Preclinical research in drug development

From bench to bedside – the long journey from the lab into the clinic

Developing a novel drug is an interdisciplinary endeavour involving a multitude of competences from biologists, chemists, computer scientists, medical staff, statisticians, and regulatory experts. Taking a compound from bench to bedside requires up to 12 years at an average estimated cost exceeding US $1 billion.1 Figure 1 summarises this long-term process (see overleaf).

Drug development starts with the identification of a “druggable” target. Bioinformatics, genetic association studies, and phenotype screening are valuable tools in the discovery of novel targets. To validate the relevance of the identified target for a particular disease, studies are performed to investigate whether target modulation is disease modifying.2 Eventually, lead compounds are obtained and their potential to interact with the target as well as their effect on the biological system is evaluated. Thousands of modifications and variations of these lead compounds are synthesised and tested during preclinical activities. Once an optimised compound is identified, this investigational new drug (IND) becomes a candidate for clinical trials involving human subjects.

Clinical trials are conducted over different phases (Phase I–IV), starting from a small number of subjects and extending to large cohorts.1 In Phase I studies, the IND is administered to humans for the first time.3 Early Phase I studies (previously Phase 0) describe first-in-human studies where a small group of subjects, usually 10 to 15 individuals, received a single, sub-therapeutic dose to obtain pharmacokinetic information without inducing pharmacological effects. The goal of these exploratory studies is to investigate whether the drug candidate performs as expected based on preclinical studies. If successful, further studies assess safety and tolerance of the IND in human subjects. These studies typically involve 20–50 healthy volunteers. Apart from determining the drug’s maximum tolerated dose by increasing the treatment dose until dose-limiting toxicity is reached (dose escalation), the drug’s most common and serious adverse effects (AEs) as well as pharmacological, pharmacodynamic, and pharmacokinetic properties are evaluated.4

Approximately 70% of drug candidates move from Phase I to Phase II, in which therapeutic efficacy of the IND in patients is assessed.5 Phase II studies typically involve several hundred patients. The study population is well defined by inclusion and exclusion criteria, and based on the dose or dose range determined in Phase I, dose response in patients and the drug’s biological activity are evaluated. Comparison of (i) pre- and
post-treatment status of patients and (ii) response of patients receiving IND and a placebo drug provide preliminary data on effectiveness. Although researchers obtain indications regarding the drug’s benefit, Phase II studies are not comprehensive enough to provide sufficient evidence. During Phase II, subjects are carefully monitored for AEs to further assess safety of the drug. Moreover, these trials commonly determine the optimum dose regimen to be used in Phase III.6

Figure 1. From basic research to approved drug

Basic research

Drug discovery
5,000-20,000 compounds

Preclinical research
250 compounds

Clinical studies
5 compounds

Approved drug
1 compound

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About one-third of tested INDs transition into Phase III having 100-500 patients and with the primary objective of confirming the therapeutic benefit of the IND as well as its safety and efficacy in the intended indication.4 Moreover, the use of different dosages and study populations and combination with other therapeutic agents are investigated to provide information regarding indications and contraindications as well as dose range and AEs. As Phase III studies include a larger cohort and have a longer duration than Phase I and II studies, they can potentially reveal rare and long-term side effects. Based on the outcome, 25% to 30% of INDs progress to the next phase.6,7

Phase IV studies are long-term and typically conducted after regulatory agency approval (post-marketing studies).5 They often involve more than 10,000 individuals of the relevant patient population and aim at gathering additional information on safety, efficacy, and new indications. Thus, Phase IV trials assess the drug’s real-world effectiveness in an extensive cohort and provide the opportunity of detecting unique AEs. In some cases, this might result in withdrawal of the drug from the market or restriction to particular uses. On the other hand, Phase IV studies may also open up new markets by demonstrating effectiveness for new indications.6,7

**Preclinical studies**

Preclinical studies aim at providing information about safety and efficacy of a drug candidate before testing it in humans. Furthermore, they can provide evidence for the compound’s biological effect and usually include both in vitro and in vivo studies. Preclinical studies have to comply with the guidelines dictated by Good Laboratory Practice to ensure reliable results5 and are required by authorities such as the FDA before filing for approval as IND. Insights into the compound’s dosing and toxicity levels are essential to determine whether it is justified and reasonably safe to proceed with clinical studies and are provided by studies on pharmacokinetics, pharmacodynamics, and toxicology.5

**Pharmacokinetics – what does the body do to the drug?**

The effect of a drug is determined by the amount of active drug present in the body particularly at the target site. This, in turn, depends on absorption, distribution, metabolism, and excretion (ADME) of the compound. Pharmacokinetics describes changes in plasma concentrations over time as a consequence of ADME. ADME profiling is critical for establishing dose range and administration schedule for subsequent phases of the clinical trial.1,4,9

Most drugs are administered orally and need to be absorbed in the gastrointestinal tract to enter the bloodstream, allowing them to be transported to their site of action. On its way to the target site, the drug reaches the liver, where first-pass metabolism takes place. Consequently, the drug concentration – and thus its bioavailability – is reduced before entering systemic circulation. Intravenous drug administration bypasses the first-pass effect, resulting in greater bioavailability. Once in the circulation, the drug is transported to different tissues. Distribution of the compound throughout the body is determined by (i) the drug’s affinity for plasma proteins, (ii) the drug’s molecular properties and polarity, and (iii) tissue vascularisation. After entering the body, drugs are metabolised to facilitate elimination. Metabolism refers to the chemical alteration of the parent drug into pharmacologically active or inert metabolites. To ensure adequate long-term dosing and appropriate steady-state concentrations of the drug, it is critical to obtain information on drug elimination from the body (clearance). Clearance is mainly achieved via the renal and hepatic routes; however, pulmonary clearance plays a major role for volatile drugs such as anaesthetics.1 Concomitant disease, lifestyle factors, and patient’s age can affect clearance and these are frequently studied in later stages of the clinical trial.8 When the rate of clearance equals the rate of absorption, a so-called steady state is reached. Typically, maintaining a stable steady state level is desirable and can be achieved through repeated dosing. Eventually, the drug
and its metabolites are excreted from the body mainly through urine or faeces.

Toxicology – it is potent, but is it safe?
To determine whether a drug is safe for testing in human subjects, preclinical toxicology studies are performed to identify the treatment regimen associated with the least degree of toxicity and thus determine a suitable and safe starting dose for clinical trials. Additionally, they can be used to establish biomarkers for monitoring potential AEs later. Starting with single-dose studies to identify organs that might be subject to drug toxicity, preclinical in vivo studies continue with repeated-dose approaches. The treatment regimen ideally mimics the intended clinical design with respect to treatment duration, schedule, and route of administration. Other studies evaluate carcinogenicity, genotoxicity, and reproductive toxicity. While the drug’s genotoxic effect is usually studied based on its potential to induce mutations in yeast-based in vitro systems, carcinogenicity and reproductive toxicity studies typically involve rats. As the tumorigenic effect of a drug may only become evident after prolonged exposure, carcinogenicity studies comprise continuous drug administration for a minimum of six months.

The ideal preclinical model accurately mimics human disease
Obtaining relevant results from preclinical studies with a high degree of generalisability requires appropriate preclinical models that are as comparable to the target population as possible. Typically, this involves a series of experiments using in vitro, in vivo, and more recently, also in silico models.

In vitro models – studying the drug in a petri dish
In vitro studies are a relatively fast, simple, and cost-efficient way of preclinical testing. Those studies utilise cell, tissue, and organ cultures, or focus on particular cell components such as proteins or other biological macromolecules. In vitro studies permit tight control and monitoring of experimental settings and often provide mechanistic evidence for the investigational compound’s mode of action. While having the potential to provide mechanistic insights, in vitro models are constrained by the fact that isolated cells may not behave in a petri dish as they would within the body where they partake in crosstalk and interaction with millions of other cells. Consequently, more sophisticated preclinical models are required to establish the investigational compound’s safety profile before transitioning to a clinical setting.

In vivo models – is the mouse the best experimental animal?
In vivo studies consider the complete organism based on various animal models. Similar to studies in humans, animal testing is tightly regulated in most countries and permission from local ethical review boards is required to ensure that no unnecessary harm is done to the experimental subjects. Recent advances have refined the use of animal models in drug development through non-invasive imaging technologies, microsampling, and telemetric monitoring. Naturally, controlling experimental settings is far more complicated for in vivo studies and, due to the complexity of the living organism, compounds may behave differently from what is expected based on results obtained in a test tube.

The choice of appropriate animal models depends on myriad criteria and requires understanding of species-specific physiology and similarity with regard to the target organ, metabolic pathways as well as financial, regulatory, and ethical considerations. Typically, in vivo studies are performed in a rodent (e.g., mouse, guinea pig, hamster) and non-rodent model to comply with FDA requirements. Mice, rats, and dogs are among the most frequently used animal models while testing in primates (e.g., monkeys, apes, etc.) is performed occasionally and typically for larger molecules.

One of the most popular animal models in pharmaceutical testing is the mouse.

The genomes of mouse and man are highly similar: 99% of all mouse genes overlap with those of humans. Additionally, genomic manipulation in this organism has become fairly simple. Nevertheless, species-specific differences in host immune response, drug metabolism, and tumour heterogeneity affect therapeutic outcomes. Differences in pharmacokinetics and pharmacodynamics among species are also not negligible and thus, mouse models often suffer from poor predictive power regarding clinical efficacy. However, lack of superior alternatives makes mouse models the gold standard for testing cancer-targeting drugs.

Classically, such mouse cancer models were limited to transplantation of cultured human tumour cells (cell lines) to immunodeficient mice such as nude or severe combined immunodeficiency (SCID) mice. Transplantation of cells, tissue, or organs from one species to another is called xenografting. In these cell line-derived xenograft (CDX) models, cancer cells are injected subcutaneously and tumour growth curves are established by measuring the size of the tumour in regular intervals. Treatment of tumour-bearing mice with a drug candidate provides information regarding its potential to reduce tumour growth and thus its in vivo efficacy. However, these cell lines have been passaged under artificial conditions that do not recapitulate the natural tumour microenvironment. Consequently, CDX models may lack similarity with human disease. To improve clinical relevance, a range of different mouse models has been developed and is used in in vivo experiments:

Patient-derived xenograft (PDX) models:
Tissue from a patient’s primary tumour is directly implanted into the animal. This strategy omits in vitro adaptation of tumour cells and, thus, these models are more similar to human disease in terms of stromal composition and tumour heterogeneity, in contrast to classical CDX models. The PDX approach is challenging; however, recent advances in sample retrieval and transplantation technology made this method feasible. To date, PDX models consist of almost exclusively subcutaneous transplants.

Orthotopic tumour models: Tumours are implanted into the organ of origin (i.e. orthotopically) to better mimic the microenvironment and recapitulate metastasis...
pathways of human tumours.14,15 Consequently, orthotopic models are more clinically relevant. Orthotopic PDX models are technically challenging and thus uncommon, while orthotopic transplantation is widely used for CDX models.11

Genetically engineered mice (GEM): Genetically engineered has given rise to humanised mouse models and provides valuable tools for translational research. Through genetic manipulation, mutations in oncogenes or tumour suppressor genes associated with human malignancies are introduced. In GEM, tumours develop orthotopically from initiation through progression in their native microenvironment recapitulating human tumourigenesis.13 Hence, preclinical studies in GEM have the potential to provide more relevant data for subsequent clinical trials.

**In silico models – the computer’s role in drug development**

Progress in bioinformatics over the past decades has made in silico studies attractive so that they often precede or complement in vitro and in vivo studies. In silico models are based on computer simulations and provide information on how an investigational compound might behave in subsequent in vitro and in vivo experiments.14 Apart from technological requirements, these computer simulations demand expert knowledge in biochemistry and molecular biology.

**Preclinical research is indispensable**

Despite all efforts to identify relevant animal models to ensure a significant translational value, drugs often show different pharmacodynamic characteristics when administered to human subjects. Thus, merely one out of five investigational drugs tested in clinical trials eventually gains approval for clinical use. Some studies even report that only nine percent of compounds passing preclinical efficacy evaluation are approved by the FDA.11 The fact that most anti-cancer drugs do not pass efficacy evaluation in Phase II and III studies suggests that currently used preclinical models fail at appropriately mimicking tumour heterogeneity, host factors, and drug resistance mechanisms.15,16 Nevertheless, preclinical research is indispensable to protect human subjects in clinical trials. Adequate design of preclinical studies and careful choice of model systems are vital to ensure relevant results that translate into applicability in clinical settings.

**Conflicts of Interest**

The author declares no conflicts of interest.

**References**


**Author information**

Jennifer Honek has a background in molecular biotechnology and holds a PhD in Medicine. She has been active as a freelance medical writer since 2015 and also works for a medtech company as a clinical trial lead.