Overview of Transgenic Mouse Models

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Hypothesis

- Disease = Genome *Environment *Time
- An inducible protooncogene or inactivated tumor suppressor gene by itself does not cause cancer
- Exposure to a carcinogen will induce cancer with reduced latency due to other induced genetic alterations

Criteria

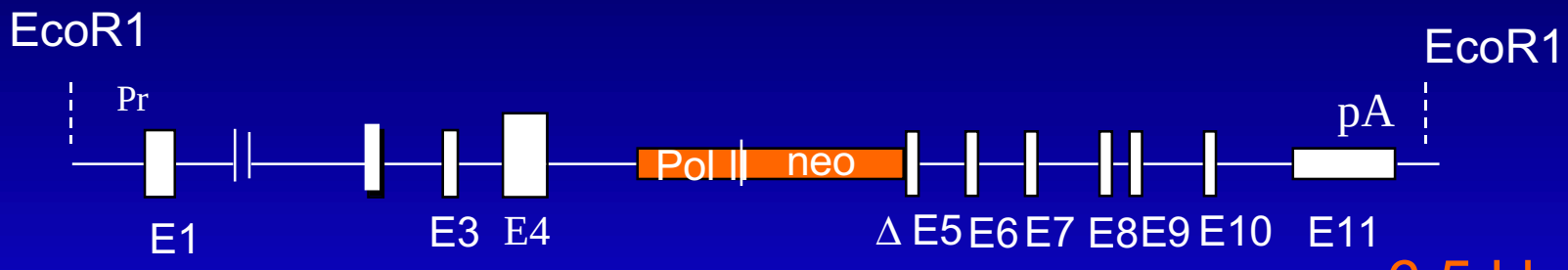
- Broad range of susceptible tissues
- Zero to low incidence of sporadic tumors
- Zero to low frequency of false negatives and false positives (i.e. accurate)
- Mode or mechanism consistent with development of human cancer

Trp53 Haploinsufficient Mouse

B6.129(N5)-*Trp53*^{tm1Brd} (N5)

Heterozygous p53 deficient Mice

(Donehower et al., *Nature* 356:212, 1992)

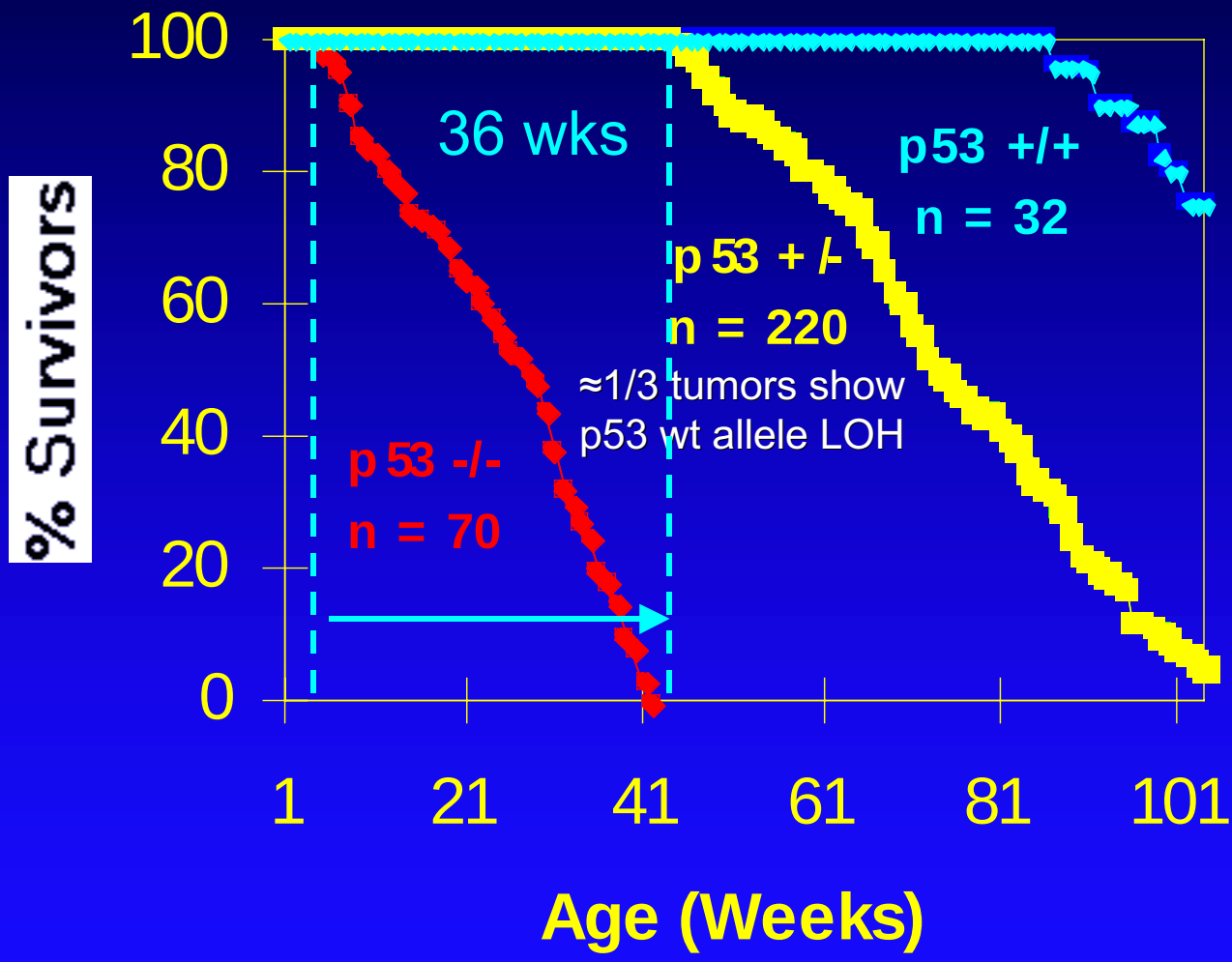


Null (paternal C11)

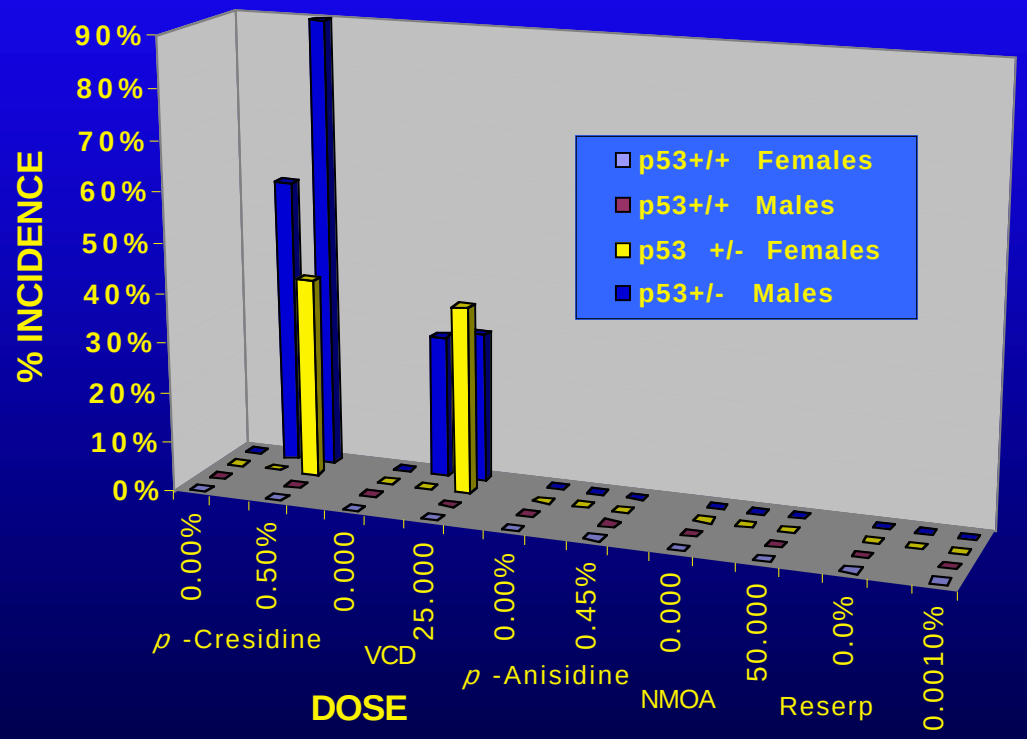
16 kb

Wild-type (maternal C11)

B6.129- *Trp* 53 (N5) Survival

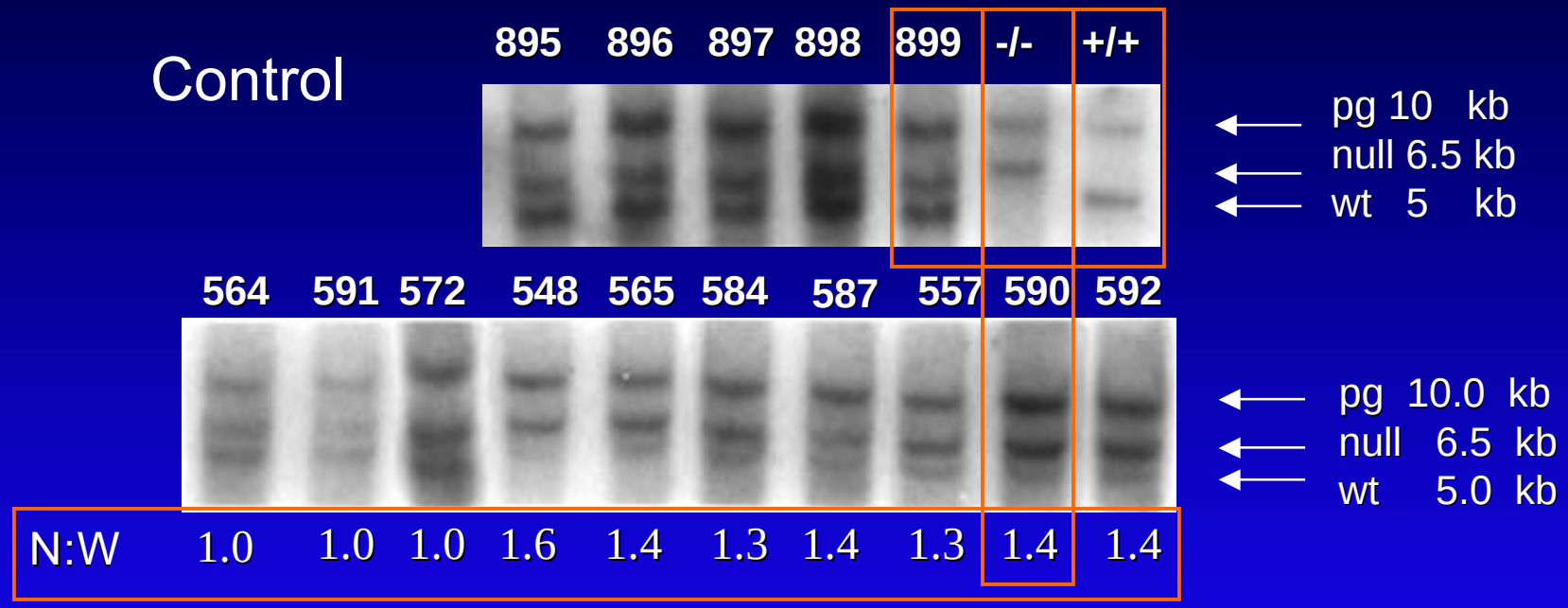


Mutagenic carcinogens rapidly induce tumors



Tennant,RW, French, JE, Spalding, JW. Env Health Perspectives 103:942-50, 1995

LOH at the p53 locus



Benzene induced lymphomas and sarcomas

French et al. Carcinogenesis 22:98-106 (2001)

Loss of the wildtype p53 allele in short term cancer studies[a] using haploinsufficient B6.129-*Trp*53 (N4-N5) mice. LOH = > 2 SD change in null/wt signal.

			F344/N		$B6C3F_1$		het p53 def		
Chemical	Mut[b]	Route	Sex	Target	Sex	Target	Sex	Result	LOH
Benzene	-; +	G, I	M,F	mul; mul	M,F	mul ;mul	M	sarc; thy lym	13/16
p-Cresidine	+;-	F,G	M,F	bl, liv; bl	M,F	bl;bl, liv	M,F	ub, liv; ub	3/19
VCD[c]	+;nt	SP	M,F	sk; sk	M,F	sk, ov	M,F	sk;sk,ov	12/24
Phenolpthal[d]	-;+	F	M,F	ad, kid; ad	M,F	thy, sar	F	thy lym	21/21
Mephalan	?	IP					M,F	thy lym	14/16
Foreign[e]							M,F	sarc	12/16
DMN[f]	+;+	G					M,F	sarc	17/22
DMBA/TPA	+;+	D			M,F	Sk	M,F	scc	10/16
Radiation (^{137}Cs)	?;+	WB					M,F	thy lym	17/20
BBN[h]	+;?	G					M,F	ub	3/20

[a] Replication of the NCI/NTP 2 year
[b] Salmonella; in vivo micronucleus assay
[c] 4-Vinyl-1-cyclohexene diepoxide
[d] Phenolphthalein
[e] transponder microchip
[f] Dimethylnitrosamine
[g] linear energy transfer (cobalt 60)
[h] N-butyl-N-(4- hydroxybutyl)nitrosamine

French et al. Carcinogenesis 22:98-106 , 2001;Venkatachalam et al. Toxicol Pathol, 29 (suppl) 147-154, 2001

Conclusions

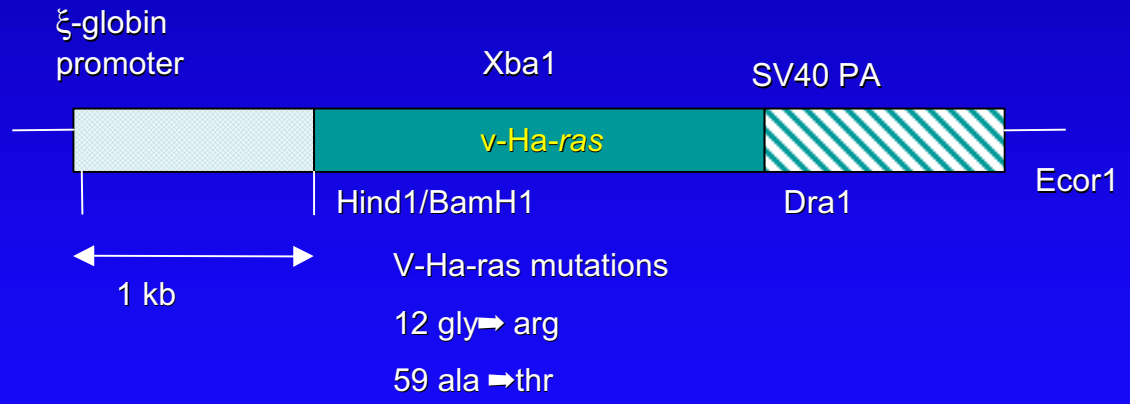
- p53 haploinsufficient mice are susceptible to mutagenic carcinogens
- LOH includes both p53 locus specific and chromosome 11 (genomic) loss

Tg.AC Mouse

FVB/N-*v-Ha-ras*^{tgLep}

v-Ha-*ras* transgene

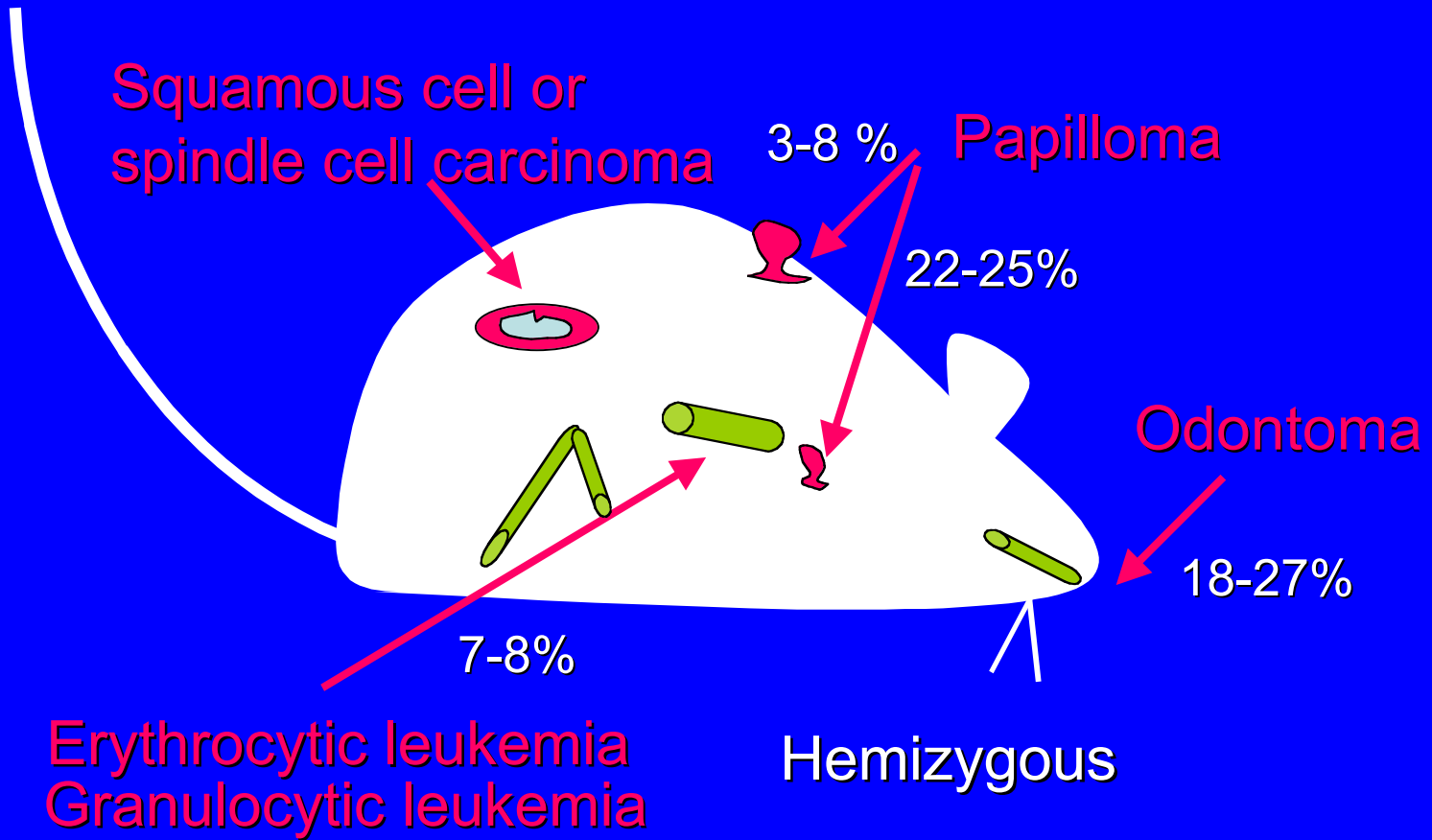
- Tripartite construction
- Ectopic (integration site) expression
- Induced and/or clonally expanded
- Reporter phenotype



Transgene Expression

- Insertion site (transcriptional domain)
- Promoter/Enhancer
- Palindromic structure
- Hypomethylation
- *v-Ha-ras* expression phenotype

Spontaneous vs. Induced Tumors

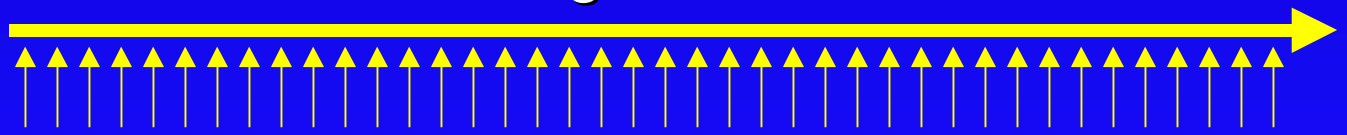


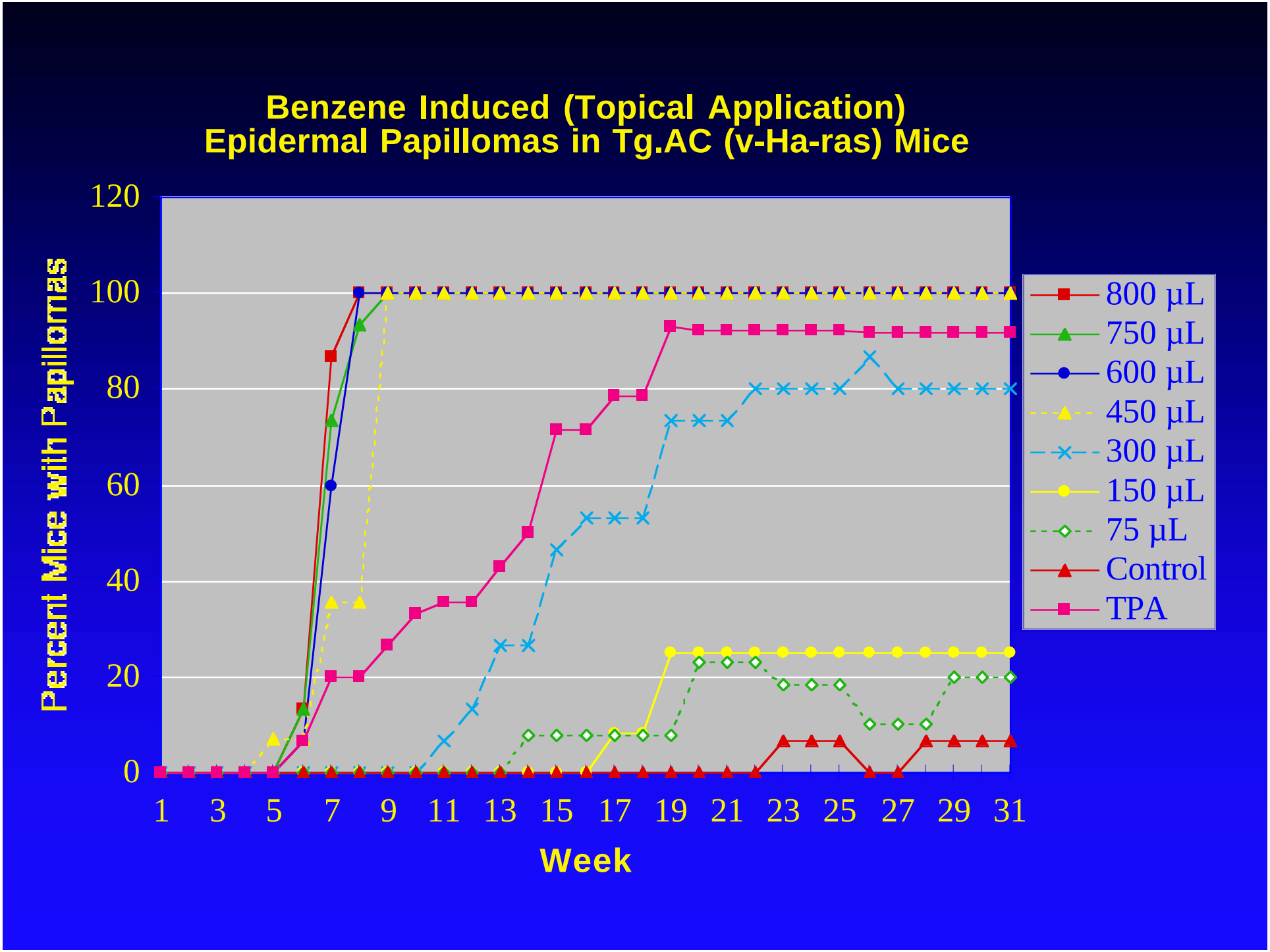
Skin carcinogenesis

- Initiation-Promotion-Progression Model

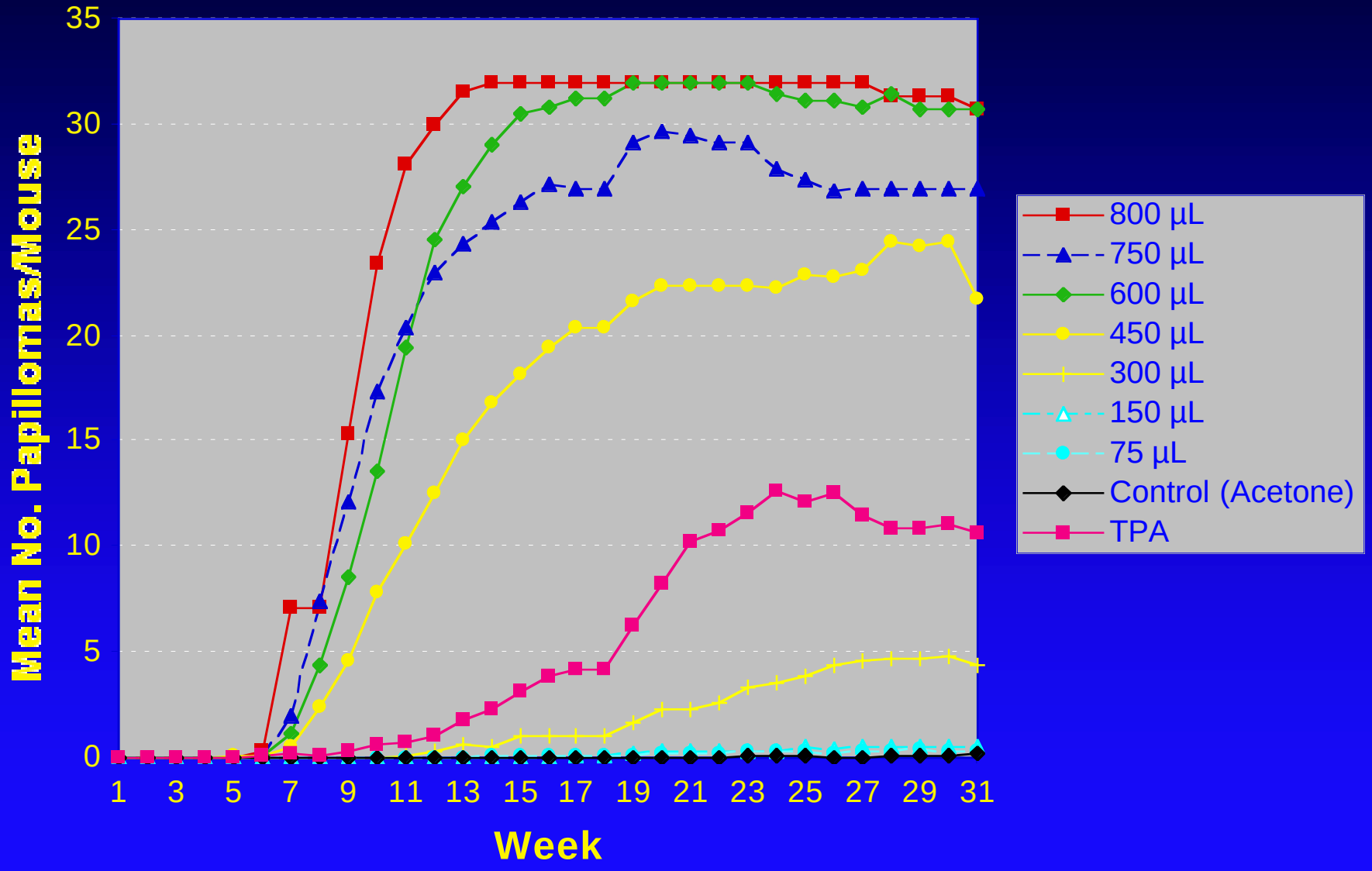
Mutagen	Test Agent/Positive	Promoter Control
Mutant *C-Ha-ras*	Clonal expansion-Papillogenesis; Trisomy-7	Acquired genetic Alterations (e.g. p16/p53); malignant conversion

- Dermal Carcinogenesis Model (26 wk vs. 104 wk)

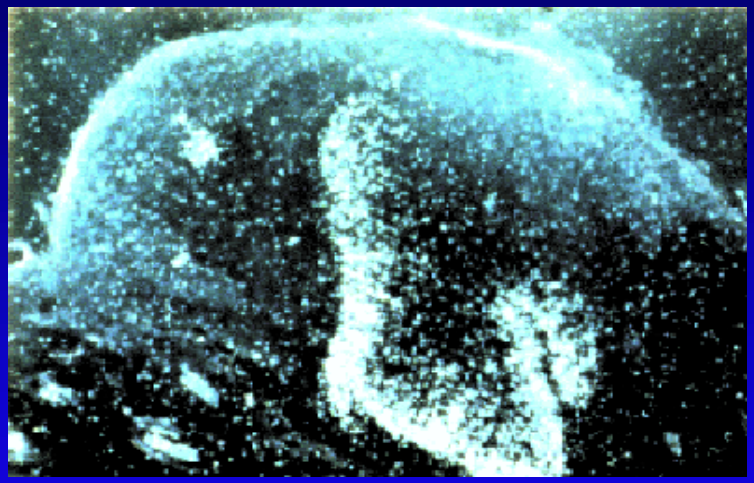



Benzene Induced (Topical Application)
Epidermal Papillomas in Tg.AC (v-Ha-ras) Mice
Percent Mice with Papillomas
120
100
80
60
40
20
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
Week
800 µL
750 µL
600 µL
450 µL
300 µL
150 µL
75 µL
Control
TPA

Benzene Induced Dose Response Curve for Epidermal Papillomas/Mouse



Transgene expression (ISH)



- Epidermal papilloma
- TPA induced
- Tg expression co-incident with BrdU incorporation

- Forestomach papilloma
- DMVC induced
- Tg expression co-incident with BrdU incorporation

FVB/N-Tg.AC vs. FVB/N skin

- Squamous epithelial cell proliferation
- Temporal sequence of changes
- Induction of papilloma & carcinoma
- Sensitivity (I-P studies)

Benign - SENCAR ≥ FVB/N >Balb/c > C57BL/6

Malignant - FVB/N > SENCAR > C57BL/6 > Balb/c

Hennings et al. Carcinogenesis 14(11):2353-8 (1993)

FVB/N-Tg.AC tumorigenesis

- Carcinogens induce and/or clonally expand transgene expressing cells -

  bulge area of hair follicle $\rightarrow$ papilloma $\rightarrow$ SCC
- Inflammation alone insufficient to drive tumorigenesis

  benzene vs. phenol; TPGDA vs. EA, rotenone
- Carcinogen potency decreases latency and increases rate of conversion from benign to malignant

  B[a]P > benzene >TPA

TPGDA vs. Ethyl Acrylate

- Topical application dose set by induction of epidermal cell proliferation in FVB/N mice
- Tripropylene glycol diacrylate (TPGDA) but not ethyl acrylate (EA) induced papillomas and malignant tumors in Tg.AC

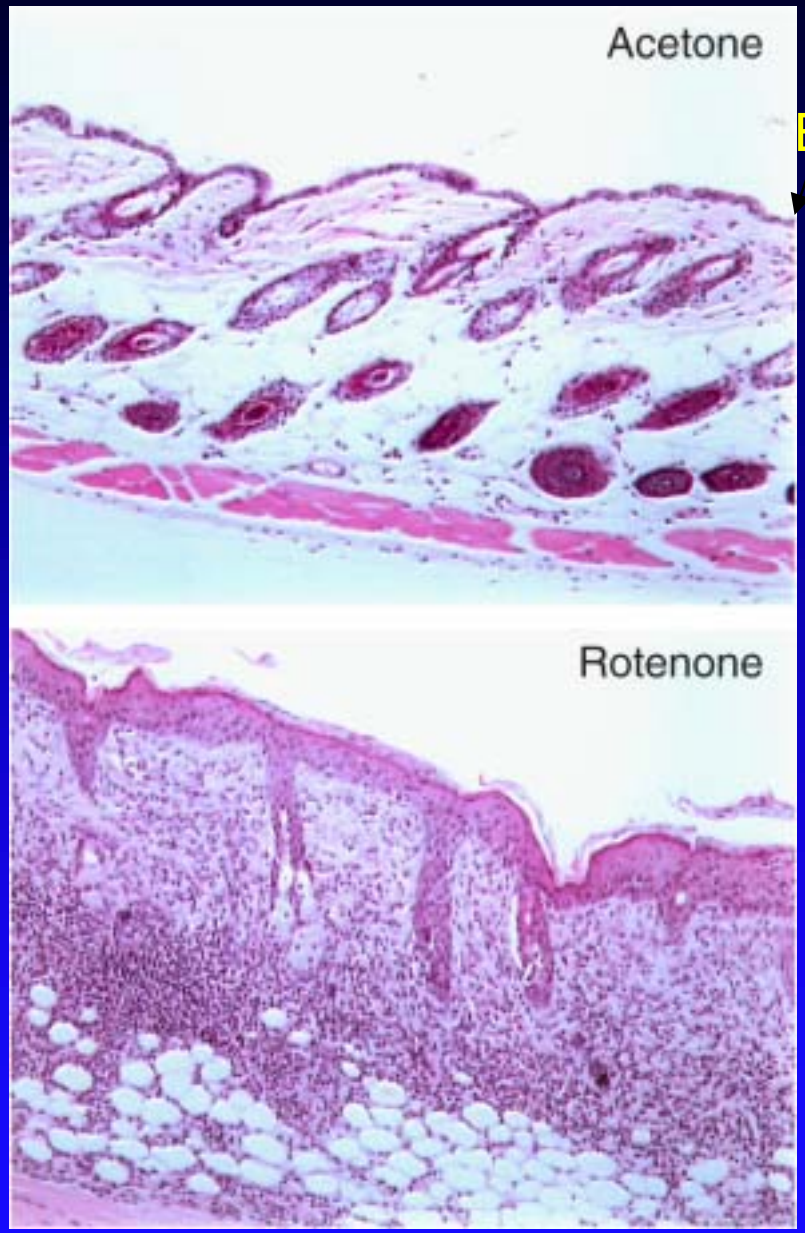
  Nylander-French & French *Toxicologic Pathology* 26:476-483 (1998)
- TPGDA & EA did not induce micronuclei in vivo

  Tice et al. *Environ Mol Mutag* 29:240--249 (1997)

Is tumorigenesis in the Tg.AC mouse skin caused by inflammation?

- induces inflammation & cell proliferation
- does not induce papillomas at doses that induce the inflammatory response
- inflammation alone is insufficient to drive tumorigenesis

Inflammation



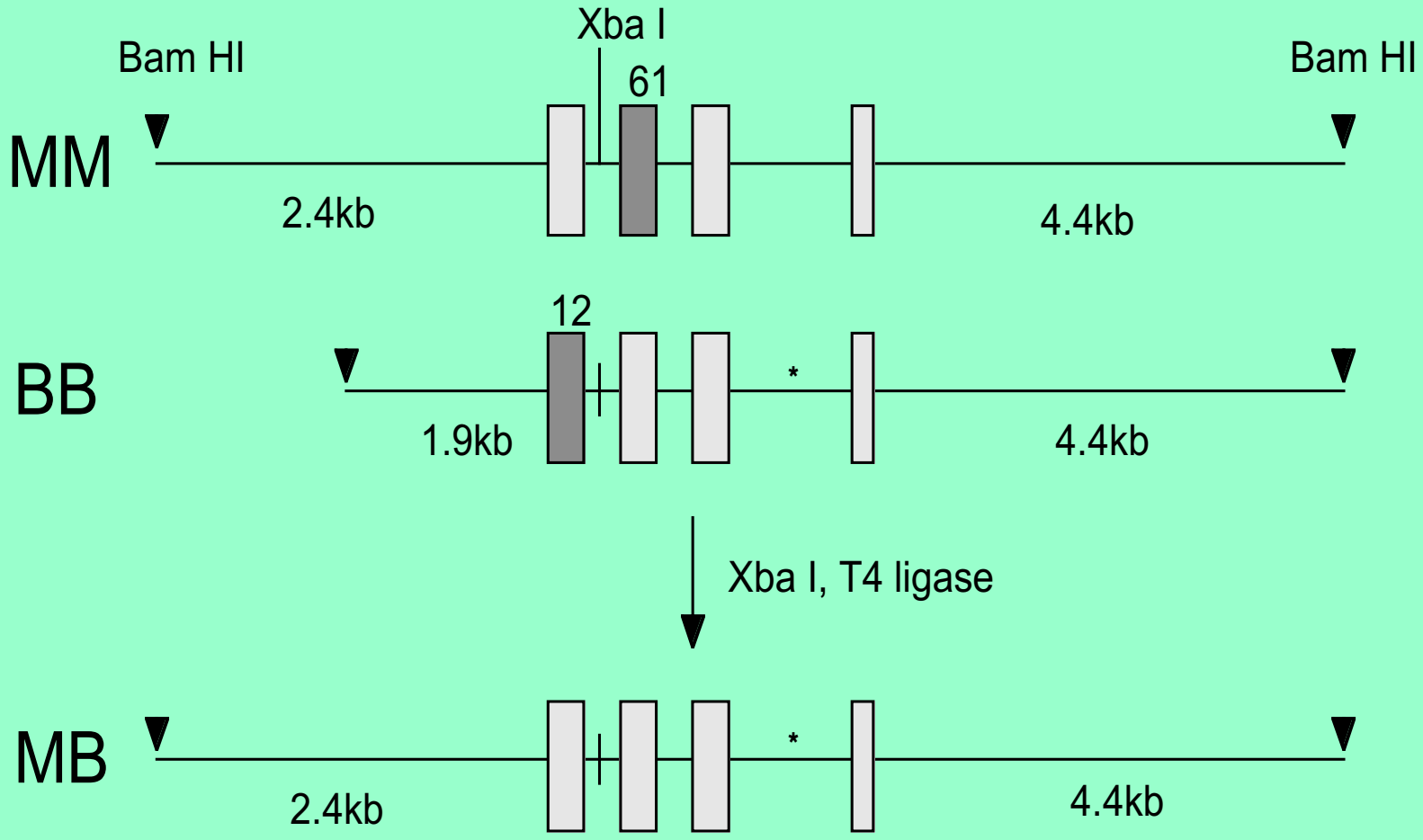
Conclusions

- Reporter phenotype
- Carcinogens induce and/or clonally expand cells expressing the transgene
- Carcinogens induce skin papillomas & carcinomas

RasH2 Mouse

CB6F1-*RasH2*^{tgCiea}

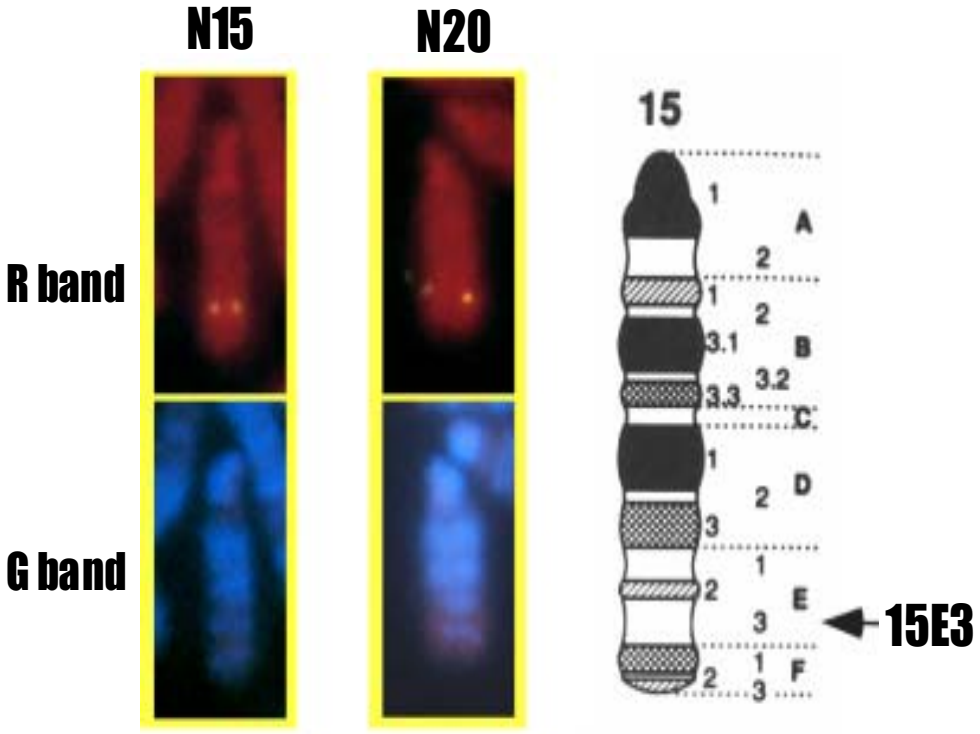
Structure of the rasH2 transgene



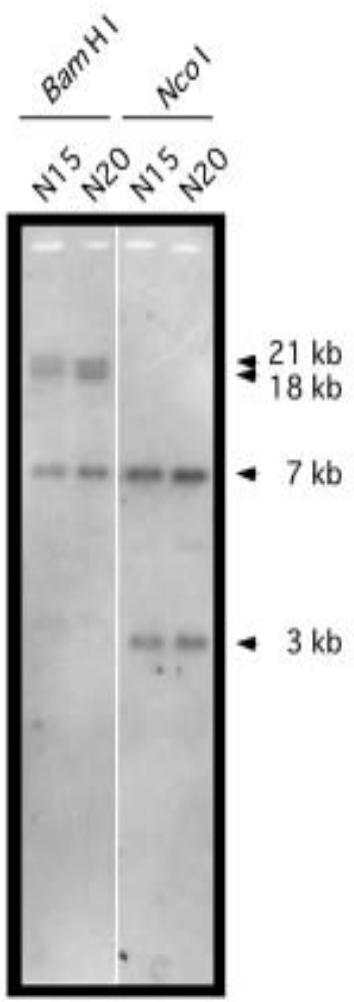
*: a point mutation in the last intron

Sekiya et al, Jpn. J. Cancer Res., 1985

Stability of Human H-ras Transgene



FISH



Southern

The rasH2 Transgenic Mouse

Expression of Transgene

- Constitutive expression in all tissues by Northern blotting/RT-PCR
- Elevated total ras p21 in tissues demonstrated by Western blotting

Point mutations in chemically induced tumors in rasH2 mice

Carcinogen	Organ	Mutations			
		Human H-*ras*	Murine H-*ras*	Murine K-*ras*	p53[a]
ENU	Forestomach	1/9[b,c]	0/9	0/8	0/9
MNU	Forestomach	12/12[d]	0/14	0/10	0/12
Urethane	Lung	14/14[e]	13/13[f]	0/11	nt

[a]: Sequencing was performed with exons 5 to 8 of p53 gene cDNA. [b]: Number of tumor with mutation/number of tumor tested. [c]: One tumor showed a CAG to CTG change at codon 61. [d]: All tumors showed a GGC to GAC at codon 12. [e]: All showed CAG to CTG at codon 61. [f]: All 13 tumors showed CAA to CTA change. nt: Not tesed.

Summary of Gene Expression in Induced Tumors

Carcinogen	Expression	Mutations		
	Human c-Ha-*ras*	Human c-Ha-*ras*	Murine H-*ras*	Murine K-*ras*
ENU	2.25 ± 0.65	1/9	0/9	0/8
MNU	2.27 ± 1.22	12/12	0/14	0/10
Urethane	2.49 ± 1.30	14/14	13/13	0/11

Mechanistic Basis for Tumorigenesis

- Point Mutation of Transgene (human c-Ha-ras)
  - spontaneous tumors: high frequency, but not in all tumors
  - induced tumors: high frequency, depending on compounds
    100% (MNU, Urethane) 10% (ENU)
- Point Mutation of Endogenous (mouse) K- & H-ras
  - induced tumors: usually not detected (high in urethane-induced lung tumor)

Comparative Analysis

Are transgenic models more accurate?

Data set for prediction (99 test chemicals)

B6.129-Trp53^{tm1Brd} | FVB/N-v-ras^{tgLep} | CB6F1-rasH2^{tgCiea}

[+]

- 47 IARC Class 1/2 NTP ROC - Known/Probable Human Carcinogens

[-]

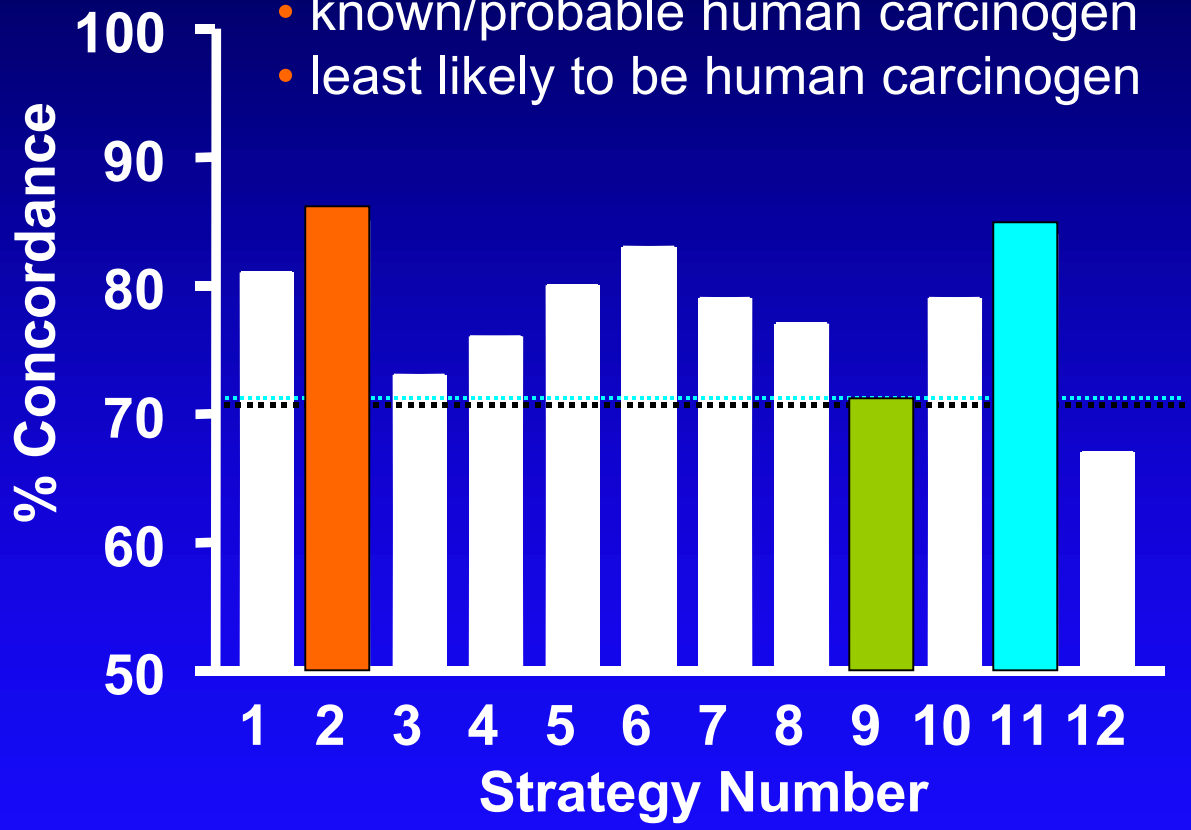
- 52 IARC Class 3/NTP ROC negative/unlisted

Least likely to be a human carcinogen

Pritchard et al. EHP (2002)

Transgenic Mouse Models Performance

Discrimination between
- known/probable human carcinogen
- least likely to be human carcinogen



1 = Trp53+/-

2 = Trp53-G

3 = Tg.AC

4 = RasH2

5 = p53-G/Ras-N

6 = p53-G/Ras-All

7 = p53-G/Tg.CA-N

8 = p53-G/Tg.AC-All

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

Pritchard et al. EHP (2002)

Conclusions

The transgenic models:

- missed few non-carcinogens -

1/59 *Trp*53; 5/51 *Ras*H2; 10/62 Tg.AC (v-Ha-*ras*)

- missed rodent carcinogens –

10/59 for Trp53; 7/51 for RasH2; 7/62 for Tg.AC

The NTP 2-year bioassays:

- missed no carcinogens – 0/58
- missed several non-carcinogens -17/58

Acknowledgements

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