Genetic Effects of Asbestos Fibers

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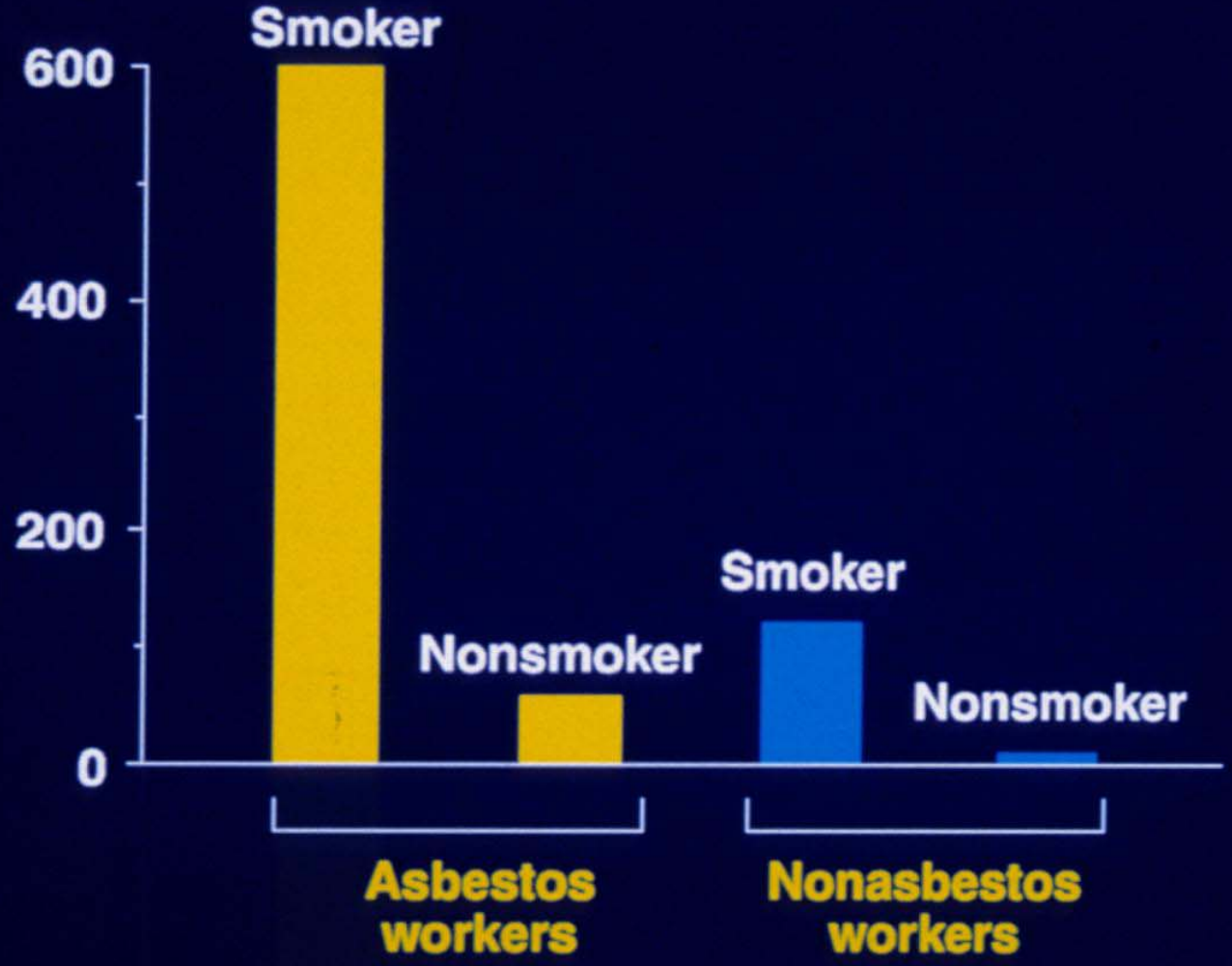
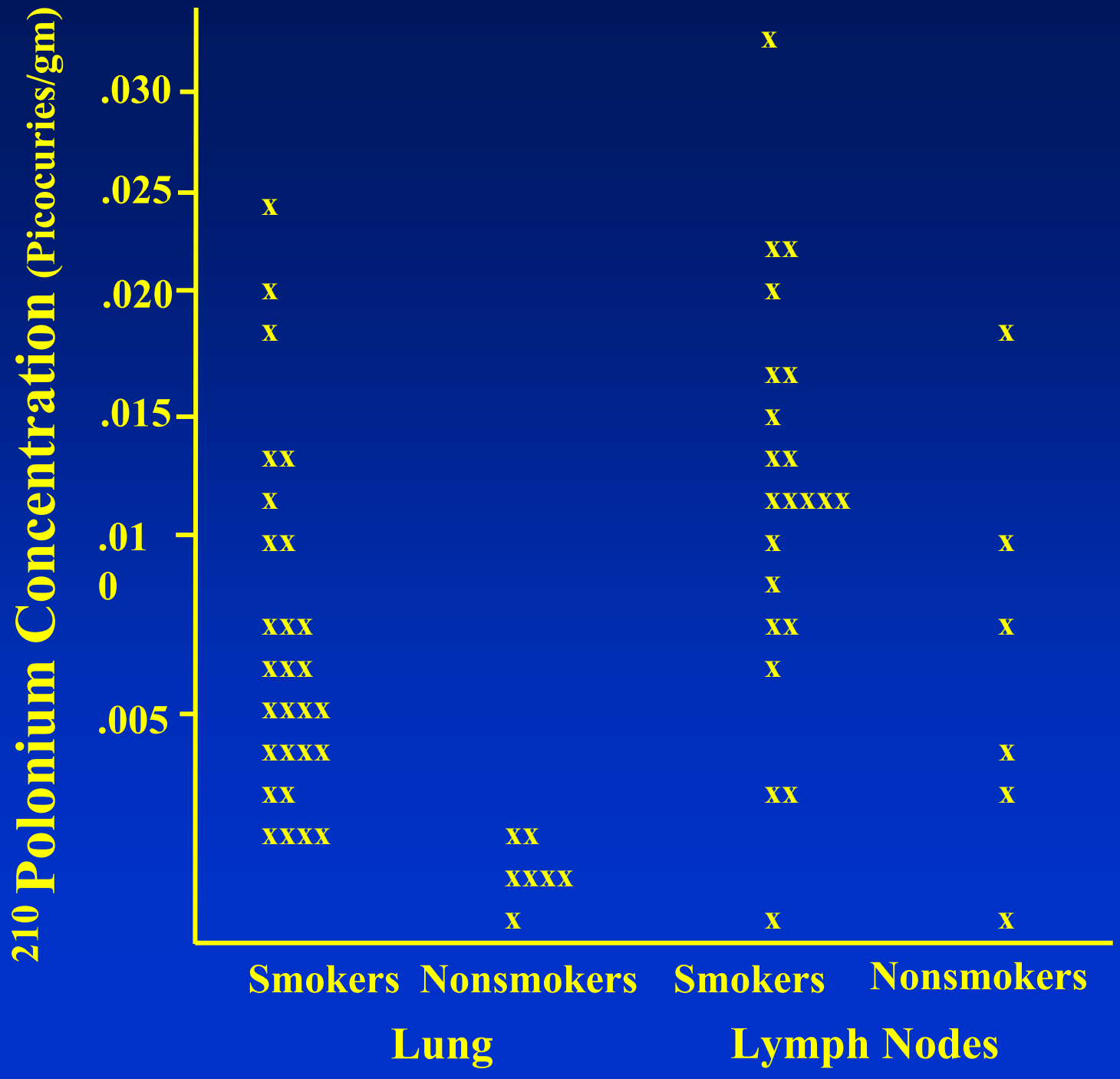
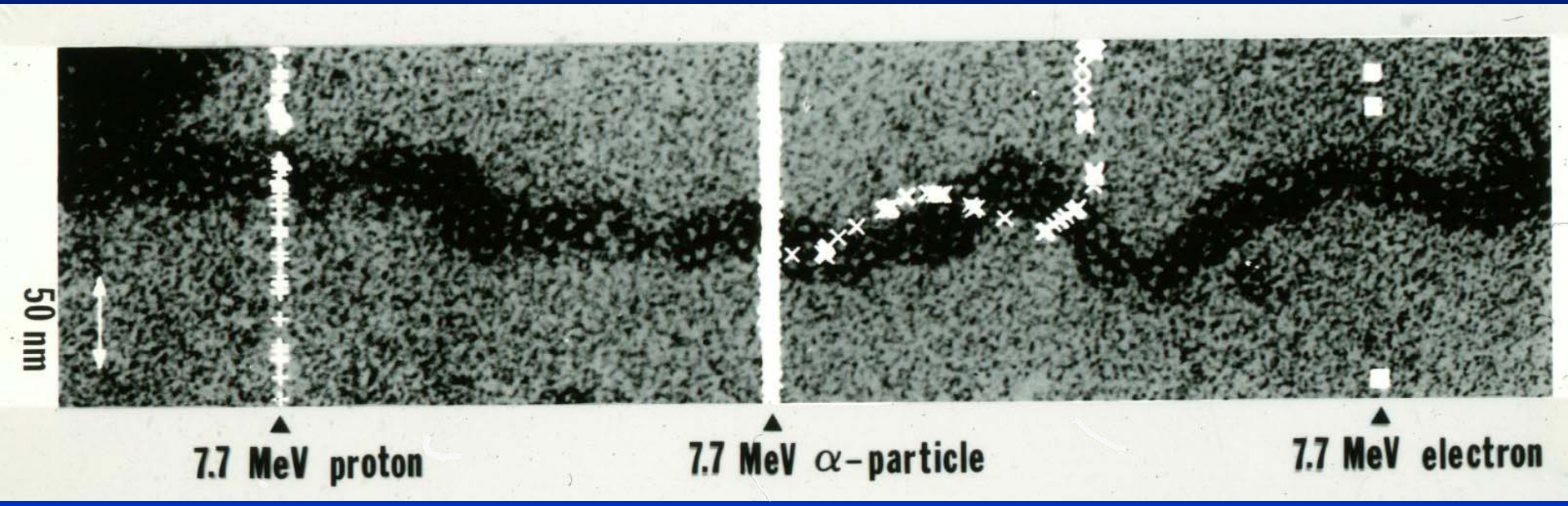
Known Carcinogens for Human Lung Cancers

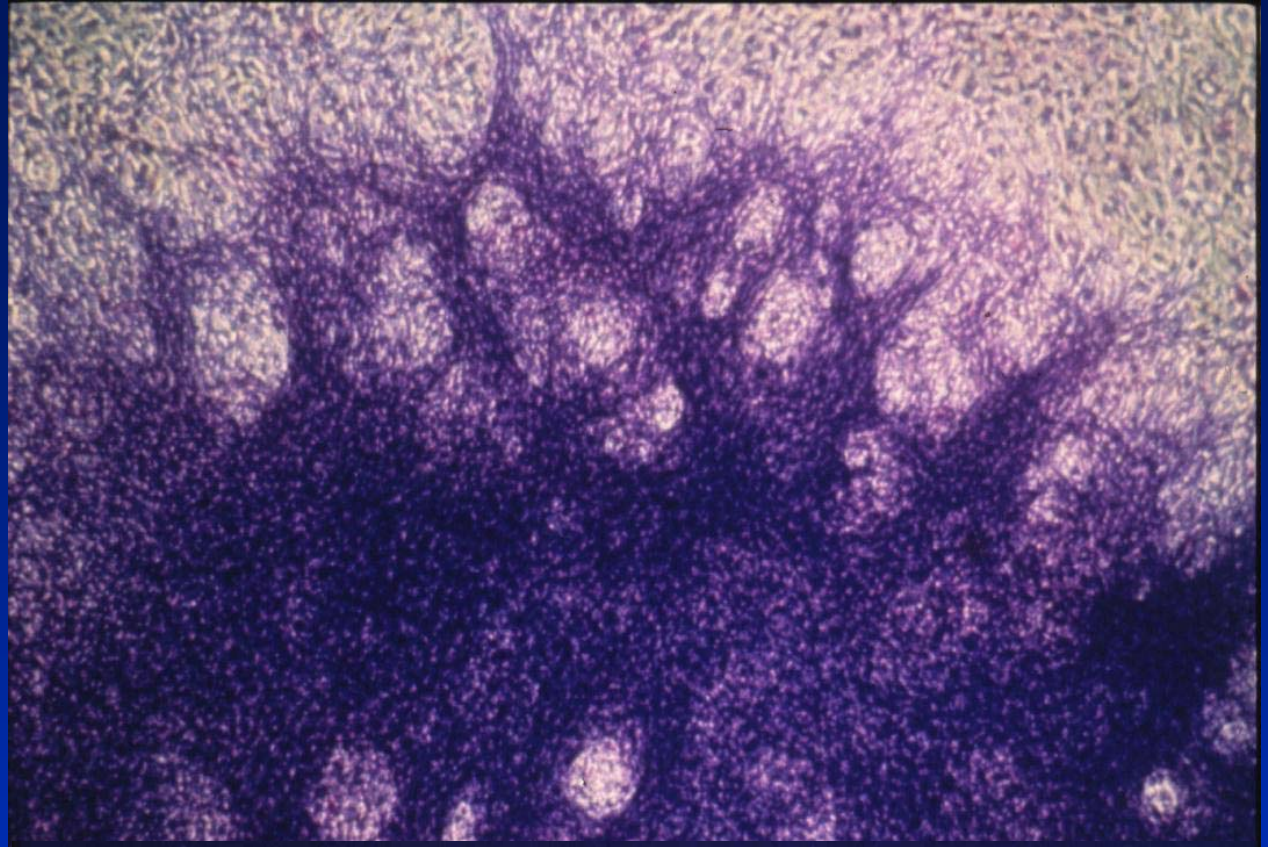
- **Tobacco Smoke**
- **Polycyclic aromatic hydrocarbons**
- **Radon**
- **Asbestos fibers**
- **Inorganic Arsenic, Nickel, and Chromium**
- **Mustard gas**

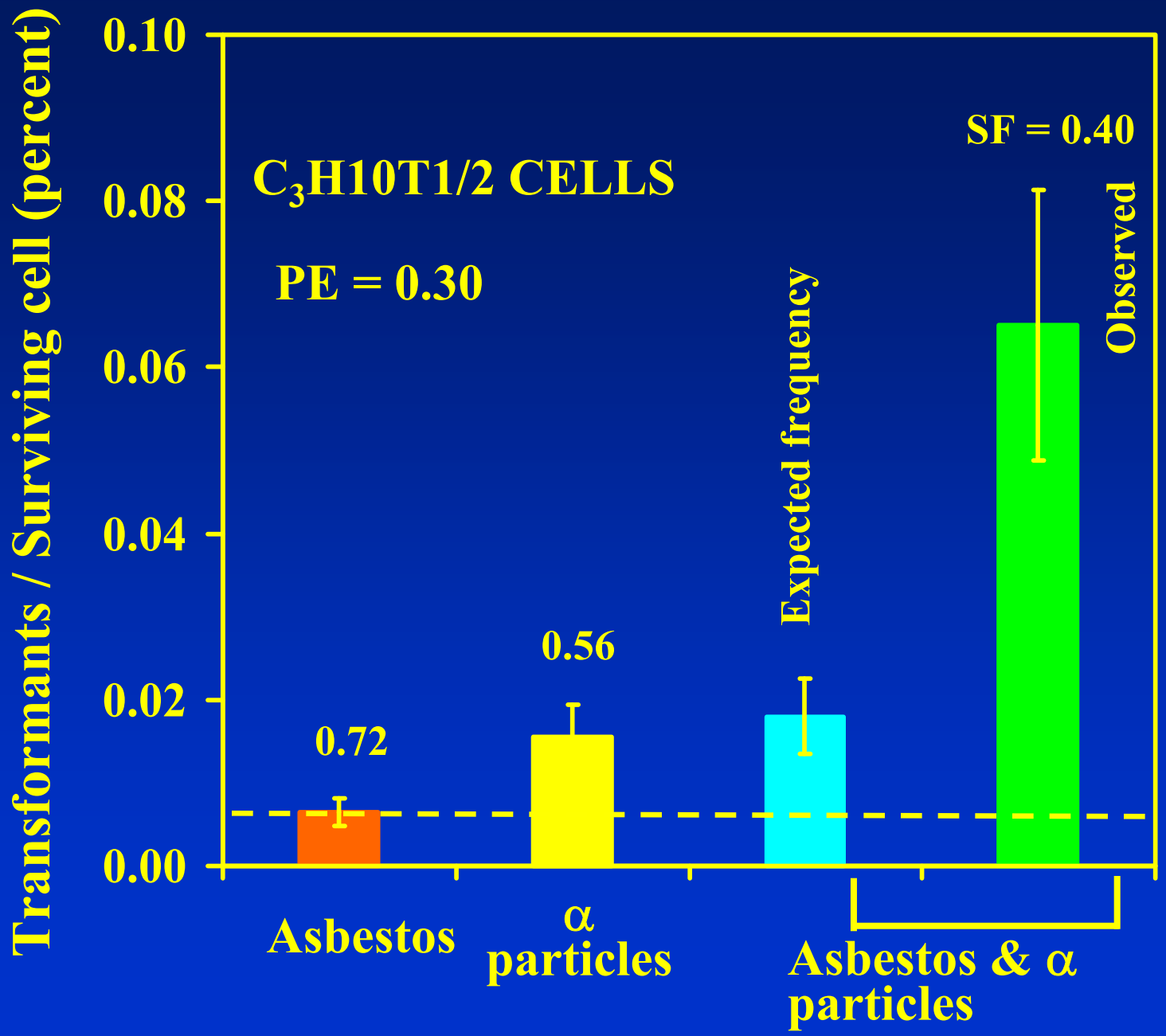
Age Standardized Lung Cancer Mortality Rates Per 10^5 Man–Years for Smoking &/or Asbestos Exposure




^{210} Polonium Concentration (Picocuries/gm)
.030
.025
.020
.015
.01
0
.005
Smokers
Nonsmokers
Smokers
Nonsmokers
Lung
Lymph Nodes


50 nm
7.7 MeV proton
7.7 MeV α-particle
7.7 MeV electron




Transformants / Surviving cell (percent)
0.10
0.08
0.06
0.04
0.02
0.00
C3H10T1/2 CELLS
PE = 0.30
SF = 0.40
Observed
Expected frequency
0.72
0.56
Asbestos
α particles
Asbestos & α particles

Tumor Induction in Spraque-Dawley Rats by Radon Gas and Various Fibers

Treatment	No. of Broncho-pulmonary Tumors	No. of Pleural Tumors	Fractions of Animal with Tumors
Radon 600 WLM	17	0	17/60
Radon plus			
Chrysotile	2	4	6/9
Acid leached	14	2	16/18
Crocidolite	5	3	8/10
Acid leached	3	4	7/10
Glass fibers	4	2	6/10

Data from: Bignon *et. al., Carcinogenesis* **4**: 621, 1983.
Monchaux *et al., IARC Sci Pub*. **90**: 161. 1989.

Morphological Transformation in Rodent Cells (I)

Model	Fibers	Foci Formation	Reference
$C_3H10T1/2$	UICC Crocidolite	- 2.3 μg/cm^2 x 2 days	Brown *et al.* '83
	Amosite	- 1.2 μg/cm^2	Hei *et al.* '84
	UICC Crocidolite	- 1 μg/cm^2 x 1 day	
	Amosite	-	
SHE	UICC Crocidolite	- 4 μg/cm^2 x 6 days	DiPaolo et al. '83
	Chrysotile	- 2 μg/cm^2 x 6 days	
	Amosite	-	
	Anthophyllite	-	
SHE	UICC Chrysotile	+ 2.0 μg/cm^2 x 7 days[à]	Hesterberg & Barrett '84
	Crocidolite	+	
	GF100	+	
	GF110	+/-	

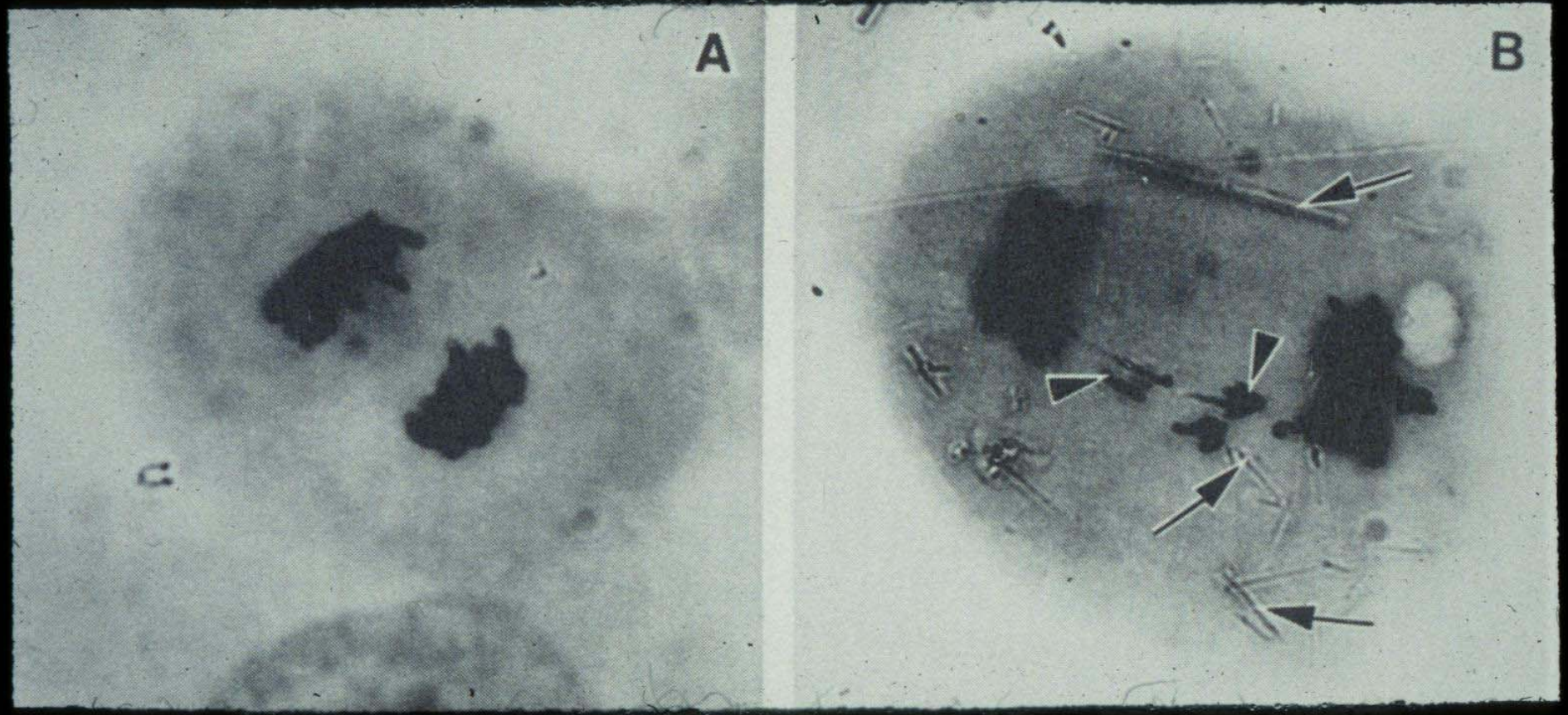
Morphological Transformation in Rodent Cells (II)

Model	Fibers	Foci Formation	Reference
SHE	UICC Chrysotile	+ 0.1 μg/cm^2 x 5 days x 2	Mikalsen *et al.* '85
	Crocidolite	+ 5 μg/cm^2	
	Amosite	+	
	Anthophyllite	+	
	GF100	+ + 5 μg/cm^2	
	GF100	- 20 μg/cm^2	
RMC	UICC Chrysotile	- 1 μg/cm^2 x 27 passage[à]	Paterour et al. '85
RMC	Chrysotile	± 5 μg/cm^2 x3 days from P_4 to P_{30}[♣]	Kravchenko et al.'98
RME	UICC Chrysotile	+ 0.4 μg/cm^2 x 45 passages	St. Etienne et al. '93
	Crocidolite	- 1 μg/cm^2 x 60 passages (controls give rise to tumor in mice as well)	

♣ Agar(+) growth at P_{16} in Rx cells , P_{24} in control

In vitro morphologic transformation of mineral fibers depends on:

- fiber dimension
- treatment time
- cell model system
- glass fibers tend to give positive data as well


A
B

In vitro Mutagenesis ~ Mammalian Genes

Cell	Fiber tested	Mutagenicity	Reference
V79-*hprt*	UICC Chrysotile	+/-	Hung *et al.* 1978
	Crocidolite (10mg/cm^2 x 24hr)	–	
CHO-*hprt*	Crocidolite	–	Kenne *et al.*1986
	Amosite (0.9mg/cm^2 x 24hr)	–	
A_L-*hprt*	Chrysotile	–	Hei *et al.* 1992
	Crocidolite (4mg/cm^2 x 24hr)	–	1990
ARL(6)-*hprt*	Chrysotile (10mg/cm^2 x 6 days)	–	Reiss *et al.* 1982
	Crocidolite (26mg/cm^2)	–	
	Amosite	–	
SHE-*hprt*	Chrysotile (2mg/cm^2 x 48hr)	–	Oshimura *et al.* 1984
-*oua*	Crocidolite	–	

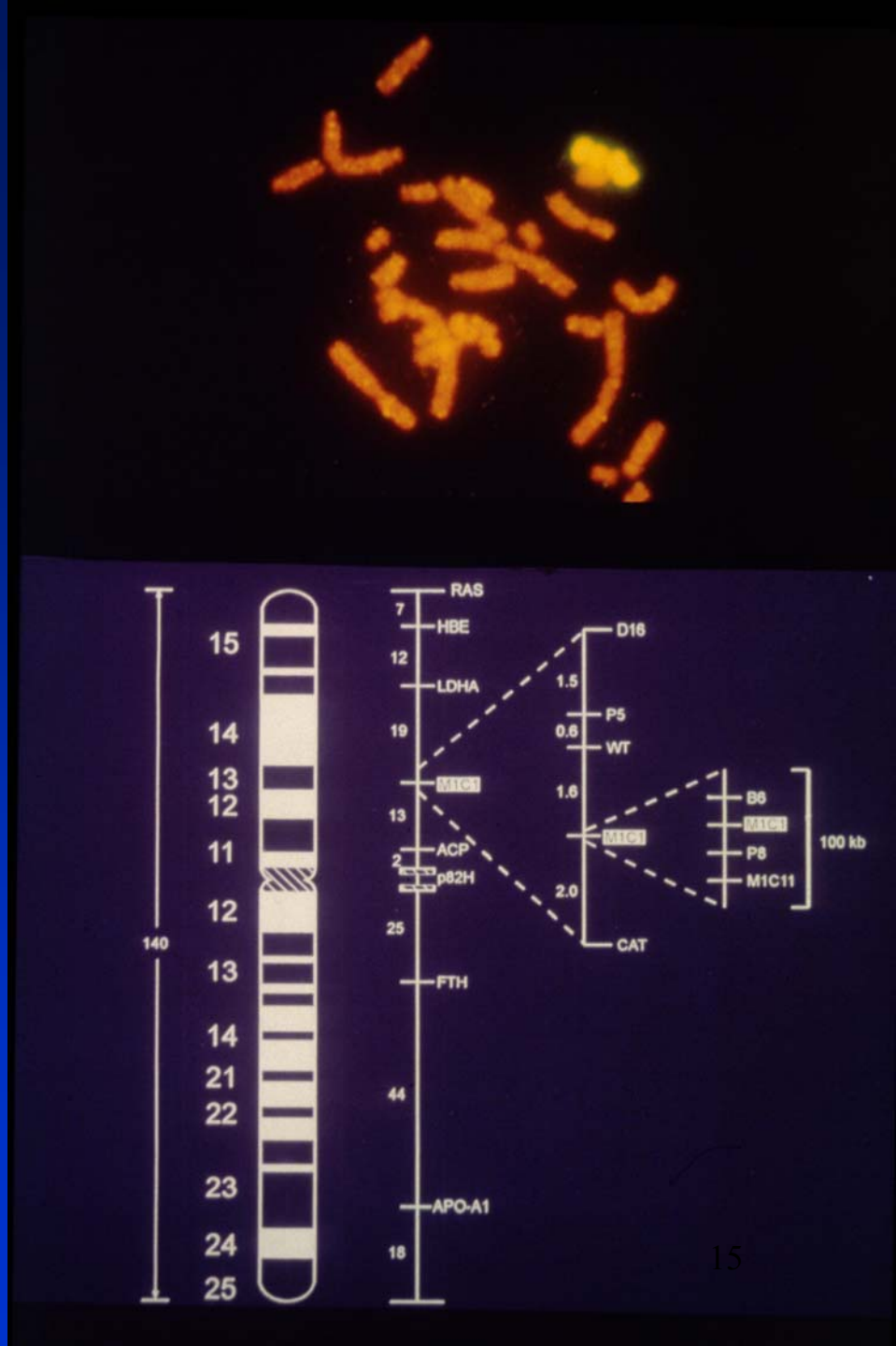
Observation:

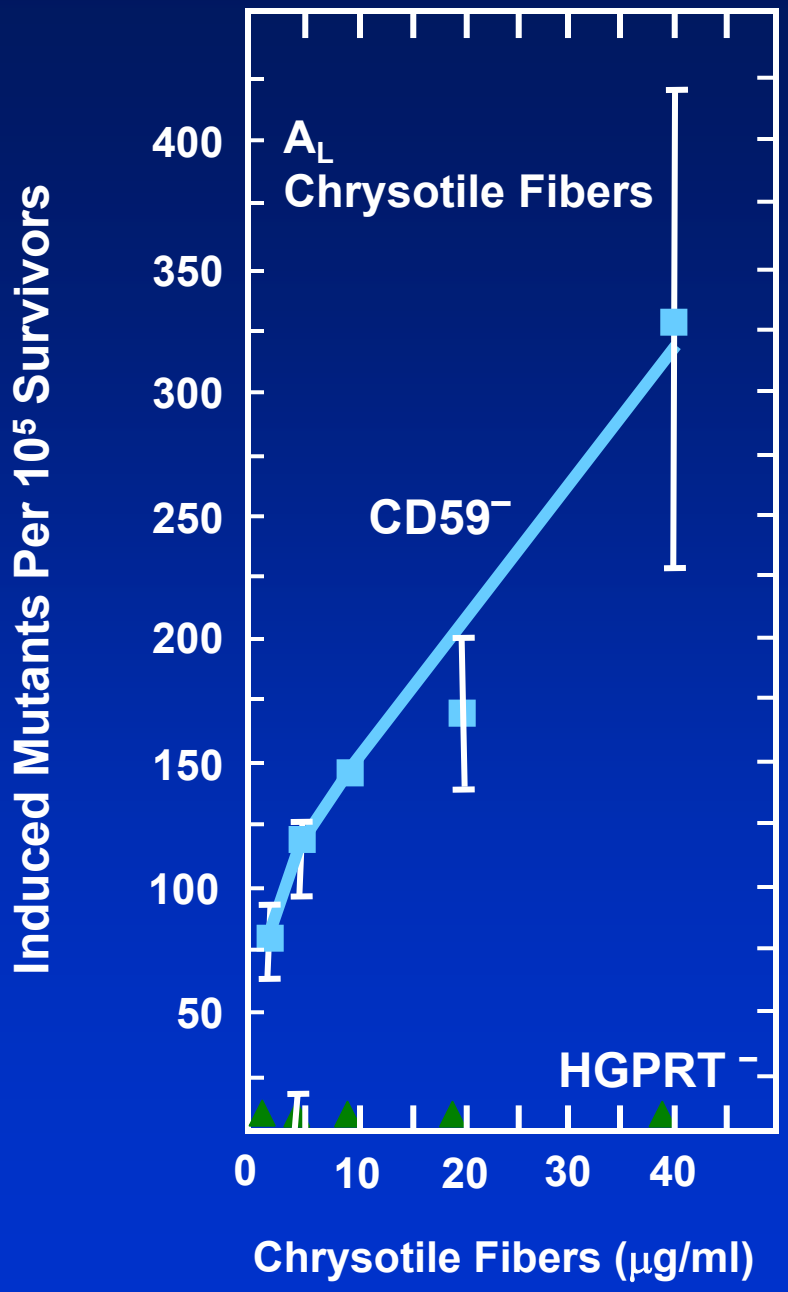
The negative gene mutation data suggest either: 1) asbestos is a non-genotoxic carcinogen;

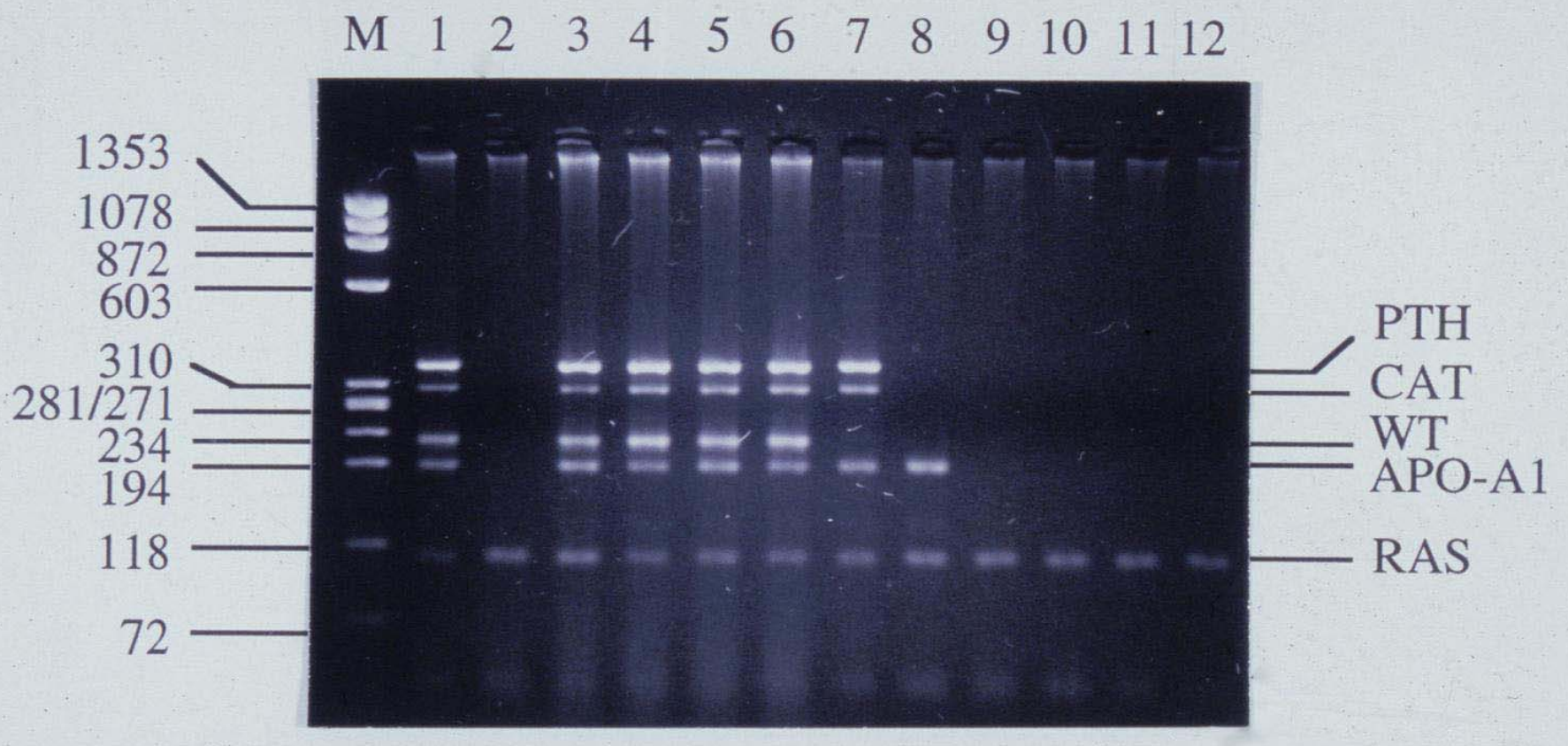
2) mutants induced at these loci are non-viable.

Given the strong evidence that fibers induce chromosomal alterations, it is likely that asbestos induces mostly large multilocus deletions that are non-compatible with survival of the mutants.

The human hamster hybrid A_L cells contain a single human chromosome 11 and a gene at 11p13.1 encodes the cell surface CD59 antigen that forms the basis of the mutagenic assay




Induced Mutants Per 10^5 Survivors
400
350
300
250
200
150
100
50
A_L
Chrysotile Fibers
CD59⁻
HGPRT⁻
0
10
20
30
40
Chrysotile Fibers (μg/ml)


M 1 2 3 4 5 6 7 8 9 10 11 12
1353
1078
872
603
310
281/271
234
194
118
72
PTH
CAT
WT
APO-A1
RAS

Data in support of asbestos-induced multilocus deletions

System	Fiber	Mutagenicity	Reference
Human lymphocyts (LOH at *Hla-A* locus)	UICC Crocidolite (400μg/ml x 72 hr)	+	Both et al. 1994
	Chrysotile	+/-	
	Erionite	+	
Rat 2λ-*lacI* gene (Homologous recombination)	Calidria Chrysotile (6.7μg/cm^2 x 3 hr)	+	Lezon-Geyda et al. 1996
V79 *gpt* gene	NIEHS Crocidolite (6μg/cm^2 x 24 hr)	+	Park & Aust 1998
	Chrysotile (30μg/cm^2)	+	

In Vivo Mutagenesis in Transgenic Animals

Inhalation studies in *LacI* transgenic mice (Rihn *et al*., **2000**):

5.75 mg South African crocidolites: 6 hr/day for 5 days;

Mutation induction factor of 1.96 was obtained (13.5 versus 6.9 $x10^{-5}$)

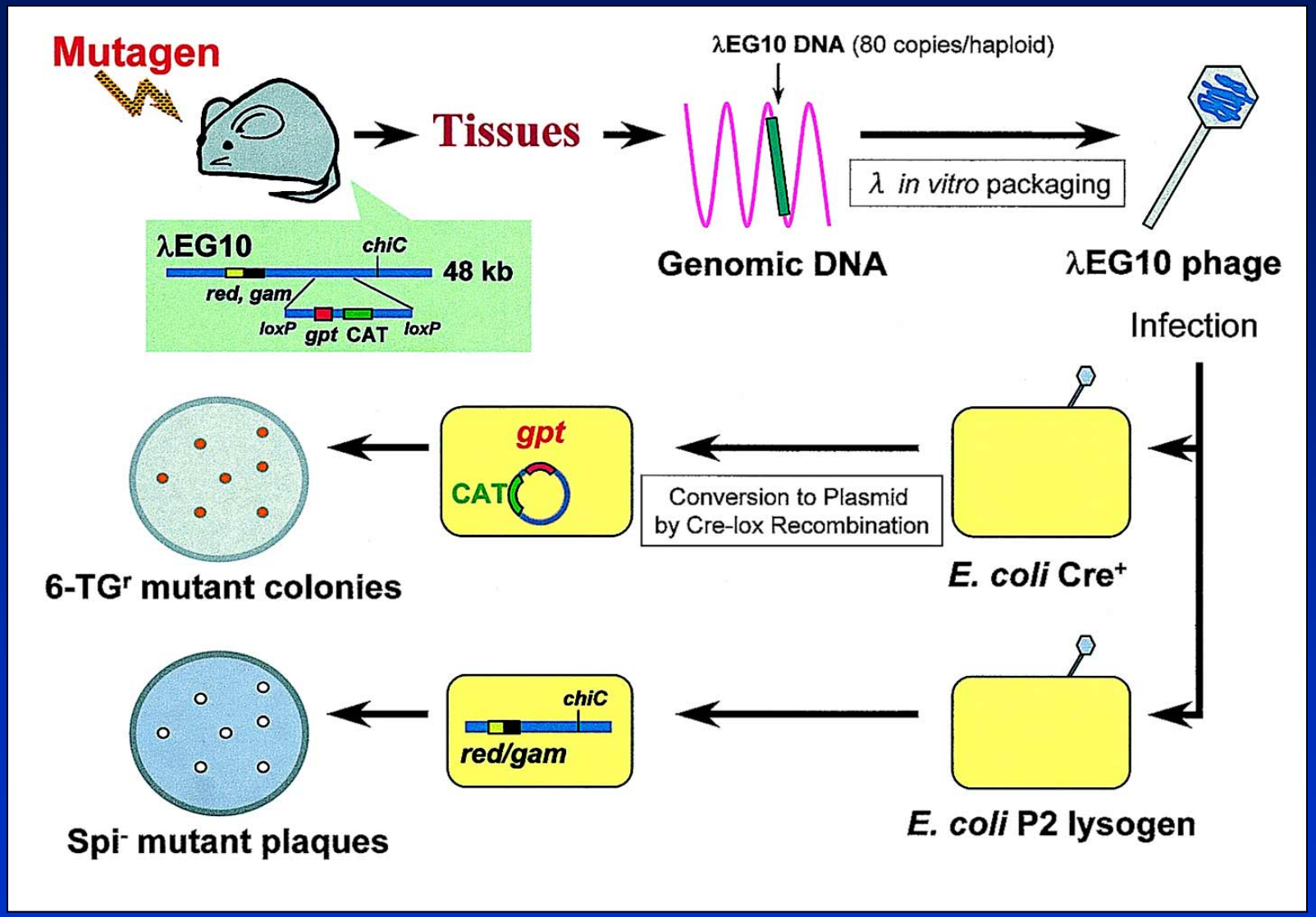
No specific mutant spectrum identified.

IP injection studies in *LacI* transgenic rats (Unfried *et al*., **2002**):

2 and 5 mg single injection of UICC crocidolites:

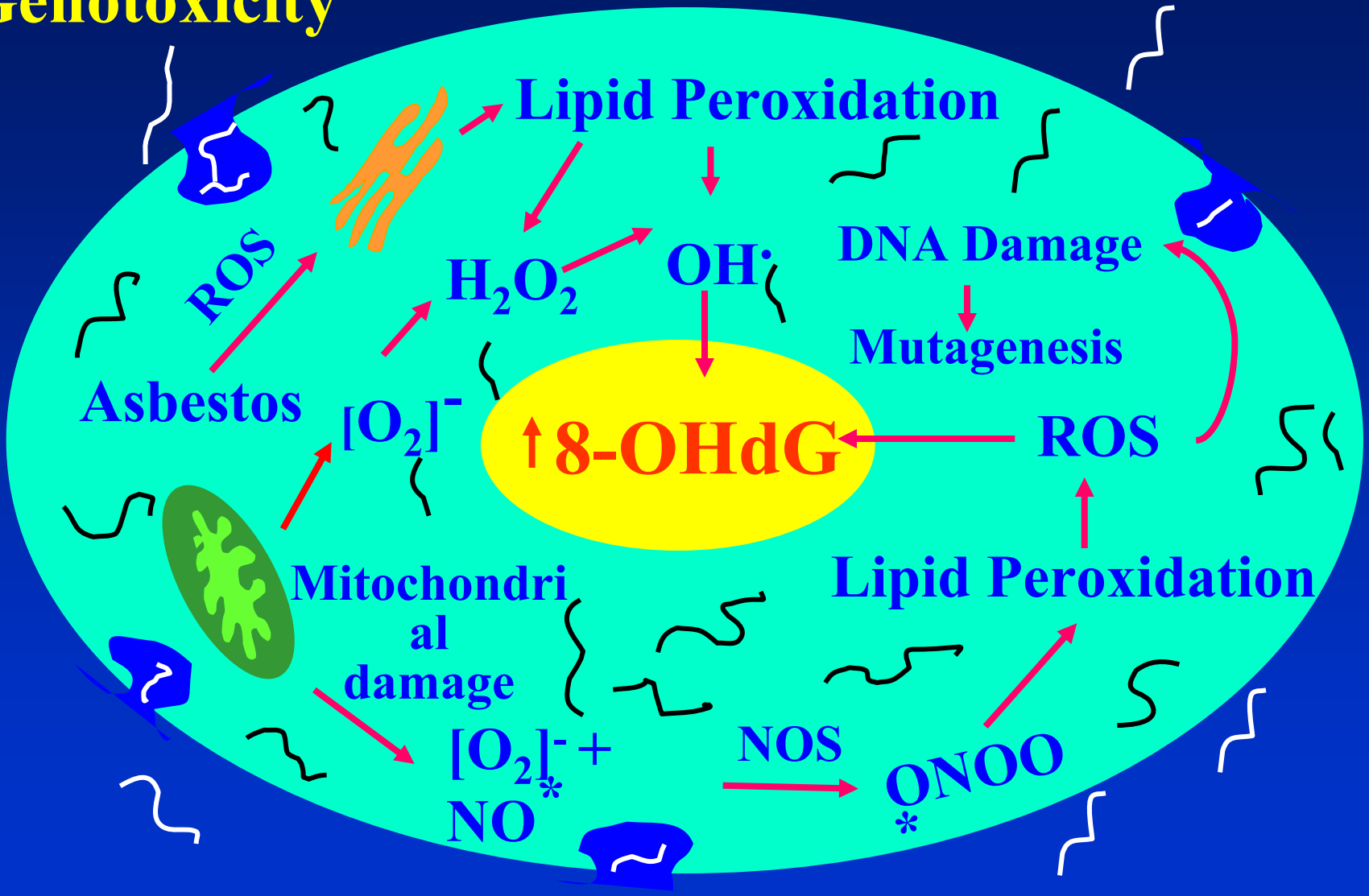
Mutation induction factors range from 1.1 to 3.2 in the omenta of animals were obtained

G→T transversion in 29% of mutants isolated

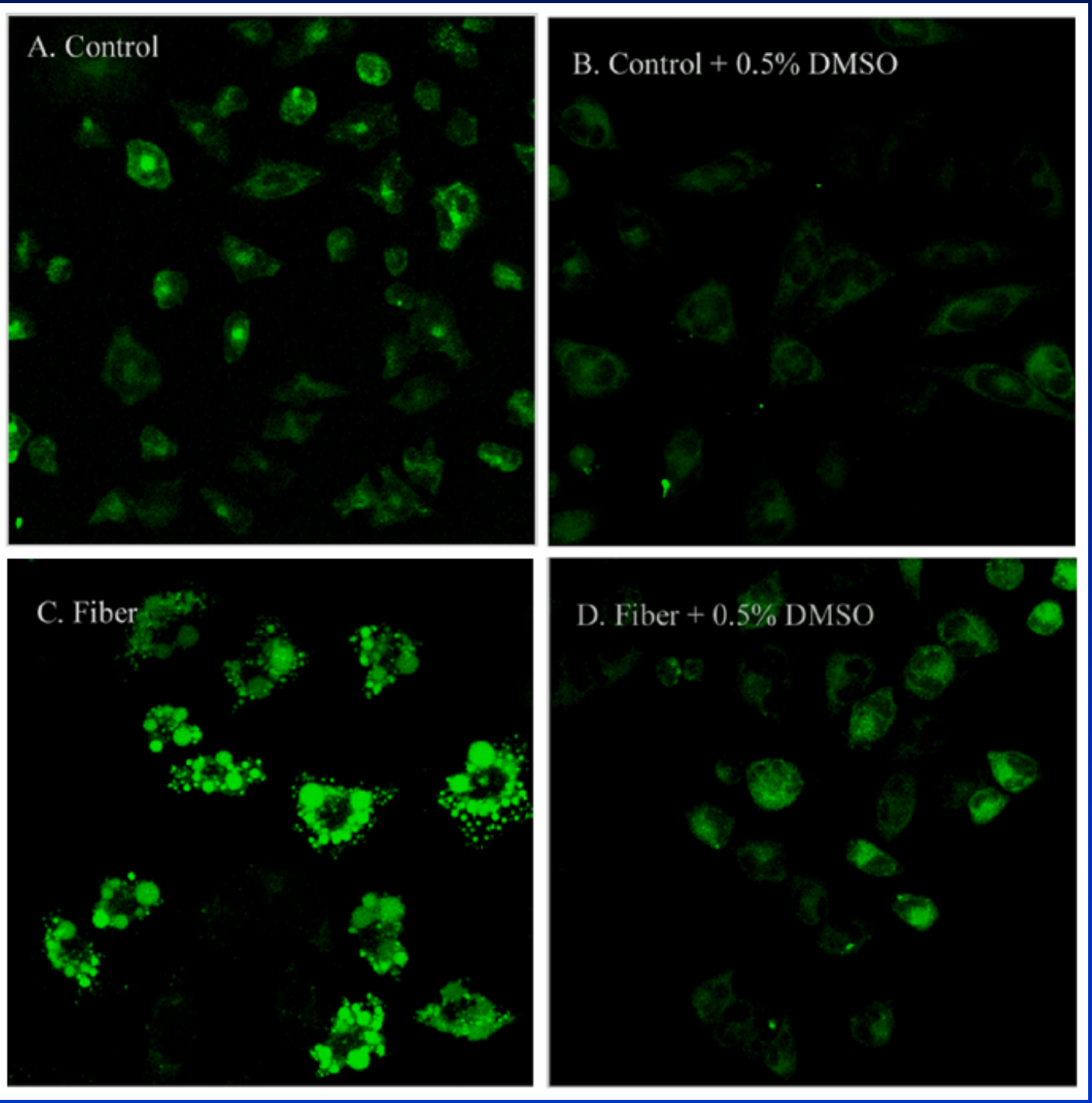


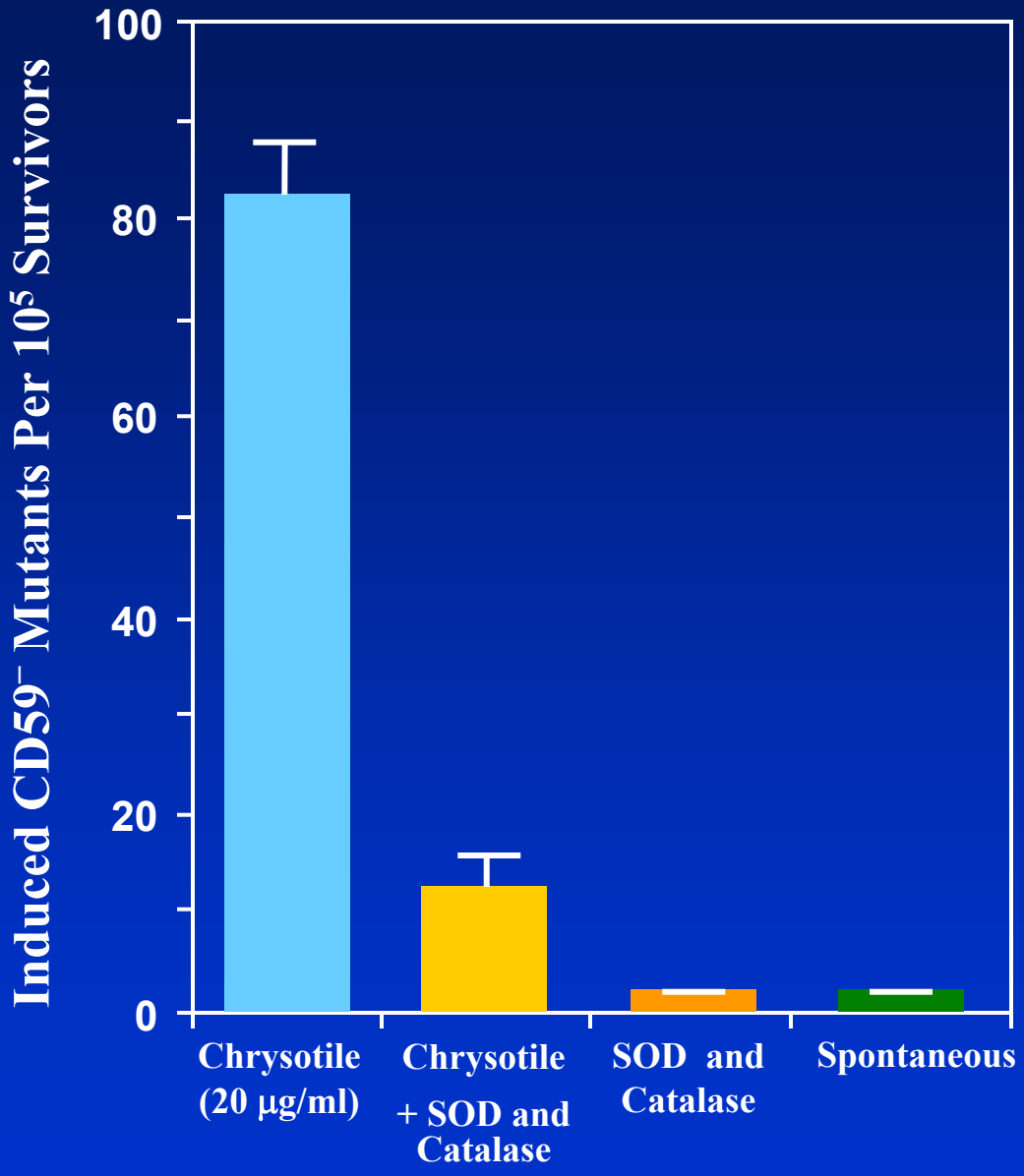
From Nohmi *et al.*, *Mutation Research* **455**: 191-215, 2000

Proposed Mechanism for Asbestos Induced Genotoxicity

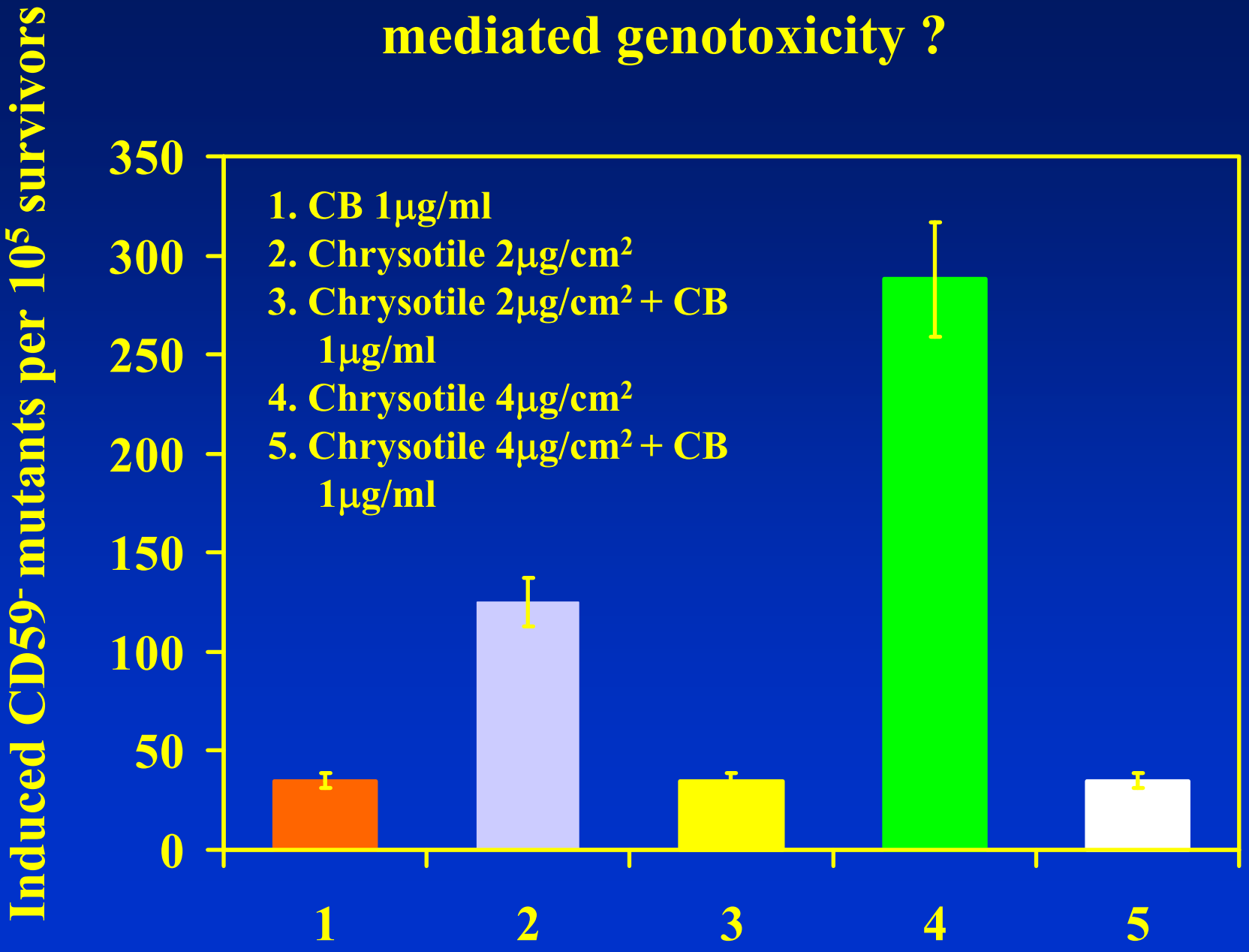


Quantification of reactive oxyradicals using CM-H_2DCFDA

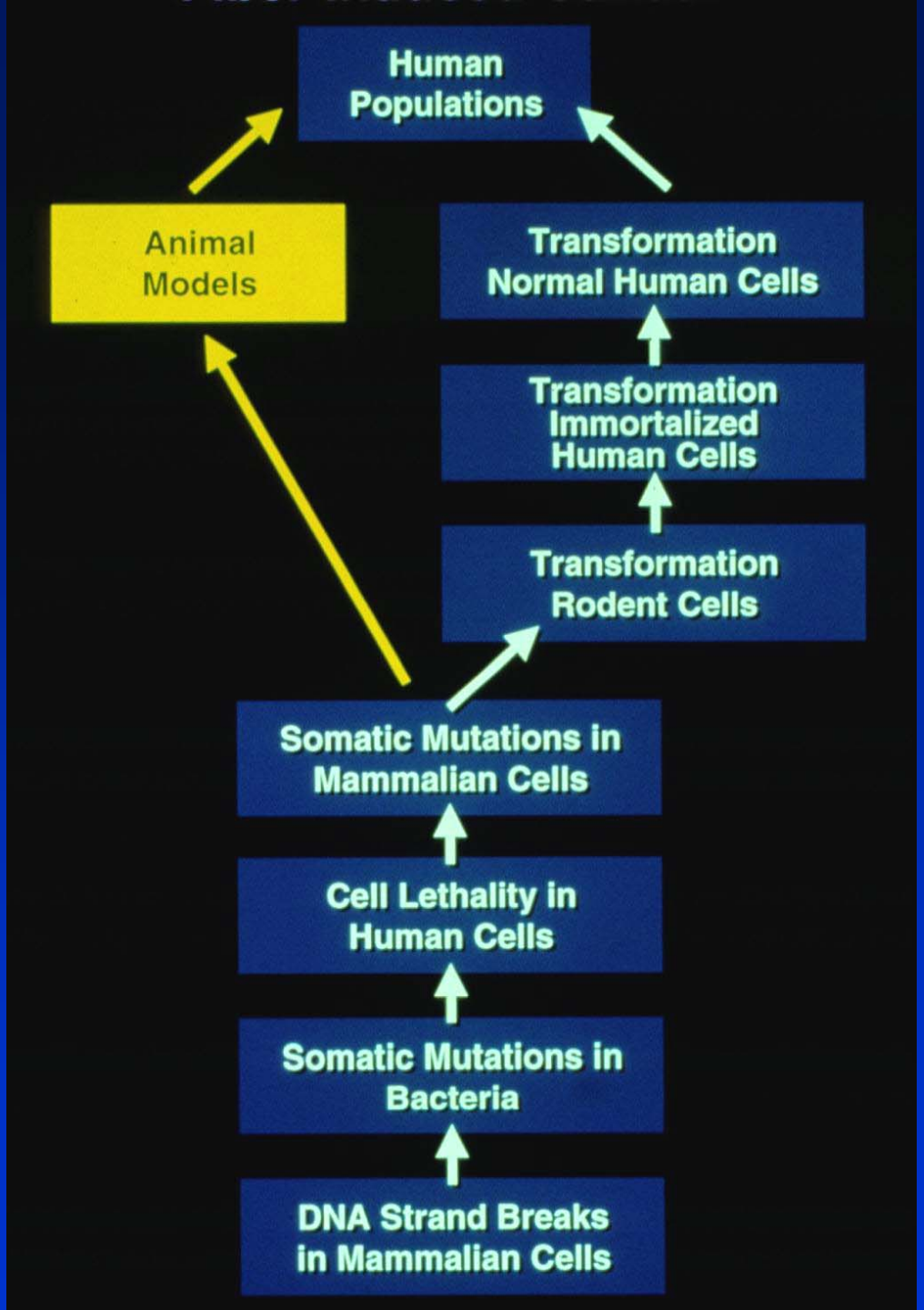


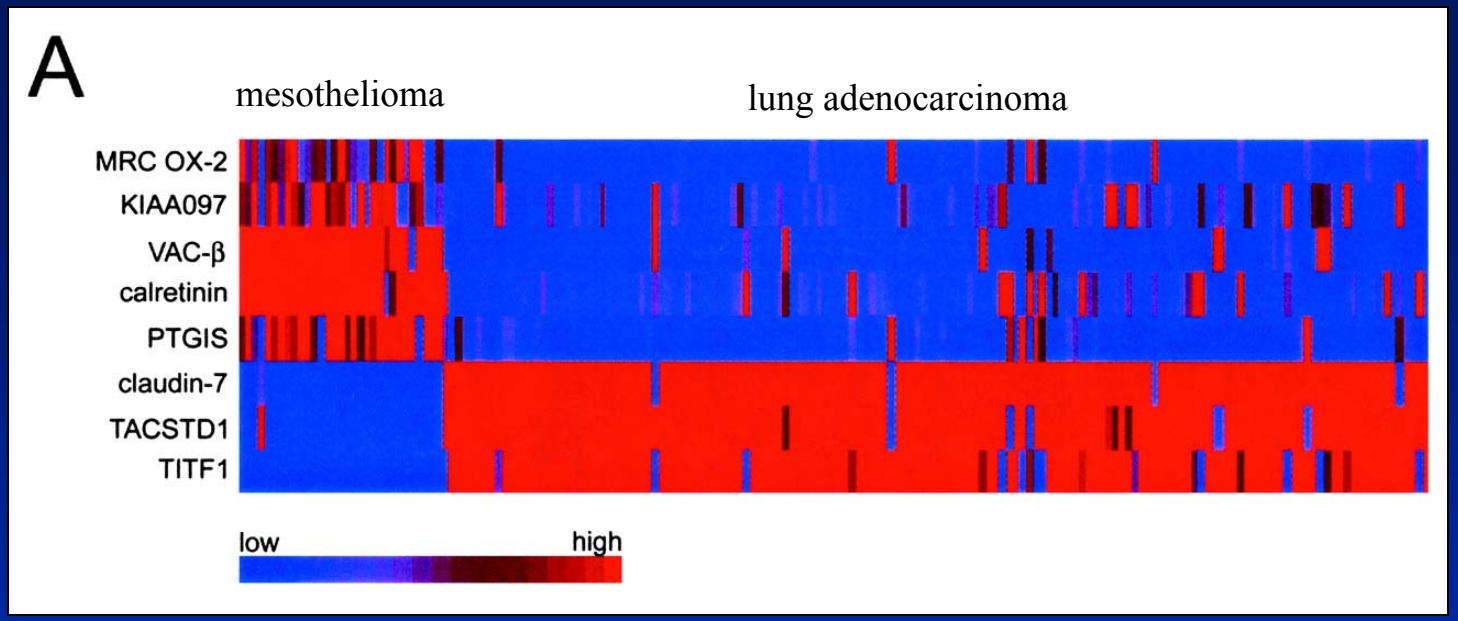

Induced CD59⁻ Mutants Per 10⁵ Survivors
100
80
60
40
20
0
Chrysotile (20 μg/ml)
Chrysotile + SOD and Catalase
SOD and Catalase
Spontaneous

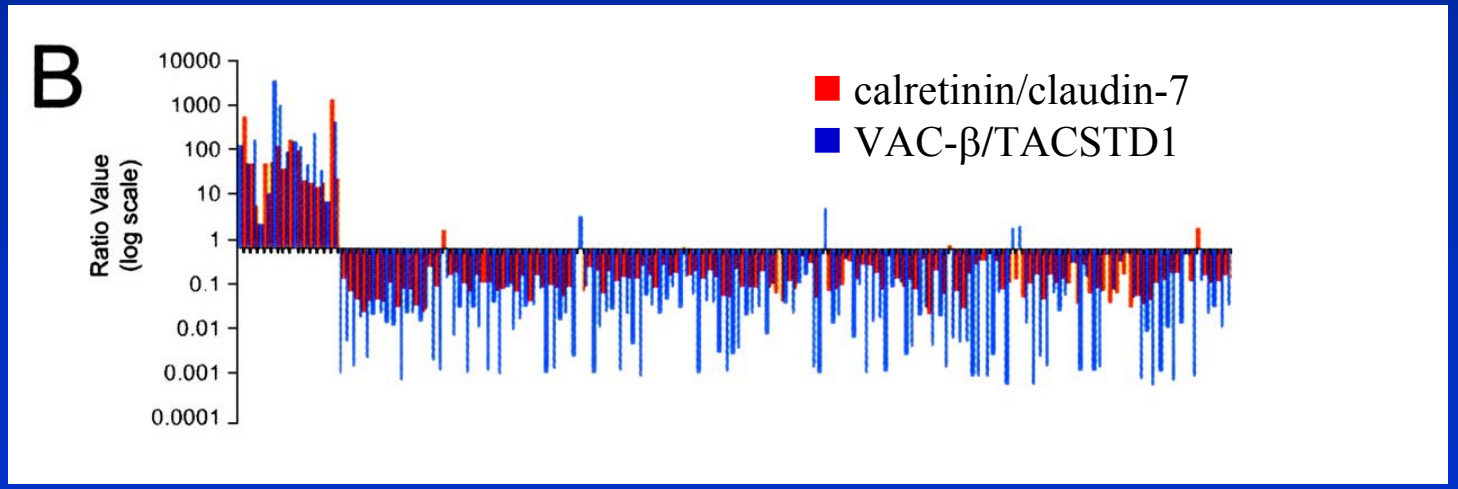
Is phagocytosis a critical step in fiber/ particle-mediated genotoxicity ?



Models available for Asbestos Carcinogenesis Studies







Gavin J. Gordon, Roderick V. Jensen, et. al.;
Cancer Research 62:4963-7, September 1, 2002

Molecular alterations in asbestos induced mesotheliomas:

Oncogenes and growth factors:

- **no mutations in K-*ras* oncogene**
- **↑ PDGF (human and mouse only, not in rat)**
- **↑ $TGF_{\beta 1}$ (not specific for tumor development)**
- **↑ TNF_{α}**
- **↑ Cyclin D1 (?)**

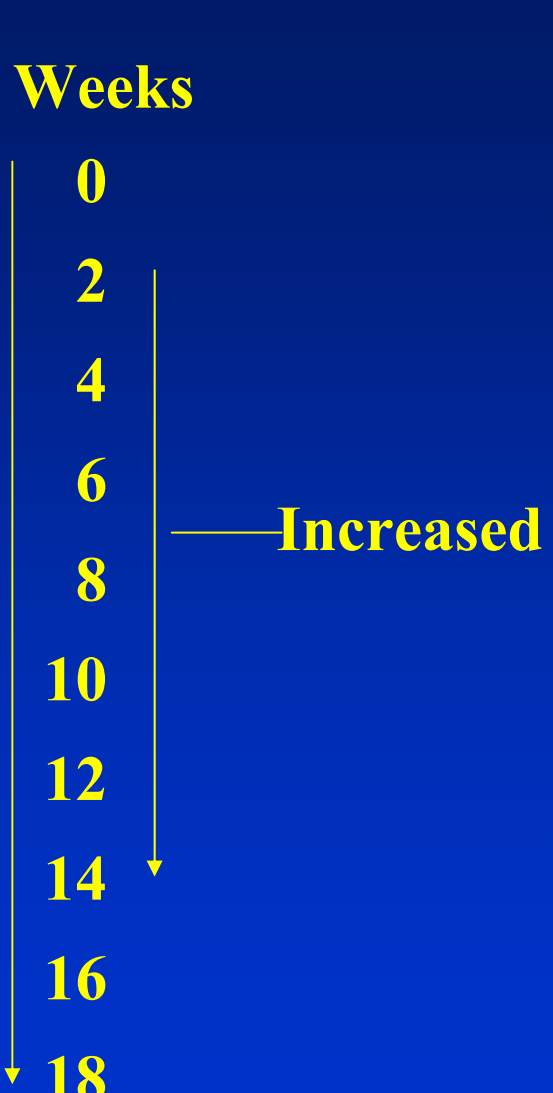
Tumor suppressor genes:

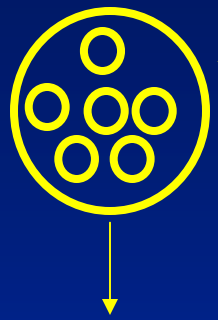
- **No changes in *Rb, WT,* and *Pten* genes**
- ***p53* gene mutation is rare, p53 protein can often be detected**
- **Allelic loss in chromosomes 3p (*FHIT*), 6q, 9p, 13q, 17q (*NF1),* and 22q (*NF2*) reported**

Transformation in Human Cells

No **primary human epithelial cells of any histological origins have *ever* been shown to be malignantly transformed by either single or multiple doses of chemical carcinogens including asbestos fibers. It is estimated that the neoplastic incidence is ~10^{-15} (Hei *et al.* 1994).**

Immortalized Human Bronchial Epithelial Cells





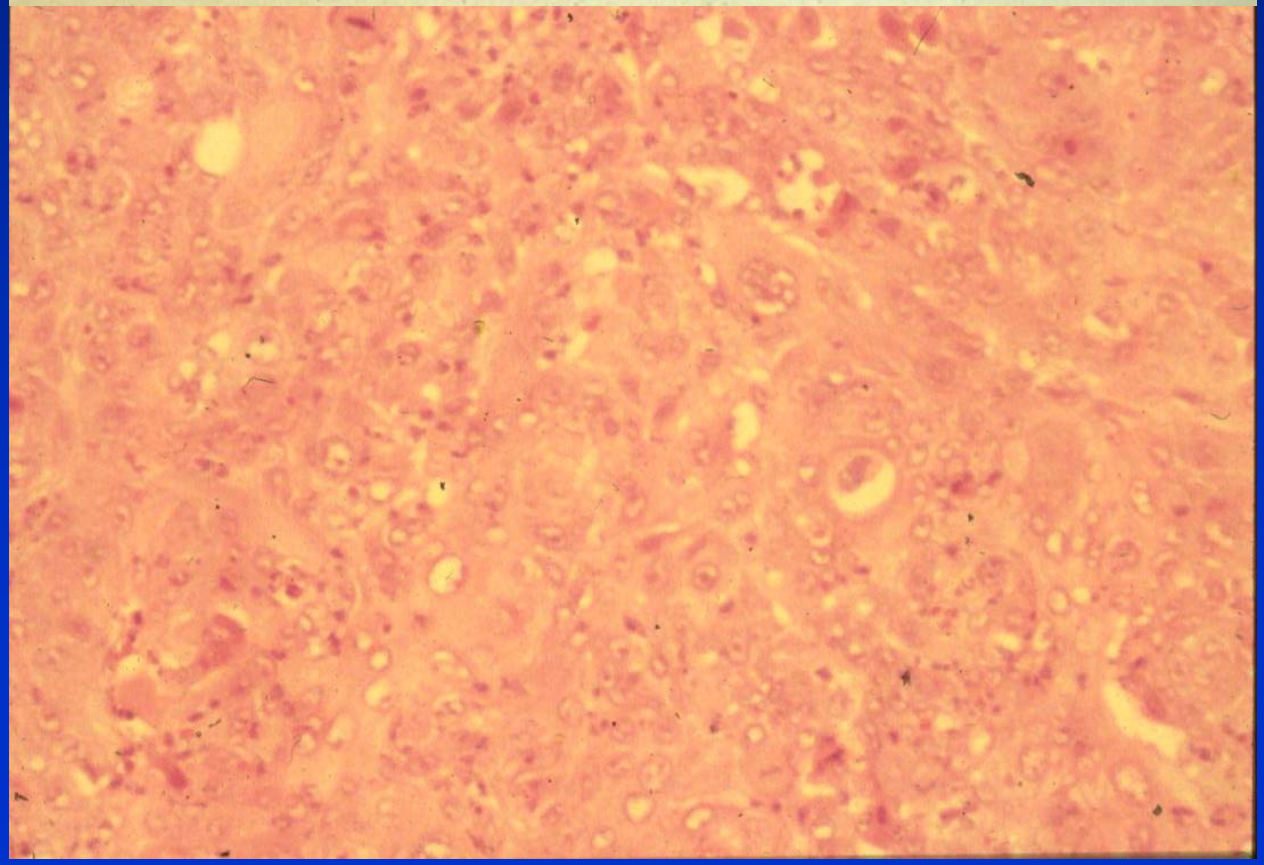
UICC chrysotiles
4 µg/cm^2 for 7 days

Saturation density
Terminal differentiation altered
Growth in soft agar
Chromosomal aberrations
Tumorigenicity

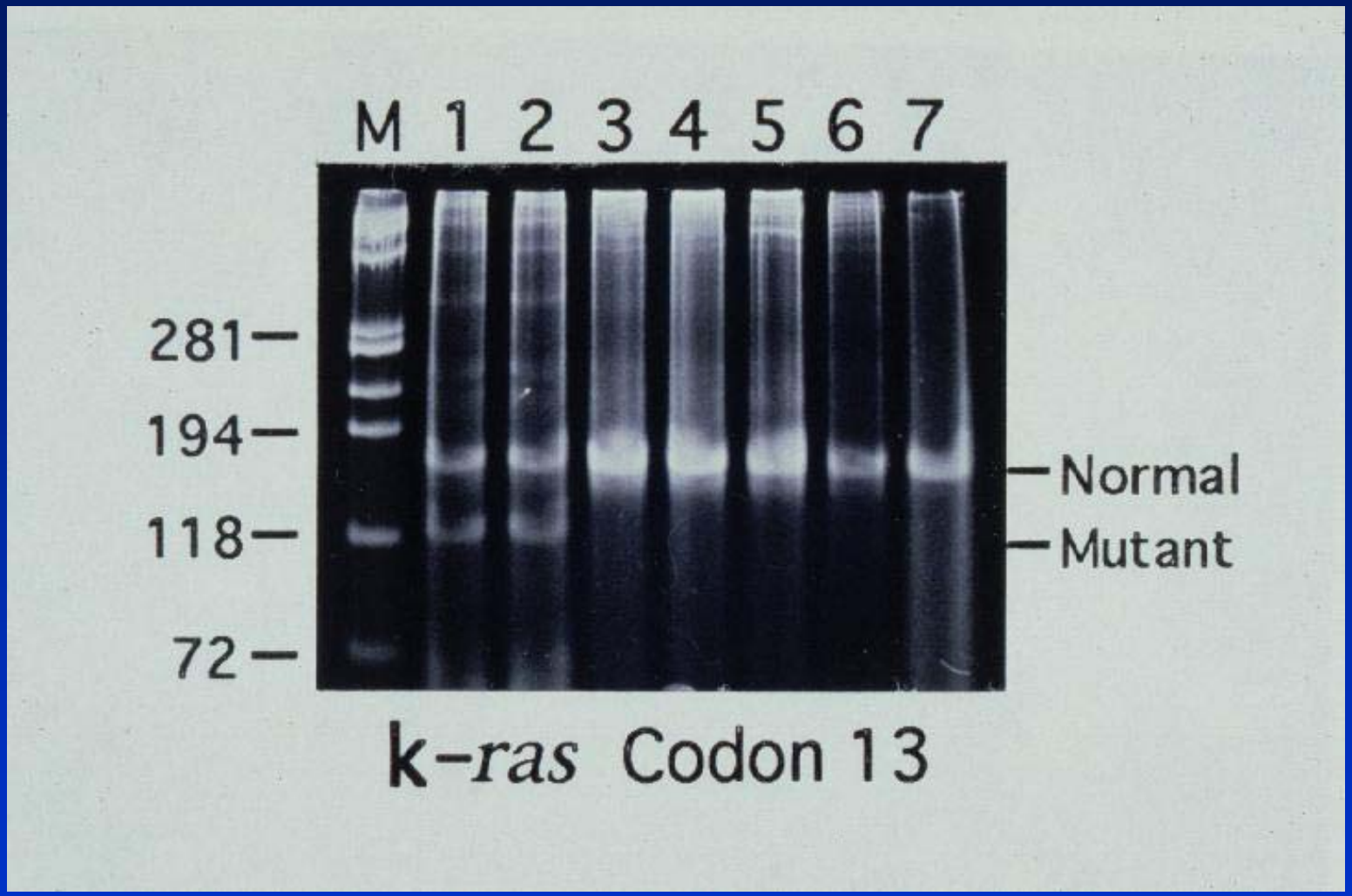
No mutation in any *Ras* oncogenes
Mutant p53 expressed
Cyclin D1 overexpressed
Telomerase activity increased
↓ regulation of *Big-h3*& *p21^{Cip1}* gene

Nude mice bearing BEP2D tumors

H & E stain of tumor showing carcinoma: consistent with their epithelial cell origin

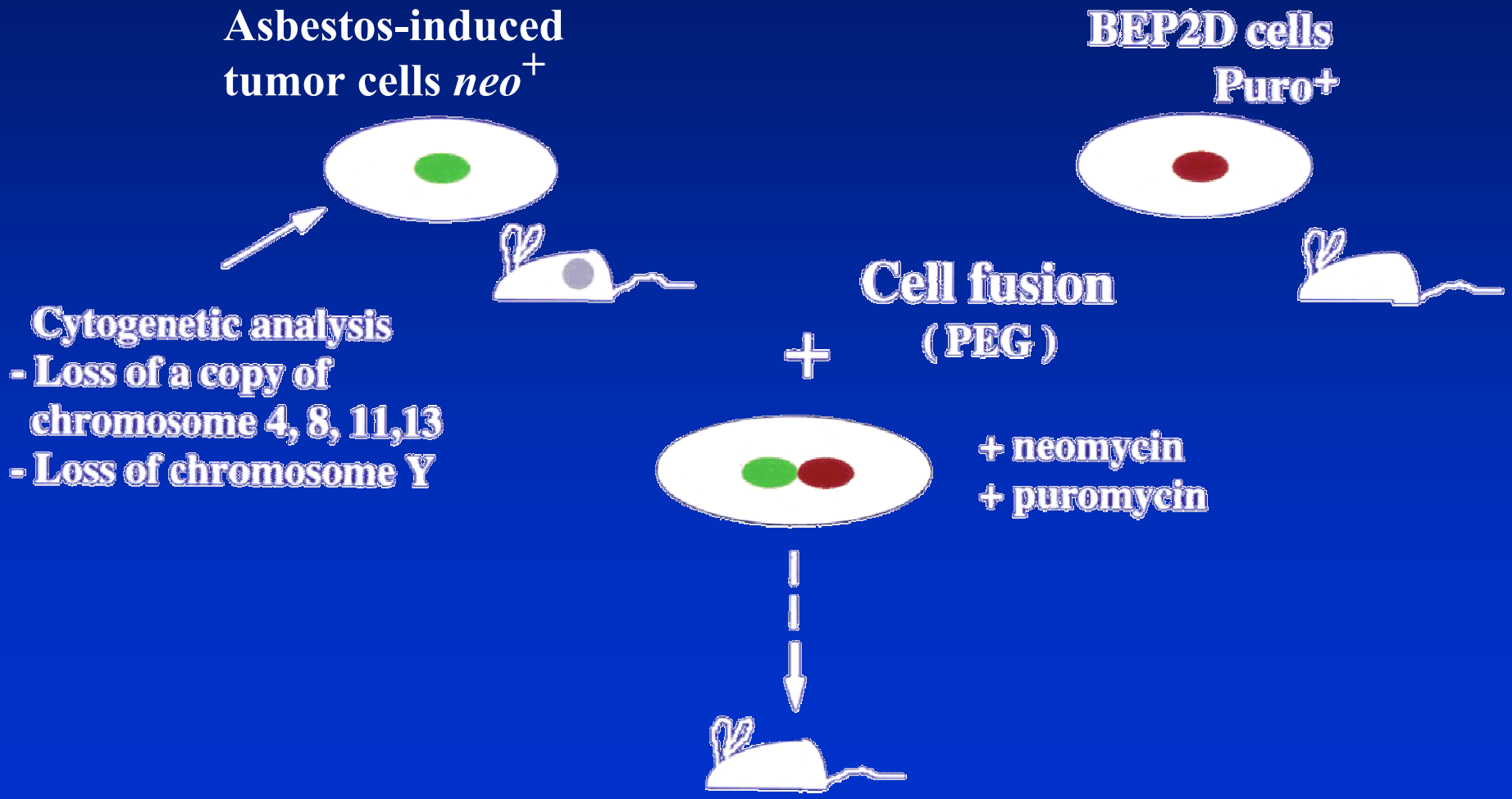


Detection of ras mutations in asbestos induced tumorigenic lung cells



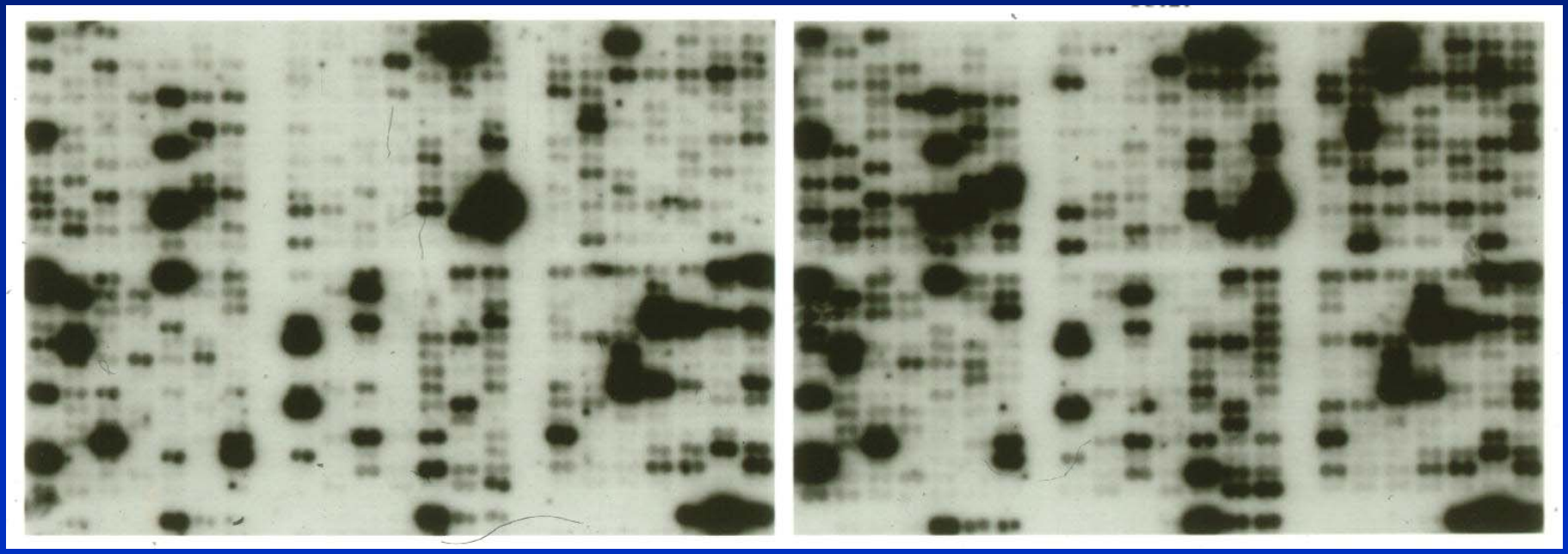
From Hei *et al.*, *Environ. Hlth. Persp.* **105**: 1085, 1997; *Oncogene* **20**: 7301, 2001

Suppression of malignancy



BEP2D

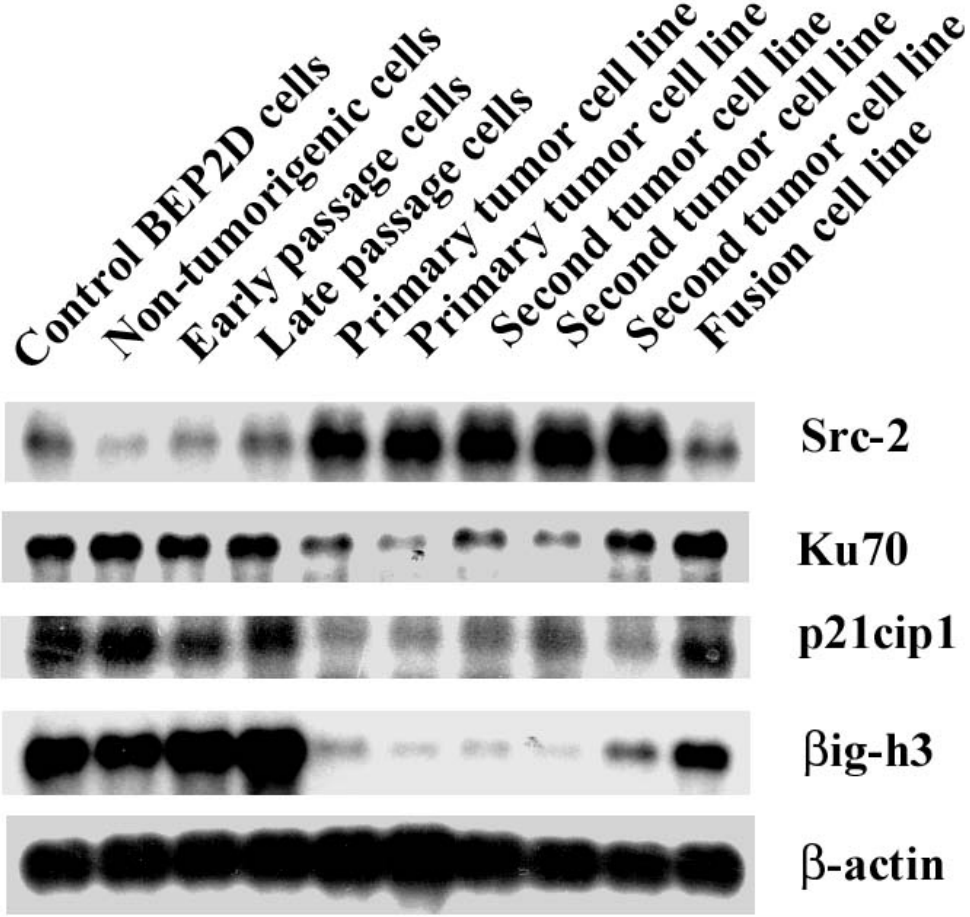
Tumor



List of differentially expressed genes between tumorigenic and control BEP2D cells based on mRNA values

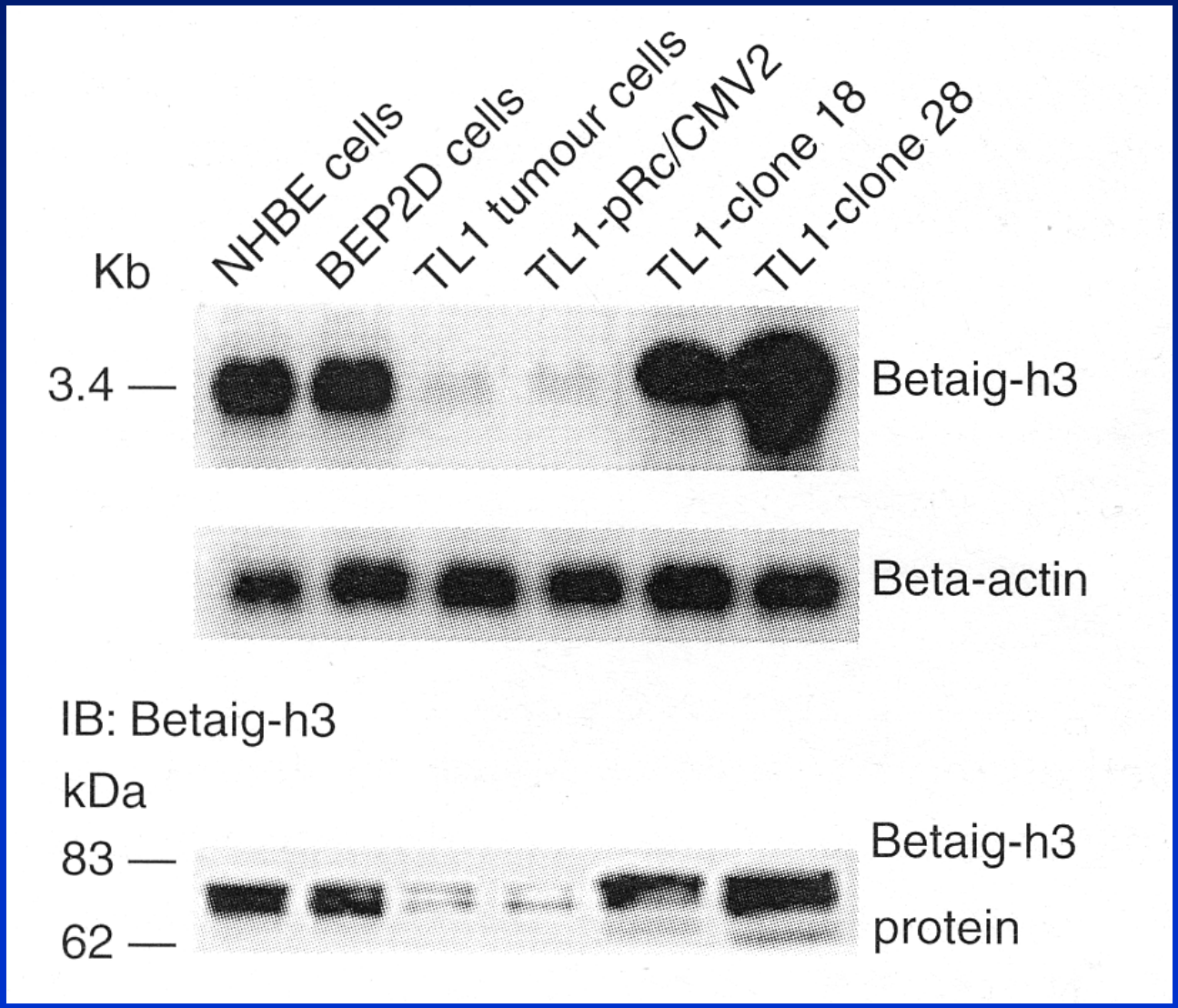
DCC	**↓ 2.9 ± 0.6**
***DNA-PK / KU* 70**	**↓ 2.4 ± 0.3**
BigH3	**↓ 7.5 ± 0.5**
HSP 70	**↓ 2.6 ± 0.5**
Cytokeratin 14	**↓ 3.0 ± 0.4**
***DNKN1A* ($p21^{C1P1}$)**	**↓ 2.7 ± 0.3**
***CDC 25B* (M-phase inducer)**	**↓ 2.6 ± 0.5**
c-*fos*	**↑ 4.8 ± 1.5**
NFκβ	**↑ 3.8 ± 0.4**
Insulin receptor pathway	
Insulin receptor	**↑ 2.2 ± 0.3**
Grb2	**↑ 1.9 ± 0.1**
Shb2	**↑ 3.7 ± 0.4**
ErK-2	**↑ 2.3 ± 0.2**

Differentially expressed genes in tumorigenic human bronchial epithelial cells induced by chrysotiles

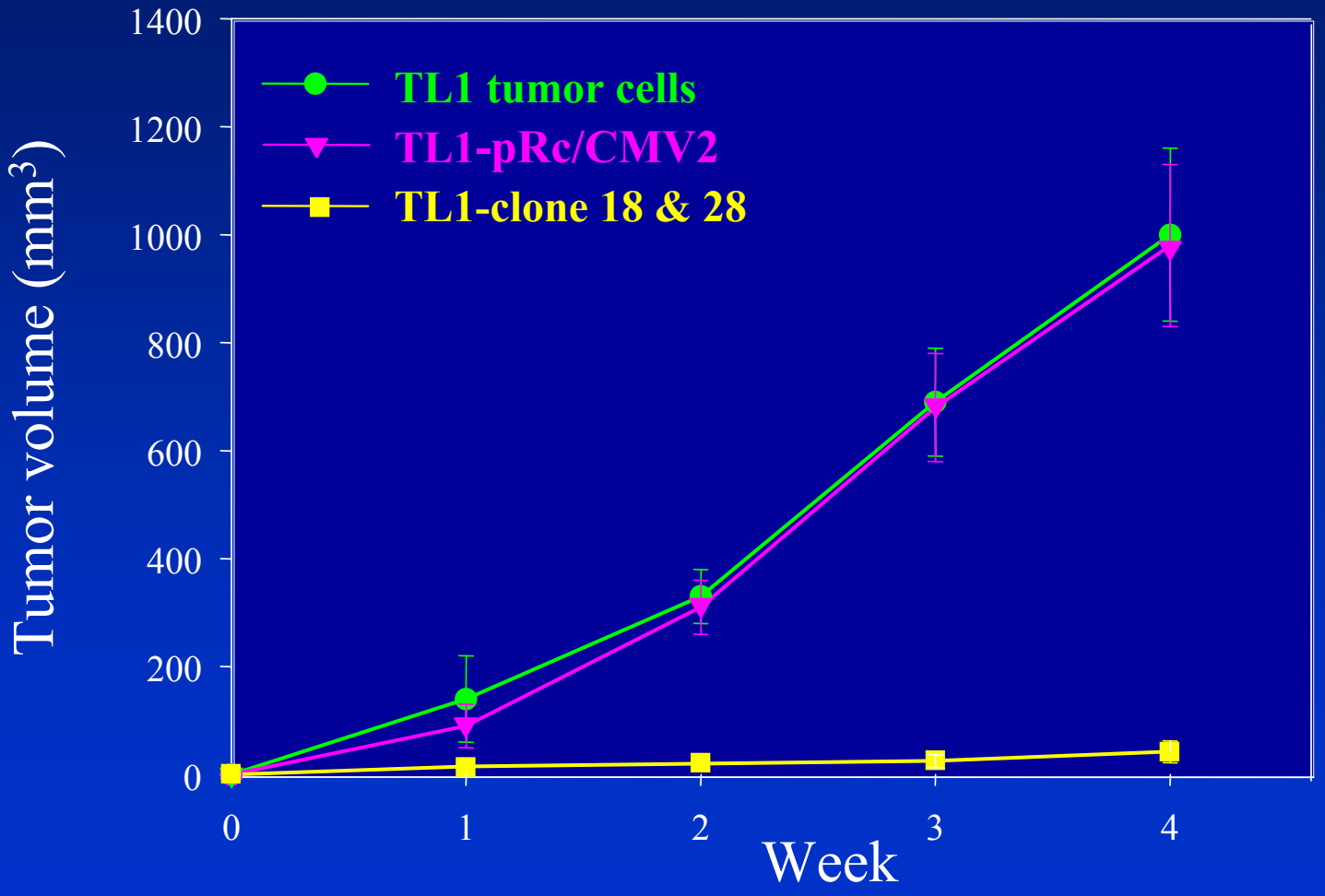


Zhao *et al. Carcinogenesis*, **21**:2005, 2000
Piao *et al. Oncogene*, **20**:7301, 2001
Zhao *et al. Oncogene* **21**: 7471, 2002

mRNA (top) and protein levels (bottom) of *Big-h3* gene in normal NHBE, control BEP2D, TL1 tumor cells and *Big-h3* -transfected tumor cells



Inhibition of tumor growth by *Betaig-h3* transfection relative to vector alone and parental TL1 tumor cells



From Zhao *et al.*, *Oncogene* 21: 7471, 2002

S U M M A R Y

- **Asbestos is genotoxic to mammalian cells based on several predicative endpoints;**
- **Reactive oxygen species plays an important role in mediating this effect;**
- **Carcinogenicity of fibers/particles is a complex interplay of many factors:** **Dose,**
  Fiber characteristic
  Fiber-cell interaction
  Cell and tissue response to foreign particles
- **Tissue inflammation is a necessary but insufficient condition for asbestos carcinogenesis**
- ***Big-H3* gene appears to be causally-linked to the carcinogenic mechanisms of asbestos fibers.**

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