

Specific Aspects and Approaches for Regulatory Evaluation of Pharmaceuticals in Two-Year Rodent Carcinogenicity Studies

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Conflict of Interest Statement

Speaker has consulted for numerous pharmaceutical companies over the past 12 years addressing issues related to drug development including the design and interpretation of carcinogenicity studies.

Lecture Outline

- Overview of two-year carcinogenicity study for pharmaceuticals
- Acceptable approaches for dose selection
- Planning approach for carcinogenicity study
- Oversight of carcinogenicity study during study performance
 - Including regulatory interaction

Abbreviations

- Carci – Carcinogenicity
- ICH – International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Overview of Two-Year Carci Study for Pharmaceuticals

- Objective
- Species and strain selection
- Route of administration
- Dose groups including control groups
- Number of animals/group
- Pathology evaluation

Objective of Carcinogenicity Testing

“The objectives of carcinogenicity studies are to identify a tumorigenic potential in animals and to assess the relevant risk in humans.”

ICH S1A

Species and Strain Selection

Rat and mouse generally used

- Rat considered more sensitive than mouse (ICH S1B)
- Other species may be used based on metabolism or biological considerations

Currently Consider

Hamsters
Marmosets

Historically Used

Macaca monkeys
Dogs

Species and Strain Selection

- Animals in the carcinogenicity study should be same as in the precarcinogenicity program
 - Species (Obvious and rarely changed)
 - Strain (Occasionally changed but would require an additional precarci study)
 - Source (Too frequently changed)
 - Supplier
 - Production facility

Species and Strain Selection

- Animals in the carcinogenicity study should be same as in the precarcinogenicity program
- Deviation can result in a “failed” carci study
 - Unexpected toxicity
 - Lack of an adequate high dose due to lack of minimal toxicity

Species and Strain Selection

Commonly used strains

- Rats
 - Sprague Dawley (Pharmaceutical, US)
 - Wistar han (Pharmaceutical, Europe)
 - F344 (Chemical and Pesticide, US and Europe)
- Mouse
 - Swiss (Pharmaceuticals, US and Europe)
 - B6C3F1 (Chemical and Pesticides, US and Europe)

Route of Administration

Relevant mode of administration should be used

- Pharmaceuticals
 - Oral pharmaceutical = gavage (rarely dietary)
 - Dermal pharmaceutical = dermal
- Environmental agents
 - Air contaminant = inhalation
 - Water pollutant = drinking water
 - Dietary contaminant = feeding

Route of Administration

- Gavage versus feed administration can result in very different toxicity and carcinogenicity profile
 - Alter MTD
 - Different C_{max}
 - Metabolism may be altered due to saturation of metabolism with high exposure at C_{max} in gavage study

Dose Groups

Number of groups will vary depending on objective and preceding toxicity data

Vehicle 1
Vehicle 2
Low Dose
Middle Dose
High Dose

Duplicate Control Groups

Examples of differences between concurrent Sprague Dawley rat control groups

Tumor	Control 1	Control 2	Sex
Adrenal Pheochromocytoma	7/60	14/60	Male
Skin fibroma/dermal fibroma	6/60	2/60	Female
Thyroid C cell adenoma	8/50	1/49	Female

Adapted from: *Toxicologic Pathology* 33:283-291, 2005

Number of Animals/Group

- Generally between 50 and 70 animals/sex/group
- Number should be based on anticipated survival to two years
 - Historical experience in the performing laboratory considering
 - Strain
 - Source
 - Anticipated losses due to effect of test agent

Pathology Evaluation Clinical Pathology

- Clinical pathology evaluation generally not included at end of study
 - Nonneoplastic effects of compound previously determined in chronic multi-dose studies
 - Complications of spontaneous disease e.g.,
 - Liver tumors
 - Chronic kidney disease

Pathology Evaluation Organ Weights

- Organ weights are not collected in carcinogenicity studies
- Organ weights at study termination are not helpful due to effects of:
 - Neoplasms
 - Spontaneous
 - Compound induced
 - Nonneoplastic spontaneous disease
 - Debilitation

Pathology Evaluation Tissue Collection

- Society of Toxicologic Pathology minimum core list of recommended tissues (*Tox Path* 31: 252-253, 2003) includes:
 - 40 tissues (see reference)
 - Organ or tissues with gross lesions
 - Tissue masses
 - Additional tissues based on study design e.g.,:
 - Nasal cavity, larynx, and tracheobronchial lymph nodes in inhalation study
 - Administration sites for IV or skin application studies

Pathology Evaluation

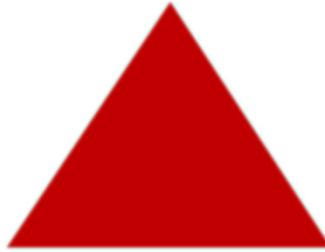
Histopathology

- Histological diagnosis of tumors is the ultimate basis for determining carcinogenicity in an appropriately designed and performed study
- Important issues:
 - Collection of appropriate tissues
 - Plan for reading tissues from “unscheduled deaths”
 - Determine approach for pathologist evaluation
 - Pathology peer review

Acceptable Approaches for Dose Selection

Approaches to Dose Selection

Critical Reviews in Toxicology 37:729-837,2007



Rodent Carcinogenicity Studies
Dose Selection and Evaluation
(US EPA 2003)

Dose Selection for Carcinogenicity
Studies of Pharmaceuticals
(ICH S1C 1997; Revised 2008)

Dose Selection—Pharmaceuticals

- “The objectives of carcinogenicity studies are to identify a tumorigenic potential in animals and to assess the relevant risk in humans” ICH S1B
- Dose selection has been standardized through the ICH process
- Dose selection guideline first developed in 1997 with subsequent revisions
- Current ICH guideline is S1C(R2) revised in March 2008

Dose Selection—Pharmaceuticals

- Carcinogenicity studies typically have three dose groups plus one or more control groups
- High dose has traditionally been selected based on a Maximum Tolerated Dose
- Doses are selected based on three-month or six-month toxicity studies that have defined multiple toxicity parameters

Dose Selection—Pharmaceuticals

Considerations for “Dose-Ranging Studies”

- Metabolic profile in selected rodent species/strain should be as similar as possible to humans
- Study data must be available from both male and female animals
- Data required from 90-day studies
- Dosing schedule and regimen should be based on clinical use
- Toxicity profile and dose-limiting toxicity should be characterized
- Changes in metabolite profile and changes in enzyme activity should be established

ICH S1C(R2)

Dose Selection—Pharmaceuticals

1) Toxicity endpoints in high-dose selection

- Continued use of the MTD
- MTD is defined in ICH as a dose that is expected to “produce a minimum toxic effect over the course of the carcinogenicity study”
- Factors for consideration:
 - No more than 10% decrease in body weight gain
 - Target organ toxicity
 - Significant alterations in clinical pathology

Dose Selection—Pharmaceuticals

2) Pharmacokinetic endpoints

- Selection of high dose may be based on a “...25 to 1 ratio of rodent to human plasma AUC of parent compound and/or metabolite”
- Approach is very useful but complex in application

Dose Selection—Pharmaceuticals

Factors that require consideration in the use of 25-fold exposure approach for setting high dose

- Adequate animal TK and human PK data should be available for parent and metabolite
 - Frequently the maximum recommended human dose or human PK at this dose is not fully defined when a carcinogenicity study must be initiated
- Similarities of metabolism may be debatable
- Selection of parent or parent and metabolite as the basis for comparison may not be clear
- Protein binding must be considered since the 25-fold should be based on free drug

Dose Selection—Pharmaceuticals

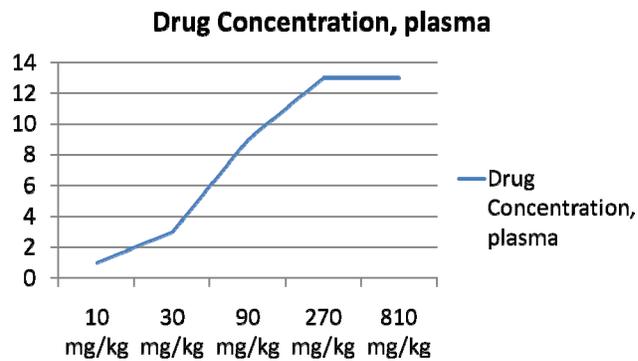
Application of a pharmacokinetic approach is best utilized when:

- Recommended human dose is clearly defined
 - Dose will not change for another indication
- Minimal metabolism occurs in humans and animals
 - A large number of metabolites complicates the application of this approach
- Minimal inter-individual variability in human exposure
- The animal to human AUC ratio is much greater than 25
 - Relatively small changes in PK from ongoing studies may change the ratio to less than 25

Dose Selection—Pharmaceuticals

3) Saturation of absorption

- High dose should not exceed the maximum absorption



Dose Selection—Pharmaceuticals

4) Pharmacodynamic endpoints

- The pharmacologic effect should preclude use of a higher dose
 - Inhibition of blood clotting
 - Hypotension
 - Neuroactive agents

Dose Selection—Pharmaceuticals

5) Maximum feasible dose

- 5% of diet has historically been a maximum feasible dose
- May also be limited by maximum gavage volume (generally 10 mL/kg)
 - Results from poor solubility
 - Other vehicles should be considered
- Acceptance of pharmacokinetic endpoints should reduce use of Maximum Feasible Dose

Dose Selection—Pharmaceuticals

Selection of middle and low doses

- Should be selected to provide insight into relevance of study results to humans.
 - Should not be specific fractions of the high dose
- Factors to consider
 - Human exposure
 - Ideally low dose should provide at least a small multiple of the human exposure
 - Linearity or lack of linearity of the rodent exposure curve
 - Mechanistic considerations
 - Threshold of minimal effects in dose range studies
 - Minimal necrosis
 - Enhanced cell proliferation
 - Alteration in rodent physiology

Planning Approach for Carcinogenicity Study

Planning for Carcinogenicity Study

- Planning for carcinogenicity studies is frequently given belated consideration
- Factors that should be considered at beginning and throughout toxicological assessment of molecule
 - Selection of species/strain
 - Metabolic profile compared to humans
 - Exposure profile
 - Sites of tissue injury

Planning for Carcinogenicity Study

Recommendation:

- Schedule data review 6 to 12 months prior to projected start of carcinogenicity study to assess data gaps
 - Toxicity data in animals and humans
 - Sites of tissue injury
 - Understanding of mechanism of injury
 - Exposure data in animals and humans
 - Metabolic profiles in animals and humans

Oversight of Carci Study during Study Performance

Study Oversight

- Carcinogenicity study requires greater monitoring than other animal studies
 - High investment in study
 - Usually critical timeline related to submission for marketing approval

Study Oversight Survival

- Important to assure that an adequate number of animals are available for statistical analysis at end of study
 - Ideally should have 15 to 20 animals in each group at end of two years
 - If survival is reduced, early termination should be considered but only with input and concurrence from US FDA

Study Oversight Survival

- Timely collection of tissue from early deaths
 - Autolysis of tissue may prevent histopathological diagnosis resulting in:
 - Reduced animals for evaluation thereby impacting statistics
 - Raise questions regarding other aspects of study performance
- Adequate collection of tissue from all animals
 - Lack of tissue reduces the number of animals examined

Study Oversight Survival

- Dealing with reduced survival takes time and therefore requires an aggressive approach
- Communication path and decision making process
 - CRO notification of sponsor
 - Internal discussions of sponsor staff and management
 - Preparation of submission of data and request for early termination to US FDA
 - US FDA consideration of request and preparation of response
 - Scheduling early sacrifice at CRO

Study Oversight Pathology Peer Review

- Not required but highly recommended
- Requires an experienced pathologist
- Process should be defined in advance but typically includes:
 - Review of all tumors and hyperplastic lesions that might be considered tumors
 - Review of all target organs
 - Review of all tissues from a subset of control and high-dose groups

Conclusion

- Design, planning and performance of Carci studies are complicated requiring close attention and special expertise
- Errors or omissions have grave consequences
 - Cost of study if a repeat is required
 - Delay in approval for marketing

ICH Guidelines

- Guideline on the Need for Carcinogenicity Studies of Pharmaceuticals S1A
<http://www.ich.org/LOB/media/MEDIA489.pdf>
- Testing for Carcinogenicity of Pharmaceuticals S1B
<http://www.ich.org/LOB/media/MEDIA490.pdf>
- Dose Selection for Carcinogenicity Studies of Pharmaceuticals S1C(R2)
<http://www.ich.org/LOB/media/MEDIA491.pdf>
- Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6
<http://www.ich.org/cache/compo/276-254-1.html>

US FDA Guidance

- Carcinogenicity study protocol submission
<http://www.fda.gov/cder/guidance/4804fnl.pdf>
- Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals
<http://www.fda.gov/cder/guidance/815dft.pdf>

Selected Publications

- Jacobsen-Kram D: Cancer Risk Assessment at the FDA/CDER: is the Era of the Two-Year Bioassay Drawing to a Close? *Toxicologic Pathology* 38:169-170, 2010
- Rhomberg LR, et al.: Issues in the Design and Interpretation of Chronic Toxicity and Carcinogenicity Studies in Rodents: Approaches to Dose Selection. *Critical Reviews in Toxicology* 37: 729-837, 2007
- Jacobs A and Hatfield K: History of Chronic Toxicity and Animal Carcinogenicity Study for Pharmaceuticals. *Veterinary Pathology* 50(2):324-333, 2013