

# EUROPEAN COURSE ON TOXICOLOGIC PATHOLOGY

Second Edition

Organized by The D.E.S.V. in Veterinary Pathology

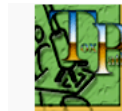
In partnership with:

The Society of Toxicologic Pathology  
& The French Society of Veterinary Pathology

VETERINARY SCHOOL OF NANTES (FRANCE)  
APRIL 23–27, 2007



TECNIPLAST FRANCE



# *Interpretation of Toxicology Results and Risk Assessments*

Jeffery A. Engelhardt, DVM, PhD, DACVP, FIATP  
Amgen  
Thousand Oaks, California





## *Outline of Presentation*

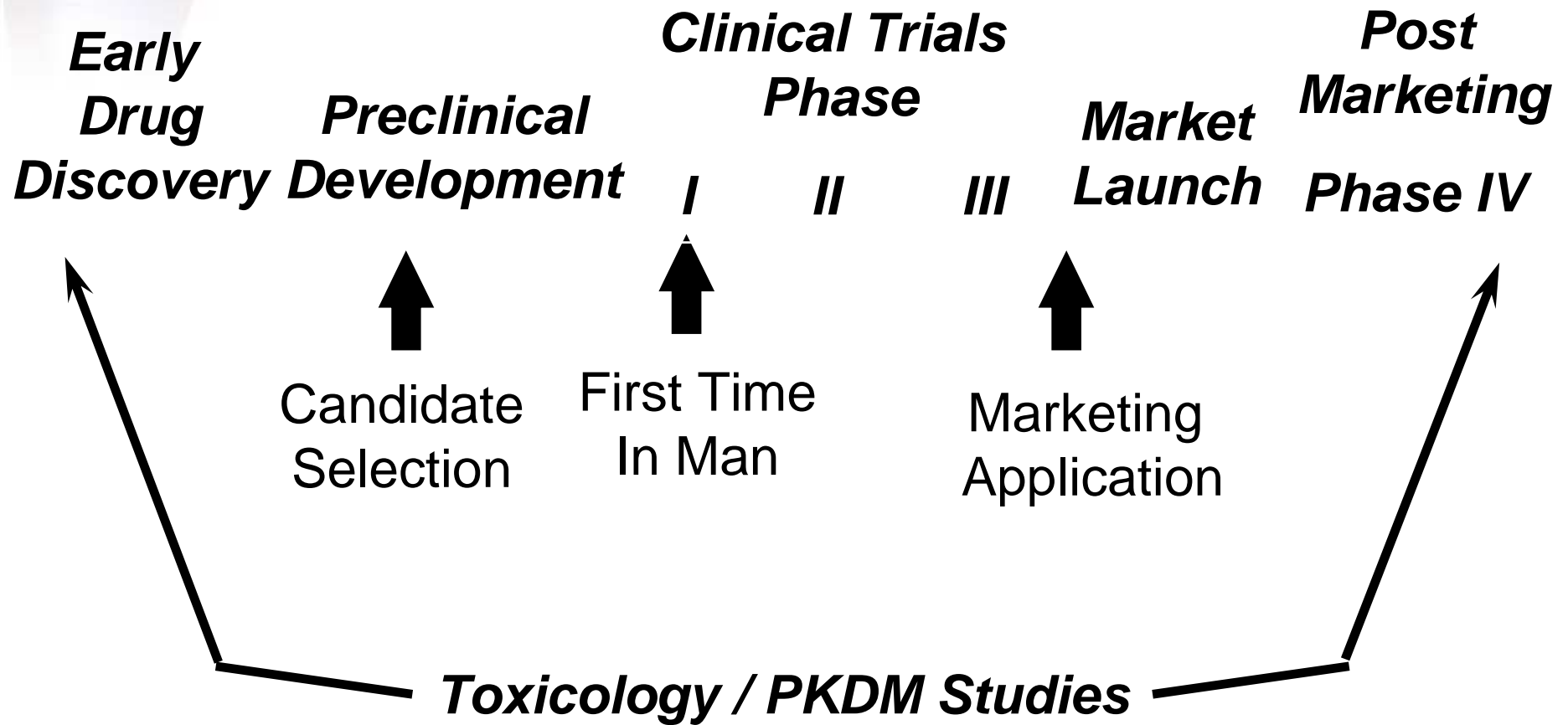
- Assessing safety of medicines
- Pathology evaluation
- Approaches to interpreting pathology data
- Pathology narrative
- Raw data and peer review
- Risk assessments



# *Assessing Safety of Medicines*



# *Drug Development Scheme*





# Goals of Nonclinical Safety Assessment

Characterisation of toxic effects

- ✓ 1 day to 2 years
- ✓ Repeated-dose toxicology studies

Clinical and/or laboratory markers of toxicity

Relationship of exposure to toxic effects

Dose dependence

- ✓ Margin of safety/therapeutic index

Estimate an initial safe starting dose

Safety data for global regulatory submissions



# *Study Design*

Specific studies needed to support clinical trials depend upon

- ✓ Human clinical population
- ✓ Intended use of the drug
- ✓ Results in pharmacology and toxicity studies
- ✓ Chemical structure of the drug candidate



## *Oversight by Regulatory Authorities*

Global regulatory agencies retain authority in determining what nonclinical studies will adequately support exposure of humans to a new pharmaceutical

Studies conducted in compliance with Good Laboratory Practise

- ✓ Satisfy FDA, OECD/EMEA, MHLW



## *Types of Nonclinical Safety Studies*

Safety pharmacology

PKDM or ADME (absorption, distribution, metabolism, and excretion)

Toxicology



# *Safety Pharmacology Studies*

*In vitro* and *in vivo* studies

Potential effects of a new drug on vital physiological functions

- ✓ cardiovascular system
- ✓ autonomic and central nervous systems
- ✓ respiratory system



# *Drug Metabolism and Disposition*

PKDM/ADME studies are primarily *in vivo* evaluations

- ✓ circulating concentration of the drug
- ✓ blood and tissue half-lives
- ✓ tissue distribution
- ✓ elimination
- ✓ biotransformation and microsomal metabolism
- ✓ placental transfer



## *Goals of Toxicology Studies*

Causal dose-response data

Clinical, laboratory, genetic, or physical alterations

*In vivo* toxicity from 1 day to 2 years

- ✓ Specific study types differ between small molecules and biomolecules



# *Staging of Toxicology Studies*

## Lead Optimisation

- ✓ Candidate identification and selection
- ✓ Dose-ranging for definitive studies

## Candidate Evaluation

- ✓ Clinical trial support studies
- ✓ Registration phase studies



## *Goals of Lead Optimisation Toxicology*

Provide pilot data to aid in the design of the definitive toxicology studies supporting FTIM

Use innovative science to define mechanisms of toxicity to allow

- ✓ Better screening paradigms
- ✓ Evaluation of human relevance
- ✓ Elucidation of On-Target versus Off-Target effects
- ✓ Identification of clinically useful biomarkers of target activation and/or toxicity



## *Categories of Toxicity*

Extension of the pharmacodynamic properties of the compound  
(ON-target)

Actions unrelated to the pharmacodynamics  
(OFF-target or intrinsic toxicity)

Activation of a physiological mechanism  
(biological toxicity)



# *On-Target versus Off-Target Effects*

## **OFF-Target Toxicity**

- ✓ High probability that the issue can be overcome through chemical development
- ✓ Improving selectivity and/or potency to the intended target could be key to success

## **ON-Target Toxicity**

- ✓ Often all that is seen with biologics
- ✓ Chemical development is unlikely to circumvent the issue.
- ✓ Solution should focus on finding the right "balance" between the intended and unintended pharmacology.
- ✓ The key to success may involve dialing out potency to the target



## *Poorly Predicted Adverse Effects*

Nausea

Headache

Dizziness

Tinnitus

Visual disturbance

Skin reactions



# *Types of Definitive Toxicology Studies*

Acute

Repeated-dose

- ✓ Generally conducted in the rat and dog for small molecules
  - Species choice depends on DM and tolerance data
- ✓ Receptor cross-reactivity for biomolecules

Carcinogenicity

Developmental

Genetic



# *Acute Toxicity Studies*

Information on overdose effects  
Minimum and median lethal dose  
Clinical signs

Part of product label and shipping classification



## *Repeated-Dose Toxicity*

Clinical and laboratory data on cumulative effects

Extensive clinical evaluation of test animals

- ✓ Physical, neurologic, and ophthalmic exams
- ✓ ECG evaluation
- ✓ Repeated clinical pathology and TK analyses
- ✓ Postmortem and histopathology examination
- ✓ Immunogenicity



# *Carcinogenicity Studies*

Potential to induce neoplasms in animals

- ✓ Not generally required for biomolecules

Not needed in advance of safety and efficacy trials

- ✓ PPAR

Large doses (MTD) are generally used

Effects may be due to exaggerated pharmacodynamics or secondary mechanisms

Lifetime exposure in rats and mice

- ✓ May include a transgenic mouse model



# *Carcinogenicity Studies*

The need to conduct carcinogenicity studies depends upon:

- ✓ Chemical structure
- ✓ Human population and intended use
- ✓ Results in other studies
  - Mutagenic or clastogenic effects
  - Proliferative lesions in shorter duration studies
- ✓ Tissue distribution and elimination
- ✓ Exposure to supra-physiologic levels
  - Biotechnology products



# *Developmental Toxicity Studies*

Effects on male and female fertility

Teratogenic potential (embryo-foetal toxicity)

Effect on peri- and post-natal development of offspring, including maternal development

- ✓ Supports inclusion of women in clinical trials



# *Genotoxicity Studies*

Include in vitro and in vivo systems

Evaluate the potential to induce mutations  
and chromosomal damage

- ✓ Bacterial mutation
- ✓ Cytogenetics
- ✓ Mammalian gene mutation



## *Summary*

Humans remain the ultimate test species

Nonclinical studies provide guidance for the investigating physician

Toxicity studies should provide a substantial challenge to the test animals

Toxicities in animals may not translate directly to humans

Animal studies are not the ultimate source of data on side effect profile



# *Pathology Evaluation*



## *Goals of the Pathology Evaluation*

Identify potential compound-related effects

- This is a descriptive and interpretive science

Name alterations, tabulate, and categorized

- Allows correlations between test material exposure and biological effects

Fulfill regulatory expectations in the evaluation of safety of medicines



## *Use of the Pathology Evaluation*

The recognition and appropriate classification of relevant changes is a critical part of the toxicologic and risk assessment of foods, drugs, chemicals, and medical devices

The interpretive power of the pathologist facilitates regulatory decision making

- Add perspective to the study findings



## *What is the Role of the Pathologist?*

Responsible for a reliable and detailed description  
of gross and microscopic changes

Consistent evaluation

Clear interpretation

Concise narrative

Facilitate informed regulatory decision making

Interpretive power versus statistical power



## *Responsibility of the Pathologist*

Provide an interface between toxicology, pathology, clinical investigation, and regulatory affairs

Target organ toxicity is important, but each organ cannot be treated as a separate entity

Separate normal biological variation and spontaneous disease from compound-induced changes

- Influence of compound on spontaneous disease



## *Responsibility of the Pathologist*

The nomenclature and severity grading system used to classify lesions may vary among pathologists

- ✓ Due to training and experience

Nomenclature used by a pathologist should be consistent within a study

- ✓ Diagnostic drift

Variation of grading among pathologists will not generally affect the overall interpretation of a study

- ✓ One pathologist evaluates all tissues from a study



## *Interpretive Power of the Pathologist*

Attribute relationship to compound

- ✓ Direct versus exacerbation of spontaneous disease

Perspective on similar lesions induced by other compounds or natural occurrence

Propose pathogenesis for toxic changes

- ✓ Pattern of tissue changes can provide clues



## *Interpretive Power of the Pathologist*

### Distinguish primary and secondary effects

- ✓ Convey subtle changes
- ✓ Need to compare treated and control animals to separate effects from normal biological variations
  - Need to know treatment group identification
    - Blinding in specific circumstances



## *Interpretive Power of the Pathologist*

Present evidence (not guesses) concerning reversibility

Reference why lesion is important or not

Ensure that important findings are understood

- By the sponsor
- By regulatory assessors



# *Approaches to Interpreting Pathology Data*



# *An Approach to Clinical Pathology Data*

Changes may be expressed in percentage (%) or fold

- ✓ Percent change is useful when the effect is less than 100%
- ✓ When the change is greater than 100%, 'x-fold' change may be used to improve readability of the report

Clinical pathology data collected in rodent studies longer than one year are only of diagnostic value for the individual animal



## *An Approach to Clinical Pathology Data*

Statistics should be used as a guide for interpretation and not as the primary criteria for importance

Individual animal values may give greater clues to compound-related effects

Group means are more useful in rodent studies

Individual values over time in combination with group means are useful with dogs or primates



## *An Approach to Organ Weight Data*

Scan mean values for statistical significance

Is the effect dose-responsive?

Inspect mean values from treated groups relative to controls to determine if a meaningful percent change is present

If body weight was affected, use relative values to determine toxicologic importance

- ✓ Certain patterns of change in organ weights are consistent with body weight loss
  - Decreased absolute weight of kidney, liver, heart, spleen, testes, adrenals, ovaries, uterus, pituitary
  - Increased or unchanged relative weight, especially to brain



# *Histopathology Data*



# *Specimen Preparation*

Tissues promptly immersed in neutral-buffered formalin

Special fixation

Trimmed at standard locations

SOP, trimming guide

Processed to minimise variability among groups

Mix treatment groups in processing and staining

Slides identified with study number, animal number, and slide number



# *Specimen Evaluation*

Pathologist should assure that standard sections of all protocol tissues and organs are present on the slides to be examined

- Missing tissues or critical portions of tissues must be recorded
- Re-cuts should be requested for critical evaluation of potential target organs

All alterations should be recorded and graded according to severity

- Compound-induced or shift in spontaneous incidence



# *Specimen Evaluation*

The pathologist must distinguish between regenerative changes and pre-neoplastic hyperplasia

- ✓ Co-existing necrosis indicates likely repair
- ✓ Age of animals
  - Older rodents (>1 year) more likely to have pre-neoplastic lesions
- ✓ Cellular atypia in adjacent tissue sections
  - Changes in nuclei indicate potential pre-neoplastic change



# *Specimen Evaluation*

Pathologist should use standardised nomenclature and diagnostic criteria

Society of Toxicologic Pathology SSNDC

ILSI monographs

Tumours must be classified to allow proper statistical analysis of carcinogenicity studies

Benign or malignant

Primary or metastatic

Fatal or incidental



## *An Approach to Histopathology Data*

Cause of death should be addressed whenever possible

Standard terminology should be used

- ✓ Read a set number of animals each day to prevent 'diagnostic drift' from subjective terminology
- ✓ Diagnostic terms should be defined for the reader



# *Pathology Narrative*



# *Pathology Narrative*

## Written pathology interpretation

- ✓ Detailed description of findings
- ✓ Clear interpretation and best judgment of importance of findings
- ✓ Concise and explicit wording
- ✓ Analysis is based on the treatment cohort rather than effects on individual animals
  - Need to consider individual effects in non-rodent species



# *Pathology Narrative*

Pathology reports must meet the needs of the customer whilst withstanding the scrutiny of peers

- ✓ What did the compound cause and are the changes important to understanding the safety of the molecule
- ✓ Can another competent pathologist arrive at the same conclusion with the same data?
  - Adverse = deleterious to the animal whether unintended or related to pharmacology
  - Toxicity does not equal adverse
- ✓ Can a non-pathologist understand the narrative?



# *Pathology Narrative*

## Correlation to other changes in the animals

- Clinical observations
  - ✓ Aid in identifying target organs and potential mechanisms of action/toxicity
- Body and organ weights
  - ✓ Primary and secondary effects on tissues
- Haematology, clinical chemistry, and urinalysis data
  - ✓ Potential markers to monitor in patients
- Metabolism and toxicokinetic data
  - ✓ Margins of exposure



## *Guidelines for Pathology Narratives*

Effects should be described qualitatively rather than quantitatively to aid understanding of importance of the findings

- Grading scales
- In text table of key findings

If there are no compound-related alterations, simply state that no effects were observed

If compound-related effects were identified, list the affected organs and then elaborate on the alterations present

- List organs in order of importance, e.g., brain before Harderian gland

Avoid excessive discussion of spontaneous lesions



## *Qualitative Grading Scale*

**Minimal (barely perceptible)**

**$\leq 10\%$  effect**

**Slight or mild (noticeable,  
but not prominent)**

**11-25% effect**

**Moderate (prominent)**

**26-50% effect**

**Marked**

**51-75% effect**

**Severe**

**$>75\%$  effect**



## Example of an In-Text Table

Table #. Incidence of Key Compound-Related Histopathological Findings in [Species] Given [Compound] for [X] Months

Lesion	Dose (mg/kg):	Number of Animals with Observation							
		0		A		B		C	
	Sex:	M <sup>a</sup>	F	M	F	M	F	M	F
Total Number of Animals:		10	10	10	10	10	10	10	10
Kidney									
Multifocal renal tubular necrosis									
	Minimal	-	-	-	1	2	3	4	5
	Slight	-	-	-	-	-	1	2	3
	Moderate	-	-	-	-	-	-	1	2
Liver									
Multifocal hepatocellular necrosis									
	Minimal	-	-	1	1	2	3	5	5
	Slight	-	-	-	-	1	2	3	3
	Moderate	-	-	-	-	-	-	1	2

<sup>a</sup>M = male, F = female.



## *Pathology Narrative and Study Report*

In-depth discussion with perspective to support safety assessment

- Statistical versus biological importance
- Relationship among changes in organ systems

Perspective on similar lesions induced by other compounds, a common mechanism of action, or spontaneous disease



# *Pathology Narrative and Perspective*

## Address all findings

- ✓ Even though some values reached statistical significance, these variations in organ weight/hematology/clinical chemistry were not considered to be toxicologically important due to the magnitude of effect, lack of dose response, or were within the range of values found in concurrent control animals
- ✓ The other lesions described in the Tables in Appendices were considered to be spontaneous alterations to the type and incidence common in (species) of the age (and strain).

## Need to lose being a 'pathologist'

- Fascinating or exciting lesions for the pathologist may be concerning to someone else



# *Raw Data and Pathology Peer Review*



## *Pathology Raw Data*

Only the signed pathology report and histological sections are considered to be raw data

- ✓ Tissues, paraffin blocks, and slides are archived

Draft tables, notes, and peer review worksheets may be discarded



# *Pathology Peer Review*

## Quality control mechanism

- ✓ Ensure accuracy and completeness in recording histopathological findings
- ✓ Control observational bias by the histopathologist
- ✓ Standardise terminology and diagnostic criteria
- ✓ Confirm target organs
- ✓ Confirm NOEL and NOAEL
- ✓ Expected in CPMP/SWP/2877/00



# Pathology Peer Review

A second evaluation of a subset of animals and tissues

- ✓ All tissues from 10% or minimum of 3/sex in high dose and control groups
- ✓ Target tissues and equivocal alterations
- ✓ Verification of cause of death

Ensures accuracy and consistency of terminology and severity grades for compound-related findings

- ✓ Necessary to determine NOEL and NOAEL

Reviews the validity of the interpretation



# *Pathology Peer Review*

## Differences of opinion

### Consensus

- Literature, consultation, pathology working group

### Non-substantive

- Minor differences in terminology or grading that have no bearing on the overall interpretation of a study

### Substantive

- Identification of important new pathologic changes or differences of a least two severity grades



## *Pathology Peer Review*

Document peer review with a signed Peer Review Certificate

- ✓ Study identification
- ✓ Purpose and process of the review
- ✓ Results of the review
- ✓ How any differences in opinion were resolved

Worksheets and all other records made during review are not retained



# *Study Reports*



## *Study Report*

Large doses are used to assure measurable effects will occur in small populations of animals

- Highest dose should cause overt toxicity
- Lowest dose should not cause any (toxic) effects (NOEL)
- NOAEL

This perspective needs to be discussed by the pathologist and study director



## *Study Report*

Describe potential pathogenesis of important findings for the regulatory assessor to understand what has occurred in animals

Facilitates appropriate evaluation of toxicity and risk assessment of a new drug

Allows clinical investigators to understand potential adverse effects in humans



## *Correlation of Animal Toxicity to Man*

Up to 71% correlation when similar organ systems are affected in both rodent and non-rodent species

63% correlation when only non-rodent affected

43% correlation when only rodent affected



## *Correlation of Animal Toxicity to Man*

Greatest correlation in predicting human adverse effects in

- Haematological system
- Gastrointestinal system
- Cardiovascular system

Least predictive for effects on skin and hepatobiliary system



## Summary

Pathology data are qualitative, subjective, and descriptive rather than quantitative and objective

- Causes interpretation differences and misunderstanding by regulatory assessors and sponsor toxicologists

Pathologists need to provide a detailed description of compound-induced effects and ensure that important findings are understood

- Use standard terminology to aid understanding by non-pathologist



## *Few Pathologists in the Agencies*

'Political Science' of agency and sponsor issues

- ✓ Compliance, interpretation, perception

Description and interpretation in the narrative

The pathology data must be reliable

How do we make pathology more reviewer friendly?

- ✓ Definitive answers in interpretation
- ✓ Explicit, consistent, and avoid jargon
- ✓ Simple concise sentence structure



## *Conclusion*

The toxicology and pathology evaluations should ensure that compound-induced alterations are presented

- ✓ Clearly
- ✓ Consistently
- ✓ Accurately
- ✓ Understandably

Importance of the findings for patient safety is explicitly identified for inclusion in the various regulatory documents



# *Risk Assessments*



## *Approaching the Risk Assessment*

A risk assessment is a succinct synthetic interpretation of relevant data to provide perspective on a toxicological finding

What do you need to know to begin

- What is the issue
- What is the purpose of the assessment
- Who is the audience



## *Know the Issue*

Need a clear, concise explanation of the issue

Need to know if the issue is

- Real or theoretical
- A risk to patients
- A class effect



## *Know the Issue*

Is it bad or not?

- Bad for the animal versus bad for a human
  - ✓ Regulatory assessors may blur this distinction

If it is perceived to be bad, need to tell the assessor why it's acceptable

Very important to frame the issue appropriately



## *Know the Purpose*

What are you trying to achieve

- ✓ Keep a clinical trial going?
- ✓ Register the compound?
- ✓ Inform internal parties?
- ✓ Inform sponsors?



## *Regulatory Realities*

### It's not always about the science

- Strict scientific discussion will not always do the job
  - ✓ Remains the foundation of all negotiations
  - ✓ Politics may drive final decisions
  - ✓ Examples

### If it is perceived to be bad, it is bad

- Need to reassure that the compound will not harm patients



# *The Global Regulatory Environment*

In spite of ICH, there remains incomplete concordance on what constitutes required studies

- ✓ Lack of local guidance documents

ICH M3 and S6 documents have apparent conflicts

- ✓ Expectations for small versus biomolecules

Contemporary regulatory case law is lacking for biotechnology products and oncolytics



## *Conclusions*

Quality of the data and interpretation in reports facilitates sound decision making internally and by regulators

Understand the point of view of the regulatory assessor

Documents and verbal interactions must be concise, clear, critical, and honest