

**【S-8】**

**Spontaneous Neoplastic Lesions and Test Article Induced  
Neoplastic Lesions in P53 Mice and rasH2 Mice**

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Several strains of genetically engineered mice are currently being used as alternatives to long term carcinogenesis studies for submission to regulatory agencies. The most commonly used mice include the p53 heterozygous knockout mouse, the Tg.AC transgenic mouse and the rasH2 transgenic mouse. Each of these models has specific genetic modifications which make them attractive for the study of carcinogenicity of novel xenobiotics.

The background and utility of each model will be discussed. Common spontaneous and induced lesions will be covered in detail. Finally, the acceptance of these models by regulatory agencies in several countries will be addressed.

### Genetically Engineered Mice in Toxicology

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### Major Players in Genetically Engineered Mice, Toxicology Division

- Tg.AC
- p53 +/-
- rash2

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### Principal Hypothesis

- Transgenic mice with specific genetic alterations critical to tumorigenesis but which are insufficient to induce neoplasms in a specified time are candidates for rapid cancer bioassays.
- Exposure to transpecies carcinogens will result in rapid induction of tumors.

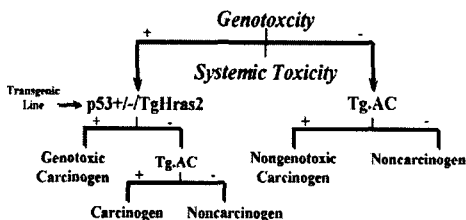
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### Protocols

- Typical protocol calls for a 6-month exposure
- All protocols are based primarily on reduced latency to tumor development
- Spontaneous/background tumors very rare prior to 12 months

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### Use of Transgenic Models in Drug and Chemical Safety Evaluation



Decision tree for use of transgenic mouse lines to complement conventional rodent bioassays (Adapted from Tennant et al, 1995)

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### *Tg.AC Transgenic Mouse*

- Mutated Harvey-ras oncogene
- Multiple copies on chromosome 11
- Expression of transgene is inducible
- Expresses in skin - genetically initiated
- Induction of papillomas - reporter phenotype
- Detects both mutagenic and non-mutagenic agents

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### *Tumors in Tg.AC Transgenic Mice Spontaneous Tumors*

- Odontogenic Tumor
- Forestomach Papilloma
- Skin Papilloma
- Skin Carcinoma
- Lung A/B Adenoma
- Lung A/B Carcinoma
- Salivary Gland Duct Carcinoma
- Erythroleukemia
- Ovary Teratoma
- Malignant Lymphoma
- Endometrial Adenoma
- Uterine Histiocytic Sarcoma
- Intestinal Carcinoma
- Subcutis Mast Cell Tumor

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### *Tumors in Tg.AC Transgenic Mice Induced Tumors*

- Skin SOA - Papilloma/Keratoacanthoma
- Skin SOA - Squamous Cell Carcinoma
- Forestomach - Squamous Cell Papilloma
- Lung - Adenoma/Carcinoma
- Systemic - Malignant Lymphoma
- Systemic - Myelodysplasia

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### *Tumors in Tg.AC Transgenic Mice Spontaneous Tumor Incidence*

	Males	Females
Odontogenic Tumors	13%	17%
Forestomach Papilloma	7%	10%
Skin Papilloma	4%	4%
Skin Carcinoma	-	0.5%

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### *Tumors in Tg.AC Transgenic Mice Spontaneous Tumor Incidence*

	Males	Females
Lung A/B Adenoma	4%	2%
Salivary Gland Duct Carcinoma	1%	2%
Erythroleukemia	1%	2%
Malignant Lymphoma	-	1%

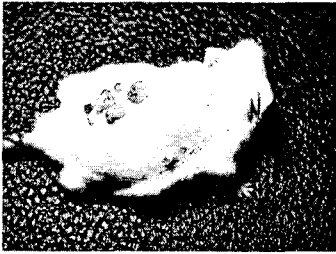
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### *Tumors in Tg.AC Transgenic Mice*

#### *Skin Tumors*

- PAPILOMA
- KERATOACANTHOMA
- SQUAMOUS CELL CARCINOMA

*TG.AC, Skin: Papillomas*



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*TG.AC, Skin: Multiple Papillomas*



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*TG.AC, Skin: Papilloma*



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*Tumors in Tg.AC Transgenic Mice*

*Skin Tumors*

- PAPILOMA
- KERATOACANTHOMA
- SQUAMOUS CELL CARCINOMA

*TG.AC, Skin: Keratoacanthoma*



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*Tumors in Tg.AC Transgenic Mice*

*Skin Tumors*

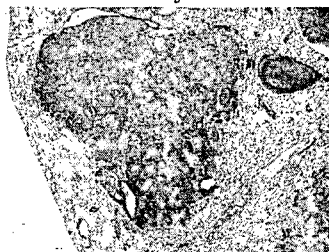
- PAPILOMA
- KERATOACANTHOMA
- SQUAMOUS CELL CARCINOMA

*TG.AC, Skin: Squamous Cell Carcinoma*



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*TG.AC, Lung: Metastatic Squamous Cell Carcinoma from Skin*



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*Tumors in Tg.AC Transgenic Mice*

- PAPILOMA, VULVA

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*TG.AC, Vulva: Papilloma*

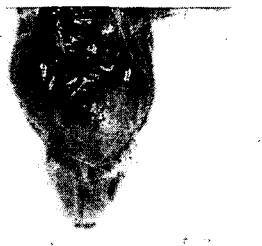


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*Tumors in Tg.AC Transgenic Mice*

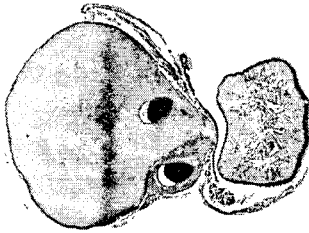
- ODONTOGENIC TUMORS

*TG.AC: Odontogenic Tumor*



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*TG.AC, Tooth: Odontogenic Tumor*



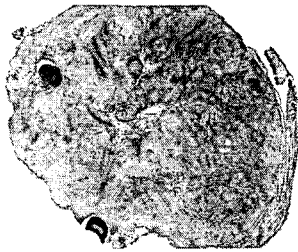
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*TG.AC, Tooth: Odontogenic Tumor*



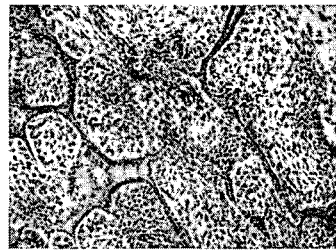
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*TG.AC Tooth: Adamantinoma*



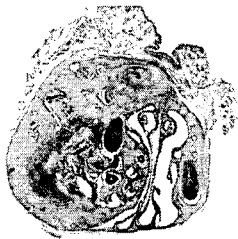
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*TG.AC Tooth: Adamantinoma*



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*TG.AC, Tooth: Odontogenic Tumor*



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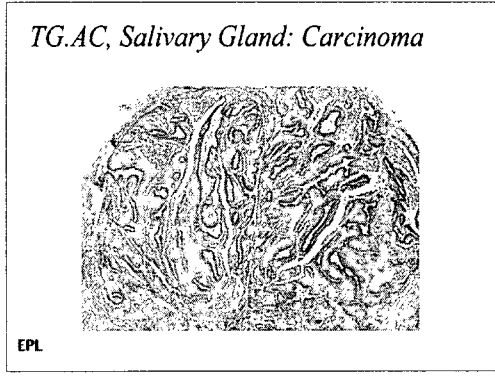
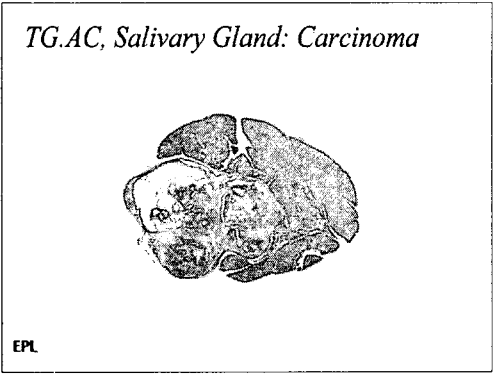
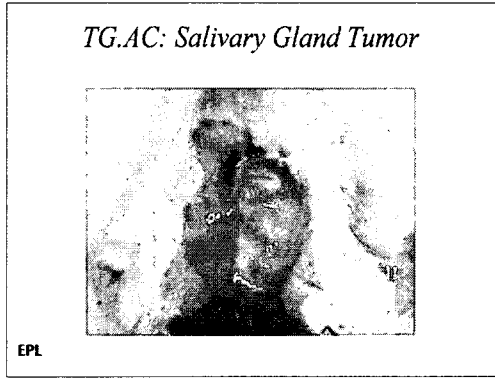
*TG.AC, Tooth: Odontogenic Tumor*



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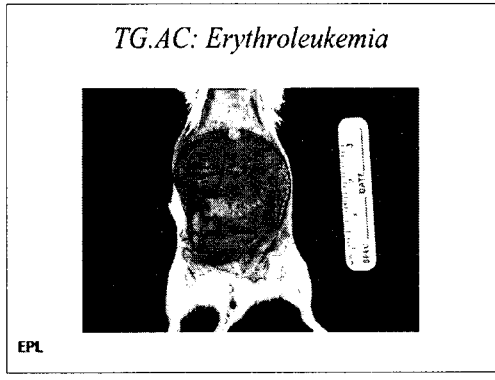
*Tumors in Tg.AC Transgenic Mice*

- SALIVARY GLAND DUCT CARCINOMA

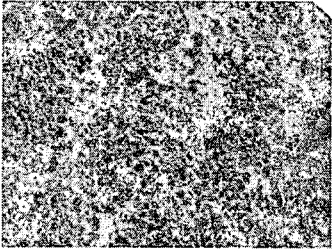


*Tumors in Tg.AC Transgenic Mice*

- ERYTHROLEUKEMIA

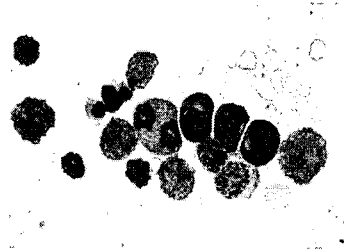


*TG.AC, Liver: Erythroleukemia*



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*TG.AC, Blood: Erythroleukemia*



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*Tumors in Tg.AC Transgenic Mice*

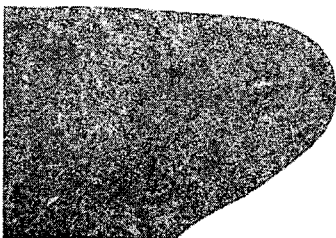
· MALIGNANT LYMPHOMA

*TG.AC, Thymus: Malignant Lymphoma*



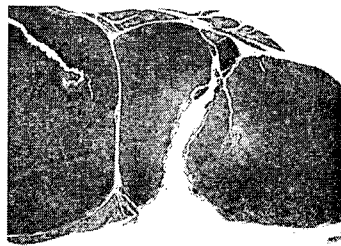
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*TG.AC, Spleen: Lymphoma*



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*TG.AC, Lymph Node: Lymphoma*



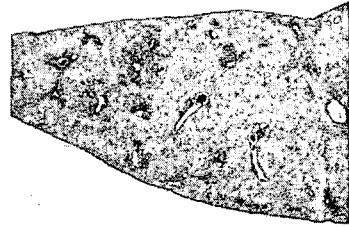
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*Tumors in Tg.AC Transgenic Mice*

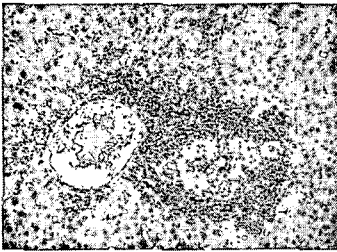
• MYELOYDYSPLASIA

*TG.AC, Liver: Myelodysplasia*



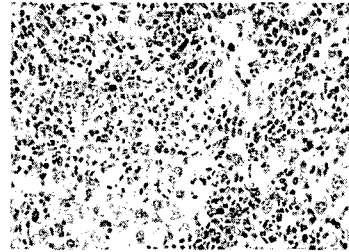
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*TG.AC, Liver: Myelodysplasia*



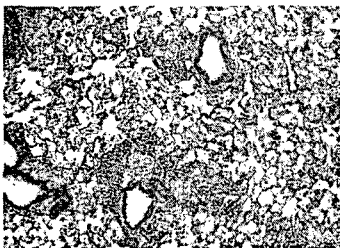
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*TG.AC, Liver: Myelodysplasia*



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*TG.AC, Lung: Myelodysplasia*

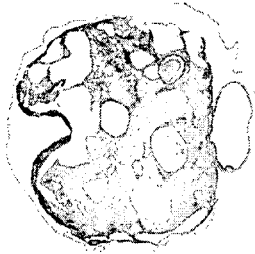


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*Tumors in Tg.AC Transgenic Mice*

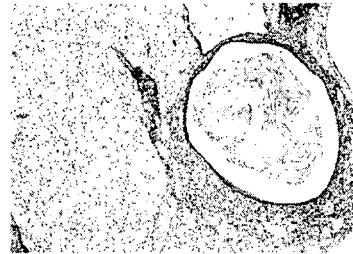
• TERATOMA

*TG.AC, Ovary: Teratoma*



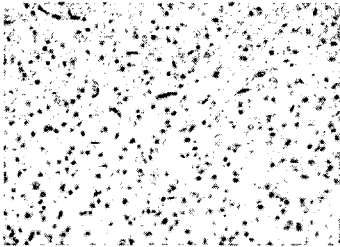
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*TG.AC, Ovary: Teratoma*



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*TG.AC, Ovary: Teratoma*



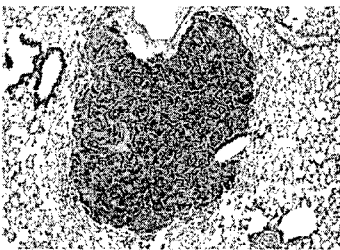
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*Tumors in Tg.AC Transgenic Mice*

- ALVEOLAR/BRONCHIOLAR ADENOMA

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*TG.AC, Lung: A/B Adenoma*

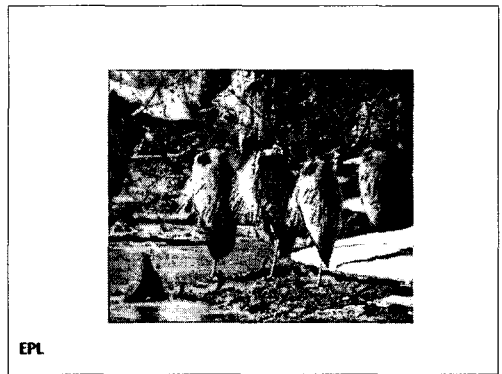
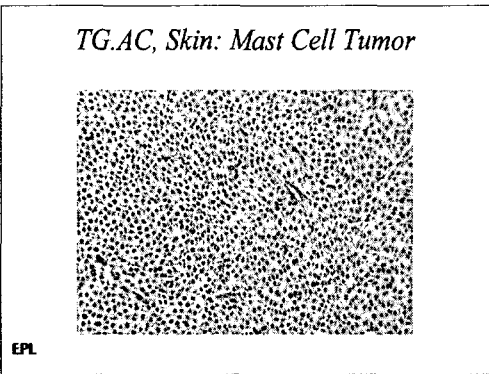
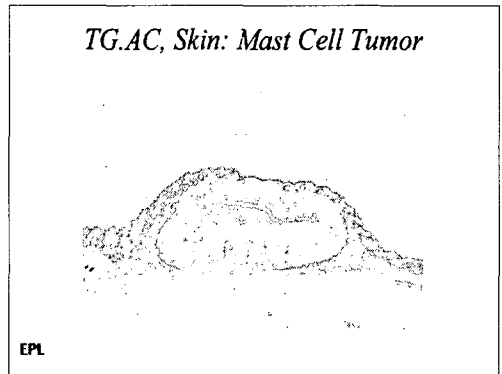
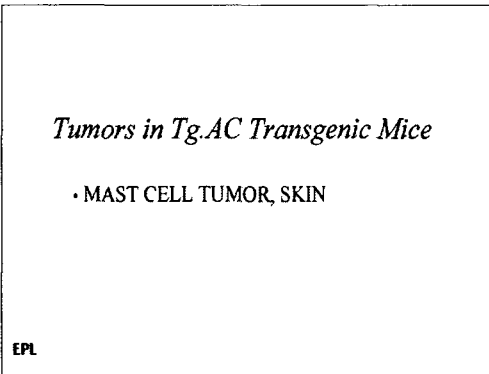
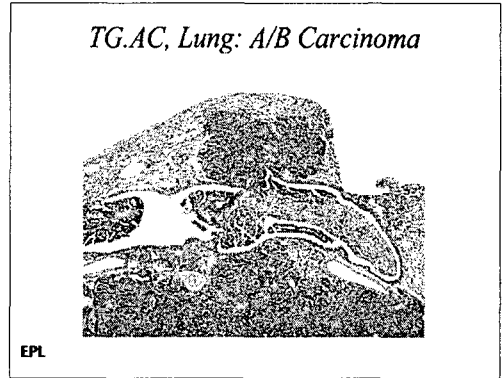
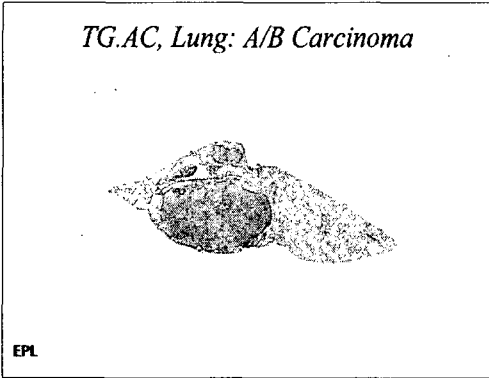


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*Tumors in Tg.AC Transgenic Mice*

- ALVEOLAR/BRONCHIOLAR CARCINOMA

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### *rasH2 Transgenic Mouse*

- Has normal coding region of human c-Ha-ras gene, Mutation in last intron
- Over-expression of gene in tumors and normal tissues
- CB6F1
- Few lesions at 6 months of age

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### *rasH2 Transgenic Mouse - Advantages*

- Carries a human oncogene
- More rapid onset of tumors
- Higher incidence of tumors
- Target tissues correspond to bioassays

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### *Tumors in rasH2 Transgenic Mice Spontaneous Tumors*

- Forestomach
  - Papilloma
  - Squamous Cell Carcinoma
- Lung
  - A/B Adenoma
  - A/B Carcinoma
- Hematopoietic System
  - Thymus, Lymphoma
  - Spleen, Hemangiosarcoma
- Uterus,
  - Hemangiosarcoma
- Integumentary System
  - Skin, Papilloma
  - Subcutis
    - Hemangiosarcoma
    - Rhabdomyosarcoma

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### *Tumors in rasH2 Transgenic Mice Induced Tumors*

- Lung Tumors
- Forestomach Epithelial Tumors
- Skin Epithelial Tumors
- Splenic Hemangiosarcomas
- Leukemias
- Urinary Bladder Tumors

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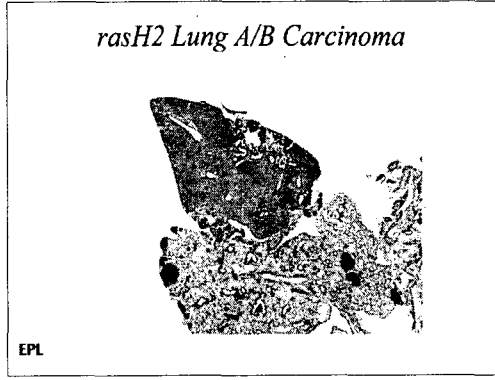
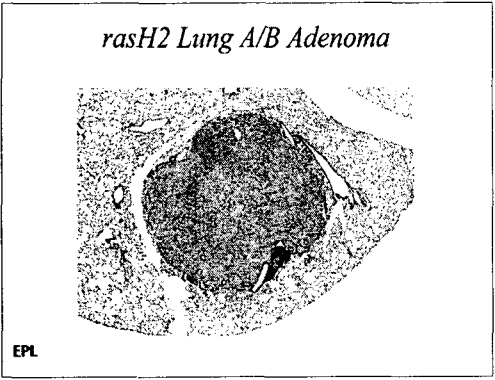
### *Tumors in rasH2 Transgenic Mice Spontaneous Tumor Incidence*

	Males	Females
Forestomach Papilloma	3%	1%
Lung - A/B Adenoma	7%	5%
Spleen - Hemangiosarcoma	4%	8%

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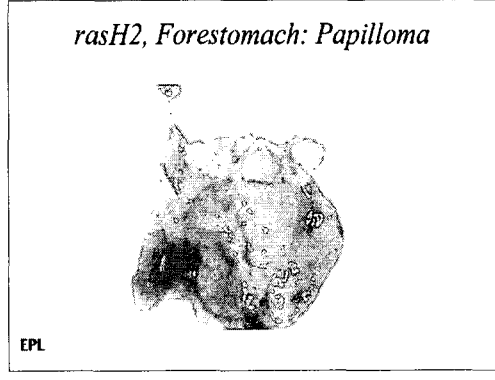
### *Tumors in rasH2 Transgenic Mice*

- LUNG TUMORS



*Tumors in rasH2 Transgenic Mice*

• FORESTOMACH PAPILOMA

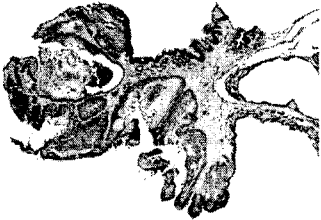


*Tumors in rasH2 Transgenic Mice*

• VULVAR PAPILOMA

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*rasH2 Vulva: Papilloma*

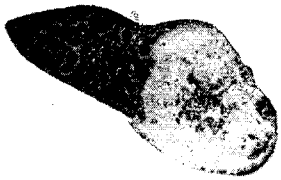


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*Tumors in rasH2 Transgenic Mice*

- SPLENIC HEMANGIOSARCOMA

*rasH2 Splenic Hemangiosarcoma*

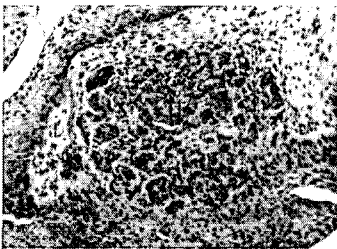


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*Tumors in rasH2 Transgenic Mice*

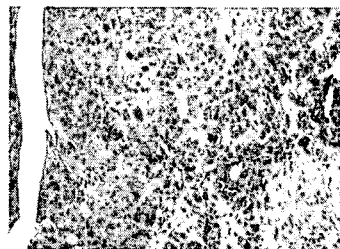
- URINARY BLADDER TUMORS

*Transitional Cell Carcinoma*



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*Transitional Cell Carcinoma*



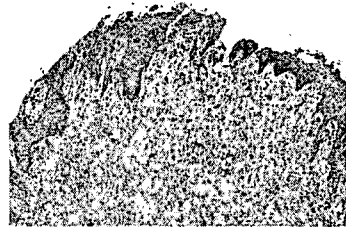
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*Tumors in rasH2 Transgenic Mice*

- SUBMUCOSAL MESENCHYMAL PROLIFERATIVE LESION OF THE URINARY BLADDER OF AGED MICE

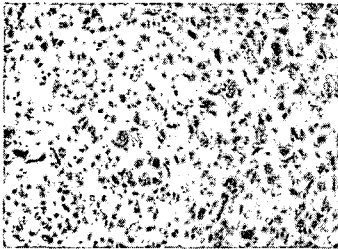
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*SUBMUCOSAL MESENCHYMAL PROLIFERATIVE LESION OF THE URINARY BLADDER OF AGED MICE*



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*SUBMUCOSAL MESENCHYMAL PROLIFERATIVE LESION OF THE URINARY BLADDER OF AGED MICE*



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*p53 +/- Knockout Mouse*

- C57BL/6
- Single wild-type p53 allele
- Inactivation of single allele results in normal phenotype
- Low sporadic tumor incidence up to one year

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*Tumors in p53 +/- Knockout Mice*  
*Spontaneous Tumors*

- Skin/subcutis Sarcoma
- Malignant Lymphoma
- Osteosarcoma
- Granulocytic Leukemia
- Meningeal Sarcoma
- Lung Adenoma
- Urinary Bladder Tumors

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*Tumors in p53+/- Knockout Mice  
Induced Tumors*

- Soft Tissues - Sarcoma
- Systemic - Malignant Lymphoma

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*Tumors in p53+/- Knockout Mice  
Spontaneous Tumor Incidence*

	Males	Females
Skin/Subcutis Sarcoma	2%	4%
Malignant Lymphoma	2%	2%
Osteosarcoma	2%	-
Lung - A/B Adenoma	-	1%

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*Tumors in p53+/- Knockout Mice*

- SUBCUTANEOUS SARCOMA  
COMPLEX

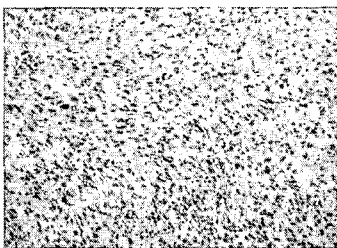
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*p53+/-, Subcutis: Sarcoma*



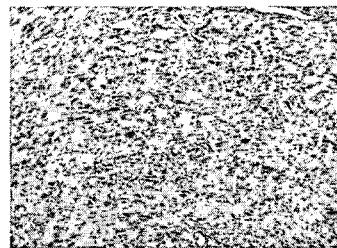
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*p53+/-, Subcutis: Sarcoma*



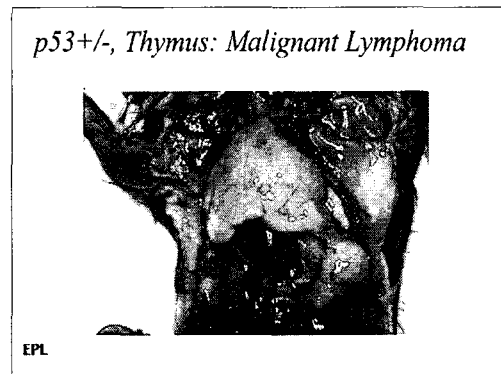
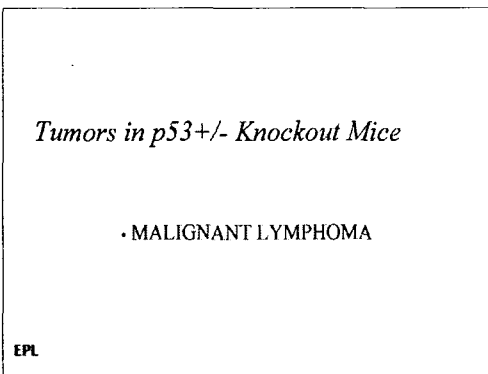
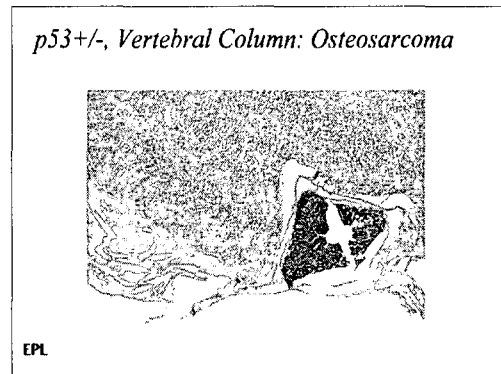
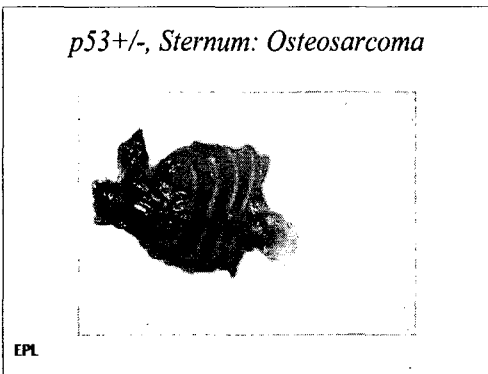
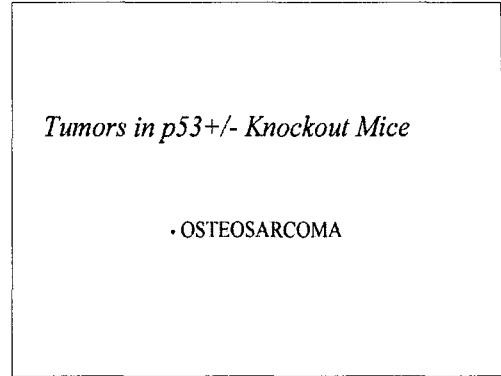
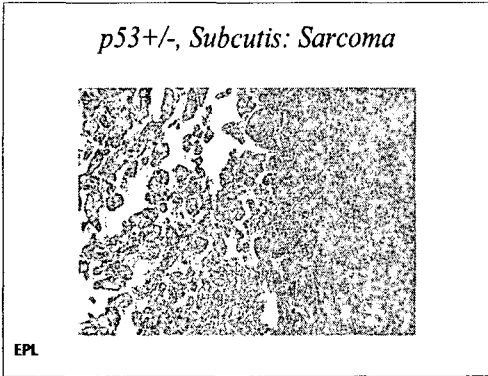
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*p53+/-, Subcutis: Sarcoma*

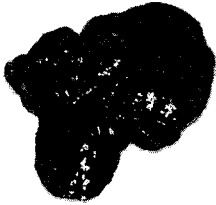


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*Pp53+/-, Liver: Malignant Lymphoma*



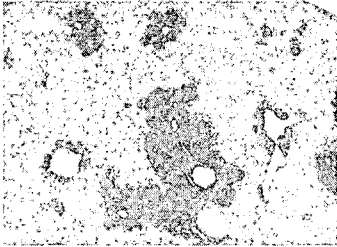
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*p53+/-, Liver, Spleen: Malignant Lymphoma*



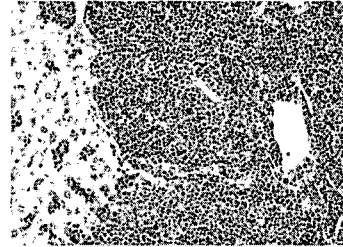
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*p53+/-, Liver: Malignant Lymphoma*



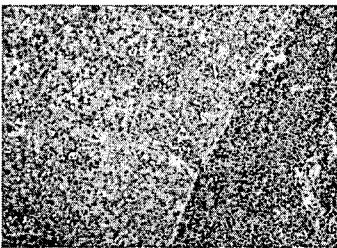
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*p53+/-, Liver: Malignant Lymphoma*



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*P53+/-, Thymus: Malignant Lymphoma*

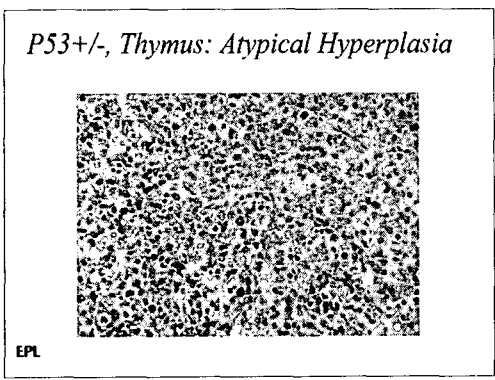
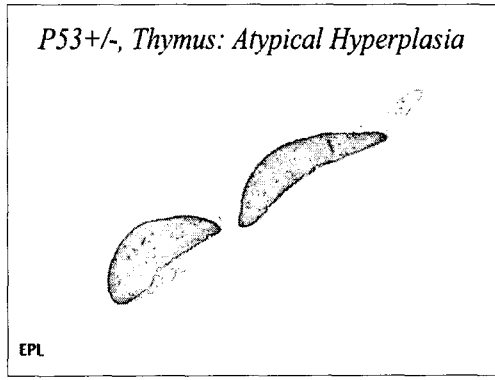
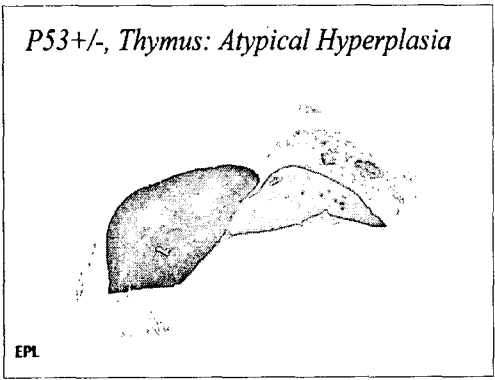
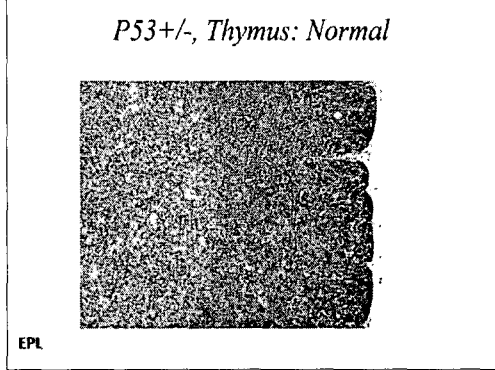
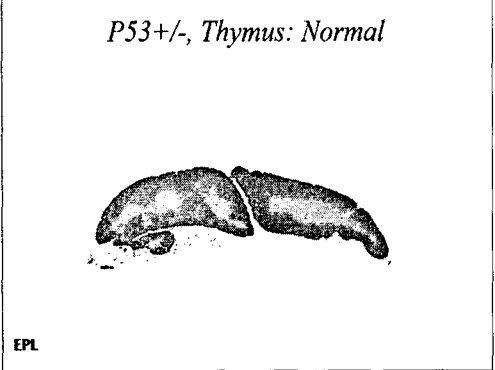


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*Tumors in p53+/- Knockout Mice*

• ATYPICAL HYPERPLASIA OF THE THYMUS

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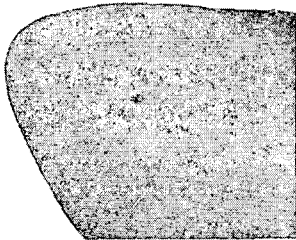


*Tumors in p53+/- Knockout Mice*

- GRANULOCYTIC LEUKEMIA

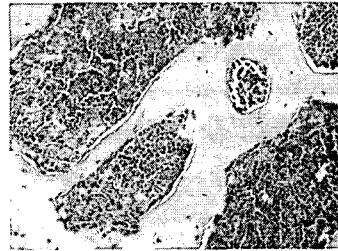
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*P53+/-, Spleen: Granulocytic Leukemia*



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*P53+/-, Bone Marrow: Granulocytic Leukemia*



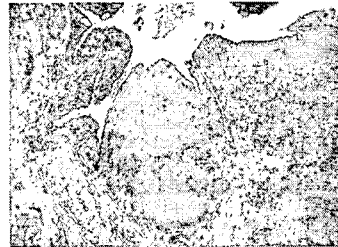
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*Tumors in p53+/- Knockout Mice*

- Urinary Bladder Tumors

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*p53+/-, Urinary Bladder: Transitional Cell Carcinoma*



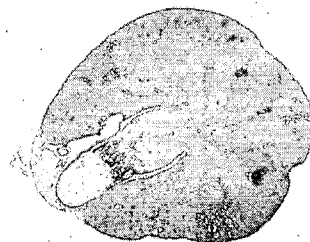
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*Renal Papillary Necrosis in p53+/- Knockout Mice*

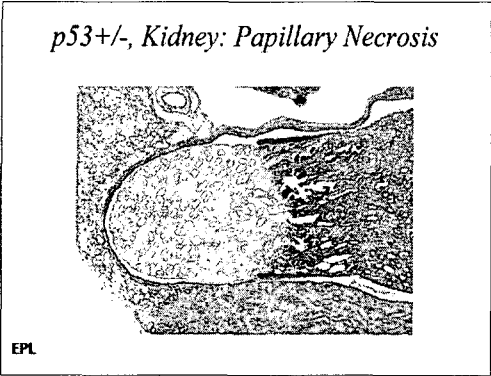
- ASSOCIATED WITH p-CRESIDINE
- VARIABLE INCIDENCE

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*p53+/-, Kidney: Papillary Necrosis*



EPL



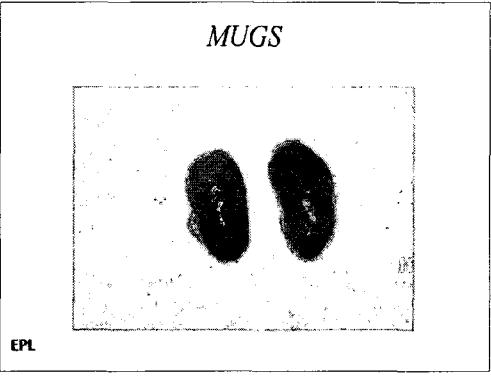
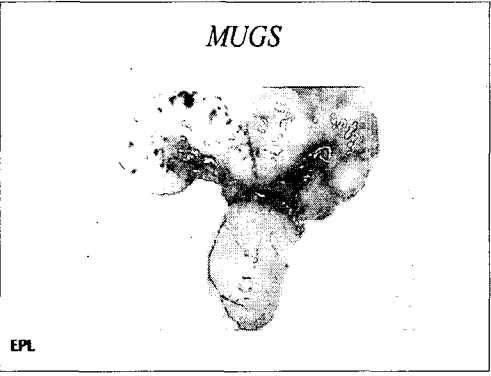
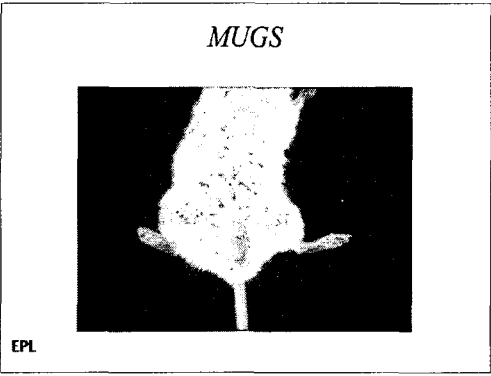
*Mouse Genital Urinary Syndrome*

- Etiology Unknown
- Reported in transgenic and nontransgenic strains
- Tg.AC
- p53 +/-

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*Mouse Genital Urinary Syndrome*

EPL





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## *Transgenic Mice at the Regulatory Agencies*

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### *FDA*

- Congressional Mandate
  - Faster Review
  - Less Regulatory Burden
- ICH Guidance
  - Approved in vivo alternative to 2nd rodent study

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### *FDA*

- Transgenic Model may be chosen as:
  - Alternative to a 2nd 2 year rodent study
  - Complimentary or confirmatory for equivocal findings
    - To set priorities
  - As an option to repeating a 2 year study
    - Inadequate 2 year study
    - Change in clinical indication or human exposure
  - To support differing completion of a 2 year study
- To assess carcinogenic potential of contaminants

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### *EPA*

- Different Levels of information needed
- Many pollutants to consider
- Emphasis on mechanistic data in new laws
- Alternatives considered
  - Transgenics
  - Medaka
  - Neonatal Mouse Model

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### *EPA*

- Does EPA accept the use of alternatives now?
  - Yes - As part of weight of evidence
  - Yes - To help understand mode of action
  - No - In lieu of tumor findings in a 2 year Bioassay for FIFRA or TOSCA

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### *Japan*

- Pharmaceuticals - either 2 rodent carcinogenicity studies or 1 rodent and 1 short term alternative test
  - Alternatives
    - Transgenic Model
    - Initiation-Promotion
    - Neonatal Mouse
- Pesticides - 2 rodent carcinogenicity tests

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### *Issues*

- What type of carcinogens do we wish to identify? or What type of carcinogens is it OK not to identify
- What is the gold standard?
- How will the results be used?
- What is the likely outcome of contemporary validation trials using genetically altered mice?
- What opportunities are afforded by the efforts?

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### *What type of carcinogens do we wish to identify? or What type of carcinogens is it OK not to identify?*

- Trans-species, multi-site genotoxic carcinogens
- Six-month carcinogens
- High-dose carcinogens
- Tissue-specific carcinogens
- Carcinogens that are mechanistically relevant to humans

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### *What is the gold standard?*

- Conventional two-year rodent bioassay
- Human carcinogens
- Organotrophism

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### *How Will Results Be Used?*

- As substitute for conventional 2-year bioassay
- As adjunct in weight of evidence
- To define dose response
- To screen chemicals prior to conventional testing
- To help define mechanisms

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### *What is the likely outcome of contemporary validation trials using genetically altered mice?*

- Déjà vu

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*What opportunities are afforded  
by the efforts?*

- A chance for progress in improving how potential human carcinogens are identified
- Stage set for not being limited to 4 cell conventional testing
- Strengthen the scientific base for risk assessment

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