

# Patient/subject biomonitoring for drug-induced genetic damage: has it's time come?



**November 6, 2007**

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**Office of New Drugs**

**Center for Drug Evaluation and Research**

**Food and Drug Administration**



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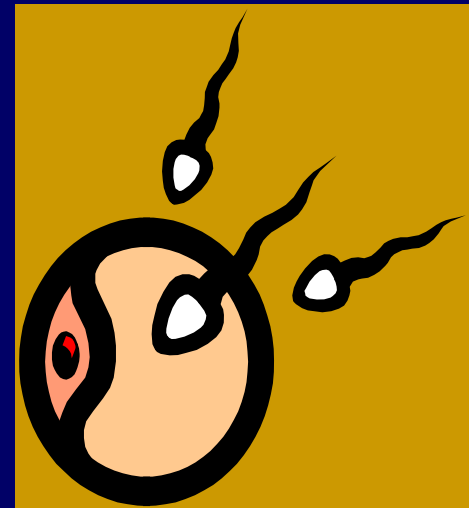
# Mutations in Somatic Cells

- Cancer
- Heart disease
- Aging



# Mutations in Germinal Cells

- Genetic diseases  
eg. achondroplasia



# Basis for belief that chemicals and drugs can induce heritable mutations in human germ cells

- Chemicals and drugs can induce somatic mutations in human cells *in vivo*.
- Chemicals and drugs have been shown to induce heritable, germ-line mutations in experimental animals, primarily the mouse.
- Spontaneous germ-line mutations are seen in humans
- Therefore, it is reasonable to assume that chemicals and drugs can induce germ-line mutations in humans.



# Why does FDA not concern itself with potential for drug-induced germ cell mutations?

- **There is no smoking gun. No agent has ever been shown to have induced a germ line mutation in a human being.**

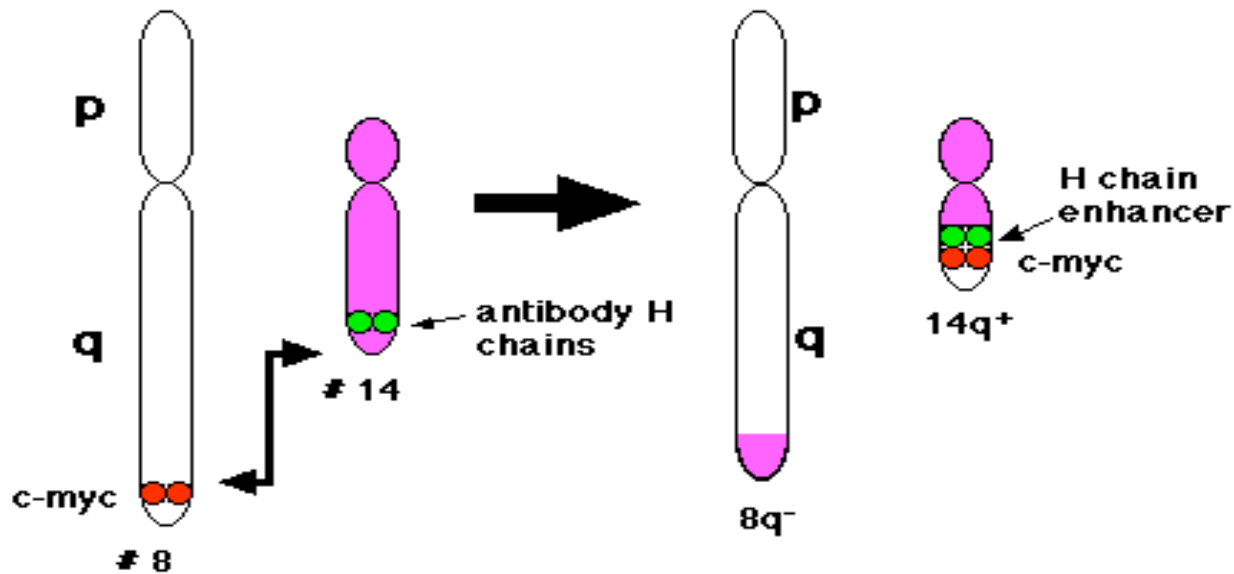


# Mechanisms of Activation/Inactivation of Cancer-Associated Genes

- Point Mutations
- Chromosomal Deletions
- Chromosomal Translocations
- Gene Amplification



# Induction of Burkitt's Lymphoma by chromosomal translocation





8q<sup>-</sup>

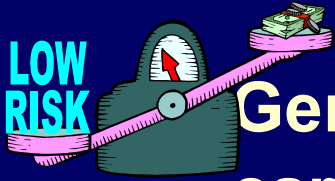


14q<sup>+</sup>



# CDER view of genotoxicity findings

**LOW  
RISK**



**Genotoxicity is seen only as a predictor of carcinogenicity prior to drug approval, most drugs will undergo carc testing but results not available until NDA submission. Many people (hundreds or thousands) including healthy volunteers will have been exposed to repeated, pharmacologically active drug doses.**

- In the past, reactions to positive findings may differed between clinical review divisions**
- For clinical trials in patients, particularly for a serious indication, positive responses can be acceptable**
- For healthy volunteers, risks must be minimal**



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# Equivocally positive *in vitro* cytogenetics assay can land a program on clinical hold

Treatment ( $\mu\text{g/mL}$ )	S9 Activation	Treatment Time	Mean Mitotic Index	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
						Numerical (%)	Structural (%)
DMSO Test Article	-	4	14.6	0.005	$\pm 0.071$	3.0	0.5
5	-	4	14.7	0.005	$\pm 0.071$	1.5	0.5
30	-	4	14.1	0.000	$\pm 0.000$	2.5	0.0
50	-	4	7.0	0.115	$\pm 0.651$	4.0	6.0**
MMC, 0.2	-	4	12.4	0.200	$\pm 0.530$	3.0	15.0**
DMSO Test Article	+	4	15.2	0.005	$\pm 0.071$	3.5	0.5
20	+	4	13.0	0.010	$\pm 0.100$	2.0	1.0
30	+	4	10.8	0.015	$\pm 0.122$	3.0	1.5
40	+	4	7.1	0.015	$\pm 0.122$	2.5	1.5
CP, 10	+	4	12.2	0.130	$\pm 0.366$	2.5	12.0**

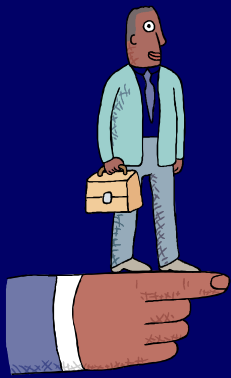




# Regulatory responses to positive genetox results

- In general, single dose studies in volunteers or “healthy patients” would be allowed regardless of genetox data.
- In the past, some divisions have required a negative Syrian Hamster Embryo (SHE) transformation assay or p53 carcinogenicity study before allowing repeat dose clinical studies





# Guidance for Industry and Review Staff:

## Recommended Approaches to Integration of Genetic Toxicology Study Results



Final Published January 2006



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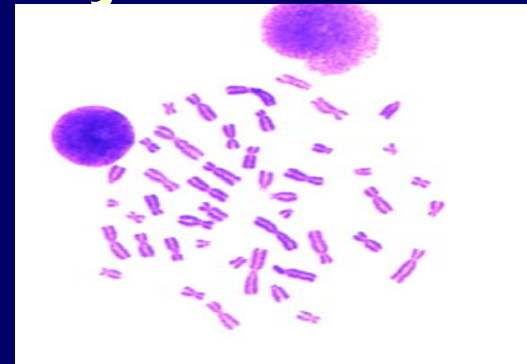
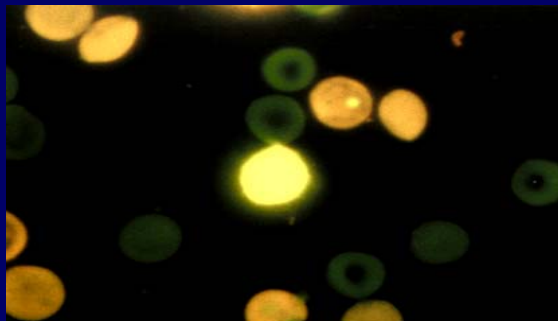
# Potential alternatives to SHE or p53

- Weight of evidence (WOE) approach
- Mechanism of action (MOA)
- Additional supportive studies bearing on WOE



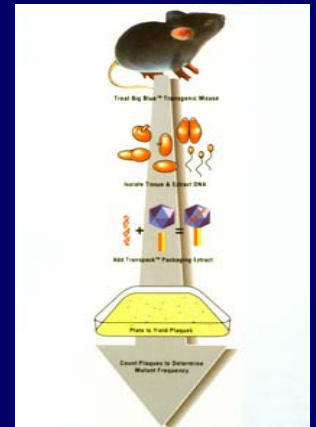
# Additional testing to support WOE

- **Genotoxicity markers from longer-term, repeat-dose studies**
  - ◆ **micronucleated normochromatic erythrocytes from mouse peripheral blood**
  - ◆ **standard or FISH metaphase analysis of cultured peripheral blood lymphocytes from rat or monkey, FISH analysis of BM**



# Additional testing to support WOE

- Complete fourth test in standard battery
- DNA adducts
- Comet assay
- Transgenic mutation assay
- Cell transformation assays
- Short-term carcinogenicity studies
- Patient monitoring



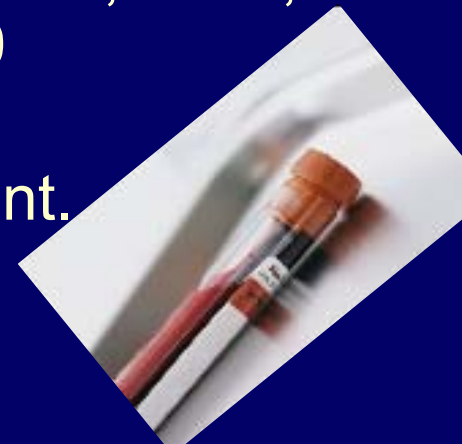
# Endpoints frequently monitored in human subjects for genetic damage primarily in PBL

- Chromosomal aberrations
- Micronuclei
- Sister chromatid exchange
- Specific locus mutations, HGPRT
- DNA adducts
- Changes in gene expression

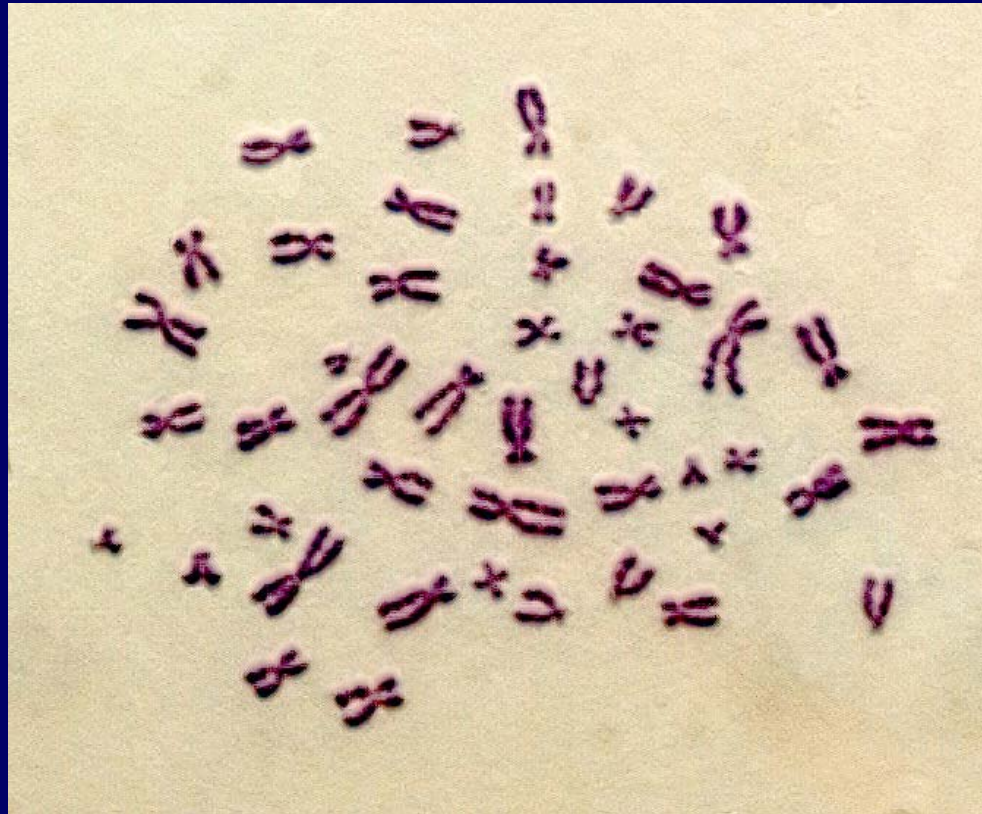


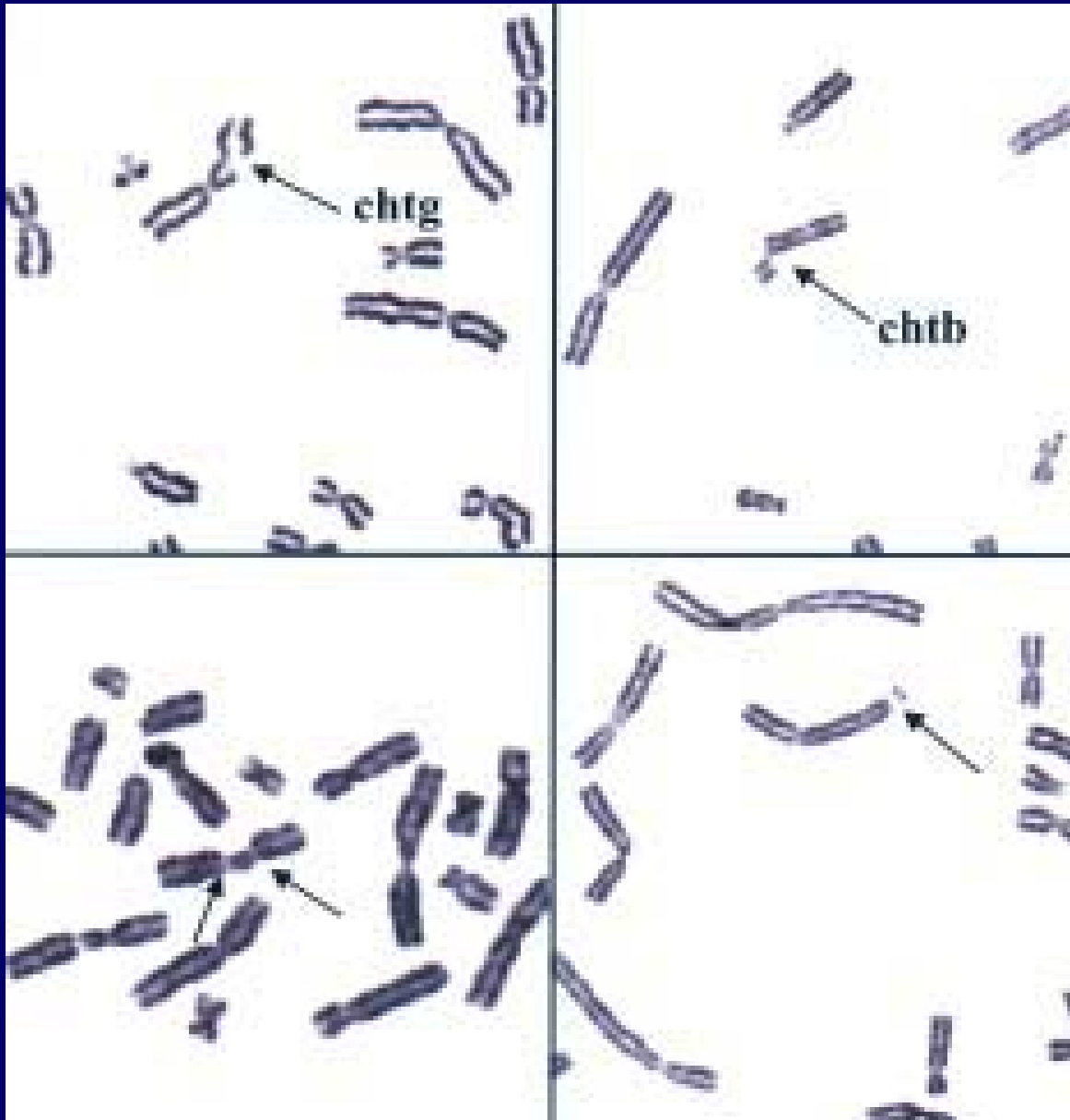
# Methodologies for metaphase analysis are relatively straightforward

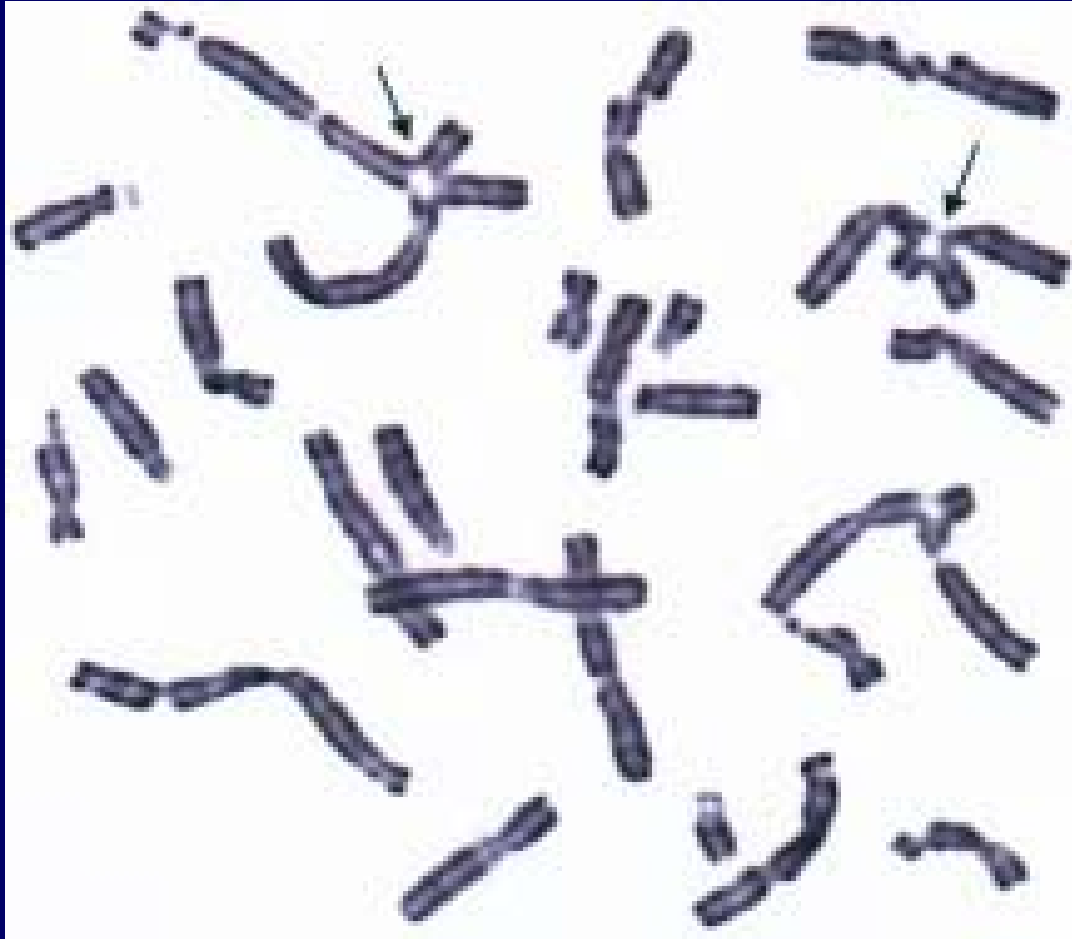
- Small quantity (2cc) of whole blood taken prior to exposure.
- Cells cultured in the presence of PHA for 48 hours.
- Cells arrested in metaphase using a 2-hour block with Colcemid.
- Cells are centrifuged, swollen in hypotonic KCl, fixed in methanol:acetic acid, dropped onto glass slides, dried, stained, mounted and scored (generally 100 metaphases).
- Process repeated after exposure to test agent.



# Typical human metaphase cell







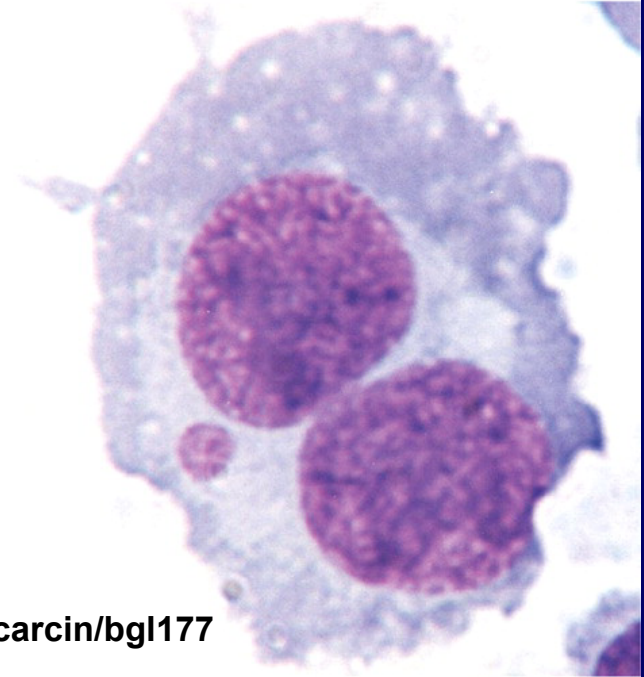
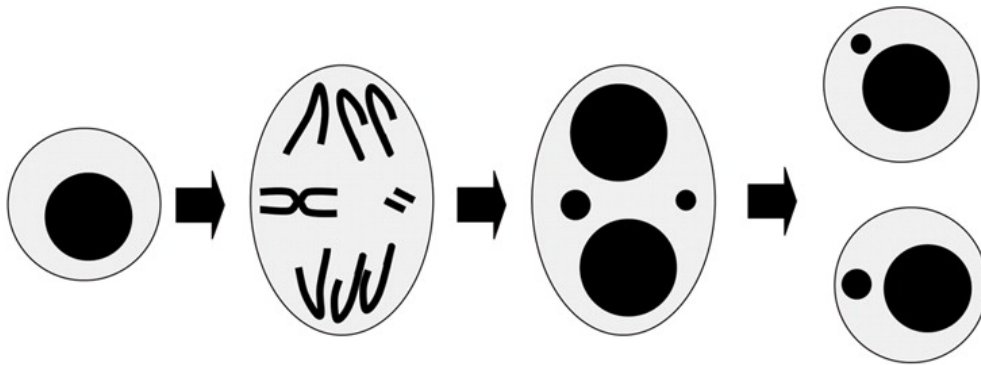
# Methodologies for micronucleus analysis are also straightforward

- Methods similar to those used for metaphase analysis.
- Instead of colcemid, cells are treated with cytochalasin B for 24 hours. Blocks cytokinesis so that cells that have replicated their DNA can be identified.
- 500 binucleated cells scored



# Schematic diagram showing the origin of MN from either a lagging chromosome fragment or a whole chromosome

Micronucleus formation - Chromosome breakage or loss



Bonassi, S. et al. Carcinogenesis 2007 28:625-631; doi:10.1093/carcin/bgl177

A)

B)



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Carcinogenesis

# Selected agents found to induce chromosome damage in human subjects

- Ionizing radiation
- Viral infection
- Smoking
- Cytotoxic cancer chemotherapy, eg. Cytoxan, adriamycin, both pts and handlers
- Ethylene oxide
- Heavy metals
- Pesticides
- Solvents
- Polyaromatic hydrocarbons
- Many others

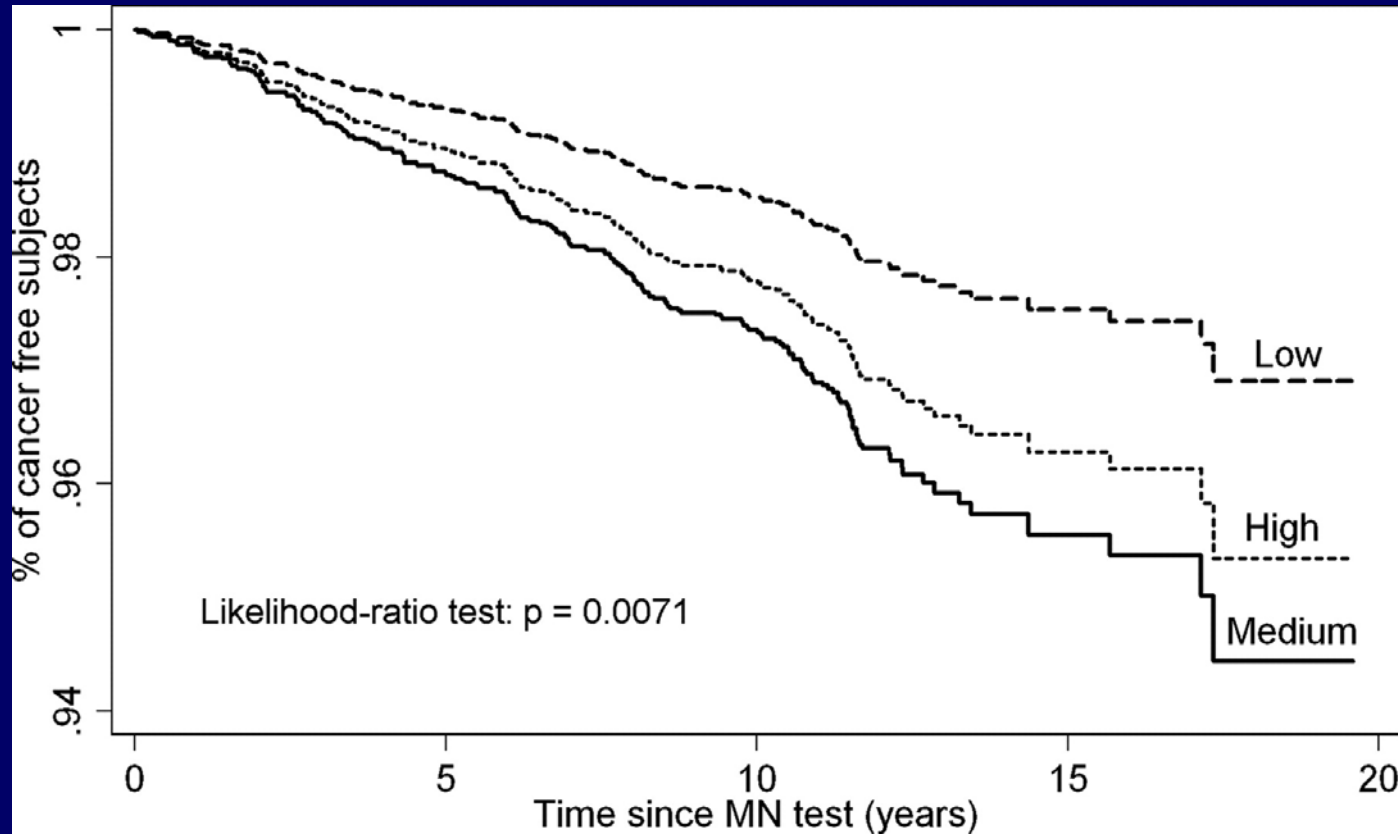


# Background frequencies of chromosomal aberrations and cancer risk

For human populations, background frequencies of chromosomal aberrations are positively correlated with risk for cancer.



## 2 Probability curves of cancer free survival by tertile of MN frequency (pooled data from the HUMN cohort)



Bonassi, S. et al. *Carcinogenesis* 2007 28:625-631; doi:10.1093/carcin/bgl177

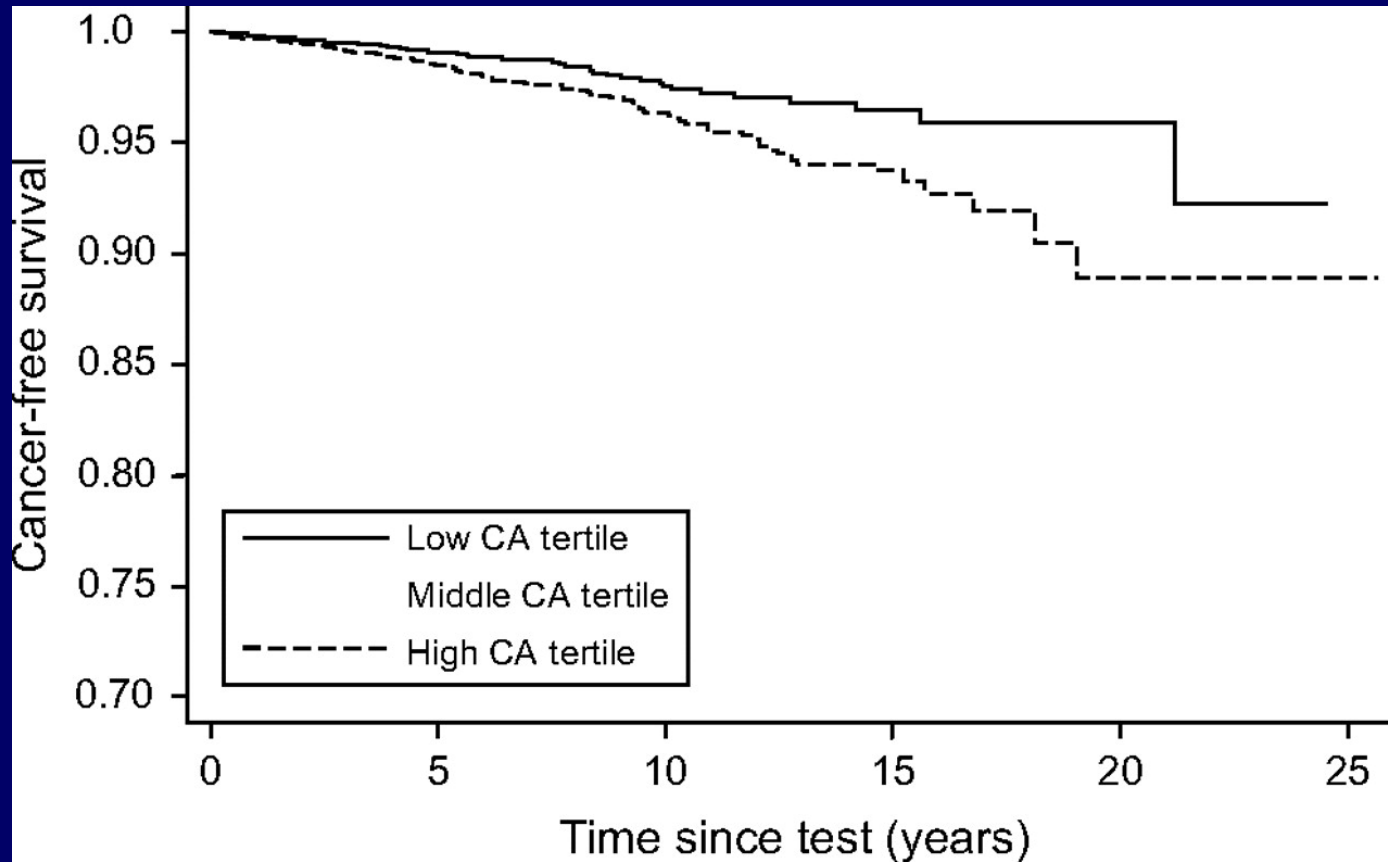


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Carcinogenesis

# Kaplan-Meier curve for total cancer incidence by frequency of chromosomal aberrations, Central Europe, 1978-2002



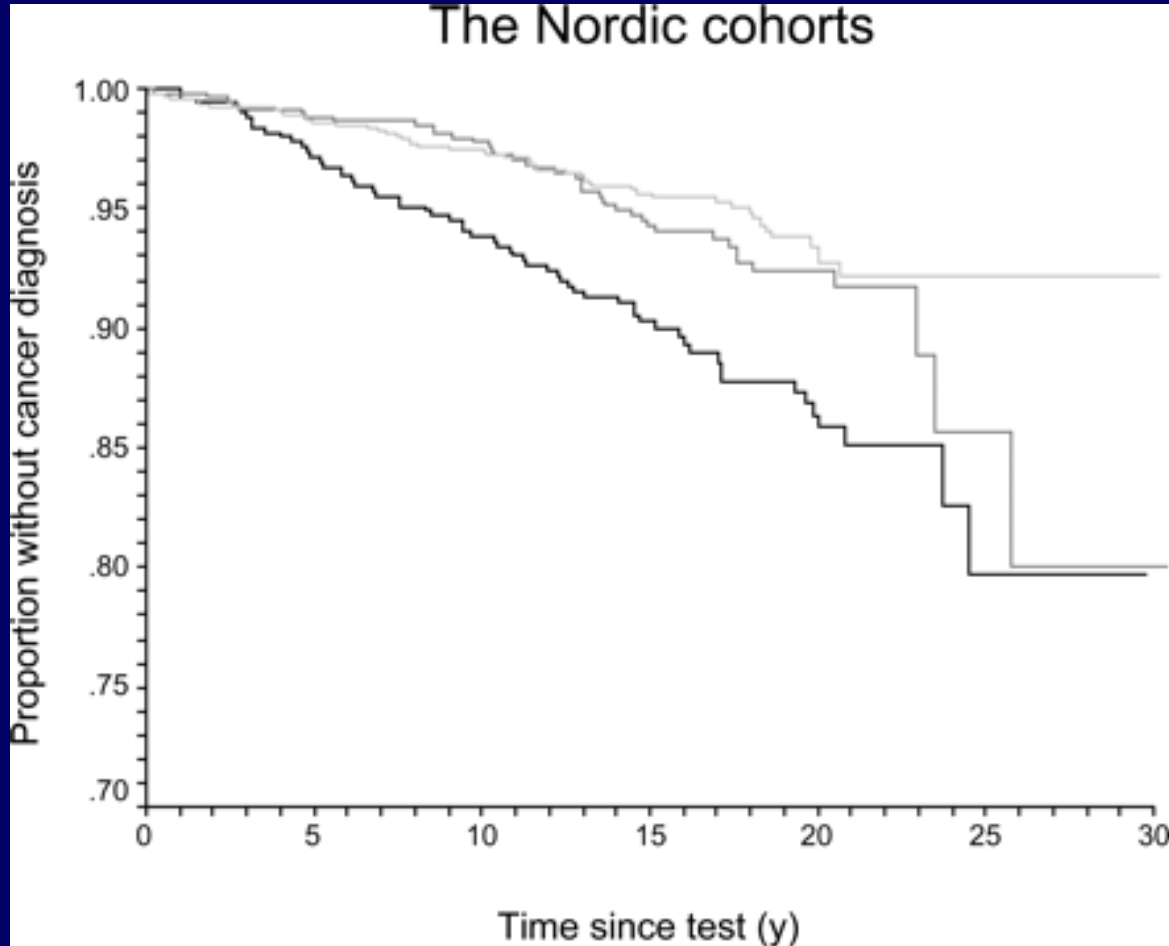
Boffetta, P. et al. *Am. J. Epidemiol.* 2007 165:36-43; doi:10.1093/aje/kwj367



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American Journal of  
**EPIDEMIOLOGY**



Kaplan-Meier curves for total cancer incidence observed in the Nordic cohorts, with respect to the chromosomal aberration results at test (*light gray curve, low; dark gray curve, medium; black curve, high*).



# Measurement of Chromosomal Aberrations, Sister Chromatid Exchange, *hprt* Mutations, and DNA Adducts in Peripheral Lymphocytes of Human Populations at Increased Risk for Cancer

by David Jacobson-Kram,<sup>1,6</sup> Richard J. Albertini,<sup>2</sup>  
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Ken Kolodner,<sup>4</sup> Saou-Hsing Liou,<sup>4</sup> Melissa A.  
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Paul T. Strickland,<sup>4</sup> Jerry R. Williams,<sup>6</sup> and  
Shuqin Xiao<sup>4</sup>



# Populations examined



- Cancer patients receiving radioimmunoglobulin therapy
- Cancer patients receiving chemotherapy
- Pharmacists preparing chemotherapy prescriptions
- U.S. Army troops stationed in Kuwait.



# Use versus abuse



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Cancer Letters xx (2005) 1–8

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## Cytogenetic effects in children treated with methylphenidate

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Received 22 November 2004; received in revised form 6 January 2005; accepted 10 January 2005



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# Study design

- Examined three endpoints in 12 children diagnosed with ADHD. Blood drawn before and after 3 month treatment with methylphenidate.
  - ◆ Sister chromatid exchange
  - ◆ Chromosomal aberrations
  - ◆ Micronuclei
- Therapeutic doses were 20 to 54 mg/day

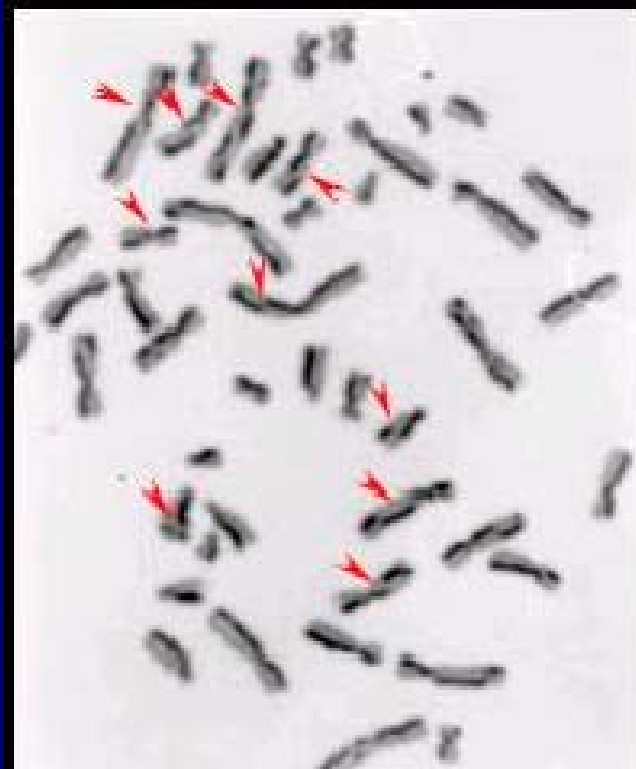


# What are sister chromatid exchanges (SCE)?

- Reciprocal exchanges of chromatid arms visualized in metaphase cells that have undergone two rounds of DNA replication in the presence of the nucleotide analogue bromodeoxyuridine.
- While the mechanism of SCE is poorly understood, increases in their frequencies are generally indicative of DNA damage.



# What are sister chromatid exchanges (SCE)?



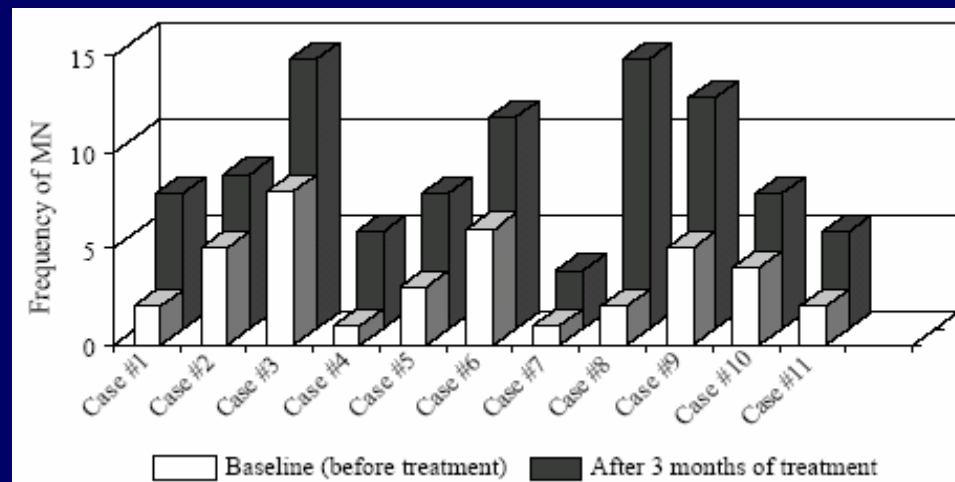
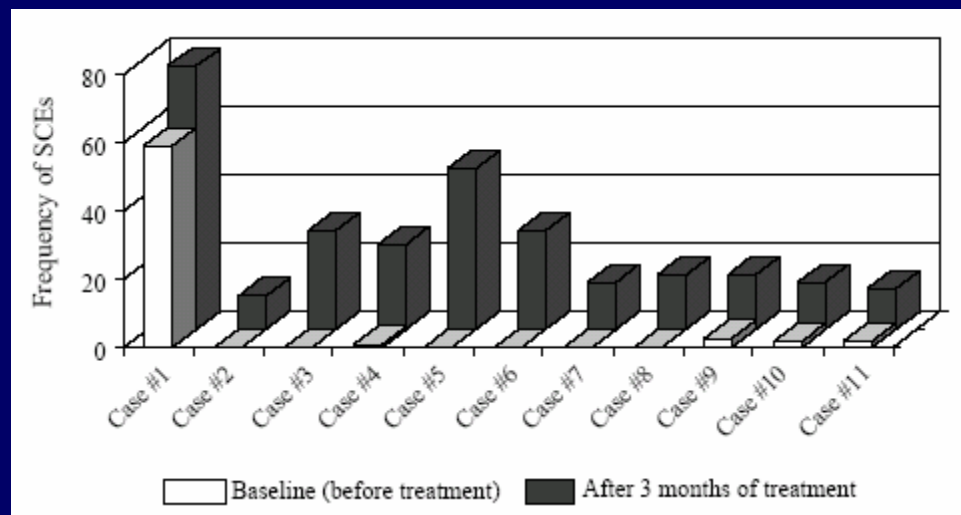
# Data summary from El-Zein et al.

Summary of the cytogenetic data

Endpoint	Mean $\pm$ SEM before treatment	Mean $\pm$ SEM after 3 months of treatment	<i>P</i> -value <sup>a</sup>
CA <sup>b</sup>	1.67 $\pm$ 0.27	5.08 $\pm$ 0.54	0.000
SCEs <sup>c</sup>	6.09 $\pm$ 5.30	26.27 $\pm$ 6.03	0.000
MN <sup>d</sup>	3.55 $\pm$ 0.68	8.46 $\pm$ 1.13	0.000



# Data summary from El-Zein et al.



# Questions Regarding El-Zein Study

## Use of unusual data presentation

- ◆ Aberrations/cell instead of % damaged cells
- ◆ Total SCE in 25 cells
- **Presence of 6 subjects with 0 SCE/cell**
- Investigators agreed to a site visit to discuss study



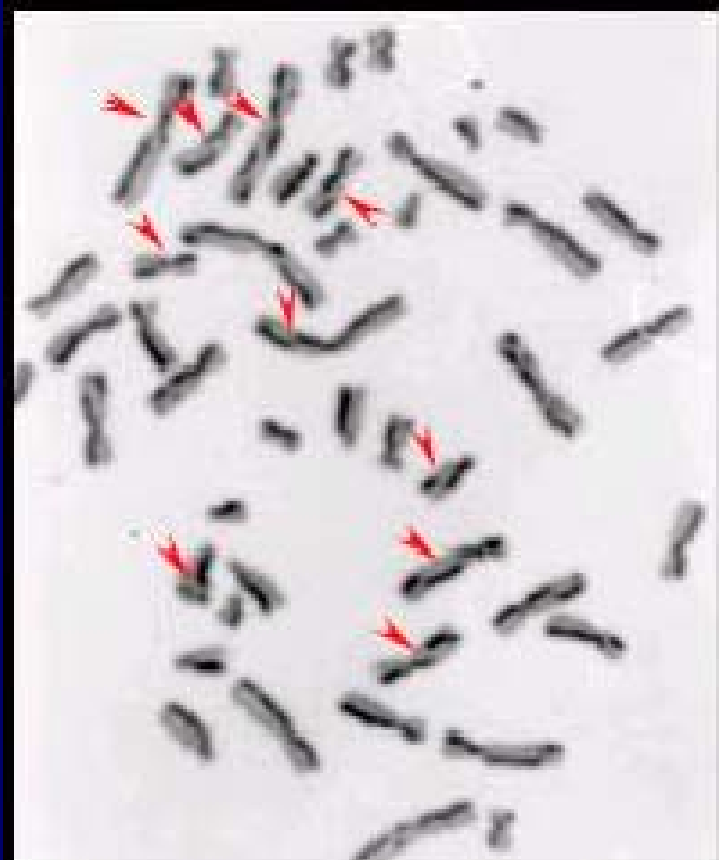
# Site visit to Univ. of Texas

- Representatives from NIEHS, NICHD, FDA and EPA site visited U of T
- Reviewed:
  - ◆ Patient selection
  - ◆ Methods
  - ◆ Raw data
  - ◆ Slide evaluation



# Effect of preparation quality on SCE frequencies

Good preparation



Bad preparation



## Does Methylphenidate Cause a Cytogenetic Effect in Children with Attention Deficit Hyperactivity Disorder?

*Susanne Walitza,<sup>1</sup> Birgit Werner,<sup>2</sup> Marcel Romanos,<sup>1</sup> Andreas Warnke,<sup>1</sup> Manfred Gerlach,<sup>1</sup> and Helga Stopper<sup>2</sup>*

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VOLUME 115 | NUMBER 5 | May 2007 • Environmental Health Perspectives



# Study design

- Prospective study of 38 pediatric patients ages 5 – 17.
- Sampled frequencies of micronucleated PBLs prior to and after treatment for 1, 3 and 6 months of treatment with methylphenidate.
- **No significant treatment related increases in genetic damage.**



# Advantages of monitoring subjects in clinical trials

- Direct information from the most relevant species.
- Knowledge of potential genotoxicity discovered early in relatively small numbers of subjects.
- If positive, duration of exposure is short and increased risk low.



# Problems with human genetic monitoring

- Unlike most other types of toxicities, genetic changes are not reversible.
- What do you tell subjects if drug-related increase is seen in the test population? Risk cannot be quantified.
- What do you tell individual with a drug-unrelated elevated frequency of genetic damage?
- Does the sponsor have an obligation to the study population if positive results are seen?
- What are the sponsors' legal liabilities in these situations?
- Anecdote



