Pharmaceutical Discovery Toxicology
Integrating mechanistic toxicology into candidate selection

CXR biosciences®
the investigative & exploratory toxicology company

www.cxrbiosciences.com
Toxicity remains a leading cause of attrition in the pharmaceutical industry. Therefore, in order to improve productivity, the identification of potential toxic mechanisms, hazard assessment and quantification of risk is shifting from the development stage to the research stage of R&D. Such research-stage toxicology is generally termed discovery toxicology.

Discovery toxicology differs greatly from regulatory toxicology (see figure, below). In the latter, a single compound is empirically characterised over a lengthy period in a series of defined regulatory studies. In the former, the pharmacological target is constant whilst chemical structures evolve over a short timeframe as a result of the design-make-test cycle. Since the aims of drug discovery are different to those of drug development, it follows that the approaches used in discovery toxicology are different from those in development (regulatory) toxicology.

CXR Biosciences® and its staff have an extensive track record in discovery toxicology, having supported the programs of leading pharmaceutical and biotechnology companies for many years. We understand the scientific and industry context in which the studies are performed, and our flexible scheduling means we can deliver quality data in the short timeframes demanded by a pharmaceutical research organisation.
Aims of Discovery Toxicology

**Aim 1:** Reduce candidate drug attrition due to preclinical toxicity by providing toxicological data and information that facilitates:
- Target selection
- Chemical series selection
- Chemical design
- Candidate selection

**Aim 2:** Provide toxicology information at candidate drug selection/nomination that permits:
- Prospective design of (and additional endpoints to be included in) regulatory studies
- Prospective risk assessment of identified toxicities
- Prospective definition of investigative toxicology studies to be performed during development
- Prospective engagement of regulatory authorities

**Discovery Toxicology – the CXR Biosciences® approach**

CXR uses a hypothesis-driven approach to:
- Review potential (e.g. on-target or secondary-target) or observed toxicity issues.
- Develop a bespoke, project-specific experimental programme.

We have a team with many years industry experience of project-based discovery toxicology, and the extensive in-house technical capabilities needed to run *in vitro* and *in vivo* mechanistic studies.

Working with CXR can provide access to expertise that may not be in-house, or access to resource to manage peaks and troughs in your discovery toxicology workload. We provide services ranging from complete outsourcing of discovery toxicology programs, to running individual discovery toxicology studies designed by the customer. In addition we can establish and run issue-specific mechanistic *in vitro* assays.

**Discovery Toxicology & Candidate Selection Services at CXR Biosciences**

1. **I. Target Safety Review**
   - Expert literature review to identify potential on-target toxicities
   - If required, design experiments to assess on-target and secondary-target toxicity risk

2. **II. Bespoke *in vivo***
   - Single dose, ascending dose TK study in rats
     - Tolerability
     - Microsampling TK
     - Select doses for repeat dose studies on basis of systemic exposure
   - Bespoke 7/14 day repeat dose study in rats
     - Compare compounds on exposure, not dose – microsampling TK
     - Standard tox endpoints
     - Bespoke endpoints based on target biology, toxicity of competitors
     - Optional toxicogenomics on key organs

3. **III. Bespoke *in vitro***
   - Issue specific, mechanistic *in vitro* assays
     - Range of assays available
     - New assays established on request

   **Candidate Selection**
   **Regulatory Tox**
   **V. Downstream investigative studies**

4. **IV. Supporting DMPK**
   - Rat CYP induction
     - Evaluate potential for rodent NGC, autoinduction of metabolism
     - 96 well plate format
     - Taqman endpoints
   - Human CYP induction
     - Evaluate potential for human DDIs
     - 96 well plate format
     - Taqman endpoints
   - In *vitro* metabolism
     - Support species selection for regulatory tox
     - Rat, dog, human, other species hepatocytes
     - Rates of metabolism, met ID
   - Microsampling PK
     - Mouse or rat
     - Minimise compound use, inter-animal variability
     - PK analysis

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I. Target Safety Review (TSR)

The aims of a target safety literature review are:

1. Identify potential on-target toxicities
2. Define the early discovery experiments required to evaluate potential target-related safety issues (possibly using tool compounds)
3. Inform the design of subsequent regulatory safety studies

At CXR, target safety reviews are carried out by our experienced toxicologists and biochemists, rather than using a purely bioinformatic approach. A target safety literature review will draw on some or all of the following sources:

- Scientific literature (e.g. tissue expression & distribution, protein function, effects in gene knockout models, effects in relevant disease models)
- Known toxicological effects (preclinical and clinical) of reference and competitor compounds
- Bioinformatics support, e.g. Ingenuity pathways analysis.

Importantly, CXRs extensive preclinical laboratory capabilities mean that we can support the entire target safety review process, by if required designing and running experiments to assess potential target-related safety issues.

II. Bespoke in vivo studies

CXR recommends the use of early, bespoke in vivo studies to:

- Confirm any expected on-target / off-target, tissue specific toxicities
- Compare potential lead candidates, and make an informed candidate selection
- Inform design of early GLP toxicity studies

Typically these programs have two stages. In the first, the potential leads are dosed in a single dose, ascending dose TK study in rats. The aims of this study are two-fold:

1. Identify acute toxicities (this will likely be the first time the compounds have been dosed at “toxicological doses” in vivo)
2. Define dose and exposure relationships, so compounds can be compared in repeat-dose study on the basis of comparable exposure rather than dose

In the second stage, compounds are compared in a bespoke repeat dose study in rats (typically 7 / 14 days), as outlined in the following diagram.
Case Study

A customer of CXR Biosciences® was in the latter stages of lead candidate selection, expected to have on-target toxicity, and had 4 development candidates to choose from.

First, a single dose, ascending dose TK study in rats (four escalating doses, n = 3 per group, single sex) was used to rapidly generate TK data (seven plasma TK points over twenty four hours generated for each animal), so that doses could be selected for a subsequent repeat dose study whereby compounds could be compared on the basis of comparative exposure rather than dose. This study also confirmed the absence of acute toxicity at the doses being tested (each group of rats was observed for 24 hours before the next group was dosed).

Of note, this study used CXRs microsampling approach, which allowed TK data to be generated in the test animals, obviating the need for a satellite TK group. As well as being desirable from a 3Rs perspective, this reduced the amount of compound needed, and the time needed to generate data – two very important considerations for a drug discovery project.

The 4 compounds were then compared in a bespoke 7 day repeat dose study in rats (single dose level per compound, n = 5 per group, single sex). Doses were chosen on the basis they would generate comparable systemic exposure. As with the preceding study, toxicokinetic blood samples were taken on day 7 using CXRs microsampling capability, allowing toxicological effects to be linked to exposure in individual animals.

The tissues for histopathology were chosen on the basis of the predicted pharmacology-dependent toxicity together with common target organs.

In addition, samples of tissues in which the target is expressed were taken for potential analysis of pharmacological biomarkers. Analysis of this type allows comparison of the different candidates on the basis of comparative pharmacodynamics effects in addition to comparative exposures.

The customer was committed to making a rapid candidate selection in order to meet their internal timelines. CXRs flexible scheduling allowed generation of quality data in the short timeframes demanded:

*Pathology may take an additional 1-2 weeks
III. Bespoke, issue specific in vitro assays

CXR has a large range of mechanistic assays available. In addition, we have the capability to establish issue-specific in vitro assays to meet the needs of individual projects.

IV. Supporting in vitro & in vivo DMPK

These assays are used to further define the toxicological and DMPK properties of potential leads, and to inform the design of the clinical trial-enabling preclinical toxicity package and early clinical studies. These assays might include:

CYP induction in rat hepatocytes.

CXR recommends testing for Rat CYP induction in order to predict issues with autoinduction (where the compound induces its own metabolism in repeat-dose preclinical studies, leading to a lowering of exposure), to explain certain liver histopathological changes and to alert for non-genotoxic carcinogenicity in the liver and thyroid.

This assay is designed to identify compounds that interact with transcription factors involved in the induction of CYP1A, CYP2B, CYP3A and CYP4A isoforms. These are, respectively, the aryl hydrocarbon receptor (Ahr), the constitutive androstane receptor (CAR), the pregnane X receptor (PXR) and the peroxisome proliferator-activated receptor alpha (PPARα). The most relevant test system is to freshly prepared rat hepatocytes.

In vitro metabolism. These studies are used to support the selection of the most appropriate preclinical species for regulatory preclinical studies, and to aid in the design and interpretation of those studies, by comparing rates of metabolism and identifying metabolites formed in different species. MIST (Metabolites In Safety Testing) guidelines encourage the identification of differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process. This is important as, potentially, a full toxicity study on a metabolite may be required if that metabolite is not present in a preclinical species at sufficient levels. Therefore it is important to use in vitro studies to predict metabolites across species and inform the choice of preclinical safety species.

CXR routinely runs comparative metabolism studies using hepatocytes or microsomes from a range of species (for example mouse, rat, dog, human, cyno, others as required) to demonstrate comparability of metabolism kinetics and to screen for species-specific major metabolites.

Typical study design:

- Fresh rat hepatocytes prepared at CXR
- 1 - 12 test compounds
- Positive controls: CYP1A1 – TCDD; CYP2B1 – phenobarbital; CYP3A1 - PCN (pregnenolone 16alpha-carbonitrile); CYP4A1 - Wy-16,423
- Vehicle control
- Endpoint: ATP assay for cytotoxicity at 9 concentrations of test compound, 48 hour incubation
- Endpoint: Taqman for CYPs 1A1, 2B1, 3A1, 4A1. Beta-actin and 18S RNA as housekeeping gene controls. Carried out for 6 test compound concentrations (chosen from ATP data)
- Define EC50 and maximal affect if data allows.

CYP induction in human hepatocytes, to assess likely drug-drug interaction (DDI) liabilities caused by induction of cytochrome P450 enzymes. In these studies, we determine the potential for test items to induce CYP1A2, 3A4 and 2B6 in human hepatocytes from 3 donors.

Typical study design:

- Hepatocytes from 3 Human donors, tested separately
- Pre-study assessment of cytotoxicity for each donor hepatocytes using ATP assay
- Test item then tested for its potential to induce CYPs in human hepatocytes in 96-well plates over 72 hours (media changed every 24 hours)
### Discovery toxicology studies are those carried out prior to selection of a development candidate.

- Reduce candidate drug attrition due to preclinical toxicity by providing toxicological data and information that facilitates target selection, chemical series selection & design and candidate selection.

### The primary aims of discovery toxicology are:

1. **Discover target**
   - Identify potential off targets that could lead to unwanted side effects.

2. **Identify mechanistic targets**
   - Understand how the drug will interact with the body.

3. **Identify and map the off-target interactions**
   - Determine the extent of the side effects.

### Discovery toxicology differs in aims and approach from regulatory toxicology.

- The study design is more flexible and exploratory.
- The focus is on identifying new targets and understanding the mechanisms of action.

### Mass spec method establishment and analysis of test item levels in those retained samples.

- Mass spectrometry (MS) is a powerful tool for measuring the concentration of compounds in biological samples.
- It allows for the quantitative analysis of small molecules, which is crucial for understanding the pharmacokinetics (PK) and pharmacodynamics (PD) of new drugs.

**Microsampling PK.** This is a rapid method of generating early PK data that minimises animal and compound use. CXR’s screening PK studies in mice or rats use a serial sampling procedure. This allows an entire PK profile to be taken from the same animal, as opposed to standard approaches where only two or three timepoints are taken per animal, requiring multiple groups to build up a whole PK profile.

Critically, because there are typically only three or four animals per group, relatively little test compound is needed. As multiple samples are taken from the same animal, inter-animal variability is also minimised. Multiple routes of administration are available, and CXR can also provide analytical method establishment, bioanalysis and full WinNonLin analysis.

### CXR Biosciences has an extensive track record and experience in discovery toxicology, having supported the programs of leading pharmaceutical and biotechnology companies for many years.

- We understand the scientific and industry context in which the studies are performed.

- Our flexible scheduling means we can deliver quality data in the short timeframes demanded by a drug discovery organisation.

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**Summary**

- **Discovery toxicology studies are those carried out prior to selection of a development candidate.**
- **Discovery toxicology differs in aims and approach from regulatory toxicology.**
- **The primary aims of discovery toxicology are:**
  1. **Discover target**
  2. **Identify mechanistic targets**
  3. **Identify and map the off-target interactions**

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**V. Downstream investigative toxicology.**

CXR Biosciences has extensive experience of toxicological problem solving. For example, we can help you design and run studies in response to regulatory questions about potential on-target or secondary-target organ toxicities. Problem resolution can identify mechanisms of toxicity, and feedback into discovery, allowing the design of molecules with reduced or removed toxicological activities.
Founded in 2001, CXR Biosciences® uses its collaborative approach and toxicological expertise to help customers large and small solve issues relating to the safety of compounds or selection of research candidates. CXR Biosciences® offers tailored preclinical services in the areas of investigative & mechanistic toxicology, exploratory & discovery toxicology and PK & metabolism. Our customers include leading pharmaceutical, agrochemical, chemical, consumer product and biotechnology companies. CXR Biosciences® is located in Dundee, Scotland, United Kingdom.

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