this preclinical stage, predicted margins to the intended target are used to guide chemistry towards efficacy and away from probable unwanted off-target effects. Safety pharmacology addresses the safety endpoints associated with the drug's pharmacology in a so called 'core battery' that looks at the cardiovascular system (heart rate, blood pressure), CNS (locomotion, reflexes, pain threshold, seizure) and respiratory system.

Genetic toxicology and carcinogenicity look at the potential of new drugs to cause cancer either through direct damage to DNA or via non-genotoxic mechanisms. A sequence of *in silico*, *in vitro*, and *in vivo* tests are used to detect and eliminate DNA-damaging molecules wherever possible. Generally, a positive in one of these assays (i.e. the compound damages DNA) would be a stop for a
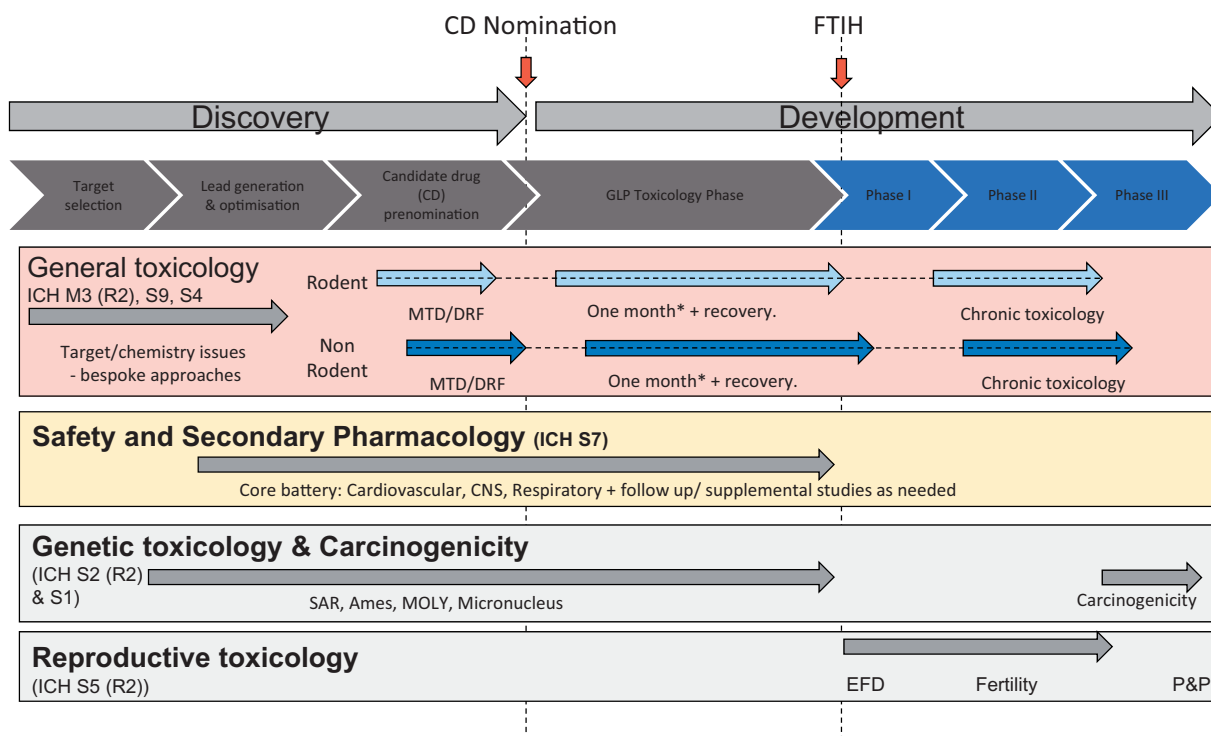
Figure 2. Navigating the regulatory framework. International Council for Harmonisation (ICH) guidelines specify the different areas of testing to be undertaken to create the 'FTIH package'. Testing is structured as general toxicology, safety and secondary pharmacology, genetic toxicology/carcinogenicity and developmental/reproductive toxicology. CD: candidate drug; CNS: central nervous system; DRF: dose range finding; EFD: embryo fetal development; FTIH: first time in human; GLP: good laboratory practice; LG/LO: lead generation/lead optimization; ICH: International Council for Harmonization; MOLY: mouse lymphoma; MTD: maximum tolerated dose; P&P: peri and post-natal; SAR: structure activity relationship; TS: target selection. *Could be different duration or cyclical dosing depending on clinical plan. (A color version of this figure is available in the online journal.)

compound unless interaction with DNA is key to efficacy, as expected with some anti-cancer drugs. Drugs that are negative in genetic toxicology testing can progress through phase I and phase II clinical trials and into phase III for some types of treatment.

Beyond FTIH, chronic toxicology studies of >3 months are generally needed to support longer term clinical dosing, since for conventional development of pharmaceuticals, the clinical trial duration cannot exceed the duration of the toxicology cover (Figure 3). This is not the case for oncology drug development; there are also other key differences in the approach to conventional versus oncology drug development especially around starting and limit doses and the need for genetic toxicology and carcinogenicity testing (Figure 3). However, if chronic dosing is intended then carcinogenicity testing is normally required for a marketing authority authorization (MAA) (Figure 3).

Although the toxicology studies conducted to support entry to phase I clinical trials generally follow guidelines, these guidelines are open to interpretation using good science and sound decision making. Also notable is that the probability of success of drug projects can be considerably enhanced by early, bespoke science aimed at derisking target and chemistry. A TSA would provide a thorough review of the likely unintended consequences of inhibiting or activating a specific target and should be used alongside the traditional thorough understanding of target biology and disease linkage.[1,2] Derisking chemistry would focus on eliminating obvious risks such as genetic toxicology

and functional interaction with ion channels associated with cardiovascular liability such as hERG.[5] It is vital that these assays are performed early in the lead optimization process while there is still choice in chemistry and in a time frame compatible with the design-make-test cycle that is a key part of lead generation, lead optimization and candidate selection.

Attrition in drug discovery and development: Prevalence of neurotoxicity

Safety-related attrition remains a major issue in drug discovery and development. The most frequent reason for candidate drugs falling out of development is cardiovascular risk; much has been done to address this over recent years with huge investments in understanding the molecular basis for arrythmia leading to the advent of hERG screening[5] and the more recent CiPA initiative.[6] However, failure due to CNS toxicity is also a predominant occurrence; Figure 4 (derived from data in an analysis of reasons for failure in the AstraZeneca portfolio[7]) shows that CNS toxicity accounts for nearly one-quarter of failures across the whole spectrum of discovery and development (Figure 4(b)); however, it is a relatively infrequent finding during GLP toxicology (Figure 4(a)).[3] As highlighted in Figure 4(c), this puts the burden of failures into clinical development where consequences are higher in terms of resources and patient impact. Interestingly, CNS toxicity peaks in drugs intended for CNS indications (Figure 4(d))

	M3	**S9**
Locator	ICH http://www.ich.org ICH Topic M 3 (R2) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals	ICH http://www.ich.org ICH Topic S 9 Nonclinical Evaluation for Anticancer Pharmaceuticals
Includes	'conventional development of pharmaceuticals and should be regarded as providing general guidance for drug development.'	'only intended to treat cancer patients with late stage or advanced disease...'
Starting doses, escalation and limits	'based on NOAEL modified by various factors' Stopping dose based on PK limit in toxicology studies	'dose escalation or highest dose should not be limited by the highest dose tested in the nonclinical studies' - starting dose based on toxic doses to avoid being too low
Duration	Clinical trial duration cannot go beyond duration of toxicology cover	Clinical trial duration can go beyond duration of toxicology cover
Differences	Requires genetic toxicology testing Requires carcinogenicity testing generally for a MAA depending on duration of clinical use	'carcinogenicity not warranted' 'genetic toxicology not warranted'
Common Aim:	Safety in clinical trials - Safety once on the market Provide what's required for the MAA Provide information for the label	

Figure 3. A comparison of key aspects of ICH M3 and ICH S9. See text for details. PK: pharmacokinetics; MAA: marketing authority authorization. (A color version of this figure is available in the online journal.)
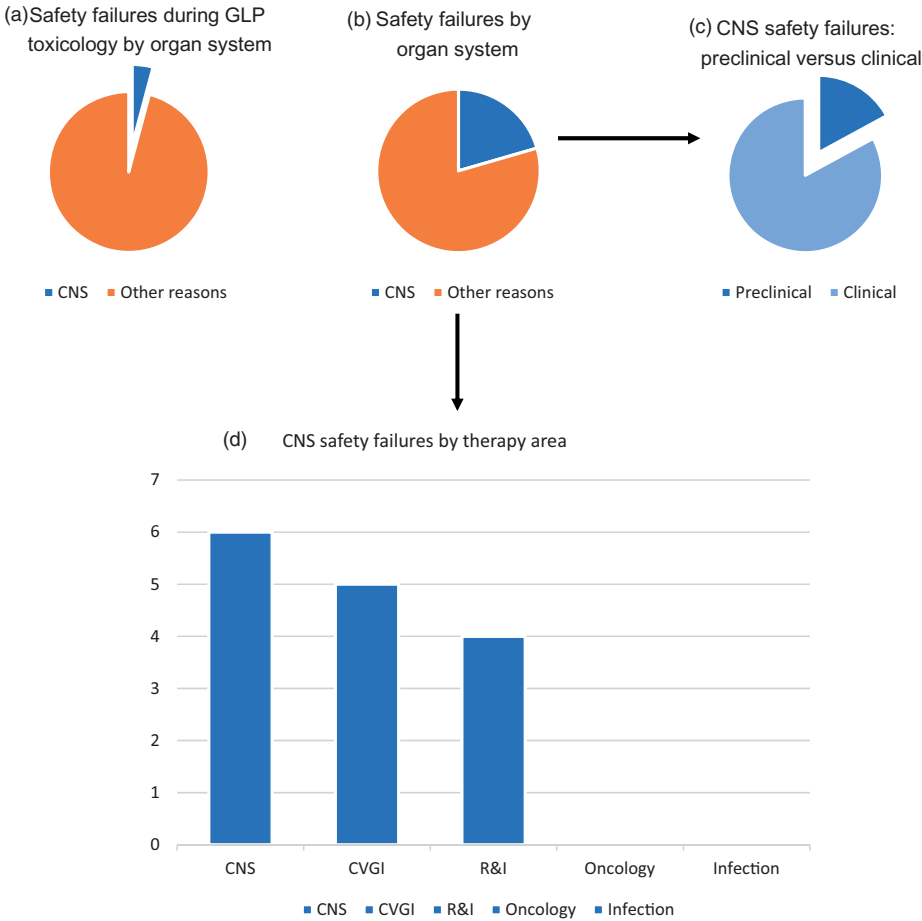

Figure 4. An analysis of reasons for attrition in drug development. (a) Safety failures during GLP toxicology testing show that CNS toxicity is infrequent. (b) Safety failures across all discovery and development stages demonstrate that CNS accounts for almost 25% of failures. (c) Clinical failures predominate over preclinical failures. The CNS therapy area predominates in the overall failure profile due to CNS toxicity but CVGI and R&I are also impacted. For original data see Cook et al.[7] (A color version of this figure is available in the online journal.)

possibly because CNS drugs are deliberately CNS penetrant, whereas this property is often avoided for other indications if at all possible. However, CNS toxicity is also frequent in the cardiovascular and gastrointestinal (CVGI) therapy areas.[7] A related study that looked at attrition data for a broader compound set from AstraZeneca, Ely Lilly, GlaxoSmithKline and Pfizer confirmed that nonclinical toxicology was the primary cause for failure in drug discovery and development.[8] However, this paper did not provide an analysis of target organ systems so cannot be used to validate or refute the AstraZeneca findings on relative frequency of CNS findings.

Neurotoxicities noted in registered drugs

As well as the issue of attrition due to neurotoxicity, many registered medicines also carry so called 'black box warnings' of neurotoxicity, a name taken from the black border around the labeling information intended to alert consumers and healthcare professionals to potential risks. The recent publication of FDALabel[9] allows an analysis of the most frequent neurotoxicities noted in NDAs. Of the around 37,000 human prescription drugs included in FDALabel, around 400 carry black box warnings for neurotoxicity and related issues. The most frequent findings were suicidal ideation and sedation followed by abuse liability, seizure/convulsion, and headache (Figure 5).

Categories of neurotoxicity: Challenges for detection and prediction

As highlighted by data on attrition and as evidenced by black box warnings for registered medicines, neurotoxicity remains a major issue in drug discovery and development. However, when considering the utility of current and future potential methods of detection, prediction, and clinical monitoring, it is important to distinguish structural from functional neurotoxicity. Broadly speaking, structural neurotoxicity is associated with tissue damage, whereas functional neurotoxicity may be associated with electrical activity (seizure/convulsions) or could be a manifestation of perturbations in higher brain function such as depression or suicidal ideation. Because of the different

Figure 5. Incidence of neurotoxicities reported in the FDALabel database.[9] FDALabel provides a concise overview of US Food and Drug Administration (FDA) drug labeling, which details drug products, drug–drug interactions, adverse drug reactions (ADRs) and contains a set of approximately 80,000 data labels. (A color version of this figure is available in the online journal.)

methodologies and endpoints studied in animal studies versus the clinic, structural damage to the CNS detected by histopathology or functional endpoints such as seizure form the majority of neurotoxicities reported in rodent and nonrodent toxicology studies. In contrast, in the clinic, the majority of neurotoxicities are functional in nature (suicidal ideation, depression, headache) with the exception of retinal/ocular toxicity, a relatively infrequent finding (2/400)[9] but one with clear structural correlates.

Current and emerging approaches to detect neurotoxicity and abnormal neurological function

Functional neurotoxicity has major challenges for detection in any model system. Issues such as abuse liability and suicidal ideation are especially challenging but other toxicities such as sedation and seizure may be more amenable to earlier detection. Current methods usually rely on observations made in the nonclinical rodent and non-rodent studies required to support clinical trials. These could be central nervous system (CNS)-related signs such as tremors or other abnormal movements, but these signs can be misdiagnosed or misinterpreted by inexperienced operators. Thus, confirmation of drug-induced seizure or seizure-like activity requires a follow-up electroencephalogram (EEG) study. Some progress has been made in in-life detection of seizure using automated video systems that record and analyze animal movements, looking for abnormalities. Nonetheless, it would be far preferable to have an earlier prediction of seizurogenic risk that could be used to eliminate liabilities early in discovery, while there are still options in chemistry. Early identification of these risks using cheaper, higher throughput and more predictive assays that both reduce and/or avoid animal use and have lower compound requirements would be preferable.

Over the last decade, several assays that are compatible with the design-make-test-analyze cycle of drug discovery have become available. Regarding seizure, two approaches offer exciting opportunities: microelectrode array is now able to detect seizurogenic signals in iPSC-derived Cortical Neural Stem Cells differentiated to electrically active cortical neurons. This offers great potential to screen for seizurogenic liability in an *in vitro* system. A second approach could be based on an understanding of the neuronal ion channels implicated in the seizurogenic response. Recently, some progress has been made in developing these *in vitro* seizure models and there is a developing interest in characterizing the ion channels both at the expressional and at the functional level. However, much of the current research on avoiding neurotoxicity is focused on structural endpoints since the associated tissue damage provides more opportunity for detection via fluidic biomarkers and imaging.

Detection of neurotoxicity: Development of fluidic biomarkers

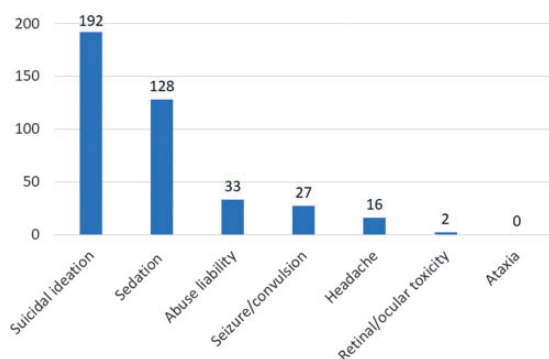Back in 2012, the Health and Environmental Sciences Institute (HESI)[10] initiated a project to enhance preclinical

detection of CNS toxicity. The development of biomarkers of neurotoxicity is a goal shared by scientists across academia, government, and industry and as such was an ideal topic to be addressed via HESI. The project goal was to determine if there are more sensitive and specific biomarkers that could help diagnose and predict neurotoxicity. These biomarkers would also be of additional use if they were relevant across animal models and also could be translated from nonclinical to clinical data. Additionally, it is relatively easy to sample fluid-based biomarkers in serum, plasma, urine, and cerebrospinal fluid (CSF) compared with taking tissues.

The HESI Biomarkers of Neurotoxicology Committee (NeuTox)[11] met on several occasions to define scope and to propose an experimental model to address the challenge. Several experimental models were considered, but on balance the committee selected trimethyl tin (TMT) in rat for a variety of reasons; the rat is the rodent species of choice in preclinical testing and the lesion induced in the rat hippocampus by TMT is well characterized.[12] The prodrug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was also considered; MPTP is a prodrug to the neurotoxin MPP+, which causes permanent symptoms of Parkinson's disease in the mouse by destroying dopaminergic neurons in the *substantia nigra* of the brain. However, MPTP is ineffective in the rat and as such is not as relevant to models of drug discovery and development. A key aspect of the project was to link the expression of the fluidic biomarkers of

interest to imaging and functional parameters but importantly to traditional histopathology endpoints (Figure 6).[12]

Rats were given a single dose of TMT and were analyzed at 2, 6, 10 and 14 days. Brain, liver, thymus, adrenal, kidney, spinal cord, and sciatic nerve tissue were sampled along with biological fluids (CSF, plasma, serum and urine).[13] Many fluid-based biomarkers were considered for analysis such as microRNAs, F2-isoprostanes, translocator protein, glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase L1, myelin basic protein, microtubule-associated protein-2, and total tau. In addition, several neuroimaging methodologies were employed including magnetic resonance imaging (MRI), magnetic resonance spectroscopy, and positron emission tomography.

Results of this study showed promising correlations between GFAP, specific miRNAs, some metabolites such as biogenic amines and phospholipids and T2 relaxation in the hippocampus measured by MRI.[13] T2 relaxation is associated with lateral ventricle volume change which in turn is associated with damage-induced fluid accumulation both in humans and in animal models. Overall, the results so far show that we have found ways to identify neurotoxic damage in fluids (CSF, plasma and serum) in this TMT-induced model of neurological damage.[13] Additional analyses including bioinformatics are underway along with analysis of other potential biomarkers arising from other studies of brain damage (see Table 1). Learnings from these studies of brain damage and of disease models offer the opportunities to improve the tools available to nonclinical toxicologists in the early detection of potential neurotoxicity as well as better ways to potentially monitor patients during clinical development.

Potential use of CNS biomarkers in the clinic – Traumatic brain injury

The Center for Disease Control and Prevention estimates that in 2013 traumatic brain injury (TBI) contributed to the deaths of some 50,000 people.[23] In 2012, more than 300,000 people under the age of 19 sought emergency room treatment for TBI resulting from sport or recreation injury. Thus, TBI is a big issue that especially impacts the younger demographic. GFAP has been proposed as a marker of TBI[24] and in a recent exciting development, the FDA has approved GFAP as a test for TBI that could be used to monitor
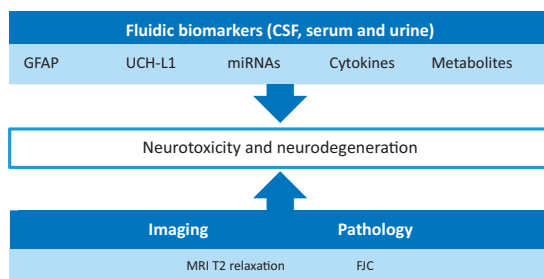

Figure 6. Correlation of biomarkers in the rat TMT model with imaging and histopathological endpoints. In MRI, magnetic pulses perturb the orientation of protons (typically hydrogen atoms) and the instrument records the time it takes for the perturbed protons to return or relax to their pre-perturbed state. Longitudinal relaxation time is referred to as T1 and transverse relaxation time as T2. Fluorojade C (FJC) is a marker for dead neurons. GFAP: green fibrillary acidic protein; MRI: magnetic resonance imaging. (A color version of this figure is available in the online journal.)

Table 1. Models and possible biomarkers of neurotoxicity, neurodegeneration and/or neurological conditions.

Disease/condition	Models	Possible biomarkers
CNS toxicity	TMT	GFAP[14]
		miRNAs[13]
Traumatic brain injury (TBI)	–	GFAP[15]
		UCH-L1[16]
Parkinson's disease (PD)	Rotenone	α-Synuclein[17]
Multiple sclerosis (MS)	Cuprizone	Myelin oligodendrocyte glycoprotein (MOG)[20]
	EAE[18,19]	
Autism	*In utero* valproic acid-exposure	Vasopressin/oxytocin[21,22]

CNS: CENTRAL nervous system; TMT: trimethyl tin; GFAP: glial F acidic protein; miRNAs: micro RNAs; TBI: traumatic brain injury; UCH-L1: ubiquitin carboxyterminal hydrolase L1; EAE: experimental autoimmune encephalomyelitis.

biochemical changes in patients and gauge the response to treatment.[25] As well as GFAP as mentioned earlier, UCH-L1 is also cited as a potential marker to be measured in serum as a diagnostic for mild TBI.[25] These markers are recommended to be used as an acute diagnostic (within 12 hours) of when a CT scan maybe required to detect concussion. It will be interesting to see if UCH-L1 is expressed in the TMT model alongside the biomarkers already detected (miRNAs, biogenic amines and phospholipids).

Potential use of CNS biomarkers in the clinic – Neurodegenerative disorders

Although the fluidic biomarkers mentioned above were detected and validated in a toxicant model, there is a possibility they could be useful in clinical development of new treatments for neurodegenerative and other neurological disorders such as Parkinson's and multiple sclerosis (MS). Currently, is it very difficult to detect a signal for efficacy for such conditions in early clinical trials; the duration of experimental new drug treatments may be limited to one month by the toxicology cover since the chronic (≥3 month) toxicology studies needed to support longer term exposure are not conducted until later in a drug development program. Additionally, patients may have advanced and complex disease conditions, having failed other therapies. Any biomarker that could provide evidence of a potential for therapeutic benefit would be very helpful in this context. But is it realistic to anticipate cross-over from biomarkers noted in a TMT toxicant model and such disease conditions? To answer this, we can look at commonly used animal models and their translation.

Animal models of Parkinson's

Parkinson's disease is a progressive disorder of the nervous system that affects movement.[26] It develops gradually, sometimes starting with a barely noticeable tremor in just one hand. But while a tremor may be the most well-known sign of Parkinson's disease, the disorder also commonly causes stiffness or slowing of movement. As with many models of neurodegenerative disease, models of Parkinson's are based on either toxicant administration or on gene deletion or addition. As with all models, they have their limitations, making it important to select the optimal *in vitro* or *in vivo* model for the question being asked where any weaknesses will not invalidate the interpretation of an experiment.

One of the most widely used toxicant models is MPTP in mice and monkeys and rotenone in rats and mice.[26] Although the MPTP neurotoxic model has advantages, one notable departure is that the degeneration of dopaminergic neurons progresses rapidly, taking days and not years as would be seen in human disease. Additionally, lesions are primarily dopaminergic and lack the typical PD proteinaceous inclusions called Lewy bodies (LBs). On the positive side, MPTP has been shown to be toxic in a large range of species. Chronic systemic exposure to rotenone in rats causes many features of PD, including nigrostriatal dopaminergic (DA) degeneration. The rotenone-administered animal model also reproduces all of the behavioral features reminiscent of human PD. Importantly, many of the degenerating neurons have intracellular inclusions that resemble LB morphologically. More recently, rotenone has also been tested in mice recapitulating the slow and specific loss of DA neurons.

In speculating that fluidic and imaging biomarkers detected in the rodent TMT study may be relevant to PD, it is worth noting that MPTP is frequently used as a model neurotoxicant as well as a model compound for inducing PD-like symptoms. These commonalities suggest that looking for the biomarkers noted in Figure 5 in models of PD and in clinical samples is a worthwhile step. Detection of UCH-L1 in CSF and the possibility of detection in serum/plasma may offer a specific biomarker of great use in PD models.[27]

Animal models of MS

MS is a serious and debilitating disease with variable progression patterns and symptom manifestation.[18] Development of effective treatment strategies is supported by qualitative *in vivo* research efforts which seek to examine related disease pathologies from cellular components up to large-scale whole system appraisal in the form of an animal model. As with other models of neurodegenerative diseases, MS can be modeled by demyelination with a toxin such as cuprizone and lyso-phosphatidyl choline (lyso-lecithine). However, one of the most widely studied models is experimental autoimmune encephalomyelitis (EAE). EAE is a term used to describe a collection of inflammatory disorders which develop upon immunization with antigens derived from CNS proteins – a process that induces an autoimmune response. It was first induced experimentally in 1933[19] and at its most basic level leads to progressive paralysis with B and T cell activation cumulating in white matter lesions.[28] Interestingly, EAE can be combined with other MS induction protocols such as cuprizone dosing. This toxin induces demyelination of the CNS and, when combined with EAE, generates an *in vivo* MS model that encompasses multiple pathological elements.[29]

Looking at other types of progressive neurodegenerative disease such as amyotrophic lateral sclerosis (ALS), miR-218a-5p, a brain enriched miRNA has been described as a clinically useful marker of ALS progression.[30,31] Notably, this specific miRNA was readily detectable in the rat TMT model described earlier.[13] These data suggest that such miRNAs could be very useful biomarkers of overall CNS toxicity and as such applicable to earlier detection of CNS signs in nonclinical animal studies.

Conclusions

Identifying neurotoxicity in drug discovery and development can improve outcomes in a number of ways, including increasing our efficiency and accuracy of diagnosis and our ability to intervene with pharmaceutical treatments. Early identification of neurotoxicity enables early intervention, which improves outcomes. Utilization of biomarkers of neurotoxicity also allows for continual monitoring of

disease states and drug efficacy and, thus, may improve disease management. From a therapeutic standpoint, detecting and predicting neurotoxicity in preclinical (testing phase before new drugs enter the clinic) and nonclinical (testing of nondrug entities at all phases or ongoing testing of drugs in parallel to clinical development) models can improve decision making during drug development. Functional endpoints such as seizure and sedation may be amenable to earlier detection with some of the *in vitro* and *in vivo* developing methodologies such as in cage monitoring and microelectrode array detection of cellular electrical activity. However, higher order brain function endpoints such as suicidal ideation and depression remain a challenge for the foreseeable future. Because of this, much of the current research is focused on improving detection of structural change via imaging and fluidic biomarkers. Studies of toxicity models have provided a panel of possible biomarkers, supplemented with learnings from damage and disease models such as TBI and MS. Reciprocally, these toxicity biomarkers may provide the opportunity for earlier detection of efficacy in clinical trials of new medicines for Alzheimer's, Parkinson's and other neurodegenerative conditions. Overall, such approaches should form part of a rationale stepwise cascade of screening to identify, mitigate and manage risk using *in silico*, *in vitro* and *in vivo* methodologies.

Authors' contributions: AW, SI and RR contributed equally to this manuscript.

DECLARATION OF CONFLICTING INTERESTS

Ruth Roberts is co-founder and co-director of ApconiX, an integrated toxicology and ion channel research company that provides expert advice on nonclinical aspects of drug discovery and drug development to academia, industry, government and not-for-profit organizations.

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