Validation of Genetically Modified Mouse Models

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Why Use Genetically Modified Mice for Cancer Hazard Identification?

- Animal welfare- reduction in group sizes from 50 to 15
- Time- 6 to 9 months vs. 2 years in life
- Cost- saving estimates range from one third to one half compared to traditional mouse study
- Mechanistic information- models exploit metabolic alterations in pathways involved in oncogenesis

History of Use of Transgenic Models for Cancer Studies at the NIEHS

- Tennant et al.
  - MMTV driven v-Ha-ras, c-myc, c-neu mammary tumor models of Leder (1993)
  - v-Ha-ras Tg.AC (ζ- globin promoter) skin tumor model of Leder (1993 -)
- French et al.
  - p53 +/- knock out mouse of Donehower (1997 -)
- Maronpot et al.
  - Tg rasH2 (c-Ha-ras expression) developed by Katsuki (2000 -)
  - TRAMP (Pb-tag) prostate cancer model
- Rao et al.
  - MMTV driven v-Ha-ras, c-myc, c-neu mammary models (prevention)
- Mahler et al.
  - PIM1 lymphoma model

History of Use of Transgenic Models for Cancer Studies in the National Toxicology Program

- Eastin et al.
  - v-Ha-ras Tg.AC and p53+/- studies of genotoxic and non genotoxic human and rodent carcinogens and non carcinogens Tox Path 26:461-473 (1998)
  - ILSI/HESI ACT collaboration Tg.AC, Tox Path 29 (Suppl.) 2001
- Dunnick et al.
  - p53 +/- (1997 -) phenolphthalein, methylphenidate
    other models- APC, p16/p19 +/-
  - French et al. p53 +/- (1997 -) phenolphthalein molecular analysis
- Spalding et al.
  - p53+/-, and Tg.AC prospective studies on nine bioassay chemicals Tox Sci. 53:213-223, 2000

Review #1

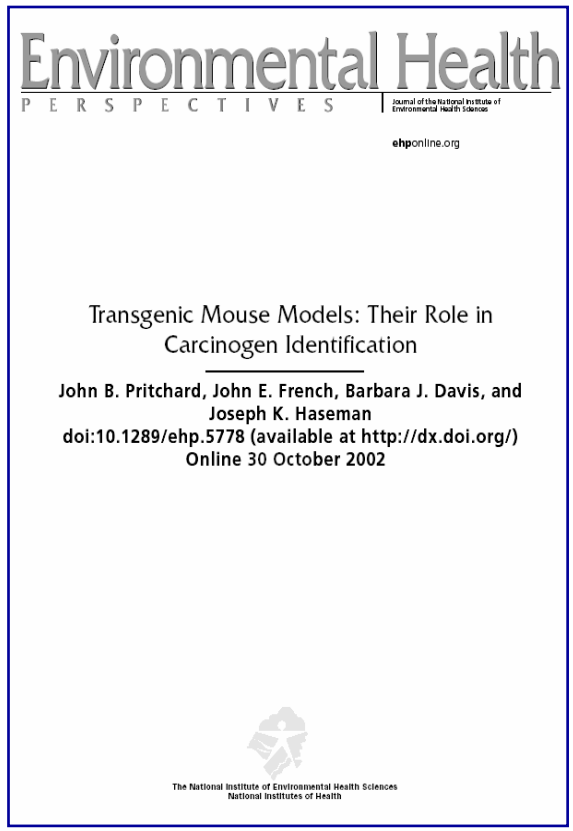
- NTP Board of Scientific Counselors, Feb. 1998
  - Conditional acceptance
    - p53+/- accepted, Tg.AC questioned
    - Noted lack of dose response information
    - Lack of understanding of “misses”
- Urged development of specific tumor site models and continued effort on these and other models for general carcinogen screens

Current NIEHS/NTP Statistics

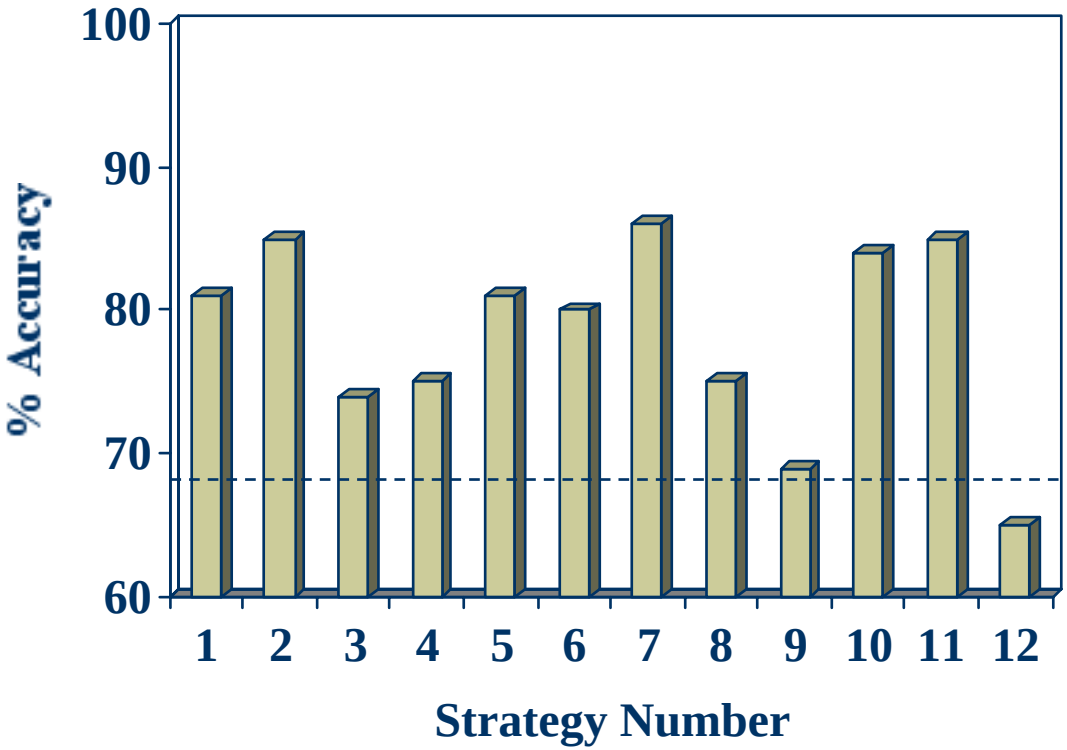
- In house and contracted studies with genetically modified mice -
  - Over 100 studies with various cancer models
  - Tg.AC, p53+/-, p16/p19 +/-, TRAMP, MMTV/neu, PIM1, rasH2, MMTV/ras, MMTV/myc, APC
  - Prevention of site specific cancer- 18 studies, 17 chemicals or mixtures- TRAMP, MMTV/neu
  - Retrospective studies, model development- 55 studies, 30 chemicals-Tg.AC, p53+/-, p16/p19+/-, rasH2, APC, MMTV/ras, MMTV/myc
  - Prospective studies- approx 30 studies, 20 chemicals

Transgenic Mouse Models

- Pritchard et al. evaluation
  - **Concordance of selected model results with IARC and ROC carcinogen lists**
  - **Design and analysis issues**



Transgenic Model Performance



1 = Trp53+/-

2 = Trp53+/- G only

3 = Tg.AC

4 = RasH2

5 = p53-G/Ras-N

6 = p53-G/Ras-All

7 = p53-G/Tg.AC-N

8 = p53-G/TgAC-All

9 = Rodent Bioassay

10 = Rat Bioassay Plus Tg.AC-N or Trp53-G

11 = Rat Bioassay Plus RasH2-N or Trp53-G

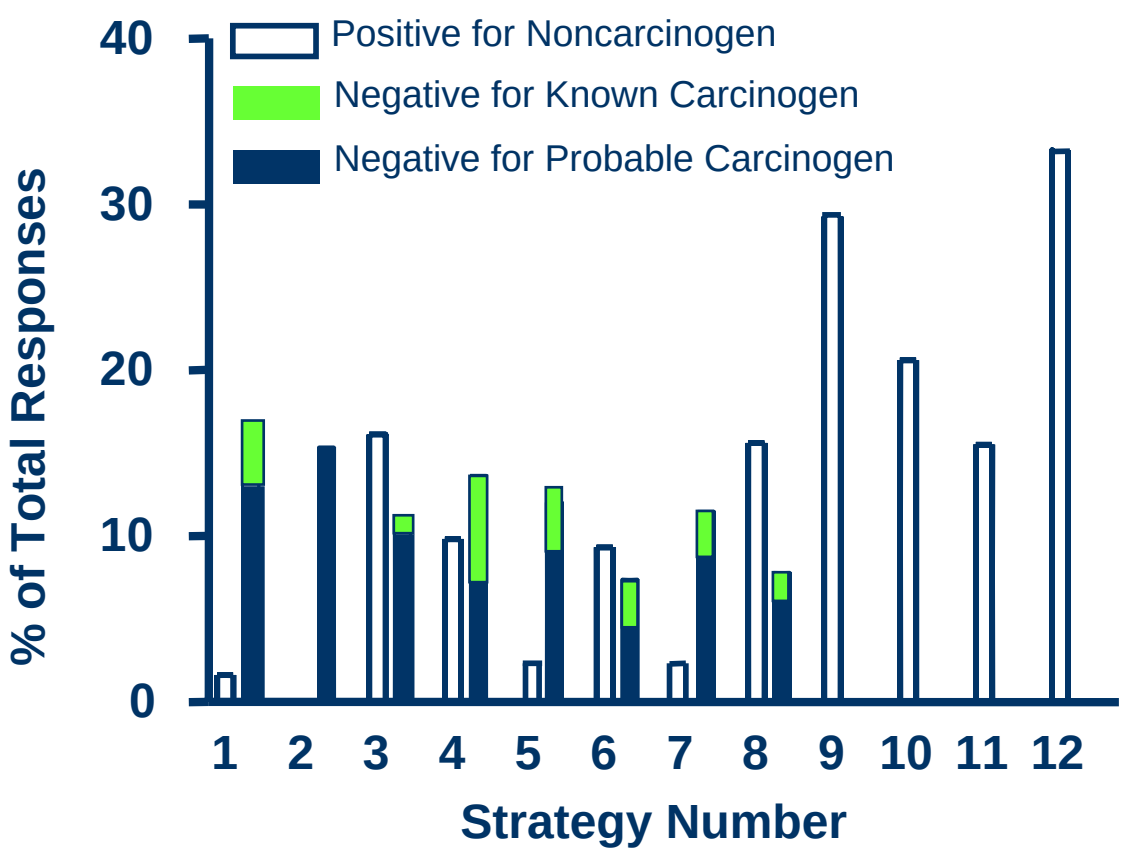
12 = Rat Bioassay Plus Genotoxicity

% Accuracy = Positive findings for IARC/ROC Known/ Suspected Carcinogens plus Negative for IARC/ROC Non-Listings

G = Genotoxic Chemicals

N = Nongenotoxic Chemicals

Is There a Pattern in the Missed Calls?



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9 = NTP Rodent Bioassay

10 = Rat Bioassay Plus Tg.Ac-N or Trp53-G

11 = Rat Bioassay Plus RasH2-N or Trp53-G

12 = Rat Bioassay Plus Genotoxicity

NTP National Toxicology Program

Review #2

- NTP Board of Scientific Counselors Technical Reports Subcommittee, Sept. 2002
  - Review of Tg.AC studies of Pentaerythritol triacrylate and Trimethylolpropane triacrylate
  - In your opinion, is there sufficient scientific evidence using this model to evaluate the potential carcinogenicity of each compound? If not what steps should the NTP take next?
- Subcommittee rejected proposed conclusion of “clear evidence of carcinogenic activity”
- Suggested that more appropriate, model-specific descriptive language be developed

Review #3

- NTP Board of Scientific Counselors- Sept. 2002
- Does the Board have recommendations regarding the issues to consider 1) in choosing a transgenic animal for mechanistic research and 2) in validating its use for screening?

- Under what conditions would the Board feel a positive result in a single or in multiple transgenic models sufficiently reflects a reasonable concern for carcinogenicity in humans? What additional research is needed to “validate” that the conditions suggested by the Board are scientifically sound?

- Under what conditions would the Board feel a negative result in a single or in multiple transgenic models sufficiently reflects little or no concern for carcinogenicity in humans? What additional research is needed to “validate” that the conditions suggested by the Board are scientifically sound?

Review #3 Questions- Sept. 2002 (continued)

- Does the Board have suggestions concerning research the NTP can support to determine if positive findings in transgenic models can be used to predict risk (level of exposure versus probability of carcinogenic response) in human populations?

- To what degree would the Board suggest that we balance further research on the development of transgenic animals for understanding mechanisms with the validation of these animals as part of a carcinogenicity screening program?

Review #4

- NTP Workshop-
  - Genetically Modified Rodent Models for Cancer Hazard Identification:
    Selecting Substances for Study and
    Interpreting and Communicating Results
- February 21, 2003, Washington, DC

Workshop Charge

- Does the scientific/regulatory community consider tumor findings in genetically modified mouse models as equivalent to tumor findings in traditional rodent cancer models? Is the answer the same for all commonly used models (Tg.AC, p53+/-, rasH2)?

- To what degree is the scientific/regulatory community confident that negative results in studies with genetically modified mouse models are equivalent to negative results in the traditional bioassay?

Address these questions after working through 12 "case studies"

P53 +/- mouse on a C57BL/6 background strain

6-month study in females; 15 animals per group. Finding: malignant lymphoma. The chemical is positive in Salmonella and in the acute mouse bone marrow micronucleus test.

	Controls	Low dose	Mid dose	High dose
Tumor incidence	**1/15**	**0/15**	**0/15**	**4/15**
p =0.039, survival adjusted trend test				
Historical control rate is 9/90 (range 0/15 to 3/15)				

Under the conditions of this 6-month oral feed study of ____, there was:

- EE of carcinogenic activity in the male P53 +/- mouse (13)
- EE of neoplastic activity in the male P53+/- mouse (5)
- EE of biological activity in the male P53 +/- mouse (0)
- NE of biological activity in the male P53 +/- mouse (1)

Based on the increased incidence of malignant lymphomas-

Hemizygous Tg.AC: on an FVB/N background strain

6-month dermal application study in females; 15 animals per group. Findings: multiple skin papillomas and several carcinomas. Multiple papillomas occurred in 5 mice in Dose Group 4 and in all mice in Dose Group 5. All of the nonneoplastic lesions were graded as minimal to mild. Chemical is negative in Salmonella and in the acute mouse bone marrow micronucleus test.

Skin	Dose Group					
	Control	1	2	3	4	5
Squamous cell papilloma	0	0	0	1	11**	15**
Squamous cell carcinoma	0	0	1	0	1	1
Inflammation	0	0	0	3	14**	12**
Hyperkeratosis	0	0	1	7**	14**	13**
Epidermal hyperplasia	0	0	1	4*	15**	15**
* p < 0.05, ** p< 0.001, survival adjusted test						

Under the conditions of this 6-month dermal study of ____, there was:

- CE of carcinogenic activity in the female Tg.AC mouse (1)
- CE of neoplastic activity in the female Tg.AC mouse (4)
- CE of biological activity in the female Tg.AC mouse (10)
- CE of tumor promoting activity in the female Tg.AC mouse (4)

Based upon the increased incidence of benign and malignant epithelial neoplasms of the skin-SOA

Question 1

- In the majority of the cases, does the scientific community consider tumor findings in genetically modified mouse models equivalent to tumor findings in traditional mouse cancer models? Is the answer the same for all commonly used models?
  - Case by case
  - Strong responses may be similar
  - Negative responses may not be similar
- Should the NTP continue doing studies in genetically modified animals at all, and if so which models should be used?
  - Yes (P53+/- or rasH2), but only if generally (sometimes??) in place of the B6C3F1 study

Question 2

- To what degree is the scientific/regulatory community confident that negative results in studies with genetically modified mouse models are equivalent to negative results in the traditional bioassay?
- p53+/-
- rasH2
- Tg.AC

Review #5

- NTP Board of Scientific Counselors Technical Reports Subcommittee, May 2003
- New technical report series- GMM reports 1 and 2
  - Review of Tg.AC, p 53+/- and P16/19+/- studies of aspartame and Tg.AC and p53+/- studies of acesulfame K

- Subcommittee accepted proposed conclusions of:
  - **“no evidence of carcinogenic activity” for the p 53+/-**
  - **“no evidence of positive response for papilloma formation in the forestomach or for tumors at other sites in male or female Tg.AC mice administered aspartame/acesulfame K in feed at concentrations up to 50,000 ppm for 9 months”**
  - **“no evidence of enhanced tumor formation in a p16/19 tumor suppressor mouse model; this model is currently uncharacterized in terms of its expected tumor response to known rodent and/or human carcinogens and noncarcinogens”**

GMM Foreward

The studiesÉdesigned and c onducted to characterize the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies.

Validation "Issues"

- Can operational characteristics be determined?
  - **Reproducibility within and across laboratories- protocol similarities?**
  - **Relevance (ability to measure or predict correctly)- sensitivity, specificity?**
  - **Limitations- designed to detect positives- what about negatives?**
- What is the"gold standard"?
  - **Human carcinogens- IARC, RoC- issue of human non-carcinogens**
  - **Rodent carcinogens**
  - **Combination- human carcinogens and "relevant" rodent carcinogens?**
- Is it possible to use "mechanisms" in a validation exercise?
  - **Is it unrealistic to compare a GMM to a wild type mouse?**
  - **Should involvement of a transgene or knockout be verified as part of study interpretation?**

Validation/Evaluation Process

- Should NICEATM be tasked to extend the Pritchard et al. analysis to examine:
  - **Comparability of protocols- impact of modifications**
  - **Consistency in study performance- data analysis**
  - **Criteria for evaluating studies**
  - **Use of GLPs**
  - **Sufficiency of replicate experiments**
    - **“Quantitative statistical analysis of variability is essential”- *Guidelines for submissions to ICCVAM, 1999***
  - **Adequacy of the model for the chemicals studied**
  - **Animal welfare considerations- do GMM studies really reduce animal use, considering animal production requirements and recent protocol modifications?**
  - **Other issues?**

Validation/Evaluation

- Is it appropriate for the ICCVAM to use its evaluation process to review the scientific validity of these transgenic mouse models?
- What are the appropriate reference test systems and/or reference data that should be used to assess the predictiveness of these test systems?
- How might information on mechanism be used in the validation process?

Validation/Evaluation

- Does there need to be refinement of validation questions asked of GMM models? For example, is it reasonable to try to validate a transgenic model against the standard two-year bioassay given that the GMM is expected to miss certain carcinogens? If not, is there a way to evaluate specific GMM models versus two-year bioassay data that takes mechanistic questions into account?

- Based on the information provided, is there sufficient information (i.e. standarized protocols with validation studies to evaluate intra- and inter- laboratory reproducibility and accuracy for a specific proposed use) on GMMs to develop a submission to ICCVAM?

US Regulatory Acceptance

- **FDA Center for Drug Evaluation and Research**
  - **As of Feb. 2003, data from 24 alternative protocols accepted and evaluated**
  - **P53 considered appropriate for clearly or equivocally genotoxic drugs (primarily in vitro clastogenicity)- 16/16 negative**
  - **Tg.AC used for dermally applied drug products- 4/5 positive**
  - **Tg.rasH2 considered appropriate for either genotoxic or non genotoxic drugs- insufficient experience to judge response**