CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

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Third Conference

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October, 2010

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Design of Carcinogenicity Studies

Peter Mann

EPL NorthWest

Thanks to Dan Morton at Pfizer, who graciously shared material for this talk

Outline

- Regulatory guidance
- Features of standard 2-year rodent carcinogenicity studies
- Model selection
- Dose and route selection
- The role of the pathologist
- Peer review
- Historical control data
- Managing “positive” findings
- Impact of positive findings for registration
- Biologics

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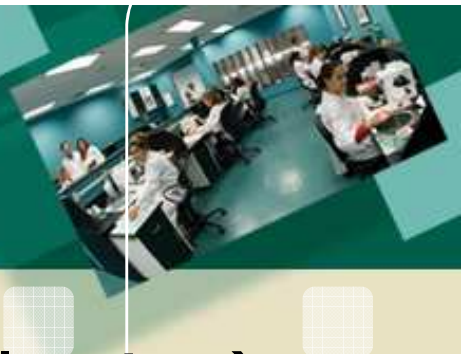
Regulatory Guidance



- ICHM3, S1A, S1B, S1C(R2), S6 (Biologics)
- OECD
- EMEA
- U.S. FDA
  - Red book - Food additives
  - Design and statistical analysis (2001 draft in use)
- U.S. EPA
- Japan

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Is Carcinogenicity Testing Required? Pharmaceuticals

- ICH S1A: Need for Carcinogenicity Testing
  http://www.ich.org/cache/compo/502-272-1.html#S1A
- Assessment of carcinogenicity is required if
  - Patients will be treated continuously for more than 6 months or drugs will be used in a frequent, recurrent, intermittent manner
  - There is cause for concern e.g. carcinogenicity findings in the class, preneoplasia in chronic studies, structure-activity indications, or extremely long exposure

Is Carcinogenicity Testing Required? Pharmaceuticals

- Carcinogenicity testing usually is not required for clearly genotoxic compounds (assumed to be carcinogenic)
- Carcinogenicity testing may not be required if the drug is intended to treat life-threatening diseases (risk benefit assessment) or when life expectancy is short
- Biologics (protein therapies) are special cases—carcinogenicity testing usually not needed.

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Is Carcinogenicity Testing Required? Pesticides, Herbicides, Food Additives

- New pesticides, herbicides, and fungicides usually must be evaluated for carcinogenic potential
- Food additives for humans and chronic use drugs for animals producing meat and milk often require testing
- OECD guidance generally addresses carcinogenicity assessment. Regional requirements vary by country.

Carcinogenicity Study Design

- EMEA Note for Guidance on Carcinogenic Potential http://www.ema.europa.eu/pdfs/human/swp/287700en.pdf
- OECD Guidance Notes for Analysis and Evaluation for Chronic Toxicity and Carcinogenicity Studies http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/NT00002BE2/$FILE/JT00130828.PDF

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Carcinogenicity Study Design

- FDA *Draft* Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079272.pdf

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Typical 2-Year Rodent Study Design

- 3 dose groups + vehicle control group
- At least 50/sex/group
  - May use up to 70/sex/group if survival is expected to be poor (<50%)
- May have an additional control group if vehicle or excipient is unusual and requires toxicity assessment
- Plan for 104 weeks of treatment (considered a lifetime study)
- Administer compound by intended route of clinical delivery (oral, IV, SC, IM, dermal, or inhaled if possible) for drugs; in-diet preferred for environmental chemicals or animal feed supplements

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Dose Selection

- ICH S1C(R2)
  http://www.ich.org/LOB/media/MEDIA491.pdf
- Doses usually based on 3-month study in the same animal model using the same route of administration
- The 3-month toxicity study often is sufficient
  - Same route of administration
  - Similar impurity profile
  - Similar husbandry conditions are desired
- Separate studies may be conducted if needed
- Human and rodent metabolite and protein binding profiles should be available to select doses

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High Dose Selection in 2-Year Studies

- Top dose
  - Maximum tolerated dose
    - "The top dose or maximum tolerated dose is that which is predicted to produce a minimum toxic effect over the course of the carcinogenicity study" (ICH S1C(R2)).
    - No more than 10% decrease in body weight gain relative to controls; target organ toxicity; significant alterations in clinical pathological parameters.
  - Nontoxic dose >25x maximum recommended human exposure based on AUC of free plasma concentrations of parent and/or metabolites (for drugs only)

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Selecting the High Dose

- Saturation of absorption—maximal exposure
- Dose-limiting pharmacology—sedation, inappetence, hypotension, seizure activity that prevents study interpretation
- Limit dose (drugs): 1500 mg/kg if
  - Human dose <500 mg day
  - Nongenotoxic
  - >10x free human AUC at maximum human recommended dose
- Maximum feasible dose
  - 5% of diet
  - Maximal gavage or injection volume
  - Local tolerance

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Assessing Major Human Metabolites

- Major human metabolites and genotoxic human metabolites should demonstrate exposures in one of the rodent carcinogenicity studies at levels at least equal to human exposure.
- If this is not possible, additional studies may be required to assess the risk of the human metabolite

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Selecting the Low and Mid Doses

- Demonstrate the range of pharmacokinetic, pharmacologic, toxic, and carcinogenicity effects at different doses—exposure differences between groups is important
- Demonstrate dose levels with no toxicity or treatment-related neoplastic findings (define thresholds)
- Geometric or linear multiples of dose or human exposures often used.
- Low dose is often chosen to approximate clinical exposure.

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U.S. FDA Carcinogenicity Assessment Committee (CAC)

- For drugs, the FDA CAC conducts a Special Protocol Assessment including dose rationale and study design.
- Sponsors must submit results of a range finding study using the same route of administration with the study plan, genetic toxicology data, human dose and exposure projections, protein binding, and human and animal metabolite data.
- No other agency reviews protocols routinely prior to study initiation.
- If the FDA agrees with sponsor or if sponsor accepts the recommendation from FDA, the FDA will consider the dose selection appropriate regardless of study outcome.

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In-Life Execution

- Clinical monitoring
  - Food consumption
  - Body weight
  - Clinical signs
  - Palpation of masses after first six months. Usually weekly, but could be less frequently. Weekly palpations provide better evaluation of tumor onset.
  - Optional for human drugs: clinical pathology, ophthalmology
  - TK—once during the study usually is sufficient.
- Euthanize animals that become moribund. Early sacrifices are better than early deaths.
- Setting euthanasia criteria in advance will speed decision-making

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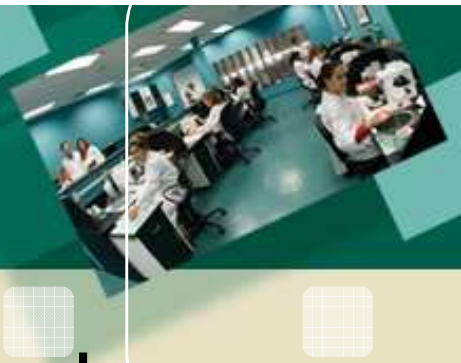
Husbandry Issues



- Equal numbers of animals from each group at each level of the rack.
- Rotate racks in room on regular basis
- Low light levels when staff are not working in the room to reduce retinal degeneration
- Dose controls, low, mid, and high in order to avoid contamination
- Collect TK samples in order of dose group.

Toxicokinetics

- Exposure must be measured within each study.
- Measuring exposure once during study at 6-12 months is sufficient.
- Usually 3-4 time points and 3-4 rats per dose per time point.
- If using main study animals, collect one sample per rat.
- Collect and analyze control samples
- Separate TK report expected

Roles of Pathologists

- OECD Draft Guidance Document on the Design and Conduct of Chronic Toxicity and Carcinogenicity Studies, (Including Histopathological Guidance) Nov 2009 http://www.oecd.org/dataoecd/62/47/44135709.pdf

Roles for Pathologists

- Evaluate clinical pathology and organ weight data (often not collected in 2-year studies)
- Ensure accuracy and consistency of necropsy findings, including correlation with in-life masses
- Examine all tissues in all study animals in all dose groups, generating neoplastic and non-neoplastic microscopic data and correlating necropsy findings to microscopic findings.
- Classify neoplasms in decedents as Fatal or Non-fatal if Peto test is used.
- Identify cause of death if possible
- Prepare the pathology narrative
- Work with the statistician to optimize statistical analyses
- Serve as peer review pathologist

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Clinical Pathology Endpoints

- Clinical pathology generally not performed for pharmaceuticals unless there is a specific program-specific reason (STP, due in 2010).
- End of study clinical pathology is not useful.
- If clinical pathology issues were not satisfactorily addressed in early studies, consider specific testing at $\leq$15 months to address the concern.
- Should blood smears and bone marrow smears be routinely collected? Usually not examined. Use judgment.

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Necropsy

- Early sacrifices and early deaths—full tissue collection. It is better to sacrifice early than to find animals dead.
- Choice of rat model influences longevity and proportion of animals surviving until scheduled sacrifice.
  - Wistar:Han rats have higher survival than SD rats
- Mass tracking—Track each mass described in life, correlate with necropsy findings, and track masses observed at necropsy through microscopic evaluation (required for Peto test).

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Microscopic Evaluation

- Examine all protocol tissues and masses in all animals from all treatment groups.
- Track microscopic diagnoses of all masses observed at necropsy.
- Correlate gross observations to microscopic findings
- Identify primary sites of neoplasms if feasible
- Classify neoplasms as “Fatal” or “Nonfatal”
  - Did neoplasm likely contribute to the animal’s early death or sacrifice?
  - Requires professional judgment
  - Not needed for animals from scheduled sacrifice
  - Required for Peto test
- Record cause of death or moribundity in animals necropsied prior to scheduled sacrifice.

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Pathologists and Statistical Analyses

- The pathologist and the statistician work together to determine which neoplasms and tissues should be combined for statistical analyses.
  - Benign and malignant neoplasms with same or similar cell of origin
  - Multi-site neoplasms—hematologic malignancies, hemangiosarcoma, muscle and bone tumors
  - Should specific hyperplastic findings or altered foci be analyzed on a study-by-study basis?

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Training for Carcinogenicity Studies

- Building experience in carcinogenicity studies in institutions that do not perform these studies is a challenge.
- Peer review offers a learning opportunity if strong oversight and mentoring is provided.
- An inexperienced peer review pathologist without close supervision places the sponsor at risk.
- Pharmaceutical and chemical companies must build this experience or hire it from CROs.

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Early Termination of Dose Groups

- If mortality is high, early humane sacrifice of an entire dose group may be appropriate.
- The FDA wants to be involved in these decisions.
- Observations from FDA correspondence
  - Sacrifice any treated group and sex when survival drops to 15 before Week 100.
  - For some programs, stop dosing affected sex in the high dose group when survival drops to 20, then sacrifice entire affected sex in the high dose when survival reaches 15.
  - Sacrifice all animals of the affected sex in all dose groups if control survival drops to 20.
  - If survival drops to 15 in the high dose group after Week 100, sacrifice all animals in all dose groups of the affected sex.
  - Early necropsy of a single dose group early does not invalidate a study

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Addressing Positive Findings

- Are the incidences of the finding in treated animals within historical control ranges?
- Is the incidence in the concurrent control group lower than the historical control mean? This may explain statistical significance in the absence of treatment effect.
- Is the mechanism for neoplasia relevant for humans?
  - Increase in rodent nephropathy→tubular neoplasia
  - enzyme induction→hepatic or thyroid neoplasia
  - Urothelial neoplasia associated with crystals
  - Hormonal effects, e.g. prolactin increases
  - Injection site sarcomas
  - Rodent-specific pharmacology

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Addressing Positive Findings

- Incidence of leiomyoma in all tissues:
  - 0/60, 1/60, 3/60, 1/60 in females—positive in trend test.
  - No effect in males. No dose response.
- Historical control data: up to 3 in female reproductive tract with mean of 1.4. Up to 4 in all organs.
- Single sex finding within historical control range suggests that the finding may not be related to treatment.

Historical data are fictional and for illustration only

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Selection of Animal Models

- High mortality in SD rats have led to use of more animals per group (up to 70/sex)
- Wistar:Han rats and F344—higher survival
- Each rat has background lesions and is prone to specific neoplasms—historical data and experience are very useful
- Wistar:Han rat is slowly growing more popular and requires only 50/sex/groups (animal welfare advantages)
- Mice—CD-1 and B3C6F1 are commonly used for 2-year studies, but alternative models are gaining ground
- Pathologists usually have a small role to play in animal selection—speak up!

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Thank you

- See STP Best Practice papers at http://www.toxpath.org/positions.asp
- Projects in progress
  - Interpretation hepatic enzyme induction
  - Clinical pathology in carcinogenicity studies
  - Pathology peer review