

## **Toxicology Skills for Drug Discovery**

### **Why is Toxicology in Drug Discovery important?**

The development of novel pharmaceuticals requires non-clinical safety studies to be performed on candidate drugs. Such studies typically assess general toxicology (as determined by *in vivo* experiments), safety pharmacology (effects on major organ systems, e.g. cardiovascular) and genetic toxicity test batteries. These studies inform progression of candidate drugs from the “discovery phase” through clinical development to regulatory submission and registration. However traditionally, less emphasis was placed on the evaluation of safety issues for projects while still in the drug design phase. Consequently, this led to a number of significant failures of candidate drugs in early development due to toxicological issues. In response to this costly attrition, many pharmaceutical companies have now invested in “Discovery-phase Toxicology” or “Discovery Safety” to identify potential hazards and to take steps to design out or significantly reduce undesirable properties at an earlier stage, with the ultimate aim of enhancing the probability of success in non-clinical and clinical drug development.

### **What does Toxicology in Drug Discovery contribute?**

Discovery safety assessment of drug projects can be considered in two broad areas: target-related safety and chemical related safety.

#### *Target-Related Safety*

Unintended adverse effects can arise as a consequence of the intended (primary) pharmacology in tissues other than tissues of efficacy. Therefore a key role for the Discovery Safety scientist is to identify potential adverse effects that may result from primary pharmacology. Careful analysis of potential target-related safety issues, together with an experimental plan to investigate the issues can provide important discovery project risk assessment data. Critically, these data may inform required isoform selectivity profile, dosing route selection, drug pharmacokinetic properties to mitigate tissue specific effects (e.g. limit exposure to the central nervous system for undesirable on-target neuronal pharmacology), or may indeed guide an early project termination decision.

#### *Chemistry-Related Safety*

A critical pillar of discovery phase safety assessment is to identify toxicities associated with a chemical series. There are a number of approaches that can be used to support chemical risk assessment. Ideally, predictive tools identify hazards to be avoided; for example it may be possible to identify a compound property or structural features that are associated with adverse effects. Such

computational tools that exploit existing toxicology information can be used to ascertain potential relationships between chemical space and toxicological response. The outputs from these analyses can be used to define project-specific, toxicology experiments to determine if the predicted toxicities are real and so influence compound design.

#### *Hazard integration and risk assessment*

Integrating target and compound safety data ranging from the molecular to the physiological underpins drug project safety risk assessment. However the patient context of this risk assessment is key: consideration of the patient co-medications and co-morbidities need to be considered when assessing the risk:benefit of the drug on symptoms, disabilities and prognosis, balanced against its unwanted effects. The Discovery Phase toxicologist is therefore required to consider primary mode of drug action from the molecules (drug targets) through the cellular and physiological effects that arise as a consequence of intended and unintended pharmacology, chemistry related toxicity and how these effects influence the predicted impact on patient tolerability. It is with this assessment that the toxicologist influences iterative drug design improvements during discovery.

#### **What are the special skills of a toxicologist in drug discovery?**

A Discovery Toxicologist or Discovery Safety scientist requires a broad understanding of the scientific principals underpinning the other main facets of drug discovery, namely biology, pharmacology, chemistry and DMPK. Toxicological risk assessment during the discovery phase requires interpretation and integration of information from all these disciplines, and others, which helps to define and provide context to the safety findings and risks. For example, toxicity of a compound is often triggered by the route of metabolism, clearance or deposition profile of the molecule. It is also clearly important for a toxicologist to be able to understand and interpret state-of-the art scientific literature on the homeostatic roles of target pathways being pharmacologically modulated. Beyond the intellectual aspects of the role, a discovery toxicologist needs to understand and apply multiple methodologies including *in silico*, *in vitro* and *in vivo* approaches, which are all core components of modern toxicology. Further, there is also an important translational aspect of the role, which may require seeking and integrating the views of disease-area experts, including clinicians, which allow the defined health risks to be contextualised with respect to the intended patient population. Below, more detailed information is provided regarding the skills and understanding required of a discovery toxicologist with respect to other drug discovery disciplines.

#### **1. Broad perspective on Biological Science: Molecular Biology, Biochemistry and *in vitro* Pharmacology**

Effective discovery phase toxicology requires a working knowledge of a broad range of biosciences, including biochemistry, molecular biology, cellular biology, pharmacology as well as the anatomy and physiology in health and disease. Understanding the biochemical nature of the interactions between drug-candidates and their intended and unintended targets (usually proteins) can guide compound selection in its earliest stages by steering chemistry towards a more selective molecule. By reducing

these unintended, or “off-target”, drug protein interactions the unintended biological activity of a compound can be minimized. An understanding of molecular biology and cell-signaling pathways surrounding these target proteins enables a discovery phase toxicologist to predict the potential cascades of molecular and cellular events following a drug-protein interaction. Combining that molecular understanding with knowledge of cell function within healthy and diseased tissues allows the toxicologist to infer the safety risk of a given drug-protein interaction in context of its use. Using this cross-disciplinary bioscience knowledge together with a broad panel of protein and cell based assays enables a discovery toxicologist to identify potential safety liabilities early in the drug discovery process.

## 2. Medicinal Chemistry

A basic understanding of medicinal chemistry principles and their application in the drug discovery process from lead identification through lead optimization to candidate drug selection is a key if the Discovery Phase toxicologist is to engage with chemists responsible for drug design. In the first instance a basic understanding of molecular complexity and an appreciation of the timescales involved in compound synthesis and medicinal chemistry project prosecution is required. Similarly, an understanding of the role of physicochemical properties in drug binding and activity alongside impacts on distribution and pharmacokinetics provides a firm foundation for consideration of the toxicological properties of the compound that may arise following disturbance of biological processes. In addition, certain structural features of small molecules are associated with adverse biological responses, therefore the discovery safety scientist should have a knowledge of the alerting structural features that might constitute hazards for further risk assessment. Finally, an awareness of computational chemistry tools and their strengths and limitations will help place such alerts into context. Clearly, the toxicologist working in the discovery space is not accountable for chemical design, however, “conversational” chemistry expertise will allow engagement with medicinal chemistry teams and understanding of the chemistry strategy and associated chemical toxicity risks such that timely risk mitigation strategies can be developed.

## 3. DMPK and in vivo Pharmacology

The Discovery Safety scientist will be responsible for designing in vivo experiments to determine lead compound (or reference compound) tolerability and toxicology. These designs need to permit the contextualisation of drug effects by reference to the pharmacology of the drug and the model system used. Specifically, exploration of effects on key organs is important: central nervous system; cardiovascular system; gastrointestinal system; respiratory system; immune/inflammatory system and endocrine system). In addition the scientist needs to appreciate compound DMPK (Drug Metabolism and Pharmacokinetics) properties. DMPK is a specialism that focuses on the properties of a drug following administration to humans or other species. These properties are broadly spread, from the structure and properties of metabolites (ie products of metabolism conducted by enzymatic machinery in the target species), through to the concentrations attained over time in plasma, blood or other

tissues following administration and further to modelling human pharmacokinetics to predict efficacious doses (PK-pharmacodynamic (PKPD) modelling) or to model markers of potential toxicity and thereby predict potentially unsafe doses (toxicokinetic-toxicodynamic TKTD modelling). Between these extremes are more subtle combinations of properties that together can begin to explain and help interpret findings such as poor efficacy in clinical studies or unacceptable toxicity (either alone or in combination with other drugs), either in humans or preclinical species.

When considering the metabolism of a drug, a number of consequences may follow, each with possible downstream implications for the safety/efficacy profile of the product. For instance, a metabolite may be toxic in itself, either through amplification of off-target toxicity through metabolism, enhanced pharmacology against the desired target, or by showing adverse events through non-reversible binding to proteins, cell membranes or organelles such as mitochondria (via activation to a 'reactive metabolite'). Interactions between the drug in question and other co-administered medicines may also occur via saturation of metabolic or other pathways responsible for removing these compounds from the body, causing an unsafe accumulation of drug or metabolites, or by direct inhibition of important drug metabolizing enzymes with the same effect.

Transporter proteins, present on most if not all cells in the body, are essential components of elimination pathways for many drugs; they are also essential for the removal of toxins from cells and tissues and for the uptake of nutrients. Consequently many drug-drug interactions and other toxic events occur at the transporter level, where inhibition of a particular transporter or group of transporters can have a profound effect on the elimination of co-administered drugs, or for the removal of toxins; either effect can cause undesired effects. Appreciation of such interactions can be key in considering the safety risk assessment in context of patient polypharmacy.

Understanding the toxicological significance of any of these interactions, the safety pharmacology, the routes and rates of metabolism or transporter function that cause them is a key skill for the discovery safety scientist and working with DMPK and pharmacology specialists to develop appropriate experiments to examine these interactions is also a critical ability. Indeed combining these insights with relevant pharmacodynamic (PD) or toxicodynamic (TD) data (PKPD or TD modelling) provide a valuable platform for a comprehensive safety risk assessment.

#### 4. Genetic Toxicology

Genetic toxicity studies are designed to detect whether a compound can induce genetic damage by a variety of mechanisms, the consequence of which includes cancer. The Discovery Phase toxicologist should be aware of the battery of assays used to predict and detect genetic toxicity hazard; as no single test is capable of detecting all genotoxic mechanisms relevant to tumourigenesis. A familiarity with computational analyses, assessment of mutagenicity in a bacterial reverse gene mutation test (such as the Ames test) and evaluation of genotoxicity in mammalian cells (*in vitro* and/or *in vivo* such

as the micronucleus test) is required. Awareness of how to respond to genetic toxicity hazards (e.g. clastogenicity, aneugenicity) and manage these risks in the context of the project aims and patient population is key.

## 5. Biopharmaceuticals and new treatment modalities

In addition to traditional small molecule drugs, biopharmaceuticals, such as monoclonal antibodies and recombinant peptides, are important therapeutics. Moreover, therapeutic agents based on oligonucleotides (e.g. anti-sense, siRNA and modified RNA) are emerging as valuable modalities. Key features of biopharmaceuticals are their clinical effectiveness, high specificity for their human target, long half-life and target coverage, and low risk for "off-target" pharmacology; consequently the toxicological profile of biopharmaceuticals as a class is largely predictable, with the principal safety focus based on target liabilities and PKPD. Similarly oligonucleotide drugs are considered relatively specific, with class effects, based on characteristic DMPK properties and biological responses to oligonucleotides relatively well defined. However, the high specificity for their human target of biopharmaceuticals and oligonucleotide drugs does require careful consideration of *in vivo* toxicological assessment: ensuring pharmacological relevance. Thus, the discovery phase toxicologist needs to be aware of the drivers for drug class selection, the similarities and differences between the various drug classes with respect to toxicity and risk assessment options including potential immunogenicity and allergenicity of protein therapeutics.

## 6. General Toxicology

Traditional toxicology aims to assess a compound in non-clinical safety studies required to support "first time in human" studies and through to marketing authorisation of a product. The development of a compound is a stepwise process involving an evaluation of both the animal and human safety information. The goals of the non-clinical safety evaluation generally include a characterisation of toxic effects with respect to target organs, dose dependence, relationship to exposure, and when appropriate, potential reversibility. For pharmaceuticals, biopharmaceuticals and newer modalities, this information is helpful for the estimation of an initial safe starting dose and dose range for the human trials and the identification of parameters for clinical monitoring for potential adverse effects.

A pre-clinical package to support first time in human studies have 3 main elements; general toxicology, safety pharmacology and genetic toxicology. The core battery of safety pharmacology studies include the assessment of the effects on cardiovascular, central nervous and respiratory systems, and generally are conducted prior to human exposure. The risk assessment for humans is based on an integration of both *in vivo* elements (either in a rodent or non-rodent species) and *in vitro* (such as secondary pharmacology data). Repeated dose toxicity studies are conducted in two mammalian species (one rodent and one non-rodent) and their design takes into account the pharmacological relevance, treatment duration, therapeutic indication and scope of the proposed

clinical trial they are to support. The intention is to characterise the drug in terms of its toxicological profile (target organs, dose dependence, relationship to exposure and reversibility). The assessment includes in-life clinical observations (including bodyweight and food intake), clinical pathology, histopathology and exposure of compound assessment. Genetic toxicity studies as discussed above are also designed to detect whether a compound can induce genetic damage by a variety of mechanisms.

A line of sight to and understanding of pre-clinical toxicology packages is a key requirement of the toxicologist working in the discovery phase since it is these data that ultimately provide the integrated risk assessment for humans required by regulatory agencies for clinical drug development. Moreover, it is important to recognise that there are distinct regulatory requirements for traditional small molecule drugs, biopharmaceuticals and newer treatment modalities. While, discovery safety experiments may sometimes employ adapted versions of these regulatory safety experiments, Discovery safety science is not simply about performing development toxicology studies earlier; rather its purpose is to provide toxicology and safety information that aids drug target selection; influences the drug design-make-test cycle and the selection of molecules with reduced toxicological liabilities. Importantly, the successful application of this approach requires hypothesis-guided experimentation. Moreover, discovery safety data enable a balanced and informed safety risk assessment and critically assist the design of the regulatory toxicology program to enable and support clinical testing; leading to improved non-clinical phase safety data prior to clinical development decision making.

### **Transferable Skills**

In terms of transferable skills, the following are essential in a dynamic, unpredictable and complex environment.

- Use of fundamental knowledge to solve new problems: problem- and context-based learning; self-driven learning and development. Continually building on existing knowledge base and skill-sets
- Good knowledge and understanding of *in silico*, *in vitro* and *in vivo* tools available
- Able to reinforce ideas through practical experimentation
- Promoting and engaging in scientific challenge across disciplines. Developing partnerships and collaborations (cross-discipline, cross-cultural, multi-centred). Effective working in geographically fractured teams with complex cultural elements; use of state of the art communication/information sharing technology
- Understanding of the external landscape of expertise, capability/collaboration partners and able to facilitate partnership through networks
- Resilience and leadership
- Energised by dynamic environment and change
- Understanding of R&D, value creation, risk, return on investment, licensing models, project management

## Core and Aligned Skills for Discovery phase toxicologists

	Core	Aligned
Molecular Biology and in vitro Pharmacology	Technical Skills, biochemistry, genomic/proteomic techniques, Cell culture. Concentration/dilution calculations Regression and curve fitting	Mathematical skills Analytical skills
<i>In vivo</i> Pharmacology	Experimental Design, dose calculations Ethics/NC3Rs. Animal handling/technical skills	Analytical skills
Pharmacology of key body systems	Central Nervous System; Cardiovascular System; Gastrointestinal System; Respiratory System; Immune/Inflammatory System; Endocrine System	Specialist systems understanding (e.g. cardiovascular system)
Disease Modelling	Experimental readouts and biomarkers Knowledge of human disease; current therapies and standard drugs; genetics of disease and how this impacts the safety risk assessment	Specialist disease understanding (e.g. oncology)
DMPK	P450s, other metabolic enzymes and transporters Aligning physicochemical properties to chemical and DMPK properties <i>In vitro/in vivo</i> scaling; Concentration/dilution calculations; Regression/curve fitting	Mathematical skills Analytical skills
PK/PD relationships	Quantitative pharmacodynamics Bioanalysis	Clinical pharmacology
Medicinal Chemistry	Appreciation compound complexity and medicinal chemistry principles. Physicochemical properties in drug binding and impacts on distribution and pharmacokinetics. Structural safety alert features. An understanding of available computational chemistry tools and their strengths and limitations	Analytical skills
Genetic Toxicology	Awareness of bacterial and mammalian mutagenicity tests. Appreciation of cytogenetic assays.	Microbiology, cell imaging
General Toxicology	Rational prediction of on-target and off-target effects. Dose ranging tolerability studies; Therapeutic window/margin. Drug specificity and non-specific effects	Analytical skills