FRONTAGE
YOUR DRUG DEVELOPMENT PARTNER

THE VALUE OF ROBUST EXPLORATORY TOXICOLOGY TESTING PRIOR TO PIVOTAL GLP STUDIES:

How to Avoid Surprises in Small-Molecule Drug Development Programs

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Introduction

Over the years, the pharmaceutical industry has made steady progress in improving the safety of drugs in development. Consequently, in 2015, only 14 percent of Phase III studies failed due to safety (compared to 55 percent for efficacy), down from 21 percent in 2013. In other words, "efforts to remove unsafe agents earlier in the development cycle are paying off."[1] Much of this success in small molecules relates to careful and methodical work done well before the Investigational New Drug (IND) application.

Preparing a pre-clinical program to support an IND application takes time and is resource intensive, which is why it is often tempting for companies to "rush to the finish line" and skip certain steps. There is ample value in the long run, however, in taking the necessary time to follow best practices in exploratory toxicology testing. An investment at the right stage will avoid unpleasant surprises that can result in otherwise avoidable costs and delays often amounting to millions of dollars and months – even years – added to the development timeline.

The following paper provides practical advice on how to usher a small molecule through preliminary toxicology testing and derive information required to design IND-enabling studies. Indeed, with a rigorous exploratory toxicity program, sponsors can mitigate the risk of unexpected challenges and optimize the Good Laboratory Practice (GLP) study plan.

The Purpose of Toxicology Studies

The overarching purpose of preliminary toxicology testing is to set the subsequent GLP studies for success. That said, exploratory toxicology testing in the non-GLP environment for small molecules can accomplish multiple goals, including:

- Narrowing the number of drug candidates for continued investment.
- Selecting the appropriate rodent and non-rodent species for pivotal GLP studies.
- Determining the appropriate starting does levels for pivotal GLP studies in animals
- Estimating the amount of active pharmaceutical ingredient (API) that will be required in GLP animal studies
- Confirming the suitability of the formulation (if it is not suitable, now is the time to consider and evaluate alternatives)
- Precluding bioanalytical challenges by confirming the appropriateness of internal standards used in preliminary plasma stability tests

Exploratory toxicology testing involves observing and documenting effects in a non-GLP environment. Subsequent GLP-compliant studies should refine the results of preliminary testing, with few surprises and, at the IND stage, permit derivation of a safe starting dose for clinical trials.plan.

The Cost/Benefit Analysis

The average overall cost to bring a drug to market approached $2 billion in 2018 and involved 12 years of development.[2] Pre-clinical work, on average, accounts for $6.2 million of that $2 billion, with the range extending from $698,000 on the low end to $20 million on the high end. Toxicology studies account for nearly a quarter of the non-clinical budget, or $1.5 million (coefficient of variation 0.9).[3]

As tempting as it may be for sponsors to skimp on their investment in non-GLP toxicology studies, a strong case can be made that it is time and money well spent. Performing robust exploratory toxicology tests up front saves time later on in the process by ensuring that studies don't have to be repeated later and that applications are not held up by regulators. Any such backtracking or delays result in direct labor-

Benefits of Thorough Preliminary Toxicology Testing

- A data package that is useful in a pre-IND meeting with regulators and provides support to the Sponsor's IND-directed plan
- Confidence in the maximum dose levels
- Confidence in the species selection
- No formulation issues
- Ability to plan for material needs
- Knowledge of how to proceed with bioanalytical validation

[1] Harrison, Richard K, "Phase II and Phase III failures: 2013-2015," Nature Reviews, Vol 15 December 2016
[2] *https://theskepticalchemist.com/process-costs-drug-development/.*
[3] *Stergiopoulos, S., Kim, J., and Getz, K., "Characterizing the Cost of Non-Clinical Development Activity," ContractPHARMA, June, 5, 2013.*

atory costs and massive lost opportunity costs. Every day that a development program is delayed diminishes the patent life, opens the door a little further to competition, and reduces the drug's revenue potential.

Elements of an Exploratory Toxicology Program

Exploratory toxicology testing takes place in a non-GLP environment. (Note: non-GLP studies should not in any way be construed as have less integrity from a scientific/validity standpoint as GLP work.) In general, an exploratory program for a typical small molecule therapeutic taken in repeat doses would involve:

- Rodent and non-rodent dose-escalation studies
- Rodent and non-rodent preliminary repeat-dose rangefinder studies (with toxicokinetics)

Dose escalation studies should establish the Maximum Tolerated Dose (MTD), meaning that the animals cannot tolerate higher doses.

In repeat-dose preliminary studies the best practice is to expose the test animals to the drug systemically for at least seven days. Toxicokinetics tests should be performed on the first and last days to determine the toxic effect of the compound over time and what happens to concentration of the compound in plasma over time. Other findings may include:

- Acute toxicity
- Poor tolerability
- Repeat dose toxicity
- Repeat dose tolerability issues
- Biochemical/metabolic alterations
- Interference with MS bioanalysis

Challenges Commonly Encountered in Pre-Clinical Toxicology Testing

When a toxicology-testing program runs smoothly and surprises are minimized, it is easy to take the efficiency for granted. However, because no two programs are exactly alike, much can potentially go wrong. The best run projects are those address the potential challenges in the following areas:

- API Supply Issues. It is important for both budgetary and logistical reasons to be able to estimate the amount of API that will be needed for toxicology testing accurately, as requirements can vary widely from less than 1 kg to almost 6 kg when low toxicity is observed in dogs. The material need not be produced in a GMP environment, but it is nonetheless very costly. (in some cases, the cost of the API used

The Toxicity Myth

There exists a common perception that the best outcome of toxicology testing is to have no findings of toxicity.

The challenge, however, is in proving non-toxicity. Doing so requires dosing animals at very high levels, which uses a great deal of API, and so can be expensive.

It is better, actually, to be able to define the point at which a drug becomes toxic so that it is relatively straightforward to determine the No Observable Adverse Effect Level (NOAEL) – the highest dose that produces no observable adverse effects. In other words, although it sounds counterintuitive, *"a little bit of toxicity may be a good thing."*

If there is no evidence of toxicity and a NOAEL cannot be identified, then the adequacy of the toxicity study can be established in a number of ways per ICH guidelines:

*The animals received the maximum feasible dose;

*A dosing limit of 1,000 mg/kg per day must be reached; or ≥2000 mg/kg if mean exposure margin is <10x clinical exposure and human dose is >1 gram per day

*The animals must be dosed at 50 times the expected efficacious human exposure; or

*Increasing the animal dose does not increase systemic exposure.

in toxicology testing can exceed the cost of the testing program itself.) The ideal situation is to have sufficient material to not only support the toxicology tests, but also to have a built-in buffer in case additional tests are required later or in case there are subsequent questions about the integrity of the lot.

Repeated synthesis of multiple batches of API is expensive because of set-up costs and the analytical requirements required to establish batch-to-batch comparability.

The amount of API that will be required can be estimated once rigorous preliminary testing is completed and the test species and protocol is known and the dosing level can be anticipated.

- Species Selection. Generally, a toxicology program will require testing in a rodent and a non-rodent species. Industry guidance states that at least one non-clinical species must demonstrate appropriate metabolic exposure, a situation that is possible only if the metabolite formed in the test species is the same as in humans and if is formed in the same proportions. Rats and dogs are appropriate choices in perhaps 90 percent of cases, but, obviously, one must know if one's compound is in the majority or in the 10 percent that require a different species.

  Good preliminary testing, including in vitro metabolic profiling studies (performed on liver cells) will determine which animal's metabolism most closely mimics the human condition with respect to the compound. Although many sponsors at this stage seek only to assess the compound's metabolic stability (the rate at which the compound disappears), it may not be enough to establish that the disappearance profile in the test animal is comparable to that in a human. The metabolic byproducts may be different, and so it is important to monitor the metabolites as well.

  ***GATHERING INFORMATION IS NECESSARY** TO SUPPORT SPECIES SELECTION*

  It is also prudent to confirm the results of the in vitro metabolite identification with in vivo testing, as soon as data are available, since in vitro metabolism might not be identical to the in vivo situation in animals and humans.

  Gathering this information is necessary to support the species selection and to defend one's ultimate choice with regulators. If this step doesn't account for all of the metabolic exposure, a sponsor may be required to backtrack and complete an additional toxicology test program with the right metabolite(s), a process that can add hundreds of thousands of dollars and more than a year to the preclinical timeline.

- Accumulation Issues. These can be identified by performing toxicokinetic tests on the first and last day of the range-finder studies.

- Bioanalytical method selection. It is important to conduct non-GLP frozen plasma stability studies for the species under consideration to identify any potential issues before toxicology testing is begun. The bioanalytical method must be validated to ensure that it is rugged enough. When it is not, it is most commonly due to an inappropriate internal standard.

  Using non-validated or poorly validated bioanalytical methods can lead to studies being rejected by regulators.

- Unexpected toxicity OR lack of toxicity. Good preliminary toxicity studies identify target organs and permit dose level selection such that a clear NOAEL can be established in GLP studies. It is a common fallacy to think that finding

Expected Results

A carefully planned and executed preliminary toxicology program will result in:

- An acceptable plan for the IND enabling studies
- Increased confidence that GLP studies will result in a successful IND application
- No unanticipated vehicle effects or formulation issues
- Proof that the bioanalytical method was robust
- Synthesis of sufficient test article, adequately characterized for the GLP studiessuccessful IND application

toxicity is unwelcome. In fact, you do hope to discover the point at which a drug becomes toxic, otherwise, you must continue to escalate the dose, using more and more material, or take other steps to justify the adequacy of the toxicity study. (See box: The Toxicity Myth)

How to Mitigate Risk in Toxicology Studies

An exploratory toxicology testing program is a pre-requisite for advancing a compound into clinical trials. The more carefully planned and executed the program, the easier, faster, and less expensive the process of advancement will be. A successful program:

- Anticipates potential problem areas and addresses them proactively. The idea is to avoid surprises which cause delays and unanticipated expense.
- Nails down the study variables before animal tests are begun. This avoids adding unnecessary cost.
- Doesn't take shortcuts. It pays to be thorough; a matter of a few weeks at this stage can save months of rework and hundreds of thousands of dollars in rework.
- Relies on laboratory specialists who have extensive experience in toxicology testing. The testing process is complex and expensive. Knowledge of how to navigate this testing stage most efficiently is gained only through years of experience and an awareness of all of the potential pitfalls.

Conclusion

Thus, there is ample latitude to make decisions (usually motivated by a sense of urgency in moving forward) that can, in the end, prove to be unwise. Experienced pre-clinical laboratories know how to avoid the pitfalls that ultimately cause rework and delays. A proper, thorough job at this stage will produce scientifically valid data that can be defended with regulators and that will support sound GLP studies. Spending the time and resources to do good preliminary testing well will pay dividends in saving time and money down the road.