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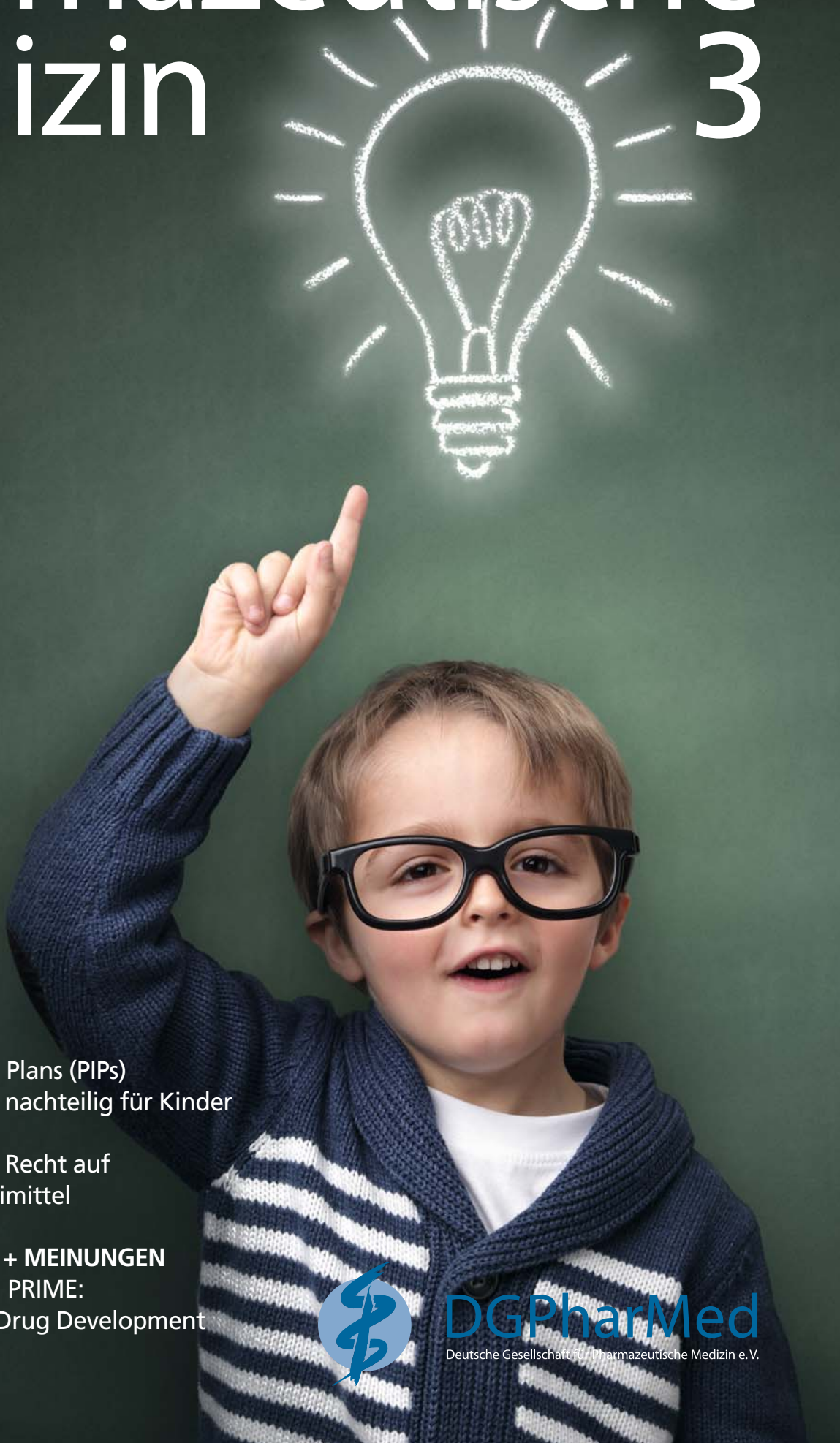

PRO & CONTRA
Paediatric Investigation Plans (PIPs)
– oft sinnlos oder sogar nachteilig für Kinder

Auch Kinder haben das Recht auf
klinisch geprüfte Arzneimittel

BERICHTE + ANALYSEN + MEINUNGEN
Adaptive Pathways and PRIME:
A True Acceleration of Drug Development
and Approval?

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First in Human (FIH) and up to Proof of Concept (POC) – an Overview

Non-Clinical Requirements for FIH and up to POC Clinical Studies

Before the first human can be exposed to a new medicine in development, the medicine has to be rigorously tested in non-clinical pharmacology, drug metabolism, pharmacokinetic and toxicology studies to provide the basis for enabling first in human (FIH) trials, supporting the selection of a maximum recommended human starting dose as well as subsequent escalating doses and identifying suitable parameters for clinical monitoring of safety. Non-clinical testing cannot be undertaken in isolation but remains closely intertwined with clinical development from the very beginning to the end of a given development. Drugs in development may either be discontinued for many reasons including those ultimately leading to unfavorable risk-benefit profiles, or finally reach approval. Regardless of the final outcome, a successful process which substantiates informed decisions in a timely manner requires close interaction with all disciplines involved. This article provides an overview on key information which needs to be established from a non-clinical perspective to support FIH trials.

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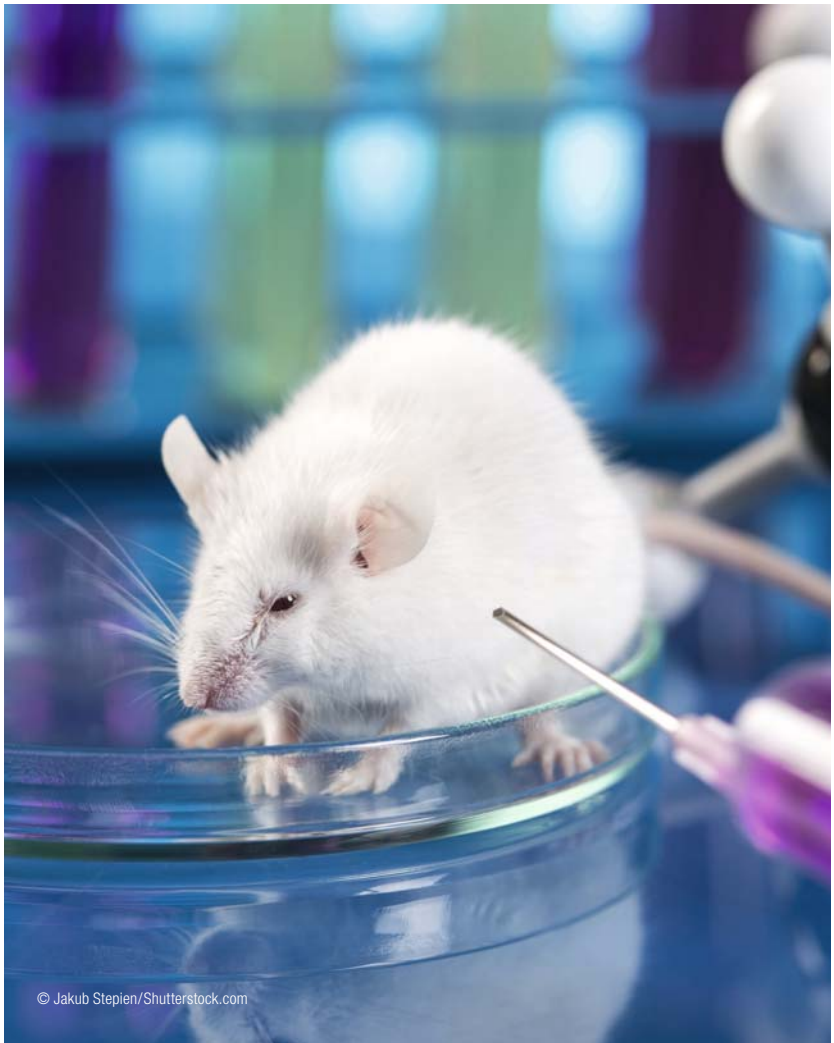

Introduction

The development of a new drug is a complicated, long and expensive process and confronts the experts in all disciplines involved with unexpected challenges during the complex process it takes to bring new medicines to the market. From understanding a disease and identifying a "druggable" target to bringing a safe and effective new treatment to patients on average 10–15 years will pass [1]. Of every 5,000 to 10,000 compounds entering the research and development pipeline only a few may reach clinical development, of which perhaps one receives approval [2]. In this article we will focus on the earlier phases of drug development, mainly the FIH (first in human) and early clinical studies including those investigating clinical POC (proof of concept). Prior to conducting any of those studies the new molecule has to be characterized in non-clinical pharmacology, drug metabolism and pharmacokinetic

GLOSSARY OF TERMS	
In vitro metabolic data	In vitro methods including cellular systems (e.g. hepatocytes, liver slices, cell lines) or preparation of drug metabolizing enzymes (e.g. tissue homogenates, subcellular fractions, isolated enzymes).
hERG (**h**uman **E**ther-à-go-go-**R**elated **G**ene)	This is a gene that codes a subunit of a potassium ion channel. This ion channel is sometimes referred to as hERG channel and contributes to the electrical activity of the heart by promoting the repolarization of the cardiac action potential.
Modified Irwin assay	A multi-parameter assessment of the nervous system function that include autonomic (e.g. salivation, lacrimation, urination defaecation), sensorimotor (e.g. touch response, reflexes), neuromuscular (e.g. posture, body tone, tremor, strength) and behavioral functions (e.g. activity level, grooming, stereotypy).
Parent compound	The pharmaceutical as given to the animal. This can be the active compound or an inactive prodrug.

(DMPK) and toxicology studies. The master guideline describing this process is ICH M3(R2) [3].

Before a new compound can enter clinical trials for the first time the side effect profile has to be characterized in in vitro and in vivo non-clinical safety studies. Those studies are aimed to identify potential target organs of toxicity, contribute in determining the starting dose in FIH trials and further escalating doses, and guide the monitoring program in clinical studies. In general, the length of clinical studies has to be supported by non-clinical studies of equal or longer treatment duration. Non-clinical and clinical development remain closely intertwined from early beginning until application for a marketing authorization.

The mission of non-clinical (syn. preclinical) development is to build a bridge from "bench to bedside", i.e. to characterize molecules—small or large—in a step-wise process to support specific phases of clinical development, i.e. to generate knowledge providing the basis for the conduct of defined clinical studies of a given scope and duration [4].

Early clinical development

Per classic definition the tolerability and pharmacokinetic properties of the new drug are evaluated in phase I clinical trials in a small population of healthy volunteers; subsequently, safety and efficacy are investigated in phase II clinical studies in more subjects exhibiting the specific disease. The combination of early phase clinical trials in combined phase I/II studies is increasingly common in order to obtain information on POC read-out as early as possible with the aim to speed up the drug development process overall and support early decision-making.

In this paper we will focus on typical non-clinical studies that need to be completed prior to first dosing in man; later aspects of non-clinical testing will be touched upon but not be addressed in detail. Every drug development program is different and has to be tailored towards the requirements of each specific molecule. This article cannot address all specific circumstances that may arise during development. Non-clinical development is driven entirely by the clinical program that needs to be supported, with the final aim to substantiate the basis for a robust risk-benefit assessment. FIH studies are initiated solely based upon the available information from non-clinical studies, and, if obtainable, from other data available for the class of compounds and/or mode of action such as from published information. One key aspect which needs to be taken into account is the predictive relevance to assess human safety.

Non-clinical studies

Non-clinical studies include primary pharmacodynamic studies intended to investigate the mode of action, DMPK studies investigating what the body is doing to the drug (i.e. absorption, distribution, metabolism, excretion) and toxicology studies investigating what the drug is doing to the body up to high systemic exposures. In this article we will focus on minimum requirements concerning non-clinical safety studies as described in the ICH M3(R2) [3] guideline. If there is cause for concern, the scope of investigations has to be expanded beyond the minimum/standard requirements. Special aspects of certain indications like topically applied compounds, vaccines, anticancer treatments, and biopharmaceuticals may be touched upon but will not be fully covered.

Non-clinical testing to support early clinical development includes a variety of different areas as outlined in Table 1 on the next page. In order to avoid duplication of non-clinical studies and to streamline the regulatory assessment process for new drug applications the regulatory authorities and the pharmaceutical industries of Europe, Japan and the United States formed the International Council for Harmonisation (ICH), formerly the International Conference on Harmonisation, to develop harmonized guidelines for the different areas of toxicity testing. The master guideline describing respective non-clinical safety studies recommended to support human clinical trials and marketing authorisation for pharmaceuticals is ICH M3(R2)

[3]. To further clarify requirements for the different areas of testing special ICH guidelines have been developed as listed in Table 1. In addition local guidelines from the U.S. American Food and Drug Administration (FDA), the European Medicines Agency (EMA) or the Organisation for Economic Co-operation and Development (OECD) may be utilized in planning appropriate non-clinical studies.

Drug metabolism and pharmacokinetics

In vitro metabolic and plasma protein binding data for animals and humans and systemic exposure data in the species used for repeated dose toxicity studies are generally required prior to initiating human clinical trials. This also includes the development of validated analytical assays to support studies in the animal species selected for safety testing and in humans. Toxicokinetic endpoints, mainly AUC (area under the curve) and C_{max} (maximum concentration achieved during the dosing interval) of the parent compound, are used to provide context for the safety studies by relating systemic exposures to toxicological findings which contributes to the assessment of the relevance of such observations for clinical safety [5]. Information about the systemic exposure allows interspecies comparisons and is much more meaningful than simple allometric scaling based on dose/body weight or surface area comparisons; furthermore, it can be utilized in determining the starting dose and escalating doses in the FIH clinical trial. There are no formal requirements on the timing of tissue distribution studies and they should be conducted on a case-by-case basis. However, it might be beneficial to investigate if a new compound reaches the site of the desired action at sufficiently high concentrations by analyzing tissue samples taken at necropsy from animals in toxicology studies.

Safety pharmacology

Per ICH S7A [7] definition studies in this category investigate the po-

Toxicology Discipline	Objectives of studies	Applicable ICH guidelines
Toxicokinetic and pharmacokinetic	Obtain in vitro metabolism and plasma protein binding data for animals and human and systemic exposure data for animal species used in repeated dose toxicity studies.	ICH M3(R2) [3] ICH S3A [5] ICH S3B [6]
Safety pharmacology	Investigate potential undesirable pharmacodynamics effects in the therapeutic range and above on vital organs or systems (i.e. cardiovascular, respiratory, central nervous system).	ICH M3(R2) [3] ICH S7A [7] ICH S7B [8]
Acute toxicity	Investigate acute high dose toxicities to predict consequences of human overdose situations. No specific studies are needed if adequate high doses are tested in dose range-finding studies.	ICH M3(R2) [3]
Repeated dose toxicity	Identify target organs of toxicity.	ICH M3(R2) [3] ICH S4 [9]
Immunotoxicity	Evaluate unintended immunosuppression or enhancement.	ICH M(R2)3 [1] ICH S8 [10]
Reproductive toxicology	Evaluate potential effects on all stages of the reproductive cycle.	ICH M3(R2) [1] ICH S5(R3) [11]
Genotoxicity	Evaluate potential DNA damage.	ICH M3(R2) [3] ICH S2(R1) [12]
Local tolerance	Evaluate local tolerance by the intended therapeutic route of administration.	ICH M3(R2) [3]
Phototoxicity	Evaluate photoirritation and photoallergic effect.	ICH M3(R2) [3] ICH S10 [13]

Table 1: Selection of relevant non-clinical safety testing guidelines.

tential undesirable pharmacodynamic effects of a pharmaceutical on physiological functions in relation to exposures in the therapeutic range and above. Safety pharmacology studies are usually required prior to entry into man. The core battery of studies investigates effects on vital functions on the cardiovascular, respiratory and central nervous systems. Depending on the results follow-up supplemental studies may be needed. Supplemental safety pharmacology studies on organ system functions not addressed by the core battery (e.g. renal/urinary system, autonomic nervous system, gastrointestinal system) may need to be considered on a case-by-case basis. The dose-levels for in vivo safety pharmacology studies in animals should define the dose-response relationship and should be compared to the pharmocodynamic effect exposure in the respective animal species and the predicted therapeutic effect exposure in human. In the absence of any adverse effects on the safety pharmacology parameters the highest dose should produce some effects to demonstrate that the compound is active in this species, however, doses in the toxic range are not recommended as they can confound the interpretation of the safety pharmacology endpoints. Therefore, safety pharmacology studies are usually planned after some knowledge about the new drug has been obtained in repeated dose toxicity studies.

In vivo safety pharmacology studies are generally performed by single dose administration. Additional endpoints may include the assessment of clinical symptoms, food consumption, body weight and, occasionally, kinetic investigations. Since those studies do not require sacrifice of the animals they may be reused following an adequate washout period between studies.

The cardiovascular system is usually investigated in freely moving non-rodents (often dogs or monkeys) that are surgically implanted with a telemetry transmitter collecting the required data (e.g. blood pressure, heart rate, and electrocardiogram). Stand-alone studies are not formally required and safety pharmacology endpoints can also be included in repeated dose toxicity studies. In the latter case the utilisation of jacketed telemetry technology is possible. In addition an in vitro assay to assess the potential of a test substance to delay ventricular repolarisation, such as the hERG (**h**uman **E**ther-à-go-go-**R**elated **G**ene) assay, is usually conducted [8].

The respiratory system is usually investigated in rats by means of plethysmography. Routine endpoints include respiratory rate, tidal volume and minute volume (derived). Additional parameters can also be investigated, such as hemoglobin oxygen saturation.

The central nervous system is usually investigated in rats by means of a functional observation battery (FOB) such as in a modified Irwin assay. Routine endpoints include motor activity, behavioral changes, coordination, sensory/motor reflex responses and body temperature. In rodents, reductions in body temperature may be the only physiological indicator of a central nervous system (CNS-)mediated effect.

Acute toxicity testing

In alignment with the principles of the three R's (replacement, reduction and refinement) aimed at reducing the numbers of animals used in toxicology research and safeguard animal welfare formal acute toxicity studies investigating the LD_{50} (lethal dose; dose lethal in 50 per cent of the animals tested) in two mammalian species using both the clinical and a parenteral route of administration are no longer required; in fact, in the UK, the use of this assay has been banned from 1991 since the method is rather crude, providing little information using large number of animals. Instead, the acute toxic poten-

tial of a new drug can be obtained from appropriately conducted dose range studies that define a clear MTD (maximum tolerated dose) and are an integral part of the repeated dose toxicity testing strategy.

Repeated dose toxicity testing

For small molecules non-clinical toxicity testing has to be conducted in two species, i.e. a rodent and a non-rodent. The rodent species are usually rats, although mice can also be considered if appropriate. And for non-rodents usually dogs, mini-pigs or monkeys are utilized. For ethical reasons non-human primates are only the last resort and whenever possible the dog or increasingly the mini-pig are selected as non-rodent species. The non-rodent species is usually selected based on the in vitro metabolism profile to assure that all major metabolites (>10 per cent of parent systemic exposure in human based on AUC) are also present in at least one preclinical species, although this knowledge only becomes available later in development and is not fully established prior to FIH. Therefore, it is not impossible that a change in the selected animal species might be required later in development, or additional studies need to be conducted testing a major metabolite in dedicated studies. While small molecules have to be tested in two animal species the situation for biopharmaceuticals is slightly different. Those compounds have to be tested in at least one pharmacologically active species [14], which in most cases is a non-human primate (non-rodent). If a biopharmaceutical is also pharmacologically active in a rodent species it has to be tested in the latter as well. However, if it is active in two non-rodent species (e.g. dog and monkey) it only needs to be tested in one of those [14].

Repeated dose toxicity studies are aimed to characterize the dose-response over the time frame evaluated, i.e. to establish a NOAEL (no-observed-adverse-effect level) and an MTD (maximum tolerated dose), identify target organs of toxicity and provide information on systemic exposures.

The most definitive information regarding the safety profile of a new compound before FIH is usually available from the repeated dose-toxicity studies. Therefore, clinical studies need to be supported by repeated dose toxicity studies of at least equivalent if not longer duration. Since the duration of treatment has an impact on the severity of adverse effects it is not possible to just plan one toxicity study of a long duration and be finished. Toxicity studies are typically conducted with three dose levels and one control group. The three doses need to span a range including a no-observed-adverse-effect dose and a dose that causes clear signs of toxicity or exaggerated pharmacology. Each repeated dose toxicity study is a dose range finding study for a subsequent study of longer duration. The very first studies need to establish a dose range in each species to be used in the subsequent GLP (good laboratory practice) studies planned to enable FIH trials. A quite common approach is to treat a small number of animals with single rising doses separated by drug-free washout periods between doses. This phase of the study determines appropriate dose levels which in the second phase of the study will be administered repeatedly to a different set of naïve animals. This early type of study is usually not conducted according to GLP. The next study would most likely be a GLP study of two or four weeks in duration, supporting a clinical trial of up to two or four weeks in duration, respectively. Routine data collected from animals may include but are not limited to clinical signs, body weight development, food and water consumption, clinical pathology (haematology, coagulation, clinical chemistry, urinalysis), electrocardiograms (ECG), heart rate and blood pressure, toxicokinetics, macroscopy at necropsy and finally histopathology. Special investigations may be necessary on a case-by-case basis. The repeated dose non-clinical toxicology studies take the longest among all non-clinical studies needed prior to moving into clinical development and therefore, are on the critical path. The study denomination indicates the in-life treatment period. The evaluation of collected data, especially the histopathology processing and evaluation, is very time consuming. For example, a routine repeated dose toxicology study in rats has four dose levels with ten male and ten female animals/group. In addition the control and high dose groups may include additional five rats/sex for the evaluation of recovery. At necropsy more than 40 tissues per animal are taken for histopathological evaluation. It is common practice in rodent studies to only evaluate the control and high dose for histopathology first and then exam-

ine any identified target organs for the mid and low dose groups subsequently. Even when this resource-saving strategy is adopted the histopathology has to evaluate about 2,000 tissues.

Immunotoxicity

New compounds need to be assessed for their ability to induce unintended immunosuppression or enhancement. Those effects can be caused by exaggerated pharmacodynamic effects with drugs intended to modulate the immune function or can be a real side effect observed for drugs that are not designed to interact with the immune system. The first risk assessment is made from data collected in the routine repeated dose toxicity studies. For example, the clinical condition of the animals may signal immune suppression such as becoming evident as an increased rate of infections. Furthermore, endpoints like haematology, organ weights and the histopathological evaluation particularly of the lymphoid organs and tissues may indicate effects on the immune system. The need for additional studies will be determined using a weight of evidence approach that includes findings from the studies mentioned above, the pharmacological properties of the drug, the intended patient population, structural similarities to known immunomodulators, the disposition of the drug and any emerging clinical information. If there is cause for concern dedicated immunotoxicity studies may be required before large-scale clinical trials. However, if a POC trial includes immunocompromized patients, the timing of specialized studies might need to be adapted. A variety of possible assays is available and needs to be selected on a case-by-case basis.

Reproductive toxicology

Those specialized studies are aimed at assessing any effects on development and reproduction (DART). Therefore, exposure must cover mature adults prior to conception and all stages of development from conception through to sexual maturity of the F1 generation. Those studies need to be conducted as is appropriate for the population that is exposed in the clinical trials. The complete package is required for marketing approval. Since the male reproductive organs are evaluated in repeated dose toxicity studies men can be included in Phase I and II clinical trials before the completion of special studies on fertility and early embryonic development. By contrast, there is particular concern for women of childbearing potential (WOCBP) due to the potential unintentional exposure of a developing conceptus. Nevertheless, in special circumstances, dedicated preclinical studies to assess any potential effects on embryo-fetal development may not be needed prior to inclusion of WOBCP if there are adequate precautions to prevent pregnancy during early short-term clinical trials.

Genotoxicity

Genotoxicity tests are designed to detect compounds that induce genetic damage and are mainly used for the early assessment of a carcinogenic potential of small molecules. Large molecules are not expected to interact with DNA and hence they do generally not need to be subjected to genotoxicity testing [12]. The standard genotoxicity test battery includes the assessment of mutagenicity in a bacterial reverse gene mutation ("Ames") test. In addition an evaluation of clastogenicity and other mechanisms of genotoxicity in mammalian cells, either in vitro and/or in vivo is necessary. Suitable in vitro tests may include the metaphase chromosome aberration assay, the micronucleus assay and the mouse lymphoma L5178Y thymidine kinase (TK) gene mutation assay. In vitro cytogenetic assays for chromosomal damage analyze micronuclei or metaphase chromosome aberrations in peripheral blood lymphocytes from human or animal origin. Alternatively, an in vitro mouse lymphoma TK gene mutation assay can be considered. As some agents can be genotoxic in vivo but not in vitro an in vivo evaluation is asked for to include aspects of absorption, distribution, metabolism and excretion. The available in vitro mammalian cell assays have been shown to exhibit a relatively low specificity in correctly identifying a negative carcinogen. This leads to a high number of false positive assays [16] [17] [18] [19] [20]. Therefore, the testing strategy includes a battery of assays designed to provide a weight of evidence approach with the two options shown in Table 2.

If an in vitro mammalian cell assay is positive, two negative assays

Option 1	Option 2
In vitro bacterial gene mutation assay	In vitro bacterial gene mutation assay
In vitro chromosomal damage assay	
In vivo genotoxicity test (usually micronuclei assay in rodent hematopoietic cells)	In vivo genotoxicity assessment with two different tissues (usually micronuclei assay in rodent hematopoietic cells and a DNA strand breakage assay in liver tissue)

Table 2: Two options for assay batteries designed to provide a weight of evidence approach.

measuring the same end points are required in vivo to demonstrate the lack of relevance of the in vitro assay.

Genotoxicity testing is usually required prior to dosing in FIH trials. One exception is the treatment of advanced cancer patients [21]. However, genotoxicity assays are not exclusively used to assess patient safety but also to classify a compound for the manufacturing process to assure the occupational safety of workers handling the compound and therefore may be conducted earlier.

Phototoxicity and photoallergy

Phototoxicity (photoirritation) is an acute light-induced tissue response to a photoreactive chemical and photoallergy is an immunologically mediated reaction to a chemical, initiated by the formation of photoproducts following a photochemical reaction.

Whilst experimental phototoxicity testing for non-topical applications is only required before exposure of large numbers of subjects it is recommended to conduct an initial assessment of the phototoxic potential in order to advise subjects on clinical trials regarding appropriate protective measures.

For a chemical to induce phototoxicity and/or photoallergy a molecule has to absorb light within the range of natural sunlight (290–700 nm), has to generate reactive oxygen species following absorption of UV-visible light and has to reach to light-exposed tissues in sufficient amounts. If one or more of these conditions are not met, a compound will usually not present a concern for direct effects. Therefore, the first step in assessing the photo reactive potential is to establish whether a compound absorbs photons at any wavelength between 290 and 700 nm. A compound that does not have a molar extinction coefficient (MEC) of greater that 1,000 $L \cdot mol^{-1} \cdot cm^{-1}$ is of no concern. A compound with a higher MEC will require appropriate protective measures of subjects in clinical trials and follow-up testing.

Safety testing of metabolites

There is no need for extra testing if any major human metabolites are formed in at least one of the two species used in repeated dose toxicity studies at systemic exposure levels equal to or greater than in humans. However, if metabolites are not formed in any of the animals used in the safety studies or are formed in humans at disproportionately higher levels dedicated safety testing of the respective metabolites is usually required prior to large-scale clinical trials [22].

Risk assessment and human starting dose

To arrive at a robust risk–benefit assessment, all of the information available from the non-clinical safety package at the time needs to be evaluated as a whole to support the selection of an appropriate clinical starting as well as escalating doses, and to identify suitable parameters for clinical monitoring of potential adverse effects. The transition from non-clinical to early clinical development is a major step and human safety is of paramount importance. Therefore, appropriate strategies for mitigating and managing risks have to be in place [23].

The risk assessment for a new medicine in development is based on the entirety of the data generated and is put into context with the exposure-response relationship. No-observed-adverse-effect levels (NOAEL) are determined and safety factors are applied when determining doses for FIH administration. For small molecules more commonly the maximum recommended starting dose (MRSD) may be established and should be put in context with the pharmacologically active dose (PAD), whereas for biologics the minimum anticipated biological effect level (MABEL) should be considered. If there is cause for concern, the starting dose for a small molecule should be reduced to a level that may not be expected to produce any pharmacological response and/or a "MABEL-like" approach be considered, if possible. The lowest NOAEL in any animal study limits the FIH dose. Some compound (e.g. CNS or cardiovascular active drugs) may only cause functional and no morphological changes. However, in most development projects the risk assessment may be based on histopathological findings seen in repeated dose toxicity studies. The human equivalent dose (HED) of the NOAEL in each animal species tested is calculated using specific conversion factors listed in respective guidelines [24].

Once the HED of the NOAEL in the most relevant species has been determined, a safety factor should be applied to provide a margin of safety for protection of human subjects receiving the initial and subsequent clinical doses. This safety factor allows for variability in extrapolating from animal toxicity studies to studies in humans resulting from:

(1) uncertainties due to enhanced sensitivity to pharmacologic activity in humans versus animals;
(2) difficulties in detecting certain toxicities in animals (e.g. headache, myalgia, mental disturbances);
(3) differences in receptor densities or affinities;
(4) unexpected toxicities; and
(5) interspecies differences in ADME of the therapeutic.

The default safety factor that should normally be used is 10 [23]. However, factors that may warrant an increase in the safety factors may include but are not limited to any of the following:

Steep dose response curve: A steep dose response curve for significant toxicities in the most relevant

species or in multiple species may indicate a greater risk to humans.

Severe toxicities: Qualitatively severe toxicities or damage to an organ system (e.g. central nervous system [CNS]) indicate increased risk to humans. Careful consideration must be given to establish whether such findings are considered to allow at all that the safety of human subjects can be ascertained. Hence, such findings may be an impediment to further development unless in circumstances where the potential benefit in disabling or fatal conditions with limited or a lack of any treatment alternatives may outweigh potential risks.

Non-monitorable toxicity: Non-monitorable toxicities may include histopathologic changes in animals that are not readily monitored by clinical pathology markers.

Toxicities without premonitory signs: If the onset of significant toxicities is not reliably associated with premonitory signs in animals, it may be difficult to know when toxic doses are approached in human trials.

Variable bioavailability: Widely divergent or poor bioavailability in the several animal species, or poor bioavailability in the test species used to derive the HED, suggest a greater possibility for underestimating the toxicity in humans. In addition, variable bioavailability in humans might be an additional risk factor.

Large variability in doses or plasma drug levels eliciting effect: When doses or exposure levels that produce a toxic effect differ greatly across species or among individual animals of a species, the ability to predict a toxic dose in humans is reduced and a greater safety factor may be needed.

Irreversible toxicity: Irreversible toxicities in animals suggest the possibility of permanent injury in human trial participants.

Unexplained mortality: Mortality that is not predicted by other parameters raises the level of concern.

Nonlinear pharmacokinetics: When plasma drug levels do not increase in a dose-related manner, the ability to predict toxicity in humans in relation to dose is reduced and a greater safety factor may be needed.

Inadequate dose-response data: Poor study design (e.g. few dose levels, wide dosing intervals) or large differences in responses among animals within dosing groups may make it difficult to characterize the dose-response curve. Such studies are not usually acceptable and a submission may be rejected.

Novel therapeutic targets: Therapeutic targets that have not been previously clinically evaluated may increase the uncertainty of relying on the nonclinical data to support a safe starting dose in humans.

Animal models with limited utility: Some classes of therapeutic biologics may have very limited interspecies cross-reactivity or pronounced immunogenicity, or may work by mechanisms that are not known to be conserved between (non-human) animals and humans; in these cases, safety data from any animal studies may be very limited in scope and interpretability.

Under certain circumstances (e.g. compound belongs to a well-characterized class, effects are easily monitored, any side effects are mild and reversible, adverse effects are consistent across animal species) lower safety factors may be possible, however, this approach needs to be well justified.

Higher starting doses are possible for anticancer drugs in order not to subject patients to lengthy dose escalation trials at non-pharmacologically active doses. A com-

mon approach in this patient population for many small molecules is to set a starting dose at 1/10 the severely toxic dose in ten per cent of the animals (STD 10) in rodents. If the non-rodent is the most appropriate species, then 1/6 the highest non-severely toxic dose (HNSTD) is considered an appropriate starting dose. The HNSTD is defined as the highest dose level that does not produce evidence of lethality, life-threatening toxicities or irreversible findings [21].

Compared to small molecules, biopharmaceuticals are very specific in targeting molecular pathways in humans and have the great advantage of eliminating much of the potential for toxicity that is not related to the primary mode of action, although recently, off-target effects are becoming more common with some types of biopharmaceuticals also. Consequently, the toxicity of biopharmaceuticals is usually more consistent with exaggerated pharmacology than with off-target toxicity that is a typical feature more frequently seen for small molecules. Therefore, side effects for biopharmaceuticals can generally be predicted based on a sound understanding of their intended function. This type of molecules has to be tested in at least one pharmacologically active species. Species which are not pharmacologically relevant must not be used for testing, as such models would not yield any meaningful information for human risk assessment. However, it may prove challenging to identify a pharmacologically relevant species. For biopharmaceuticals, it is considered that the minimal anticipated biological effect level (MABEL) is more appropriate to determine a human starting dose rather than the NOAEL as for small molecules, due to their propensity to exhibit effects primarily associated with exaggerated pharmacology. Generally, a NOAEL-derived human starting dose would be expected to be higher than a MABEL-derived dose. An impressive example is the toxicity of TGN1412 tested by TeGenero in a FIH trial with six healthy male volunteers. The human starting dose was calculated based solely on the NOAEL in monkeys and found to cause a massive cytokine release syndrome with multi organ failure requiring intensive treatment and supportive care by the intensive care unit in all subjects dosed [25]. Subsequently, the European Medicines Agency's Committee for Medicinal Products developed a guideline describing the MABEL approach for high-risk medicinal products [23]. Besides results from in vivo experiments, in vitro data such as receptor binding and occupancy should be included and the human starting dose should be calculated based on integrated PK/PD (pharmacokinetic/pharmacodynamic) modelling [21]. The respective MABEL dose for TGN1412 would have been 100 times lower than the maximum recommended starting dose calculated from the NOAEL used in the TeGenero FIH trial. The first dose used in this trial led to > 90 per cent receptor occupancy, whereas the MABEL dose would have resulted in ten per cent receptor occupancy [26] [27][28].

On the quest to develop new medicines researchers explore new modes of action in order to bring novel drugs to patients in need. Exploring new routes also opens the possibility of inducing serious adverse reactions in FIH clinical trials. This certainly was the case with TGN1412, a novel and first in class immune stimulator also referred to as a "superagonist". Regulatory requirements laid out in applicable guidelines are only guidance documents that assist in establishing the general principles and scientific standards that should be met. The development of a substance-specific appropriate testing strategy, however, falls within the responsibility of the sponsors as only they will have the full understanding of their investigational medicinal products.

Conclusion

Non-clinical and clinical development remain closely intertwined from start to end and a successful process requires close interaction with all disciplines involved. Early pharmacology, DMPK and safety studies form the basis for enabling FIH trials, supporting the selection of a maximum recommended human starting dose as well as escalating doses, and identifying suitable parameters for clinical monitoring of safety. There are, however, circumstances which call for a particularly cautious approach, including safety profiles which are characterized by features such as novel therapeutic targets, pharmacological disease models of limited predictivity for humans, steep dose and/or exposure responses, evidence of non-linear pharmacokinetics, poor predictivity of systemic exposure for target exposure levels, severe, non-monitorable, irreversible or such toxicities with a lack of premonitory signs, and also the—before first testing in humans—often unknown relative sensitivity of the animal species used in safety testing compared to humans particularly with respect to off-target effects. It is prudent to assume lower sensitivity of the test species compared to trial subjects, particularly when there is an apparent lack of toxicity, which—for small molecules—will more likely be a function of the dose rather than an intrinsic property of a given medicine in development, consistent with the testing paradigm of toxicology first stated by Paracelsus. For biopharmaceuticals, higher doses may be associated with signs of exaggerated pharmacology rather than off-target effects, although more recently, such effects are observed more commonly with molecules falling in this category also. |

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