Successful Drug Development Despite Adverse Preclinical Findings
Part 1: Processes to Address Issues and Most Important Findings

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Abstract: Unexpected adverse preclinical findings (APFs) are not infrequently encountered during drug development. Such APFs can be functional disturbances such as QT prolongation, morphological toxicity or carcinogenicity. The latter is of particular concern in conjunction with equivocal genotoxicity results. The toxicologic pathologist plays an important role in recognizing these effects, in helping to characterize them, to evaluate their risk for man, and in proposing measures to mitigate the risk particularly in early clinical trials. A careful scientific evaluation is crucial while termination of the development of a potentially useful drug must be avoided. This first part of the review discusses processes to address unexpected APFs and provides an overview over typical APFs in particular classes of drugs. If the mode of action (MoA) by which a drug candidate produces an APF is known, this supports evaluation of its relevance for humans. Tailor-made mechanistic studies, when needed, must be planned carefully to test one or several hypotheses regarding the potential MoA and to provide further data for risk evaluation. Safety considerations are based on exposure at no-observed-adverse-effect levels (NOAEL) of the most sensitive and relevant animal species and guide dose escalation in clinical trials. The availability of early markers of toxicity for monitoring of humans adds further safety to clinical studies. Risk evaluation is concluded by a weight of evidence analysis (WoE) with an array of parameters including drug use, medical need and alternatives on the market. In the second part of this review relevant examples of APFs will be discussed in more detail. (J Toxicol Pathol 2010; 23: 189–211)

Key words: adverse preclinical finding, hazard identification and characterization, risk evaluation and management, mode of action, safety ratio, weight of evidence

Introduction and Overview

Adverse preclinical findings (APFs) can arise anytime and often unexpectedly during the development of a drug candidate. They can fall in any of the following categories:

- Functional APFs, covered essentially by safety pharmacology testing¹ ², including in particular
  - QT prolongation³ ¹⁰
  - Immunotoxicity incl. immunostimulation¹¹ ¹⁵
  - CNS-related symptoms, such as seizures⁹ ¹⁶
- Genotoxicity including mutagenicity¹⁷ ²²
- Morphological toxicity such as cardiotoxicity or nephrotoxicity²³ ²⁸
- Carcinogenicity¹⁷ ²², ²⁹ ³¹
- Reproductive toxicity: Functional and gross pathological observations²² ³⁶

The above categories of APFs are essentially related to the methodology of investigation. An APF can belong to more than one category, e.g. have functional symptoms and result in morphological changes.

Figure 1 is an example of a subtle morphological APF. What do these vacuoles in the pancreatic β cells mean? Is this finding insignificant or the first sign of a major issue? A lot can go wrong at this point¹⁷ including e.g. ignoring the finding as not significant or becoming hyperactive. There is no general answer to the question what these vacuoles mean for further development of the drug candidate, as will be explained below.

¹ Not much published literature is available on this subject. Examples of drugs with functional adverse preclinical CNS findings are listed in the Physicians’ Desk Reference PDR¹⁶. Some examples are also discussed below under “Evaluation of various types of adverse preclinical findings – Functional effects”. 
This review outlines proven processes for dealing with unexpected APFs in order to optimize the chances of successful development of drug candidates or—if necessary—to create a scientific basis for their early termination. This first part of the review addresses strategic aspects regarding the “troubleshooting” approach—others prefer to call it the “problemsolving” approach. In the second part some examples are discussed in more detail. Drug development is a complex process. The accurate prediction of human drug toxicity remains a major challenge in drug development. Therefore, toxicologists and toxicologic pathologists need to be prepared to address unexpected APFs. Toxicity studies are designed to produce toxicity at least at the high dose. Absence of APFs may mean for example that dose selection was wrong or that the drug candidate has no major therapeutic value, or that the model used to detect toxicity is not valid. The examples used in this review are mainly from drug development; however, the same approach is also meaningful for chemicals or food additives.

Processes in Case of Adverse Preclinical Findings

—Overview

Guidance

The guiding principle of those involved in developing and administering drugs is still “Primum non nocere” (above all do not harm), as formulated by Hippocrates almost 2,500 years ago. Simultaneously the aim must be to bring value adding drugs to the market for the benefit of patients and the company. Over 90% of withdrawals of marketed drugs are due to clinical toxicity, particularly hepatotoxicity and cardiovascular toxicity, which underlines the importance of a careful and intelligent preclinical and clinical safety assessment before registration and marketing of new drugs. As rare APFs may only be noticed once drugs are widely used, post-marketing surveillance is important and provides also new insights for the development of further drug candidates.

Organization

Whenever possible, an experienced company-internal associate should take the lead and the responsibility for resolving issues in connection with an unexpected APF. This is primarily a scientific issue, but business aspects must also be taken into account e.g. regarding the financial resources a company is willing to invest in view of the potential benefits of the drug candidate for patients and the company. If internally no “troubleshooting” leader is available, a trusted external expert with the necessary business sense can be commissioned with the task. The company management, often at several levels depending on the issue and its anticipated impact, generally likes to be kept informed and to take major decisions e.g. regarding resources (manpower, money). However, the team leader and the team must be empowered with relevant decision making competence within company-defined limits and must have adequate resources.

Good management of the various steps for assessing the human relevance of APFs is a key success factor and involves various steps as listed below. These steps need not necessarily be taken in sequential order and a specific approach tailored to a particular problem is recommended. The various steps frequently involve:

- Assembling a team of in-house specialists and external experts, e.g. drug development consulting services. Experts from universities recognized for their achievements in the scientific field related to the APF in question can be helpful.
- Determining in a first step the potential relevance of the APF, e.g. by collecting information about similar drugs as available from literature, through the USA freedom of information acts, from the scientific community including consultants, etc.
- Examining the options at the current state of knowledge, in essence whether to:
  - Abandon development, e.g. for the following reasons: The risk/benefit ratio of the drug most likely is too small, the investment at stake is still minor e.g. because the drug candidate is in an early phase of development, or follow-up drug candidates are available
  - License drug candidate out, as other companies might be interested in developing the drug candidate in different indications or are willing to take a higher development risk
  - Review the therapeutic indication of the drug candidate, since e.g. a harder indication for a more serious disease could justify a higher therapeutic risk (see also below under risk-benefit evaluation), or a different therapeutic indication might reduce the therapeutic dose

Fig. 1. Beta-cell vacuolization in the pancreas of a SIV 50 rat. H&E, lens 25×.

The initiative to assess APFs must be kept in-house but consulting with external specialists is often necessary and very helpful.
• Try to resolve the issue, which is addressed below in more detail
• Setting up a plan for issue resolution including tentative objectives, a time frame, budget limits and potential exit points, among other aspects. If a back-up drug candidate is available, one may consider starting to develop it while still working on the first drug candidate. However, if the issue is due to a class effect (see below), similar problems are to be expected with a follow-up drug candidate of the same chemical and/or pharmacological class. In such as case it may be worthwhile to run short-term screening tests to determine the relative potential of further drug candidates to cause the APF in question, thereby supporting the selection of the optimal drug candidate
• Securing continuous support from the upper company management through reliable reporting
• Making sure that also members not belonging to the core team are regularly updated and kept interested in contributing their knowledge to resolve the issue
• Contacting authorities in the event that an application for clinical trials with an investigational new drug (IND) exists or that clinical trials are ongoing. Generally authorities need to be informed within 15 calendar days after the sponsor’s receipt of the respective APF information (see respective guidelines of the various regulatory authorities). However, it is not always necessary to file an adverse event (safety) report; it may be sufficient to inform the authorities about the findings and currently planned measures. It is understood that the latter may have to be updated later following further insight into the issue
• Conducting the necessary activities including scientific evaluation and producing the necessary documents. The end product of “troubleshooting” activities ideally is a scientific story which explains the unexpected APF. It is advisable to consider publishing the results in a recognized peer-reviewed journal, as this will increase the credibility of the conclusions. Unfortunately, sometimes not all aspects of an APF can be fully explained by scientific data, as will be shown in the second part of this review. Also this must be discussed openly and addressed in the conclusions
• Obtaining agreement with authorities and continuing with or terminating development. Even if not all aspects of the APF can be explained, risk evaluation and precautions for risk management frequently allow proceeding with clinical development, until a final risk evaluation becomes possible based on good human data

relevance of the finding for man and safety ratios, which then serve as a basis for risk management. This step-wise approach is well established for environmental agents \cite{45-48} and useful also for drugs \cite{28}.

There are four major steps to deal with unexpected APFs

1. **Hazard identification**: Recognition of a suspected APF
2. **Hazard characterization** particularly regarding dose-response, severity, and reversibility of the APF
3. **Risk evaluation**: Essentially an intellectual process to determine if and under which conditions the drug may be used in man
4. **Risk management**: Implementation of precautions for the use of the drug in man

An example regarding the various steps in dealing with APF is the following:

1. **Hazard identification**: An increased incidence of thyroid follicular tumors is seen in a mice lifetime bioassay.
2. **Hazard characterization**: The working hypothesis is that, in the absence of genotoxicity, these tumors are likely to be of epigenetic origin and related to the hormonal control of the thyroid. Deep frozen sera of the 13 week dose-finding study are investigated, and decreased thyroxin (T4) and increased thyroid stimulating hormone (TSH) levels are found. Liver and thyroid weights are increased. An additional 4 week investigative study shows increased T(4) uridine 5’-diphosphogluconosyltransferase (UDP-GT) activity.
3. **Risk evaluation**: The drug candidate is an inducer of the microsomal enzyme UDP-GT. Increased glucuronidation of T4 increases T4 excretion and lowers T4 serum levels, thus leading to TSH elevation. TSH stimulates the proliferation of thyroid follicular cells, which in the lifetime bioassay is associated with an increased incidence of thyroid follicular tumors \cite{50}.
4. **Risk management**: Human beings are known to be less susceptible to hormonal imbalance, though high TSH levels e.g. in case of a Hashimoto goiter lead to benign thyroid hyperplasia or thyroid adenoma also in humans \cite{50}. Therefore, thyroid hormones and TSH are monitored in clinical studies to make sure that therapeutic doses no changes occur.

The above steps sometimes blend, that is they may not necessarily be conducted strictly sequentially. APFs considered minor, e.g. clinical chemistry findings without morphological correlate, need not to be investigated in a complex manner, and one can proceed directly to risk evaluation (e.g. calculation of safety ratios, see below), and risk management (e.g. limitation of the starting dose in new clinical trials and monitoring of the respective enzymes).
1. Hazard identification

Hazard identification means recognition and qualitative assessment of unexpected APFs keeping in mind that sensitivity of preclinical safety test systems is more important than specificity. In other words: The test systems are designed not to miss potential APFs, but in return may generate insignificant or false positive findings.

Some basic questions to answer during the hazard identification step are:
- Is there indeed an adverse effect?
- What else is known about the drug in question?
- Were there other relevant findings?
- Is the study technically valid?
- Is the model valid?
- Were there other modifying factors?

Is there indeed an adverse effect?

In preclinical safety studies essentially incidences, severity levels, and time of onset (as far as possible) of lesions in dosed and control animals are compared. Each finding must be examined regarding its relationship with treatment. Distinction of treatment-related lesions vs. artifacts: The following findings are often histological artifacts: CNS vacuolation, terminal or post-mortem acute renal tubular necrosis and collapsed lungs particularly in dogs mimicking interstitial pneumonia. Many more morphological artifacts can be encountered and have to be recognized as such.

Examples of “artifacts” because of experimental conditions are: Carcinogenicity at cytotoxic doses, positive “genotoxic” findings in in vitro assays at high concentrations, and teratogenicity at doses toxic to pregnant test animals. In an effort to decrease the incidence of irrelevant positive results, the regulatory authorities, in consultation with the scientific community, have agreed e.g. on dose selection for carcinogenicity studies and have started to revise the International Conference on Harmonization (ICH) guideline on genotoxicity testing currently available as draft guideline S2(R1). Another experimental artifact can be light-induced retinopathy in albino rodent; this artifact may be difficult to distinguish from drug-induced retinopathy, if dosed animals were constantly closer to the light source than control animals.

Distinction of treatment-related lesions vs. spontaneous alterations and naturally occurring variations: Laboratory animals show spontaneous alterations, partly related to species, strain, age, housing condition, diet, infections, and other factors. Such alterations are seen in many different organs. They can also be due to embryonic remnants and misplacement of tissue. Examples are spontaneous seminiferous tubular atrophy/hypoplasia particularly in dogs, seasonal arrest of spermatogenesis in hamsters, immature sexual organs especially in dogs, mammary estrous cycle changes, or vascular alterations. More rare examples are e.g. spongiosis hepatitis, as described in the next section, or retinal gliosis, a lesion originally described in humans. The latter can occur spontaneously e.g. in rats and be mistaken as an induced lesion. Historical control data are very important when evaluating lesion incidences of dosed and control animals. The best historical control data are inhouse data of the last five years. If such data are not available or not sufficient, external data from the same strain and breeder can be used, while literature data can serve for confirmation.

Misdiagnosis: Also for an experienced toxicologic pathologist it can be quite challenging to distinguish e.g. an age-related lymphoid thymus hyperplasia in female mice from a malignant lymphoma. Another source of error may be related to changes of the definition of diagnostic terms over time, which can lead to differing interpretations: For example, cystic degeneration or spongiosis hepatitis in rats was assumed to be (pre-)neoplastic in the past, but is now known to occur also as spontaneous or secondary/reparative non-neoplastic lesion. To minimize misdiagnoses, a review of pathology data by a second experienced toxicologic pathologist and a discussion between the study pathologist and the review pathologist is beneficial to assure quality of the histopathological evaluation.

Data handling: Statistical tests are necessary, but do not tell anything about the biological relevance of a lesion. Testing at the 5% limit means that of one hundred statistical tests on normally distributed homogenous data pairs such as liver weights of high dose and control animals, 5 tests turn out positive by chance reflecting only normal biological variance. Therefore, toxicologists and toxicologic pathologists must have some basic understanding of statistical tests, know the limits of their application, and be able to correctly interpret test results. False positive statistical results can be particularly awkward in the case of rare findings or a slightly increased incidence only in a (high) dose group. As already pointed out above, historical control data for any type of toxicity studies are indispensable to analyze such findings and to show that the statistically significant “deviation” is within the historical control range.

Another fallacy derives from dividing lesions into different subcategories, such as in the following example: The incidence of pulmonary squamous cell carcinomas in the control, low, mid, and high dose groups is 0, 0, 0, and 3, respectively. These data suggest a carcinogenic effect in the high dose group. However, there were also broncho-alveolar carcinomas with group incidences of 4, 3, 3, and 1, respectively. If both carcinoma types are pooled to give 4, 3, 3, and 4 respectively, then no treatment-related effect is present. For a guideline of combining neoplasms see.

Review of earlier studies: It is mandatory to review earlier, often shorter-term studies with the drug candidate or related drug candidates for subtle changes, which might have been missed or dismissed as not significant. With hindsight and knowing what one is looking for, it is always easier.

What else is known about the drug in question?

Exaggerated pharmacological action: Could the
observed APF be a consequence of an exaggerated pharmacological action at high doses of the drug candidate, e.g. neuropathy, if healthy animals are treated with hypoglycemic drugs? Is the APF mediated by a receptor that is responsible for the pharmacodynamic effect? Is that receptor relevant to man, similarly distributed, and similarly responsive in man?

Drug class effect: Some classes of drugs are known to induce APFs (Table 1). Information can be obtained e.g. from the scientific literature, through personal contacts directly from the scientific community or through the US freedom of information acts. Some of these class effects are known to be relevant to man, such as cytotoxicity of most anticancer drugs in rapidly proliferating tissues. Others are known to be more or less species-specific, such as many endocrine effects seen in test animals. Still others are of somewhat uncertain relevance for man, such as vasculitis observed with phosphodiesterase (PDE) IV inhibitors, and need to be assessed on a case-by-case basis (see also second part of this review). Class effect information is crucial to assess the relevance of an APF to man, to decide about potential precaution measures to be taken in clinical trials and to anticipate how registration authorities might regulate the drug in question. Also if a class effect is known not to be relevant to man, a new drug candidate showing the same APF has to be assessed carefully. As will be discussed in the second part of this review, peroxisome proliferation was originally and correctly regarded as not being relevant to man, but new peroxisome proliferator-activated receptor (PPAR) agonists are much more potent. Therefore it can not be excluded that APFs observed with new PPAR agonists are relevant to man. Already at this early stage, it may be useful to calculate risk/benefit ratios (see below under risk evaluation) based on structural similarities and/or therapeutic class in case of a suspected class effect.

Were there other relevant findings?

Particularly in case of unexpected APFs it is important to correlate in-life observations, clinical pathology/chemistry data, pathology findings, and any other observation of relevance made during the safety study. Clinical chemistry data often come from earlier time-points and can provide information on the time course of a target organ lesion. Pathology findings to be correlated include organ weights, lesions of other organs with potentially systemic consequences such as in case of the hepato-renal syndrome, organs of the endocrine system (see second part of review), or early lesions including preneoplastic lesions. It is clear that also findings from other studies, such as results from pharmacological or mechanistic studies, need to be taken into account.

Is the study technically valid?

Important issues to be examined include dose selection especially in rodent bio-assays, purity of the test substance, especially if after a change of the substance batch new toxicities are observed, and study conduct including e.g. tissue sampling. It is of utmost importance that control and dosed groups are treated in the same way with the only exception of dosing. This also includes that control and dosed groups should be necropsied and processed by the same team. Slide evaluation should be done by the same study pathologist. If for time-constraints two pathologists share the task, then splitting by dose must be avoided by all means. If necessary, then splitting should be by sex and the two pathologists must take some time to also examine typical slides of the other sex.

Is the model valid?

Animals are not humans and some particularities of test species commonly used in preclinical safety studies are summarized below. The endocrine regulation of rodents is markedly different from that of human beings, e.g.

- Rats lack high-affinity thyroxin-binding globulin
- The estrogen/progesterone ratio is 1:100–200 in rats, but 1:1 in women
- The sexual endocrine system of old rats is progesterone dominated, while that of menopausal women is just waning with a natural decrease of estradiol and progesterone production by the ovaries
- Prolactin has a trophic effect on rat mammary gland, but induces lactation in women
- Increased luteinizing hormone (LH) leads to Leydig cell (LC) tumors in rats, while human LCs are much less sensitive

Anatomical particularities of rodents include rodent-specific organs such as forestomach, Harderian gland (eye region), Zymbal’s gland (ear), and preputial/clitoral gland. Tumors occurring exclusively in rodent-specific organs are often regarded as not relevant to man. However,

- Tumors in the rat forestomach might indicate, for example, a risk for esophageal tumors in man
- The similarity of Zymbal’s and preputial/clitoral glands of rats to human sebaceous glands must be kept in mind.

Target organ concordance between test animals and human beings need not be a prerequisite when evaluating animal tumor findings with regard to their relevance to humans. Therefore, also the significance of tumors in unique rodent tissues must be addressed using the mode of action/human relevance framework approach (see risk evaluation below).

Mice are known to have a high incidence of spontaneous tumors particularly in lungs, the liver, Harderian and adrenal glands, the hematopoietic system, and ovaries. Rats show high incidences of mammary gland and pituitary tumors. Also, differences in absorption-distribution-metabolism-excretion (ADME) parameters between test animals and man regarding e.g. metabolite pattern, (organ) accumulation or distribution of drug-metabolizing enzymes, may play a role. Differences are also found between different strains of the same species. For example, the incidence of the following rodent tumors is strongly strain- and possibly partly also breeder-influenced:
• LC tumor incidence: 88–96% in F344 rats, below 10% in Sprague Dawley (SD) rats, 1–2% in Long-Evans rats
• Mononuclear cell leukemia incidence: 20% in F344 rats, rare in SD rats
• Mammary gland tumors, the incidence of which varies widely between different strains, possibly due to endocrine differences and viral infections

Despite the above facts the value of in vivo toxicology studies to predict for many significant human toxicities is established, but the prediction of human risk based on animal data needs to take all necessary parameters into account.

**What other modifying factors have to be considered?**

Many factors can influence the outcome of a study, including the following conditions:

- Quality of animals, particularly of non-rodents and among them especially of monkeys.
- Age of the animals. As age can have a potentially important effect on drug safety, new drug candidates, unless they can not be used for children, need to be tested also in juvenile animals.
- Husbandry, including e.g. light intensity and diet.
- Feeding
  - Feeding ad libitum, which actually means...
overfeeding, significantly increases tumor incidences of the pituitary, the mammary gland, and the lung in rats and of the liver in mice, but may decrease the incidence of uterine tumors in rats. Overfeeding also increases the incidence and severity of degenerative diseases, including nephropathy, which may impact on metabolism of xenobiotics, and the incidence of myocarditis, polyarteritis, and prostatitis. Overfeeding has been shown to shorten the life span of test animals.

- Contaminations of feed, water or the air and impurities in the drug substance

2. Hazard characterization

This step serves in particular to characterize and quantify the observed APF in more detail.

The most important objectives of the hazard characterization step are to obtain additional data, as far as needed and possible, on:

- Dose response, including exposure at the maximal tolerated dose (MTD) and at the no-observed-adverse-effect level (NOAEL) in the most sensitive species relevant to humans
- Early markers for the APF, which can be used in early human trials of the translational medicine phase as well as in later trials to monitor humans (risk management, see below) for the occurrence of the respective adverse effect(s)
- Mode of action (MoA)

Hazard characterization often involves additional experimental work. The amount of efforts invested depends on other factors especially on the stage of development of the drug candidate (past investment at stake), the proposed indication (risk-benefit considerations, see also below), available alternatives internally (follow-up drug candidates) and on the market (available therapeutic alternatives), the nature of the APF, and the conviction within the company regarding viability of the drug candidate.

Additional investigations of available samples

Samples may be available from the study in question or previously conducted studies and allow e.g. the following additional investigations:

- Hormone measurements in blood/serum samples particularly in case of findings in endocrine organs
- Assessment of gene or mRNA expression and/or marker proteins or protein patterns in blood or tissue samples
- Morphological investigations e.g. by electron microscope (EM) or by immunohistochemistry (IHC) on tissue samples regarding
  - Proliferative cell type
  - Measurement of cell proliferation, e.g. by proliferating cell nuclear antigen (PCNA), and of apoptosis
  - Subcellular details partly also visible on EM pictures of formalin fixed tissue, e.g. to prove or disprove the presence of phospholipidosis
  - Molecular epitopes, which are reasonably well preserved even after many years in wet and paraffin-embedded tissue
  - Morphometry for numerical or volume changes, in particular to establish a NOAEL

Tailor-made mechanistic studies

It is crucial that the additional studies are relevant to resolving the APF issue in question. Therefore, it is necessary to

- Keep the design simple and the objective including what to (dis)prove in mind
- Avoid experiments yielding results which may not be interpretable, e.g. because of lack of experience, lack of historical data, unproven method, etc.

Tailor-made mechanistic studies can have various purposes, namely to better characterize the lesion in question and/or to test one or several hypotheses about the pathogenesis of the observed APF. They serve to obtain properly sampled and prepared material for investigation by often more sophisticated methods, such as

- EM investigations on glutaraldehyde-fixed tissues
- IHC investigations on fresh tissue for detailed cell kinetic studies including cell proliferation, single cell necrosis, and similar investigations
- Flowcytometry for cell typing, e.g. in connection with immunotoxicity issues
- Laser scanning cytometry or confocal laser scanning microscopy for a detailed assessment of cellular structures
- Microdissection followed by special analyses, especially -omics investigations (see next bullet point)
- -omics investigations for DNA, RNA, and protein expression. These investigations can be useful to find biomarkers for testing of follow-up drug candidates and in clinical trials, but identifying and validating a biomarker can be resource intensive and
detailed investigation of ADME parameters including e.g. metabolites or organ accumulation. Additional analytical work is also necessary to support Phase III clinical trials, if in earlier clinical studies human metabolites are observed at exposures greater than 10% of the total drug-related exposure and at significantly higher levels than the maximum exposure in the toxicity studies. This does not apply for metabolites which are not of toxicological concern, such as most glutathione conjugates. For details see163, 164

Tailor-made mechanistic studies allow an investigation of early findings and of their development over time, e.g. by sequential necropsies in a time-course study or by using newer imaging techniques on live animals165. Investigation of early lesions is important, as early lesions are often characteristic for the toxicity observed, while later lesions represent a more general reaction of the biological system to injury.

Toxicity generally starts with functional impairment e.g. of selected cellular membranes of specific cells, which results in subtle structural changes such as microvesiculation (accumulation of water e.g. in mitochondria or lysosomes). If the toxic insult persists, water accumulation increases and becomes visible at the light microscopic level as vacuoles in the primarily affected cells. At this stage cell organelles might be disrupted and the target cells might eventually die resulting in non-specific inflammation dominated by leukocytes and phagocytic macrophages, which may also damage other organ cell types. Lymphocytes generally appear somewhat later, but stay longer. In subchronic studies the end stage of target organ toxicity may be replacement of specific cells by non-specific scar tissue, which may also impinge upon other organs, such as e.g. in the hepatorenal syndrome83. Tumors are often preceded by signs of increased cell proliferation (see below) or by preneoplastic lesions, particularly in livers84–86, 166.

To investigate if an APF is reversible, selected animals are necropsied after a treatment-free period at the end of a repeat dose study generally on a routine basis. It is important to keep in mind that early toxic lesions may disappear under continuation of exposure to the offending drug candidate because of adaptation and regeneration. E.g. one or two weeks after acute tubular necrosis kidney tubules might appear normal with exception of tubular basophilia in hematoxylin&eosin (H&E) sections, a tinctorial change which is characteristic of regenerated tubular cells. With progressing severity of a lesion, the likelihood of complete repair decreases. However, regenerative capacity is high especially in liver and kidney. If the lesion might have regressed to some degree or if it appears useful to investigate the details of the recovery process, tailor-made studies may be conducted with a longer recovery period and possibly with investigations at various time points during the recovery period.

In vitro studies with cell or tissue cultures, organ slices or the perfused target organ can be useful to investigate metabolism, effects on subcellular organelles, receptors, or gene expression. Such studies also serve to obtain data on dose-effect relationship at subcellular or molecular levels. For this purpose human cell lines are available.

**Increased cell proliferation**

Increased cell proliferation is generally associated with tumors in lifetime bioassays80, 167–169, but this is not always the case170. Increased cell proliferation is seen particularly in the following conditions:

- Subacute to chronic cytotoxicity with increased cell death, which must be avoided in lifetime bioassays by correct dose selection

- Chronic tissue irritation e.g. in case of
  - Crystals or aggregates with proteins and lipids in the urinary system. A well-known example is saccharine, which at very high doses formed bladder calculi leading to urinary bladder carcinoma in male rats171, 172. A more recent example is the occurrence of urinary bladder carcinomas following treatment with certain PPAR agonists, as discussed in more detail in the second part of this review
  - Solid state carcinogenicity e.g. at subcutaneous injection sites especially in rats or at implantation sites of microchips173 or transponders174, particularly in mice
  - Age-related “spontaneous” lesions, e.g. chronic progressive nephropathy, particularly following exacerbation by xenobiotic treatment, which seems to slightly increase renal tubule cell neoplasms175

- Increased physiological stimulation of cells leads to cellular hypertrophy (increase in size, generally with proliferation of cellular organelles) and hyperplasia (increase in cell number). The best known examples are the hormonally-mediated tumors in rodents, which are frequent in bioassays and generally without significance to man92.

For an overview of various types of epigenetic (not genotoxicity-related) carcinogenicity see Table 2.

### 3. Risk evaluation

The most important parameters for assessing the relevance of an observed unexpected APF for man are

- Mode of action (MoA) of the drug candidate eliciting the APF
- Safety ratio (also called safety factor or safety margin) between the highest non-toxic exposure in the most sensitive and relevant animal species and the therapeutic drug exposure of man
- Weight of evidence (WoE) based on the above and further parameters as appropriate

**a. Mode of action**

Understanding the MoA as established during step 2 of
Table 2. Epigenetic Carcinogenicity is Generally without Relevance to Man Unless Mentioned in the Table, with Selected References

| Pathogenesis                                      | Examples of compound(s) and, if appropriate, target organs and details regarding MoA                                                                                                                                                                                                 |
|--------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cytotoxicity                                      | Many drugs at cytotoxic doses for prolonged periods of time\(^{291}\). Note: Man is less sensitive to tumorigenicity associated with increased cell proliferation. Chemicals binding to \(\alpha_2\mu\) globulin in renal tubular cells leading to kidney tumors in male rats, which produce significant amounts of \(\alpha_2\mu\) globulin under testosterone\(^{92, 292}\). |
| Chronic irritation                                | Drugs which are irritating at subcutaneous injection site\(^{167}\). - predispose for urolithiasis such as saccharine, PPAR agonists, etc.\(^{172, 295}\). Note: Man is less sensitive to tumorigenicity caused by chronic irritation. |
| Induction of drug metabolizing enzymes           | Phenobarbitalone and similar inducers of drug metabolizing enzymes\(^{52, 180, 227, 271}\) leading to adaptive hepatocyte hypertrophy, hyperplasia, and finally tumors.                                                                                                                             |
| Induction of peroxisome proliferations           | Liver tumors with “early” hypolipemic drugs, such as clofibrates\(^{273, 274}\).                                                                                                                                                                                                             |
| Stimulation of adipocytes (??)                   | Lipo- and fibrosarcomas in rats and hemangiosarcomas in mice with new PPAR agonists\(^{296, 297}\).                                                                                                                                                                                             |
| Hormonal stimulation                             | Drugs with hormonal action lead to hypertrophy, hyperplasia, and tumors in the target organ\(^{92}\).                                                                                                                                                                                          |
| Hormonal imbalance                               | The dopaminergic mesulergine reduces the number of LH receptors in Leydig cells (LC) resulting in increased LH and LHRH levels. Note: Not relevant to man tolerating large increases of LH, e.g. in case of the Klinefelter syndrome\(^{94, 120}\). |
| Decrease of hormone receptors                    | The dopaminergic mesulergine reduces the number of LH receptors in Leydig cells (LC) resulting in increased LH and LHRH levels. Note: Not relevant to man tolerating large increases of LH, e.g. in case of the Klinefelter syndrome\(^{94, 120}\). |
| Block of hormone production results in increased stimulation of target organ | H2-blockers lower gastric HCl resulting in increased gastrin levels leading to stimulation of enterochromaffin-like cells (ELC) with hyperplasia and carcinoids particularly in rats\(^{272, 298–302}\). Note: H2-blockers are basically not considered to be tumorigenic in man, but may lead to ELC hyperplasia\(^{301}\). Therefore, some residual risk can not be excluded\(^{304, 305}\). Antithyroid agents lower T3 and T4 thus increasing TSH which leads to thyroid tumors particularly in rats being sensitive to inhibition of thyroid peroxidase\(^{50, 91, 294}\). Note: High levels of TSH in Grave’s disease (M. Basedow) are not associated with thyroid tumors in man. |
| Unknown                                          | \(\beta_2\)-agonists e.g., salbutamol or terbutaline induce hyperplasia of salivary glands and mesovarian leiomyomas in rats\(^{92, 307}\). Can be suppressed by \(\beta\)-blockers such as propranolol.                                                         |

For additional references see text.
addressing APF is the optimal basis for predicting the relevance of an unexpected APF for man and, if necessary, for managing and minimizing human risk. This has increasingly been emphasized again in recent years in a number of publications in particular regarding the relevance of tumors in rodent bioassays for man177, 176–183, but also for other types of toxicities176, 182. Examples showing how the elucidation of the MoA helped in risk evaluation are given in the second part of the review.

b. Safety ratios

The safety ratio is defined as follows:

\[
\text{Safety ratio} = \frac{\text{Animal NOAEL exposure}^{**}}{\text{Max (anticipated) human exposure}^{***}}
\]

* The safety ratio is often called “safety factor” and in some regions “safety margin”. However, the latter is sometimes also used for the ratio between exposure at NOAEL and exposure at LOAEL (low observed adverse effect level, within or between species). Therefore, it is advisable to clearly define these terms when using them in a report or publication.

** Exposure at NOAEL of the most sensitive species relevant to humans regarding the particular APF134.

*** If the anticipated human dose is not well known, as it is often the case during very early phases of development, also the calculation of the safety ratio between animal NOAEL exposure and exposure at pharmacodynamically effective doses in the same species is helpful. For clinical trials the safety ratios between animal NOAEL exposure and exposure at the human starting dose is relevant.

The safety ratio is an important quantitative means to set the first human doses to be tested in early clinical trials in man185, 186. As a basic rule and on practical grounds, a safety ratio is estimated by a safety (or uncertainty) factor of 10 for extrapolation of animal to man and an additional factor of 10 for inter-individual variation in man, resulting in a total safety ratio of 100. However, this comfortable safety ratio is frequently not achieved, and a safety ratio of 10–20 is often acceptable.

According to the FDA guidance for industry on estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers185, usually the NOAEL from the most relevant animal studies should first be converted to a human equivalent dose (HED) using standard factors presented in Table 1 of the guidance and taking into account the body surface. Using sound scientific judgment, a safety ratio (called safety factor in the guidance) should be applied to the relevant HED to arrive at the maximum recommended starting dose (MRSD). This guidance says that in general a safety ratio of at least 10 should be considered.

High safety ratios are less important for life-saving indications and/or elderly target patient populations particularly in the following context:

- Marginal increase of tumor incidence
- No therapeutic alternatives on the market or significant therapeutic or safety advantages over available alternatives
- MoA is well understood and/or the issue is manageable e.g. by using early biomarkers in clinical and in outpatient institutions.

Conversely, safety ratios are important in case of APFs which may be relevant to man and irreversible, such as neuronal degeneration or toxicity to the reproductive organs leading to sterility.

The calculation of a safety ratio is only possible under the assumption that below a limit or threshold dose the drug candidate does not induce toxicity. Since many decades thresholds are accepted for general toxicity and epigenetic tumorigenicity, but in recent years also increasingly for genotoxicity187–190. However, others argue that covalent binding of genotoxic drugs or of parts/metabolites thereof to cellular macromolecules like proteins and DNA may not be repaired completely: therefore some effects may persist and accumulate with repeated exposure191. The discussion is ongoing, but according to the 2006 European Medicines Agency (EMA, former EMEA) guideline on the limits of genotoxic impurities in drug substance a “threshold of toxicological concern” (TTC) value of 1.5 μg/day of a genotoxic impurity resulting in a mathematical excess cancer risk of <1 in 100,000 over lifetime exposure is considered to be acceptable for most pharmaceuticals192. This allows calculating an acceptable daily intake based on the expected daily dose. Higher limits may be justified under certain conditions such as short-term exposure.

Low safety ratios including safety ratios below 1, which means significant animal toxicity at dose levels below anticipated human doses, do not necessarily stop development, in particular in case of central nervous (CNS) drugs. CNS-related APFs such as sedation, ataxia, convulsions or death may actually require a slowly increasing dose regimen to allow the healthy test animals to adapt to the treatment. To proceed to early clinical trials in case of a low safety ratio, the APF should be relatively easy to monitor in man and be recognizable early, before permanent toxic damage is inflicted. In addition, an earlier than usual completion of the preclinical program is mandatory in most cases, as will be discussed for CNS drugs in more detail in the second part of this review.

c. Weight of evidence (WoE)

The WoE evaluation is the conclusion of the risk evaluation process, similar to evidence-based human medicine193, 194. The WoE as used in this paper is established on a case-by-case basis using a multifactorial, multidisciplinary approach181, 195–198. The WoE evaluation is a summary of the relative importance and causal relationships of various aspects of the particular issue, including dose/exposure-response, metabolism, tissue distribution, severity, reversibility, (anticipated) relevance to man, (anticipated) relative
sensitivity of man, and means to look for early signs of adverse findings in man. In addition, the WoE evaluation also takes into account the conditions of drug use and the overall therapeutic context, such as

- The severity of the disease condition and how the new treatment option impacts on quality of life and survival
- The novelty of the drug in question, i.e. whether there is a true medical need for this drug and/or whether it has a relevant new mode of action
- The availability of alternative therapies and their relative safety and efficacy as compared to the drug in question
- The target population, in particular its age
- The intended duration of use

A WoE analysis and conclusion is a process, which has to be continued throughout drug development. Typical milestones are first trials in humans, each time when testing larger populations, escalating doses, extending the duration of treatment in clinical trials, and finally before submitting registration documents to the approving authorities. In case of significant findings for a drug on the market, the WoE analysis and conclusions need to be repeated for the specific case.

For example: A WoE conclusion that a tumor response in rodent bioassays does not preclude administration of the drug candidate to humans, may be based on several of the following facts:

- In comparison with control animals only slight increase in tumor number, no shift to less-differentiated tumor type, no earlier occurrence of tumors, no reduction of the life span of test animals
- No clear dose-response (e.g. highest incidence of the questionable tumor finding in the mid dose)
- Or in other words: The tumors in dosed animals appear and behave similar to tumors of control animals
- A sufficient safety ratio
- Absence of genotoxicity allowing to adopt a threshold concept of the carcinogenic mechanism

On the other hand a real carcinogenic alert exists, if one or the other genotoxicity test is positive and/or one or several of the following conditions are observed: Marked dose-related increase in tumor incidence, early onset of tumor formation potentially associated with early pre-neoplastic lesions and/or shift to a less differentiated tumor type in comparison with control animals, potentially associated with decreasing longevity of the treated animals. The WoE approach should also take into account decreased incidences of certain tumor types and weigh them against increased tumor incidences.

Many drugs are on the market which are carcinogenic in laboratory animals, generally rodents, but are found to be safe in humans. For overviews see e.g. 181, 200, 201. Often the situation is not as clear as given in the above examples and—as shown in the second part of this review—there are also new drugs on the market e.g. with animal tumors and positive genotoxicity tests.

Evaluation of various types of adverse preclinical findings

Functional effects: This section is limited to functional toxicity without morphological correlate as e.g. seen in safety pharmacology studies or occurring in toxicity studies with certain drug candidates. Low safety ratios do not a priori preclude successful development, though additional safety data are generally needed and special precautions should be applied for first-in-man trials.

Human diseases caused by a regulatory imbalance are relatively frequent, affecting e.g. the endocrine system (over- or underproduction of hormones), the cardiovascular system, in particular the blood pressure, or the metabolic system (e.g. human metabolic syndrome). Drug candidates developed for such disorders actually disturb the corresponding regulatory system in healthy test animals and may therefore be associated with dose-limiting and marked APFs. E.g. anti-diabetes drugs can lead to hypoglycemic brain damage or drug candidates overstimulating neurons can result in neuronal degeneration, an effect also observed in humans at excessive doses.

As mentioned, test animals are often very sensitive to drug candidates intended for treatment of disorders of the central nervous system (CNS) and react with tremors, decreased activity, sedation, recumbency, loss of balance and ataxia, seizures, and also death already at relatively low doses, occasionally below the intended therapeutic dose. Such APFs are usually without histopathological correlates, dose-limiting, and reversible on cessation of dosing. Frequently the severity decreases with continuing dosing and an escalating dose regimen can help to achieve acceptable exposure in preclinical safety studies. Many drugs on the market show such CNS signs at relatively low doses in test animals, including clozapine, haloperidol, risperidone, bupropion, tricyclic antidepressants or acetylcholine esterase (AChE) inhibitors such as rivastigmine and benzodiazepines (see respective drug information in the PDR).

Another important group of functional APFs is related to the cardiovascular system. Drug candidates that promote QT prolongation potentially associated with the much feared torsades de pointes, include the antiarrhythmics quinidine, disopyramide, procainamide, sotalol, dofetilide, and ibutilide as well as methadone, thiordizine, and haloperidol. The relevance of QT prolongation found in preclinical safety tests needs to be established in specific clinical trials and does per se not preclude further drug development. Drug candidates with positive inotropic activity increase heart rates in dogs, resulting over time in morphological alterations including myocardial necrosis. For more detail see second part of this review.

Adverse clinical chemistry findings in laboratory animals, such as moderate elevation of selected serum enzymes without significant morphological alterations, do not prevent progression of a drug candidate into clinical trials, as the corresponding parameter can be monitored in man. However, clinical chemistry investigations provide useful biomarkers, particularly to screen series of drug candidates before start-
Morphologic effects: Morphologic toxicity falls into one or several of the following basic pathological reaction patterns of biological systems. Regressive alterations include in particular cytotoxicity which may progress to cell death. Almost any drug tested at high enough doses is cytotoxic. A sufficient safety ratio (a quantitative measure) is needed for continuing development, unless the MoA suggests that the APF is qualitatively not relevant to man. It depends upon the intended indication what constitutes a sufficient safety ratio (risk-benefit evaluation, see also Fig. 3 below).

Progressive alterations are hypertrophy, hyperplasia, and neoplasia. Hypertrophy and hyperplasia generally indicate adaptation to cope with increased workload e.g. following induction of metabolic enzymes especially in the liver, stimulation of target organs by hormones (see below), or glomerular and renal tubular hypertrophy following treatment with diuretics and other drugs. To assess the human relevance of a proliferative APF understanding of the responsible MoA is crucial, especially in case of potentially epigenetic tumors arising as a result of prolonged cellular stimulation in the absence of genotoxicity. A sufficient safety ratio is needed, unless the MoA is clearly irrelevant to man. Human evidence to trace the emergence of proliferative changes in man can generally only be obtained by invasive techniques including biopsies and is impracticable.

Inflammation can result from different types of injury. For instance exacerbation of background infections needs to be taken into account when treating test animals with immunosuppressive agents or when toxicity decreases the well-being and therefore potentially the resistance against infections. Some drug candidates lead to inflammation of organs or organ systems by unknown MoA, e.g. PDE IV inhibitors to vasculitis, as discussed in more detail in the second part of this review. However, inflammatory foci can also be the consequence of toxic injury e.g. around hepatocellular or renal papillary necrosis. It is important to understand the pathogenesis of inflammation, which provides at least some understanding of the MoA. Safety ratio calculations can support risk evaluation.

Metabolic changes manifest themselves often in form of storage of endogenous substances, e.g. fat, and may be the consequence of subtle toxicity. Storage diseases in the strict sense are e.g. phospholipidosis resulting from an impaired clearance of membranes (more detailed discussion in the second part of this review). Such changes may occasionally also be accompanied by functional impairment of the affected organ. Accumulation of administered drugs or metabolites can sometimes be seen as pigment deposits in the respective organ, often without impairment of organ function.

Primary circulatory and respiratory disturbances are less frequent. The latter can occasionally be a consequence of phospholipidosis. Drug-induced arteritis in laboratory animals is often without clinical dysfunction. Circulatory and respiratory disturbances including morphological changes can result from exaggerated pharmacological action of drug candidates as well: Particularly the aforementioned high sensitivity of dogs to drug candidates with positive inotropic activity is well-known.

Malformations as toxicity endpoint are important in reproductive toxicity studies. Maternal toxicity needs to be taken into account to determine the relevance of potential developmental toxicity evidenced as fetal malformations and/or embryofetal toxicity. The pathogenesis of malformations is practically always unknown, unless due to cytotoxic drugs; however, for obvious reasons the latter need not to be tested in reproductive studies. Safety factors are of little help for the risk evaluation of potential teratogens: The drug candidate has to be labeled as potentially teratogen, if developmental toxicity is observed in the absence of maternal toxicity. Well controlled large human studies proving the absence of human teratogenicity can not be conducted for ethical reasons. Pharmaceuticals are classified according to their potential fetal risk according to animal and, where available, human data and the potential benefit of the drug for mothers. The categories range from “no fetal risk based on human data” to “proven human fetal risk outweighing the potential benefit for the pregnant mother”. The definitions of these pregnancy categories differ between regulatory agencies. Malformations will be discussed in more detail in the second part of this review.

Affected organelles: If unexpected morphological APFs are noted, EM investigations may be used to investigate early subcellular changes. EM investigations can also serve to select drug candidates with low potency for inducing a specific APF known to occur with a particular class of drugs, e.g. antidepressant drug candidates with a low potential for inducing phospholipidosis. Newer methods including gene expression and fluorescent microscopy can partly replace the more resource intensive EM investigations. EM and other methods may support drug candidate selection and may help avoiding longer animal studies. However, fortuitously detected subcellular changes without functional consequences and/or progression to histopathological alterations are not relevant and therefore such sophisticated investigations are not needed in routine toxicity studies.

Not infrequently drug candidates have marked effects on specific cellular components. Best known is the effect of inducers of drug metabolizing enzymes associated with hyperplasia of the smooth endoplasmic reticulum (SER), the site of enzymes metabolizing xenobiotics, particularly in the liver. SER proliferation occurs in dose-dependent fashion and leads to cellular hypertrophy and hyperplasia with increased liver weight. It can be recognized histologically in H&E sections by a clearly eosinophilic cytoplasm. In electronmicrographs the proliferation of the SER is easily seen, also in formalin-fixed tissues from routine toxicity studies. SER hyperplasia and the associated phenomena are reversible upon cessation of treatment. However, in lifetime studies marked SER proliferation is generally associated with liver tumors in rodents because of sustained increased hepatocyte stimulation as explained above. Induction of
metabolic enzymes occurs to a greater extent in rodents than in man and liver tumors due to SER proliferation are without much relevance to man. However, enzyme induction may have significant consequences for the pharmacodynamics of drugs.

Lysosomes are particularly abundant in cells specialized in phagocytosis such as leukocytes and macrophages. If ingested material, often cellular membranes, cannot be degraded, phagolysosomes accumulate in the cell or in the draining lymphatic organs, the spleen, the reticuloendothelial system of the liver or in other organs. This results in a storage-type disease. If the accumulation is mild, it can resolve. Excessive accumulation of phagolysosomes can impair the physiological functioning of the cell and also lead to single cell necrosis. This type of storage disease is therefore of some concern to regulators and will be discussed in more detail in the second part of this review. Its significance to man needs to be evaluated on a case-by-case basis. A sufficient safety ratio is important.

Peroxisomes are particularly abundant in liver cells and are rich in peroxide reducing enzymes. Peroxisome proliferation is relatively easy to induce in rodent livers. At least one factor contributing to the resulting liver tumors is an increased production of hydrogen peroxides. The older PPAR α agonists such as clofibrate were not considered to be carcinogenic in humans at therapeutic doses. However, today’s PPAR agonists in development are much more potent than fibrates. In rodent carcinogenicity studies PPAR α and dual α/γ agonists induce tumors at multiple sites, in multi-species and strains of both sexes. Though the known PPAR agonists are not genotoxic in the standard ICH genotoxicity battery, they have to be labeled as “probable human carcinogens” according to the USA Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) criteria. For a more detailed discussion of PPAR agonists see second part of this review.

In recent years mitochondrial toxicity has received some attention and is increasingly being implicated in drug-induced clinical idiosyncratic toxicity. However, the study of chemical effects on mitochondrial respiration dates back many decades. Mitochondrial toxicants are partly known to inhibit or uncouple oxidative phosphorylation, thus leading to oxidative stress and inhibition of DNA replication, transcription or translation. Mitochondrial alterations need to be assessed on a case-by-case basis.

Summary of the risk evaluation process

A decision chart for dealing with unexpected APFs is shown in Fig. 2.

The risk evaluation process includes both a qualitative and quantitative analysis: The qualitative evaluation answers the question if the observed APF can be relevant to man in principle. For this purpose, the establishment of the MoA is crucial. The quantitative analysis, that is the calculation of safety ratios, becomes important when the APF is not (entirely) test species-specific or when the MoA of the APF is not (entirely) clear.

Safety ratios in conjunction with other information have to be included into a complete WoE analysis. An important part of the latter is also an assessment of the risk-benefit ratio as depicted in Fig. 3.

A greater benefit of drug treatment in case of a severe disease and/or missing therapeutic alternatives justifies a higher treatment risk. The limit between acceptable and unacceptable risk-benefit ratio is blurred. Therefore the package insert can only make the treating physician aware of the therapeutic risk. If the risk is considerable, the treating physician then has to make his/her own decision based on a case-by-case evaluation regarding the patient and the clinical conditions and then choose the optimal treatment solution under the given circumstances.

Additional evidence, if and to which extent an APF is relevant to man, often comes from translational medicine.
with first-in-man clinical trials. The ultimate proof may only become available with long-term clinical follow-up studies and post-marketing drug monitoring, which is also addressed in the next chapter. However, clinical observations do generally not allow verifying in humans potential genotoxicity or teratogenicity observed in preclinical development: Genotoxic effects are generally too weak and cancer may, if at all, develop only after many years of exposure. Ethical barriers do not allow exposing pregnant women; if on rare occasions pregnant women were exposed to potentially teratogenic drugs, generally no final conclusions are possible.

4. Risk management

Risk management means to take precautions to minimize the risk for man196, e.g. by

- More carefully monitoring patients with increased risk for adverse reactions e.g. because of chronic kidney disease in case of drugs mainly excreted by the kidney
- Excluding women in child bearing age in case of potentially teratogenic drugs, unless they are under contraceptive therapy and the expected benefit outweighs the potential risk
- Carefully selecting the initial doses for the first-in-man clinical trials185
- Escalating the dose in clinical studies with particular care
- Increasing clinical monitoring, e.g., serum and urinary biomarker measurements throughout treatment
- Adequately instructing health professionals
- Employing an effective post-marketing surveillance program246

Risk management is a task of the physicians responsible for clinical trials on behalf of the drug company and as actual trial leaders in hospitals. Once the drug is on the market, risk management is a task of the treating physicians in hospitals and in outpatient institutions. However, toxicologists and toxicologic pathologists are required to contribute their knowledge based on preclinical data of the drug, particularly for setting dose limitations and suggesting monitoring activities in early clinical trials, but also when it comes to increase doses in later human trials or when signs of potentially new toxicities (in the clinical environment often called “side-effects”) are observed during the post-marketing phase.

Recently, the USA Food and Drug Administration (USA FDA) has published a draft guidance on risk evaluation and mitigation strategies (REMS)247, 248. This document requires e.g. a communication plan to health care providers, measures to assure safe use of the drug in question, an implementation plan for such measures, and a timetable for submission of follow-up assessments.

Conclusions

Drug development is complex and not infrequently the path to market is paved with obstacles. Toxicologists and toxicologic pathologists play an important role in drug development and have to contribute to the well-being of patients who will be treated with a new drug. They also have to avoid being overcautious and promoting premature termination of a potentially useful drug. Drug development is routine in many aspects, but becomes challenging when sound scientific judgment is needed. The toxicologic pathologist with training in medical sciences is well equipped to contribute significantly to hazard identification, hazard characterization, risk evaluation, and proposing measures for risk management. This paper has outlined possible processes to resolve unexpected APFs and has provided an overview over typical issues encountered, from functional toxicity to morphological toxicity, genotoxicity, carcinogenicity, and reproductive toxicity.

This first part of the review emphasizes that “trouble-shooting” is a task which must be managed from within the company, though external experts might be able to contribute significantly to solutions. The clarification of the pathogenesis of an APF, that is the MoA of the drug candidate leading to this APF, is important in the process to evaluate the relevance of the finding to man. In addition, the calculation of safety ratios provides a quantitative measure for the potential risk to man particularly in early clinical trials. Identification of early markers of toxicity adds further safety to these studies in man. Additional tailor-made mechanistic studies need to be carefully planned, taking into account potential hypotheses regarding the MoA. The methods employed must be well established in the facility running investigative studies and historical control data must be available to assure that the results are interpretable. The conclusion of the risk evaluation process is an analysis of the WoE of the various parameters characterizing the APF, taking into account also the context of the drug use, medical need, and alternatives on the market.

In the second part of this review a number of APFs will be discussed in more detail, in particular with regard to their MoA and their relevance to man. Examples will cover toxicity, both of the functional and morphologic type, genotoxicity, tumorigenicity, and reproductive toxicity. The examples will also show that the MoA of drugs leading to APFs can sometimes not entirely be clarified, but that a well founded risk evaluation may still be possible.

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