Preclinical Drug Development

A guidance prepared by

Pharmacelsus®
CONTRACT RESEARCH ORGANISATION

From a 2004 NIH Summit Workshop:

“A major reason for the tremendous cost of drug development is the high rate of drug candidate failure during clinical testing. The withdrawal of drug candidates in late-stage testing has a significant impact on driving up the expense of bringing drugs to market and reducing the number of drug candidates moving through the pipeline. It is recognized that failure to detect drug toxicities in preclinical testing contributes to drug candidate failure during clinical phase testing.”
1 Introduction

Many drugs entering clinical trials still fail in late stage development due to unexpected drug toxicities (~22%), lack of biological activity (~31%), or poor pharmaceutical properties (~41%). The pharmaceutical industry has recognized the urgent need to decide already in the early preclinical development stage when a drug candidate is sufficiently characterized and capable to be selected for human testing. This way, greater value can be generated while minimizing escalating development costs. To achieve this, sophisticated testing strategies have to be used at a very early stage to streamline hit-to-lead generation and to identify the most promising lead. Once the lead has been selected, a comprehensive drug development plan must be designed and executed. Both tasks, lead generation and designing a drug development plan, require years of hands-on experience and an extensive network of experts in drug profiling, regulatory issues and clinical trials.

Before any clinical trial can be initiated, a number of non-clinical studies need to be conducted that provide the evidence necessary to support the safety of administering the compound to humans. This is required by the regulatory authorities of the region in which the trial will be conducted (i.e. FDA/EMA). The sponsor must first submit data showing that the drug is reasonably safe for use in initial, small-scale clinical studies.

Important considerations for determining the nature of such studies and their timing with respect to clinical trials include:

- Targeted disease area (chronic disease, cancer, immunocompromised etc.)
- Duration of drug exposure and total drug exposure
- Characteristics of drug (t_{1/2}, biodistribution, bioavailability, serum binding, ADMET etc.)
- Special population targeted (older patients, children, pregnant women etc.)
- Route of administration (intravenous, intramuscular, oral etc.)

The scope of preclinical studies largely depends on the type of compound (e.g. small molecule vs. biotech-derived substance) and the disease area targeted (e.g. chronic vs. life-threatening disease). A comprehensive Drug Development Plan should always be in place prior to initiating any preclinical work.

*It is of utmost importance to take into account the requirements of all drug development stages including large-scale manufacturing, storage, formulation, cost of goods, regulatory and marketing aspects. Failure to do so will inevitably result in significant time delays, higher development costs, reduced return on investment (ROI) and possibly project termination.*

The following paragraphs will highlight selected preclinical studies of critical importance. It should be noted that the list is an excerpt only and does not cover the entire range of testings required. A detailed list can only be prepared with a deeper understanding of the project. Professional management of the entire drug development process and detailed documentation of all preclinical activities are a prerequisite for any successful drug approval.
2 Pre-clinical drug screening *in vitro*

Generally, many interesting drug candidates are identified by pharmaceutical companies based on highly specialized and target-specific assay systems. The vast majority of these “hits” still requires further evaluation and selection. It is necessary already at an early stage in drug development to optimize the subsequent hit-to-lead generation and to screen emerging lead candidates by choosing meaningful in vitro assay systems. The rising costs of clinical drug development make it necessary to keep the numbers of drug candidates entering clinical trials as small as possible. The modules listed below exemplify the plethora of available screening systems designed to simplify lead generation, identify drug toxicities, reduce unwanted drug-drug interactions, and describe metabolic and pharmacokinetic profiles of drug candidates:

Relevant physico-chemical properties are usually determined in the very first drug development phase and can include aqueous solubility, chemical stability in aqueous solutions, lipophilicity, protein binding and permeability through artificial (PAMPA) membranes. These properties will ultimately predict drug absorption, distribution, metabolism, excretion and toxicity (ADMET) and are of fundamental importance for the success of this particular drug.

The second module that needs to be addressed covers all ADMET parameters. One of the most widely used assay systems to predict permeability through biological membranes is the blood-brain-barrier and the Caco-2 assay. The latter one can be performed uni- or bidirectional and yields important information on the potential of a drug to achieve systemic exposure. A mechanism counteracting drug absorption (thus avoiding intracellular accumulation of therapeutic drug concentrations) should be evaluated at the same time, namely the activity of the drug efflux pump Pgp, coded by the multi-drug resistance gene (MDR).

Since metabolic stability and/or drug metabolism are a pivotal contributor to drug efficacy, assays using liver microsomes of different species are an indispensable component in any drug screening process. Furthermore, if drugs are believed to be metabolized, regulatory authorities generally require identification of the metabolite(s) and description of the corresponding metabolic pathways. This can be done by performing cytochrome P450 phenotyping, inhibition, and induction assays in combination with mass spectrometry as analytical tool.

In preclinical drug testing a focus should be on the generation of early human data, since approaches using animal models are not always predictive of the in vivo effect in humans due to differences in P450 isoforms, phase II enzymes or drug transporters. Although adverse drug effects can be seen in other systems, the clinical success rate could be increased by integrating the human factor into the development screening plan from the beginning. The liver, representing the major organ for metabolism, has to be mainly involved into this strategy. Ideally, hepatocytes, subcellular fractions like microsomes or S9, liver slices or recombinantly expressed single P450 isoenzymes, should all be originating from the relevant species in order enhance prediction accuracy.

The FDA prefers isolated hepatocytes, which represent the complete range of metabolic liver activity. Although recombinant P450 isoforms or liver microsomes indeed enable the characterization of potential inhibitors or the identification of specific pathways, hepatocytes are considered as the most suitable model with best extrapolation to clinical trials. However,
for intrinsic clearance screening, liver microsomes are preferred for they represent a simple system that can be adapted to high throughput screening (HTS).

A reasonable screening strategy therefore focuses on the route of human metabolism, the identification of P450 isoforms that are involved, and the analysis of inhibitory or inductive interactions. In a practical approach, compounds are ranked regarding their intrinsic clearance in human liver microsomes, followed by a species comparison to provide an indication of the species that probably best mimics human metabolism in vivo. NCEs, displaying a potential for a direct phase II metabolism should be investigated in S9 fractions for their conjugation potential.

Screening for P450 inhibition can be performed in a three-tier approach. Inhibition screening often includes CYP3A4 and CYP2D6 at a few test concentrations on recombinant P450 isoforms initially. In a second step, concentration-response curves for CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are performed using recombinant P450s. A final characterization, including concentration- and time-dependent inhibition, is subsequently performed in hepatocytes.

Since P450 inducers bear a risk to cause liver toxicity, e.g. due to the formation of toxic metabolites, and lead to a reduction of systemic drugs levels, P450 enzyme induction studies should not be underestimated and are recommend at least for lead compounds, even though a positive result will not necessarily prevent a drug from entering first-in-man trials. As recommended by the FDA, induction studies should be performed on fresh or cryopreserved hepatocytes from at least three donors. Cryopreserved hepatocytes are widely accepted as they present better reproducibility. The most valid screen is the determination of enzyme activity using probe substrates, whereas mRNA quantification can only provide information in case the potential inducer is not a P450 inhibitor at the same time. Again, one should be careful when extrapolating induction from rat or dog studies since the results for distinct isoforms can differ substantially.

Last but not least, in vitro cytotoxicity assays using biological endpoints such as fractional survival, membrane integrity, cell cycle distribution and metabolic activity will generate valuable information on the toxic pattern of a particular drug.

Prior to engage in expensive and time consuming screening programs, an abbreviated test package that covers several key aspects of physico-chemical and ADMET properties should be executed. This not only saves time and resources but also streamlines and facilitates identification of the most valuable candidates with the best chances to enter further clinical development.

3 Pharmaceutical technology

The rules and regulations for the development of small molecules and biotech-drugs are fundamentally different. The pharmaceutical technology comprises all steps of drug manufacturing. For biotech-drugs, this includes gene synthesis, expression system, fermentation technology, the entire line of downstream processing, and the packaging of the final drug compound for clinical use. For small molecules, pharmaceutical technology also comprises chemical synthesis, purification methods and drug identification. For biotech drugs, significant reductions in cost of goods can be accomplished by carefully selecting expression systems, streamlining R&D efforts, facilitating technology transfer to contract manufacturers,
and monitoring production. A large number of pharmaceutical companies engage in strategic alliances with contract manufacturers, which often involves the sharing of cutting-edge technologies as well as pivotal intellectual property. In order to efficiently deal with these complex technological environments, involvement of a management team with proven track record is indispensable.

4 Pharmacological drug profiling

This area contains all information related to the characterization of the drug intended to enter clinical trials. It is highly recommendable to develop standard operating procedures (SOP) and validated techniques, although not required in early clinical testing, to document reproducibility and reliability. The goal of pharmacological drug profiling is to present as many relevant data as possible to facilitate the risk-benefit determination by regulatory authorities and to obtain permission to start with the clinical drug development process. The following are components of the pharmacological drug profiling required by regulatory authorities:

4.1 Chemistry, manufacturing and control (CMC)

The discussion of safety issues in the CMC section is typically brief. However, for biotech-derived products, a more detailed description might be warranted. Studies include but are not limited to:

- Identity: what substance are we dealing with?
- Purity: what is the percentage of purity?
- Impurities: what impurities are included at what percentage?
- Antigenicity: can neutralizing antibodies be induced?
- Immunogenicity: can the drug induce unwanted immunological responses?
- Strength: what is the concentration of the drug substance?
- Additives: what inactive substances are in the final formulation?

Acceptable limits and analytical methods used to determine identity, purity, impurities, and strength need to be developed. Since the drug substance needs to be recovered from body fluids such as serum, adequate tools must be available to allow accurate detection and quantification of the drug. This task is often extremely difficult due to binding of the drug to serum components or drug metabolism and requires a large amount of experience and sophisticated analytical tools. Furthermore, biotech drugs need to be characterized regarding post-translational modifications such as phosphorylation and glycosylation pattern.

4.2 Physical, chemical or biological characteristics

A list of all biological activities with reasonably perceived relevance to the indication area has to be listed. This also includes expected induction of fever, allergic reactions, pyrogenicity, hypersensitivity, and cytokine induction. Additionally, biomarkers should be developed to demonstrate the desired biological effects in vivo. These surrogate endpoints become more and more important in providing “clinical proof of concept”, the major milestone in early clinical development. Due to the nature of the drug, biotech-derived compounds require significantly more characterization of their reasonably perceived biological effects in vivo.
4.3 Source and method of preparation

It needs to be addressed whether the product is manufactured by chemical synthesis (for peptides) or fermentation (for proteins), what fermentation organisms (bacteria, yeast, CHO, COS etc.) and fermentation media has been used, and whether animal products are contained in the media. This section is usually easier to address for small-molecules.

4.4 Removal of toxic reagents

Since many processes contain unwanted or even toxic by-products (e.g. antibiotics or pyrogens in biotech drugs), a detailed description of the methods used to remove those components from the system has to be provided. It is important in this regard to know what type of toxic reagents the regulatory authorities look for, to incorporate that knowledge into the manufacturing process (e.g. avoiding antibiotics) and to anticipate difficulties upfront. All impurities need to be documented and, if a risk can reasonable be associated with it, characterized.

4.5 Quality controls

Methods need to be listed documenting how the quality control of the product is monitored. The use of SOP, internal standards, and validated methods is highly recommended. The degree by which quality control and quality assurance is implemented directly reflects on the value of the work conducted. It is recommended to outsource QC and QA or at least to conduct external audits to avoid conflict of interest and to assure the highest level of quality possible.

4.6 Formulation

Prior to initiation first fermentation and drug manufacturing steps, a decision needs to be made regarding the final formulation of the drug product that will be administered to animals and, subsequently, to patients. It is very time consuming, costly, and often detrimental to change formulation in midst of ongoing clinical trials. A deep understanding of clinical trials is warranted to avoid dead end developments.

4.7 Sterility

This seems to be a rather uncritical step but potential problems down the line should be anticipated early on in development. The choice of final container (glass or plastic), storage condition (frozen, refrigerated, or room temperature), and storage form (lyophilized, in solution) to name a few all influence sterility testing efforts and need to be determined upfront.

4.8 Stability information

Stability studies should be designed in order to address the following areas of interest: stability in solution, stability in final container (e.g. glass vial), stability at different storage temperatures (-20°C, 4°C, 20°C, and 37°C), stability after repeated thaw-freeze cycles, stability in regards to material contacted (e.g. glass, rubber, plastic). It is recommended to
include as many relevant biochemical parameters as possible including mass spectrometry, SDS-PAGE, immunoassays, HPLC, and protein quantification assays.

5 In vivo Pharmacology and Toxicology

5.1 Preclinical Drug Optimization

During the hit-to-lead generation phase, many hit compounds are investigated to filter out potential failures and select the most promising candidates. Afterwards, an extensive lead optimization process follows with the objective to synthesize a small number (1-5) of lead compounds with improved biological activity, reduced side effects, and advantageous physiochemical and metabolic properties indicative of good pharmacokinetics and pharmacodynamics. These compounds are then deemed suitable to enter early stage clinical testing in humans.

5.2 Pharmacology, Biodistribution, and Toxicology

Due to the high cost associated with preclinical toxicology studies in animals, it is highly recommended to first perform non-GLP dose-range finding and pilot studies. This will allow the sponsor to reduce the amount of animals and study drug needed for the GLP-study, resulting in significant cost savings. As mentioned previously, the selection of appropriate protocols and testing sites are crucial for the success of this very important requirement of any preclinical data package. If clinical studies are intended to be conducted under the regulatory authority of the FDA in the US, studies in two animal species (one rodent and one non-rodent) have to be completed. The EMEA generally requires only one species although it might be advisable to add a second species anyway in preparation of a regional expansion of trials at a later stage. This will greatly facilitate and speed up approval by the FDA.

5.3 Biomarker identification

In recent years, biomarkers have increasingly been used as surrogate endpoints, replacing classical endpoints such as patient survival and tumor progression. The identification of disease-specific biomarkers is of greatest importance and should be implemented as early as possible. This task is one of the most difficult endeavors on the molecular level and requires a specialized expertise in the field of proteomics and the use of high-tech mass spectrometry equipment. If biomarkers cannot be identified, special attention needs to be paid to the development of alternative markers for biological activity.

6 Concluding Remarks

Forward looking project and risk management, deep knowledge of regulatory requirements and hands-on experience in clinical drug development are undoubtedly the most time- and cost saving components in the entire drug development process. It is highly recommended to involve a professional management team right from the beginning that can support the design of a drug development plan, coordinate the different phases of drug development, identify experienced contract research organizations (CRO), provide regulatory support, and control the efficient and timely execution of the DDP.
7 List of selected documents released by regulatory authorities

7.1 EMEA [http://www.emea.eu.int/]

- General considerations for clinical trials (ICH E8)
- Impurities in new drug substances and products (ICH Q3AR)
- Stability testing (ICH Q1AR, ICH Q1B, Q5C)
- Stability testing for biotech products (ICH Q1AR, ICH Q1B)
- Test procedures and acceptance criteria for small molecules (ICH Q6A)
- Test procedures and acceptance criteria for biotech products (ICH Q6B)
- Good manufacturing practice (GMP) for active pharmaceutical ingredients (ICH Q7A)
- Preclinical safety evaluation of biotech-derived pharmaceuticals (ICH S6)
- Immunotoxicity studies (ICH S8)
- Position paper on non-clinical safety studies to support clinical trials with a single microdose (CPMP/SWP/2599/02 Rev 1)
- Impurities in new active substances (directive 75/318/EEC)
- Production and quality control of medicinal products derived from recombinant DNA technology (directive 75/318/EEC)

7.2 FDA [http://www.fda.gov/]

- Guidance for industry: Exploratory IND Studies (January 2006)
- Guidance for industry: Clinical trial endpoints for the approval of cancer drugs and biologics (April 2005)
- 21 CFR 312.23(a)(10), (11) and (b), (c), (d), and (e): content and format of INDs for phase I studies of biotech drugs
- guidance for industry: IND meetings for human drugs and biologics: CMC information