Drug Discovery and Development: assessment and impact of neurotoxicity

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Abstract:	The discovery and development of new drugs is 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 the broader implications of this work in terms of relevance to other models of CNS damage and degeneration such as Parkinson's Disease (PD), traumatic brain injury (TBI) and multiple sclerosis (MS). Differences and similarities in in vivo models of disease and toxicity present challenges and opportunities to find common biomarkers, especially in the context of the drive to move away from animal testing towards in silico, in vitro and humanized models.

Drug Discovery and Development: assessment and impact of neurotoxicity

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Abstract

The discovery and development of new drugs is 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 the broader implications of this work in terms of relevance to other models of CNS damage and degeneration such as Parkinson's Disease (PD), traumatic brain injury (TBI) and multiple sclerosis (MS). Differences and similarities in *in vivo* models of disease and toxicity present challenges and opportunities to find common biomarkers, especially in the context of the drive to move away from animal testing towards *in silico*, *in vitro* and humanized models.

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).

Drug Discovery and Development: an overview

Small molecule drug discovery begins with the selection of a target of interest (target selection; TS) based on data to support 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 characterise 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. Hits are selected and confirmed in lead generation (LG); these leads are then optimised (LO) 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, hERG and the potential to form reactive metabolites since at this early stage any liabilities can be designed out in an iterative design-make-test cycle. 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 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.

The majority of FTIH clinical trials are conducted in healthy male volunteers and are aimed at establishing tolerance, kinetics and pharmacology (proof of mechanism). 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 and pharmacology. 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 standardise 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.). In practical terms, testing is structured as general toxicology, safety and secondary pharmacology, genetic toxicology/carcinogenicity and developmental/reproductive toxicology (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 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 would be a stop for a 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 authorisation (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 target safety assessment (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 optimisation 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 optimisation and candidate selection.

Attrition in drug discovery and development

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 4B); however, it is a relatively infrequent finding during GLP toxicology (Figure 4A). [3] As highlighted in Figure 4C, 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 4D) 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]

Detection of neurotoxicity

Identifying neurotoxicity 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.

Back in 2012, the Health and Environmental Sciences Institute (HESI)[8] 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)[9] 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 characterised. [10] 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 5). [10]

Rats were given a single dose of TMT and were analysed at 2, 6, 10 and 14 days. Brain, liver, thymus, adrenal, kidney, spinal cord and sciatic nerve tissue was sampled along with biological fluids (CSF, plasma, serum and urine). [11] Many fluid-based biomarkers were considered for analysis such as microRNAs, F2-isoprostanes, translocator protein, glial fibrillary acidic protein, 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, magnetic resonance spectroscopy, and positron emission tomography.

Results of this study showed promising correlations between green fibrillary acidic protein (GFAP), specific miRNAs, some metabolites such as biogenic amines and phospholipids and T2 relaxation in the hippocampus measured by Magnetic Resonance Imaging (MRI). [11] 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. 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. [11] Additional analyses including bioinformatics are underway along with analysis of other potential biomarkers arising from other studies of brain damage (see below).

Potential Use of CNS Biomarkers in the Clinic – Traumatic Brain Injury (TBI)

The Center for Disease Control and Prevention estimates that in 2013 TBI contributed to the deaths of some 50 000 people. [12] 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[13] and in a recent exciting development, the FDA has approved GFAP as a test for TBI that could be used to monitor biochemical changes in patients and gauge the response to treatment. [14] 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. [14] 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. 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 ($\geq$3 month) toxicology studies needed to support longer term exposure are not conducted until later in a drug development programme. 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. [15] 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. [15] Although the MPTP neurotoxic model has advantages, one notable departure is that the degeneration of dopaminergic neurons progress 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's 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. [16]

Animal models of multiple sclerosis

Multiple sclerosis (MS) is a serious and debilitating disease with variable progression patterns and symptom manifestation. [17] 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 modelled 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 immunisation with antigens derived from CNS proteins - a process that induces an autoimmune response. It was first induced experimentally in 1933[18] and at its most basic level leads to progressive paralysis with B and T cell activation cumulating in white matter lesions. [19] 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. [20]

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. [21, 22] Notably, this specific miRNA was readily detectable in the rat TMT model described earlier. [11] These data suggest that such miRNAs could be very useful biomarkers of overall CNS toxicity.

Animal models: future perspectives

One of the major challenges in drug safety testing is the drive to replace animals; as well as the 3Rs (reduce, refine, replace) imperative [23], animals do not always predict human responses both in terms of efficacy and safety/toxicity. Can we do better? Significant progress has been made over the last 20 years or so in in silico and in vitro replacements, with several tests now validated and in use. [24] Importantly, much of this progress comes from collaborative projects between industry, CROs, government and academia since all stakeholders are needed to achieve a workable outcome. [25] Despite this progress, in vivo work still forms the basis of regulatory decision making today for new drugs both in the US and in Europe (Figure 6). It is interesting to speculate on how this might change over the next 25 years in Pharma but also in the other sectors of agrochemicals, chemicals and personal care. It seems reasonable to suggest that in silico/in vitro (ISIV) methods may be used for some aspects of drug safety testing by 2028 with further progress to animal studies only on a 'for cause' basis by 2043. It seems likely that societal pressure will drive faster replacements in other sectors (agrochemical, chemical and personal care) with perhaps a complete shift to ISIV alone by 2043. As scientists, we may imagine this will take place in a step wise manner as new ISIV tests become available. However, in this context it is worth noting that the European Union introduced a near-total ban on cosmetics-related animal testing and the sale of cosmetics tested on animals from 2009. [26] This step is likely to have follow on implications for testing of cosmetics in the US and also on the use of animals for testing in other sectors.

Although this transition away from animal testing poses a challenge, it also offers an opportunity to move from observational studies in animals to mechanism-based systems. New and emerging technologies such as big data, artificial intelligence and microphysiological systems are all likely to play a part in the evolution of testing strategies.

Author Contributions Statement

AW contributed expertise and authored the section on animal models of neurodegeneration. SI contributed expertise and authored the section on biomarkers including table 1. RR developed the main conceptual ideas around drug development and attrition due to CNS toxicity and authored the sections on opportunities for cross-discipline learning between toxicity, neurodegeneration and conditions such as TBI.

Conflict of Interest Statement

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 organisations.

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Figure 1. The drug discovery and development paradigm. See text for details. TS: target selection; LO: lead optimization; LG: lead generation; GLP: good laboratory practice; CD: candidate drug.

Figure 2. Navigating the regulatory framework. See text for details. 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.

Figure 3. A comparison of key aspects of ICH M3 and ICH S9. See text for details. PK: pharmacokinetics; MAA: marketing authority authorization.

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 demonstrates 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., 2014. [7]

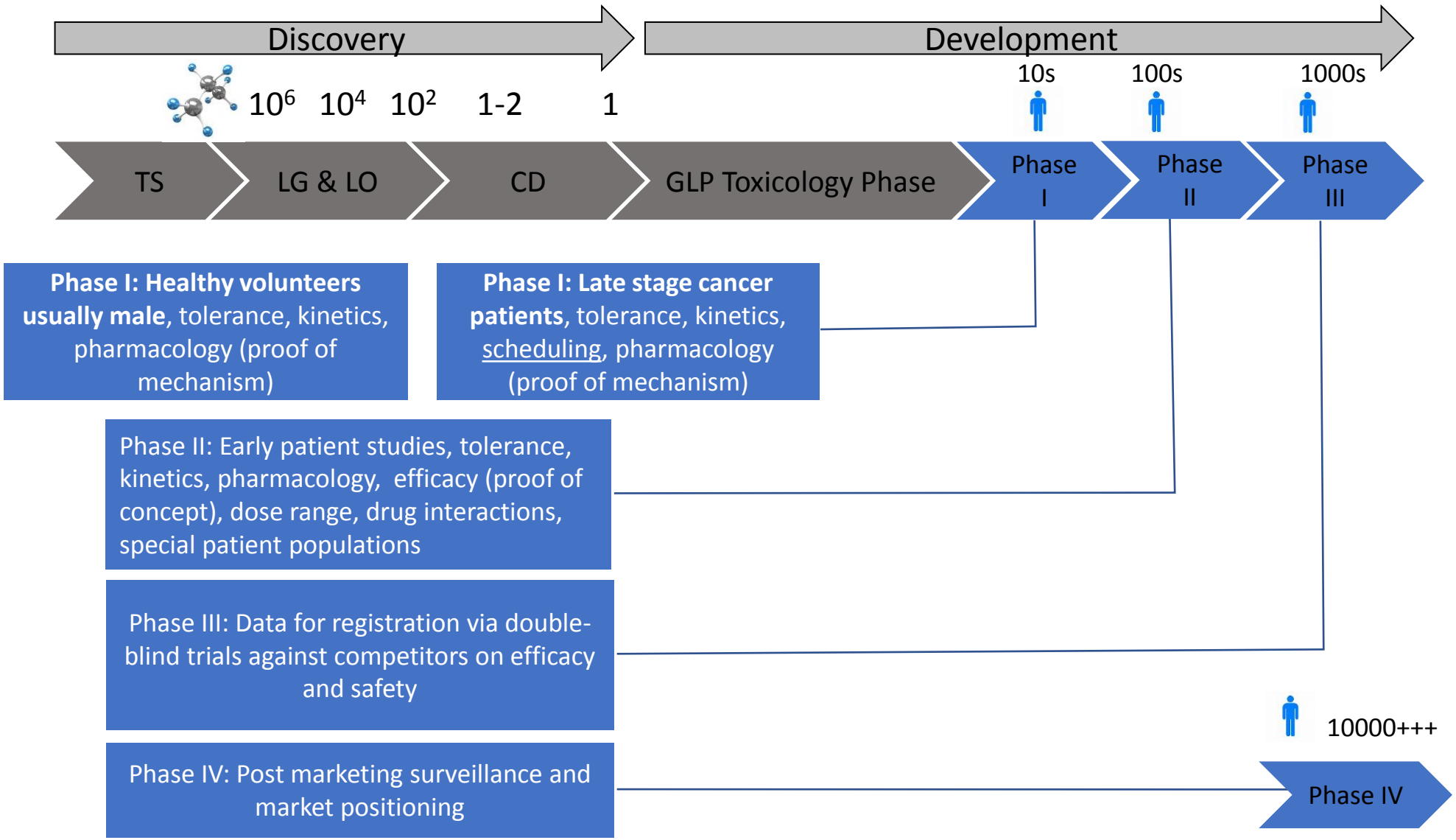
Figure 5. Correlation of biomarkers in the rat TMT model with imaging and histopathological endpoints. GFAP: green fibrillary acidic protein; MRI: magnetic resonance imaging.

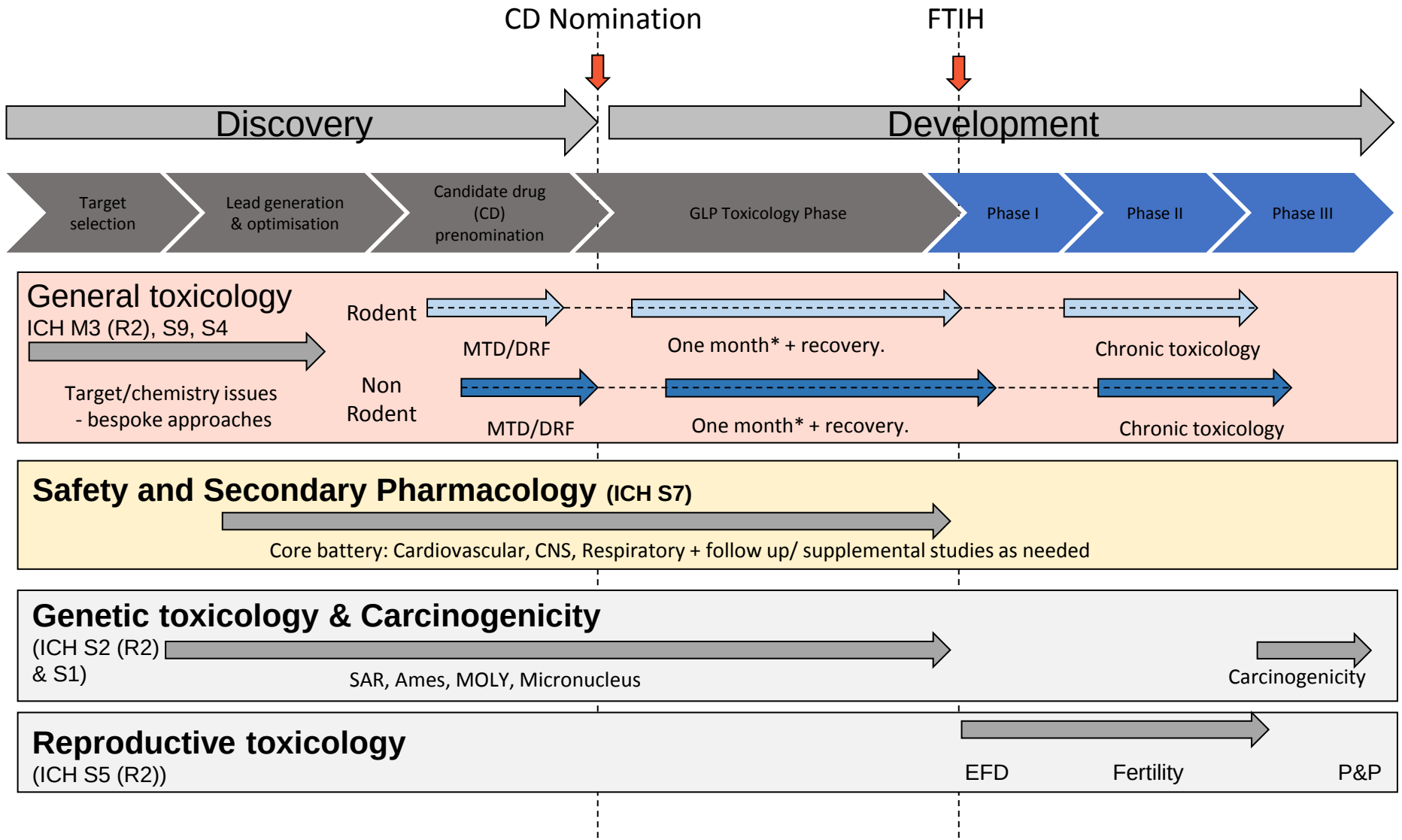
Figure 6. A projected evolution over 25 years of the basis of regulatory decision making in the pharma, agrochemical, chemical and personal care industries. In silico/in vitro (ISIV) methods may initially supplement animal data. Next, ISIV methods would form the basis of testing with animal studies only on a 'for cause' basis. Finally, ISIV could replace testing in most if not all situations.

Table 1 – Models and Possible Biomarkers of Neurotoxicity, Neurodegeneration and/or Neurological Conditions.

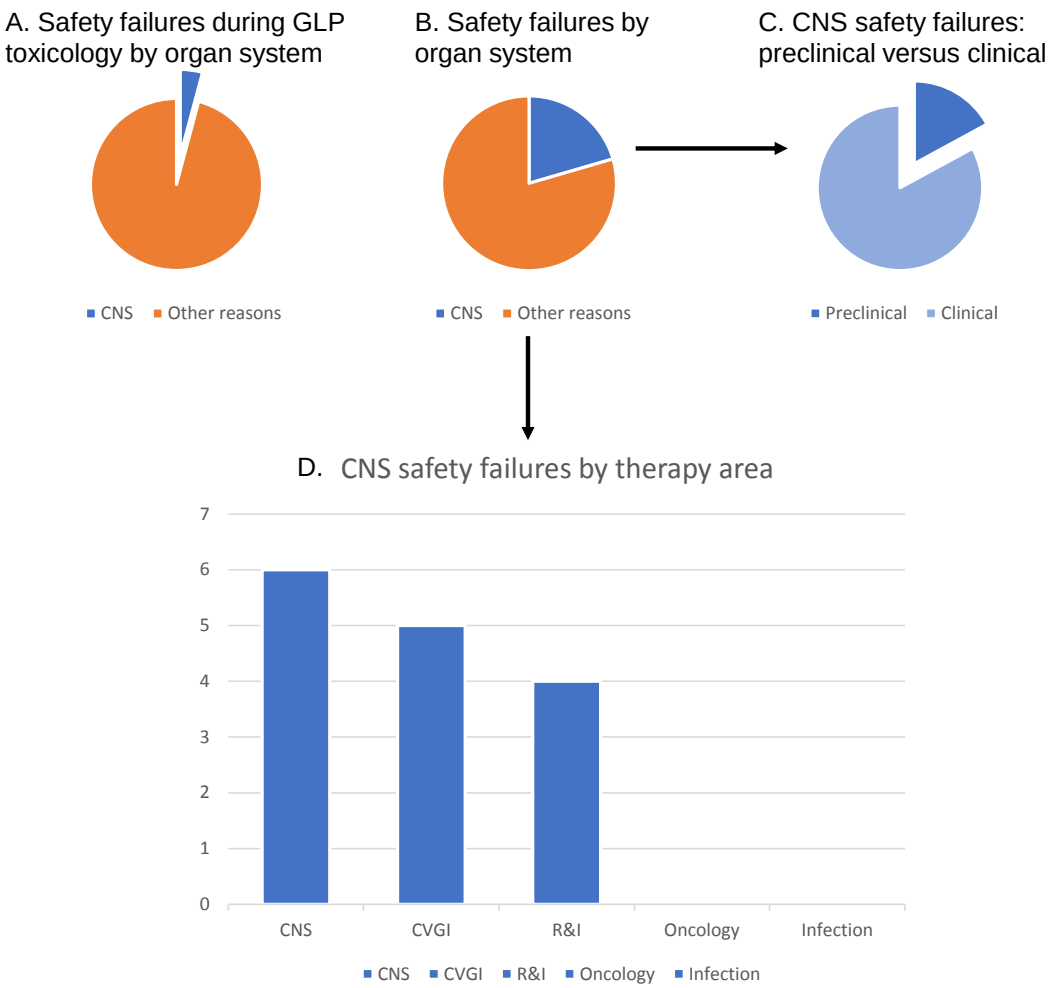
Disease/Condition	Models	Possible biomarkers
CNS toxicity	TMT	GFAP [27] miRNAs [11]
Traumatic brain injury (TBI)	-	GFAP[28] UCH-L1 [29]
Parkinson's Disease (PD)	Rotenone	a-Synuclein [30]
Multiple Sclerosis (MS)	Cuprizone EAE [19, 20]	myelin oligodendrocyte glycoprotein (MOG) [31]
Autism	*In utero* valproic acid-exposure	Vasopressin/Oxytocin [32,33]

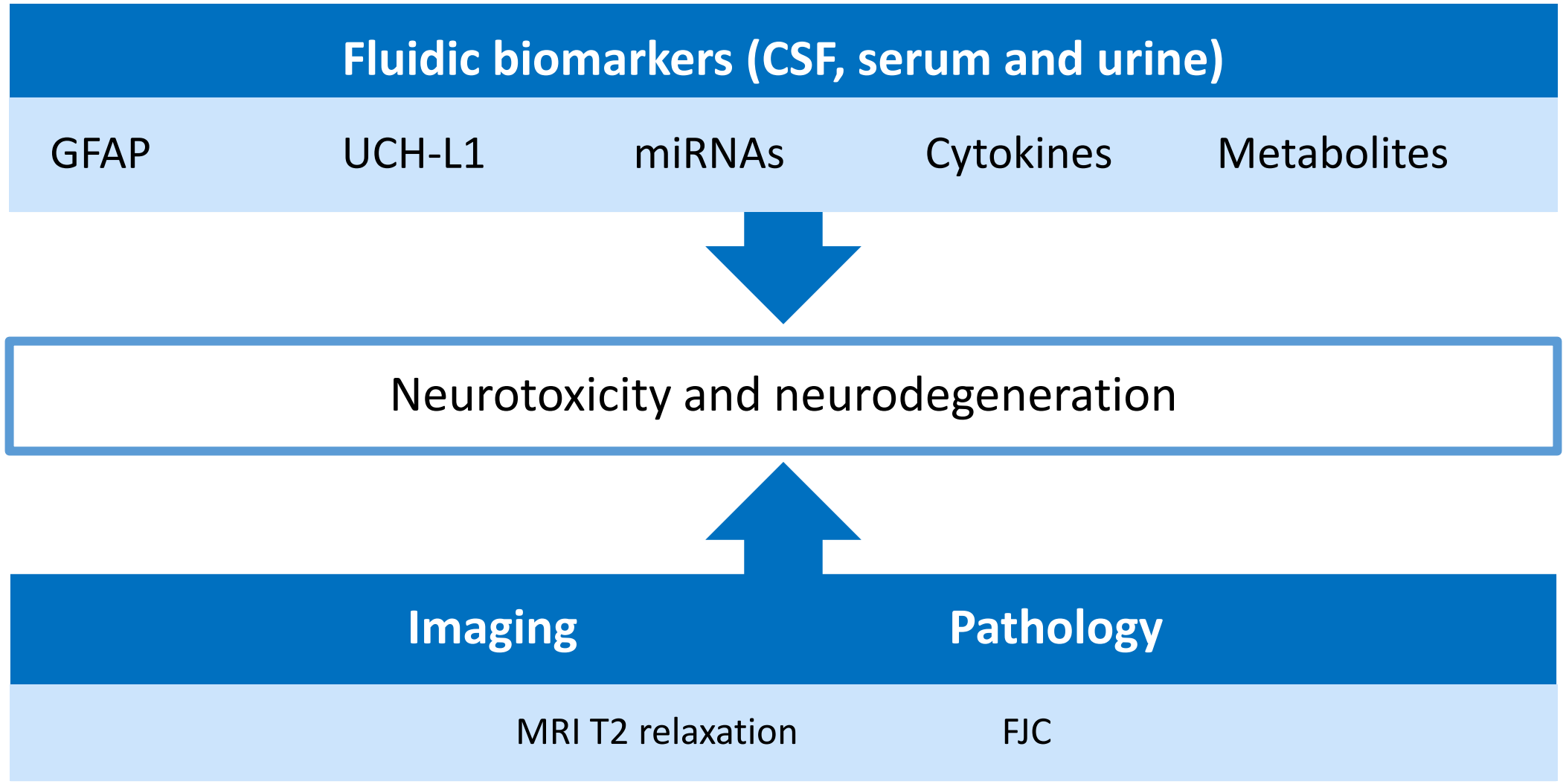
CNS: Central nervous system; TMT: trimethyl tin; GFAP: Glial F Acidic Protein; miRNAs: micro RNAs; TBI: traumatic brain injury; UCH-L1: ubiquitin carboxyterminal hydrolase L1; EAE: experimental autoimmune encephalomyelitis.


Discovery
Development
10^6 10^4 10^2 1-2 1
10s 100s 1000s
TS
LG & LO
CD
GLP Toxicology Phase
Phase I
Phase II
Phase III
Phase IV
10000+++
Phase I: Healthy volunteers usually male, tolerance, kinetics, pharmacology (proof of mechanism)
Phase I: Late stage cancer patients, tolerance, kinetics, scheduling, pharmacology (proof of mechanism)
Phase II: Early patient studies, tolerance, kinetics, pharmacology, efficacy (proof of concept), dose range, drug interactions, special patient populations
Phase III: Data for registration via double-blind trials against competitors on efficacy and safety
Phase IV: Post marketing surveillance and market positioning


CD Nomination
FTIH
Discovery
Development
Target selection
Lead generation & optimisation
Candidate drug (CD) prenomination
GLP Toxicology Phase
Phase I
Phase II
Phase III
General toxicology
ICH M3 (R2), S9, S4
Target/chemistry issues - bespoke approaches
Rodent
MTD/DRF
One month* + recovery.
Chronic toxicology
Non Rodent
MTD/DRF
One month* + recovery.
Chronic toxicology
Safety and Secondary Pharmacology (ICH S7)
Core battery: Cardiovascular, CNS, Respiratory + follow up/ supplemental studies as needed
Genetic toxicology & Carcinogenicity
(ICH S2 (R2) & S1)
SAR, Ames, MOLY, Micronucleus
Carcinogenicity
Reproductive toxicology
(ICH S5 (R2))
EFD
Fertility
P&P

	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	


A. Safety failures during GLP toxicology by organ system
CNS
Other reasons
B. Safety failures by organ system
CNS
Other reasons
C. CNS safety failures: preclinical versus clinical
Preclinical
Clinical
D. CNS safety failures by therapy area
7
6
5
4
3
2
1
0
CNS
CVGI
R&I
Oncology
Infection
CNS
CVGI
R&I
Oncology
Infection


Fluidic biomarkers (CSF, serum and urine)
GFAP
UCH-L1
miRNAs
Cytokines
Metabolites
Neurotoxicity and neurodegeneration
Imaging
Pathology
MRI T2 relaxation
FJC