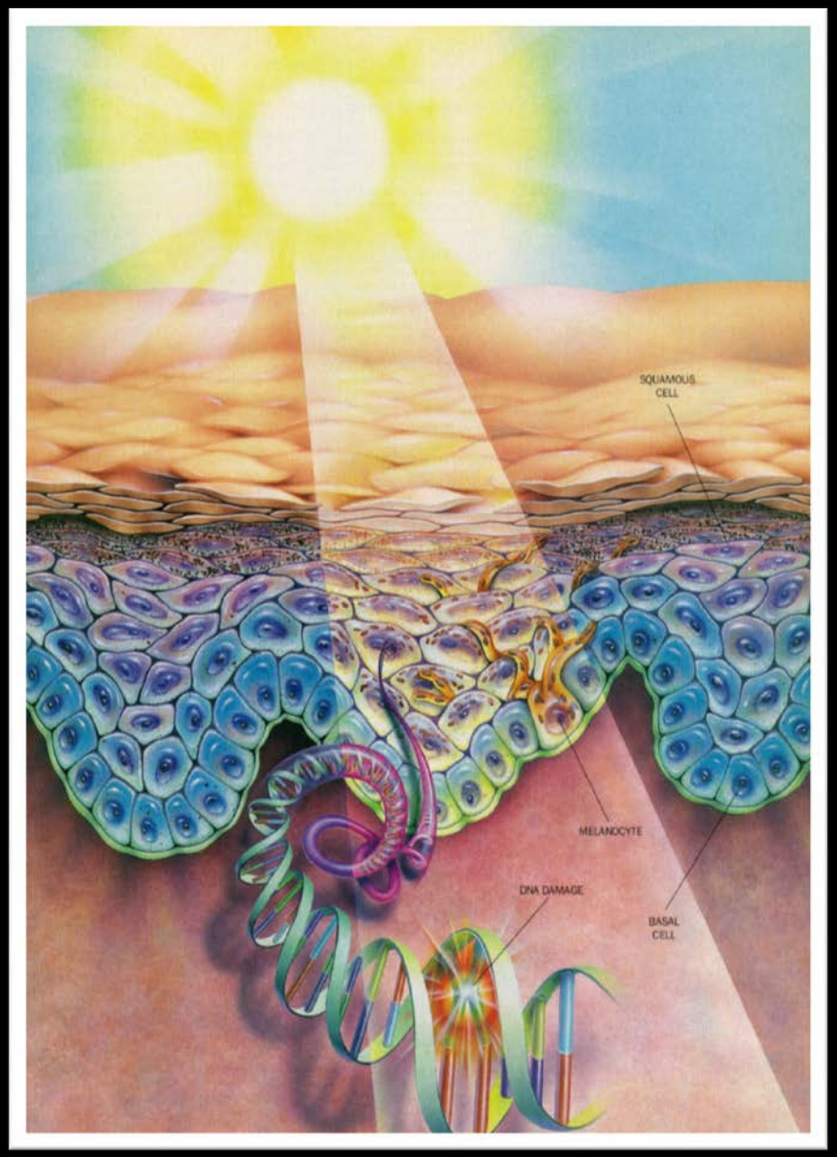


Do the Steps between Genotoxin and Cancer Create Dose or Duration Thresholds?

Douglas Brash
Yale School of Medicine

Outline

Cancer is not just mutations

Are there dose thresholds?
for stress signaling, survival, mutation

Melanoma: dose thresholds & single exposures

Linearity, biphasic dose responses, & thresholds in toxicology

I. Cancer is Not Just Mutations

The mutation conundrum

If a carcinogen's role is to mutate N genes, then: cancer $\propto$ Dose ^{N}

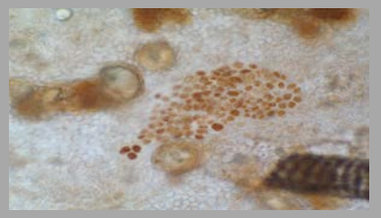
Gene

But human and rodent cancers are:

cancer $\propto$ Dose $^{2\text{-}3}$
Duration $^{5\text{-}6}$

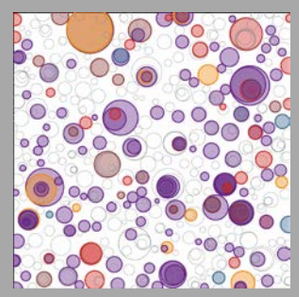
So some biology is happening.

The Cancer-Cell Loop



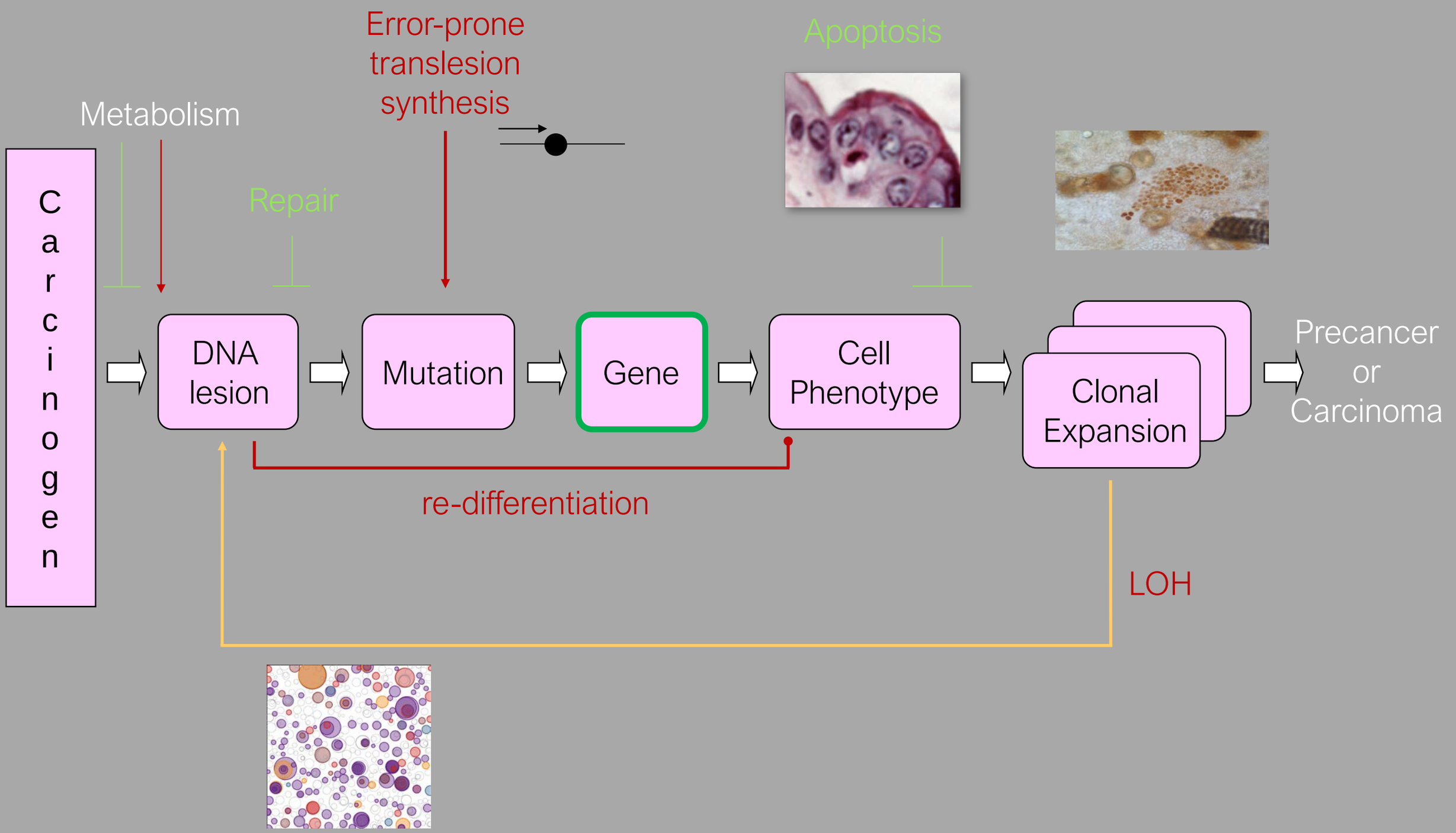
Carcinogen → DNA lesion → Mutation → Gene → Cell Phenotype → Clonal Expansion → Precancer or Carcinoma

10^{-5} - 10^{-3} / gene per cell generation



Ziegler et al, Nature 372:773,1994

Modulators of the cancer-cell loop



Carcinogens are also differentiation agents

Table 1 Inducers and non-inducers for normal cell markers in MGI^+D^+ myeloid leukaemic cells

Type of compound	Inducers	Non-inducers
Peptide hormones	MGI	Erythropoietin, nerve growth factor, insulin, ubiquitin, thymopoietin, interferon
Steroids	Dexamethasone, prednisolone, hydrocortisone, oestradiol	Progesterone, testosterone, epitestosterone, androstenedione, cortisone
Lectins	Concanavalin A, phytohaemagglutinin, pokeweed mitogen	
Polycyclic hydrocarbons	Benzo(a)pyrene, dimethylbenz(*a*)-anthracene	Benz(*a*)anthracene, dibenz(*a*, *c*)anthracene, dibenz(*a*,*h*)anthracene, phenanthrene
Other compounds	Lipopolysaccharide, lipid A, mitomycin C, dimethyl sulphoxide cytosine arabinoside hydroxyurea, thymidine, 5-iododeoxyuridine, 5-bromodeoxyuridine, 5-fluorodeoxyuridine, nitrosoguanidine, actinomycin D, adriamycin, daunomycin, X-irradiation 12-*O*-tetradecanoyl-phorbol-13-acetate	Colchicine, vinblastine, Na butyrate, cycloheximide, dibutyryl cyclic AMP, dibutyryl cyclic GMP, cordycepin, deoxyglucose, ouabain, ionophore 23187

L Sachs, Nature 274:535, 1978

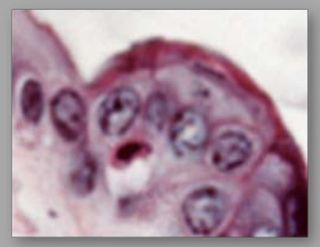
Modulators of the cancer-cell loop

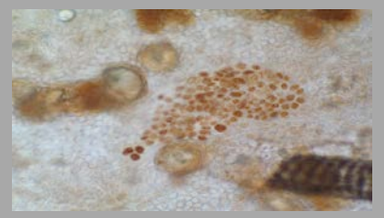
Metabolism

Repair

Error-prone translesion synthesis

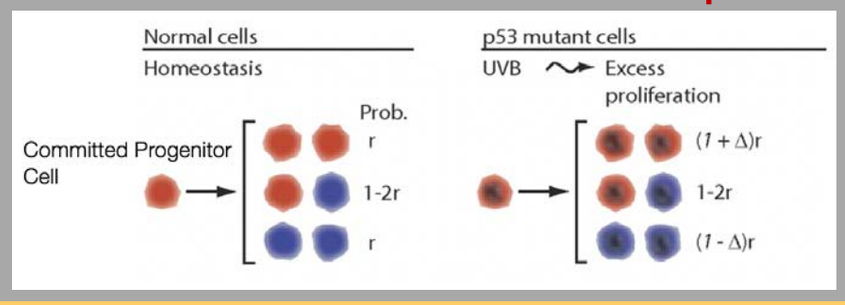
Apoptosis[R]
UV as a selection pressure



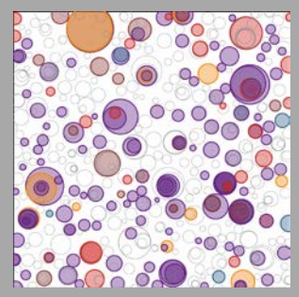


Carcinogen → DNA lesion → Mutation → Gene → Cell Phenotype → Clonal Expansion → Precancer or Carcinoma

Cell-Fate Alteration

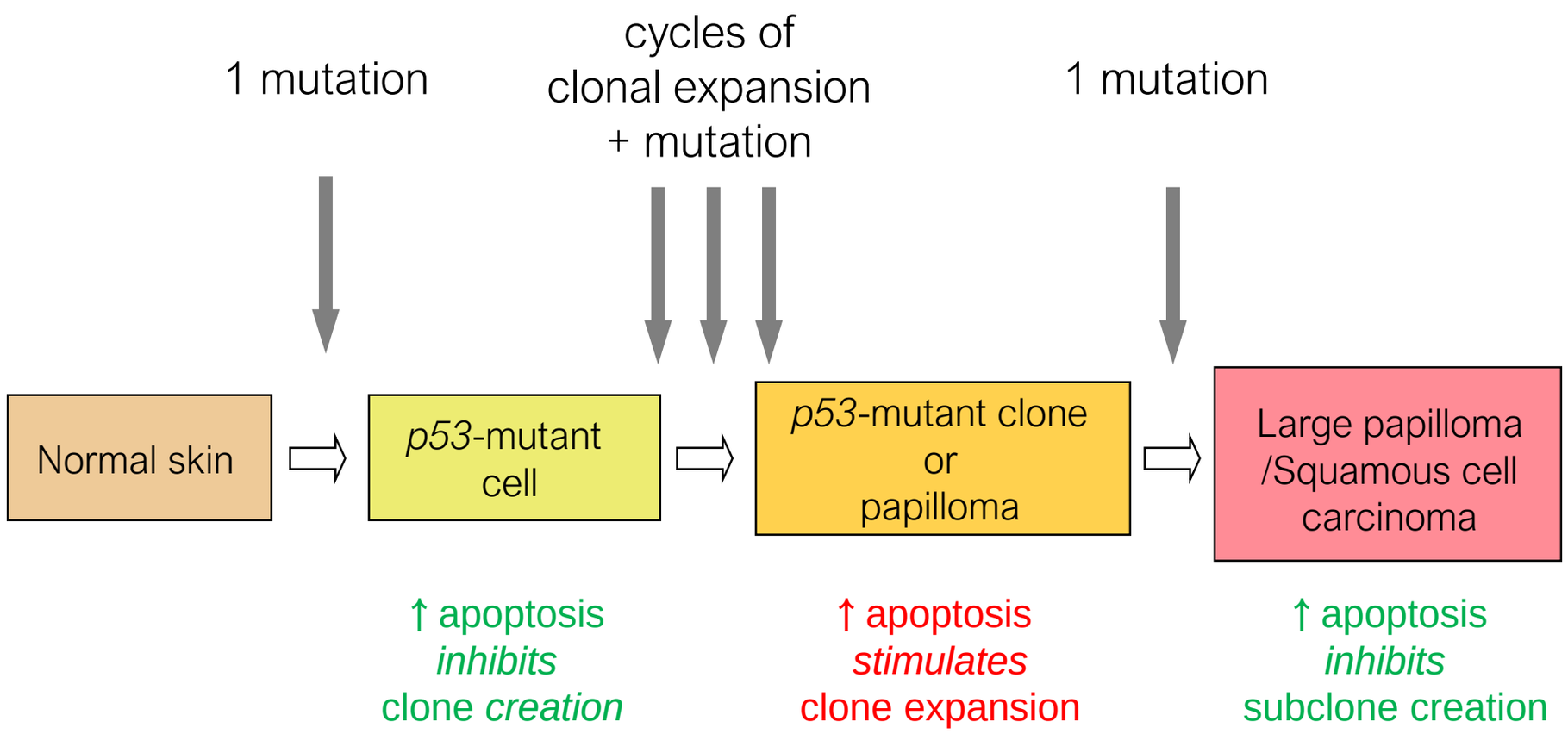


LOH



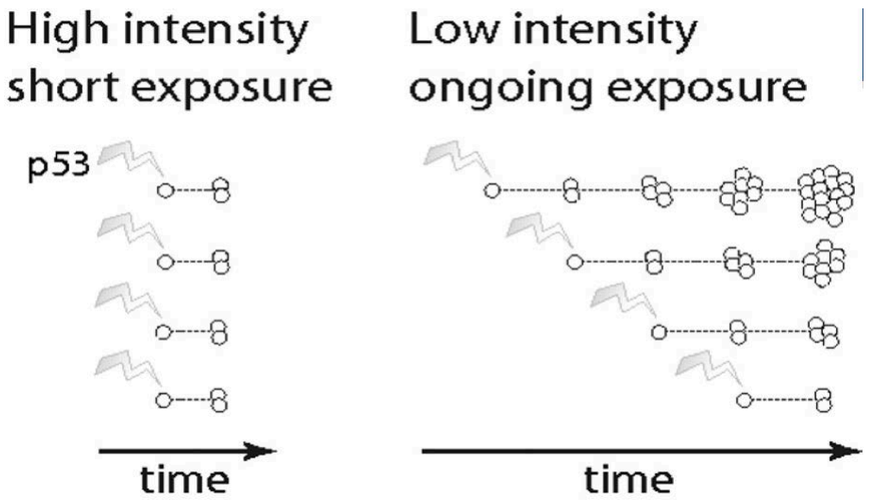
Zhang et al, Carcinogenesis, 26:249, 2005 Klein et al. PNAS 107:270, 2010; Parsons, Mutat Res. 777:1, 2018

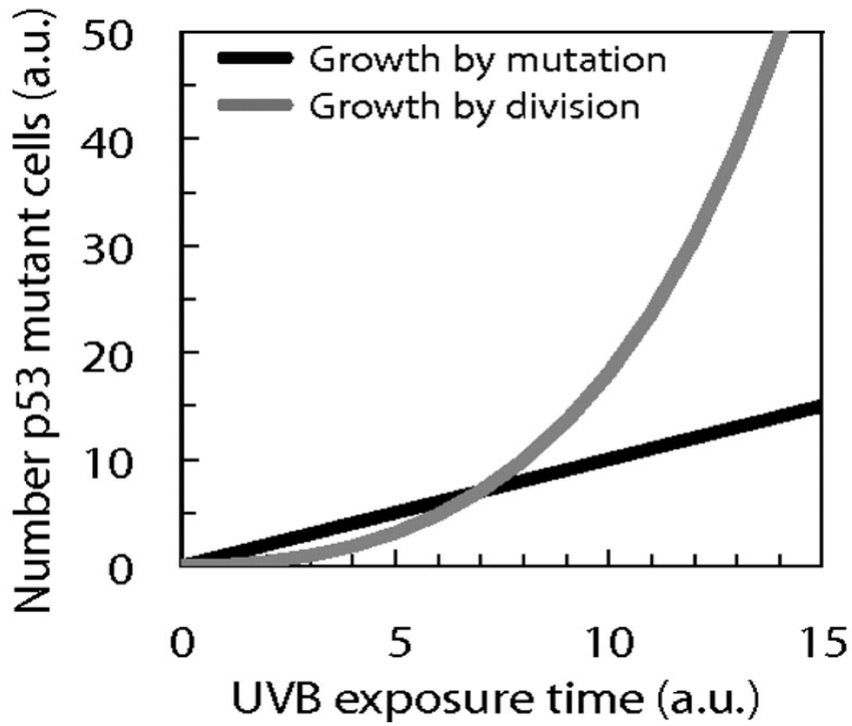
The apoptosis inhibitor *Survivin*:
> steps dependent on mutation
< steps dependent on clonal expansion



Zhang ... Brash, Grossman Carcinogenesis, 26:249, 2005

The importance of exponential growth





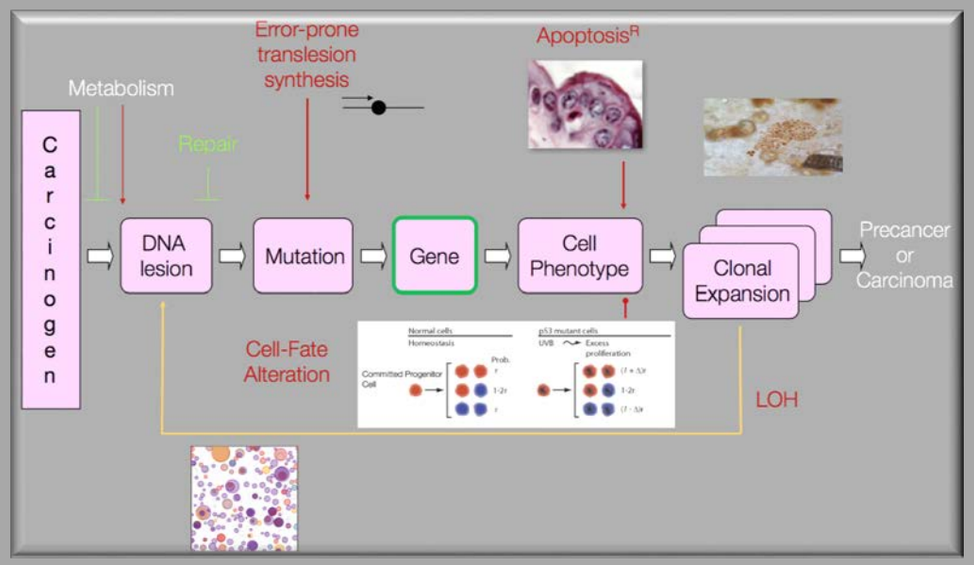
Human & rodent cancers depend weakly on dose & strongly on number of exposures.

Klein et al. PNAS 107:270, 2010

Modulators of the cancer-cell loop

Tumor promoters
Cell proliferation state

Polyclonality of CA



Immune surveillance

Microenvironment
Inflammation
Microbiome

Conclusions 1

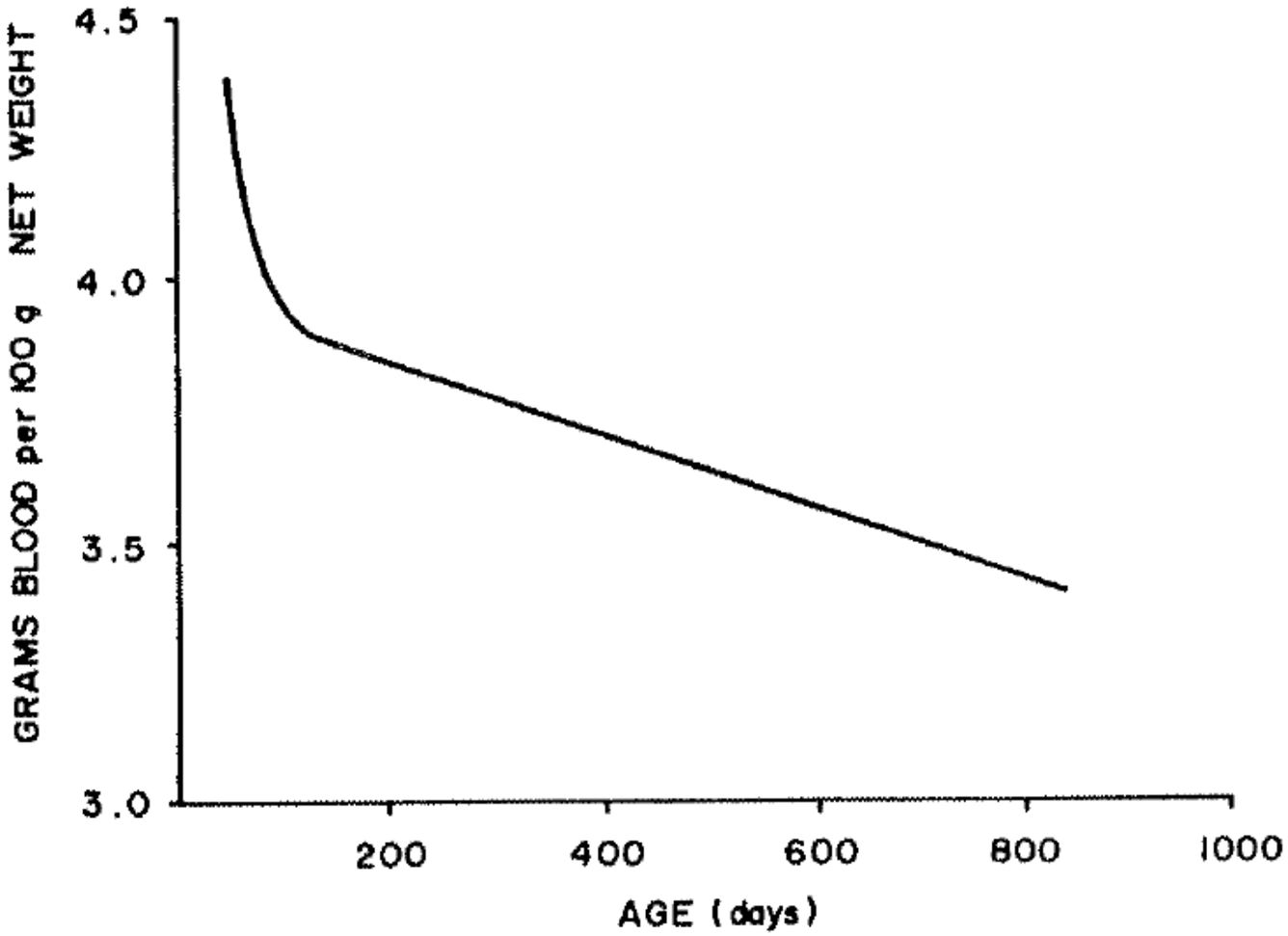
- Biology offers no reason to *expect* a simple dose-response that is linear or even monotonic.

- WB Cannon's homeostasis implies that toxins will often be opposed, giving low-dose regions less impact per dose.

- Homeostasis possibilities:
  - partial
  - complete (gives a threshold)
  - overshoots (ie beneficial)
  - clears out past toxic events
  - Is a matter of experiment.

Homeostasis depends on age

A decline in homeostasis is what characterizes aging.

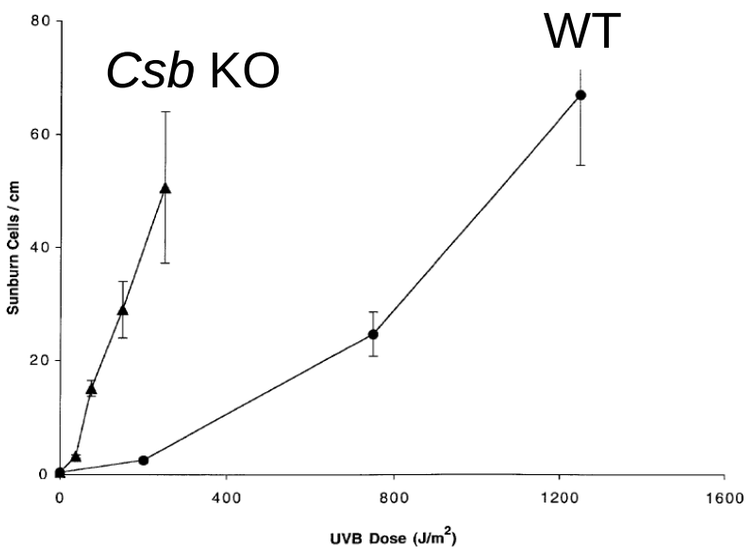


II. *Are* There Dose Thresholds?

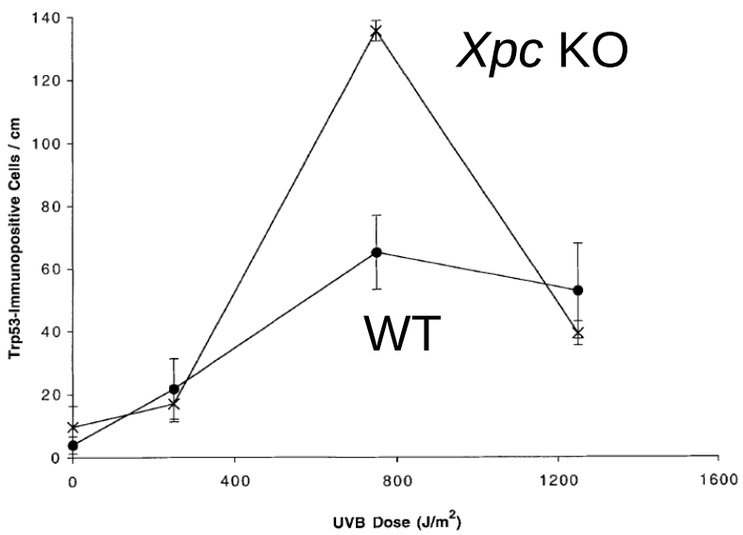
= harmless below a certain dose

(for signaling, survival, mutation)

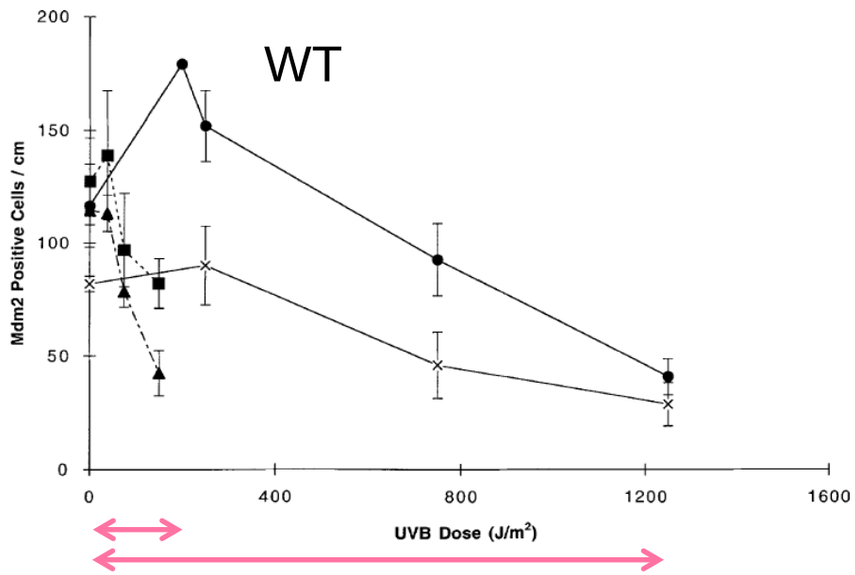
UV signaling dose-responses



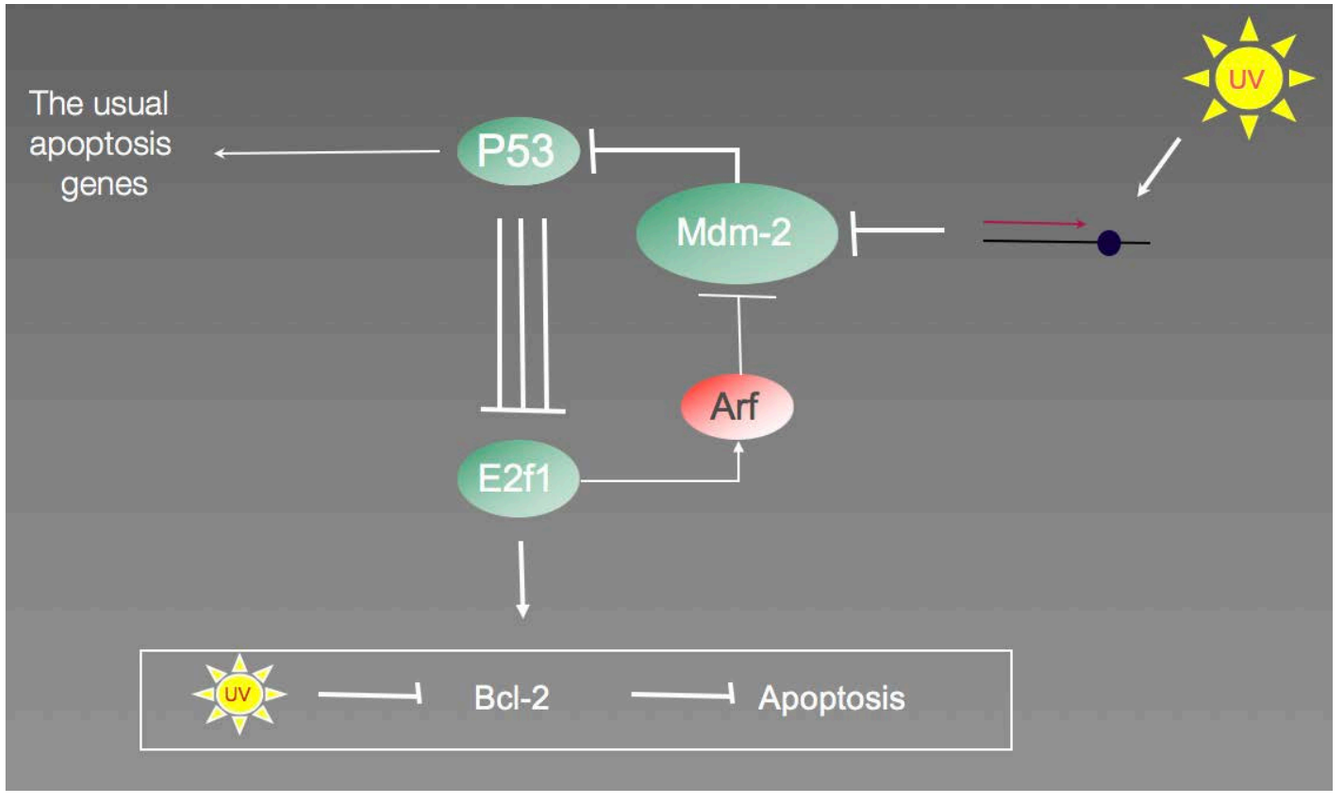
Apoptosis



Trp53 induction



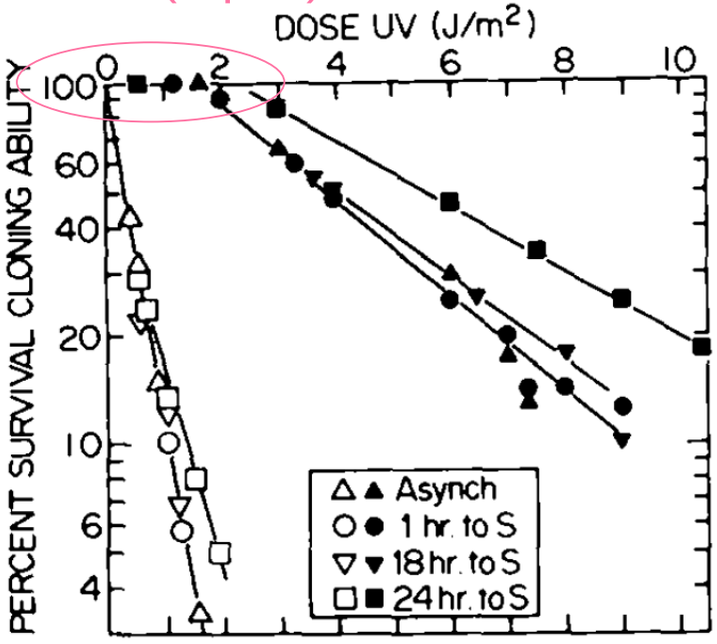
Mdm2 induction



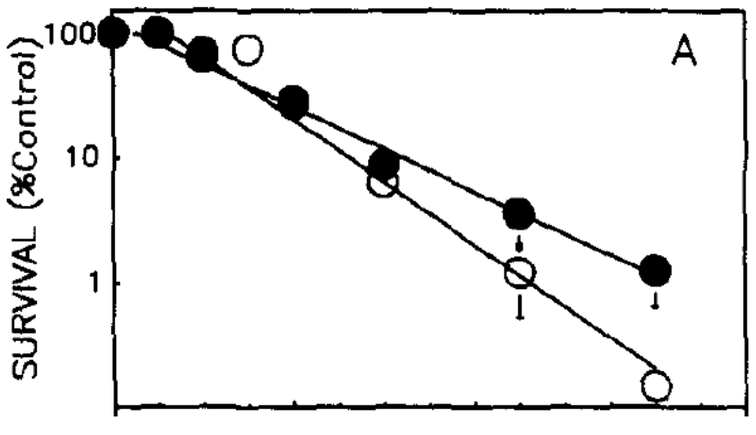
Survival dose-responses

homeostasis (repair) broken cell

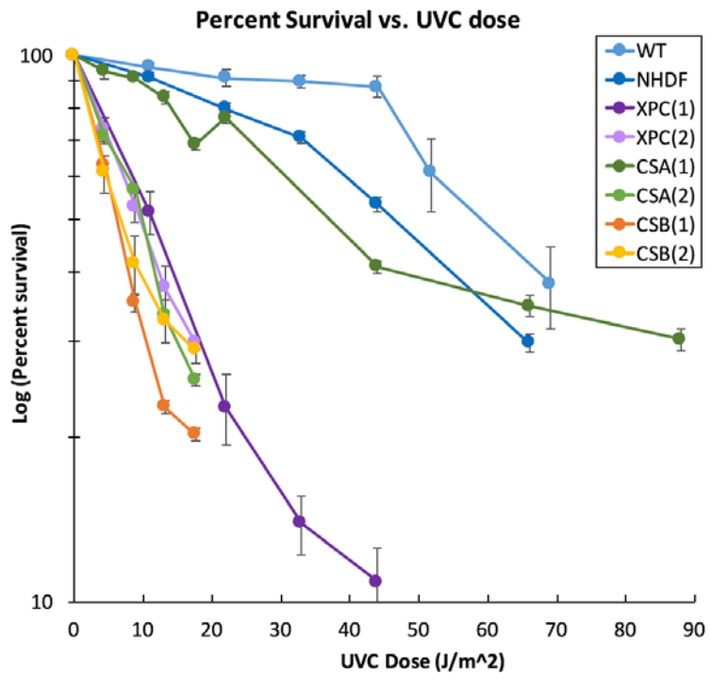
log

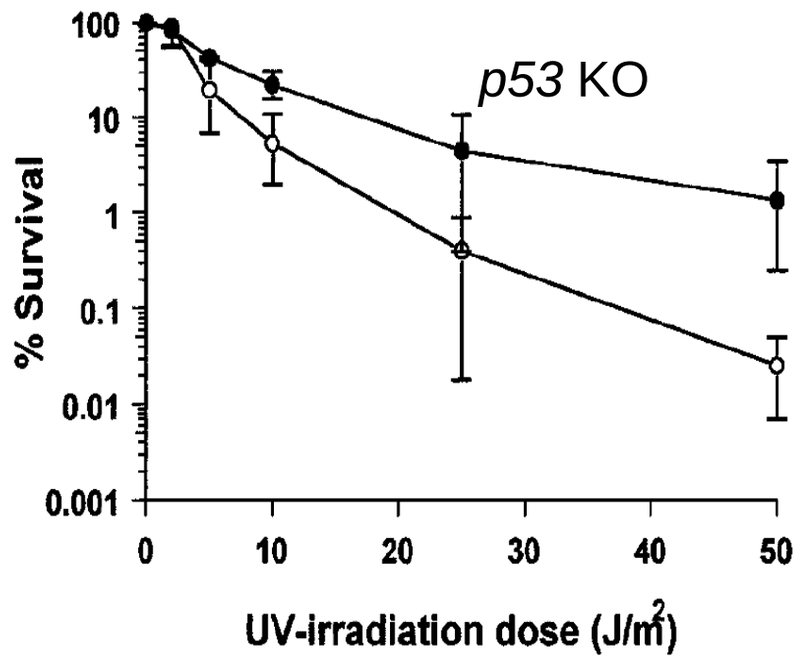


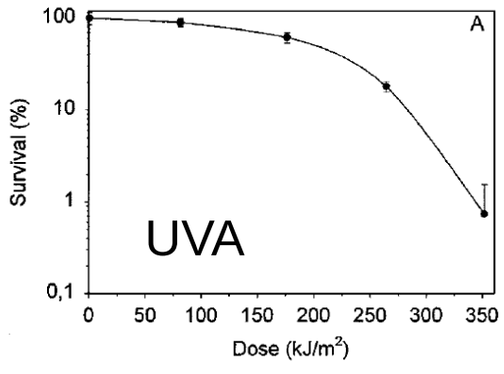
2.5 5 10 15 J/m2

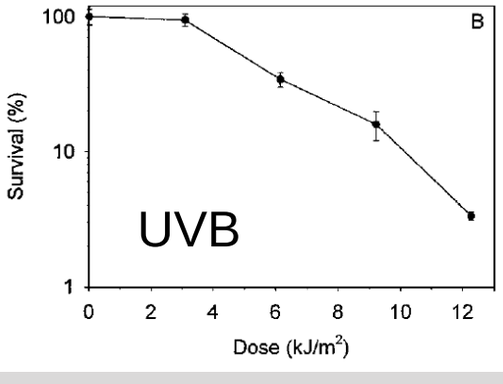


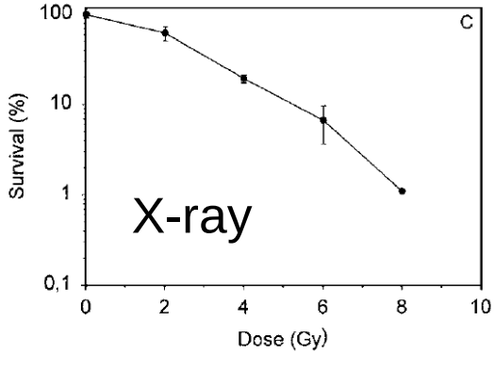
UVC melanoma & HeLa

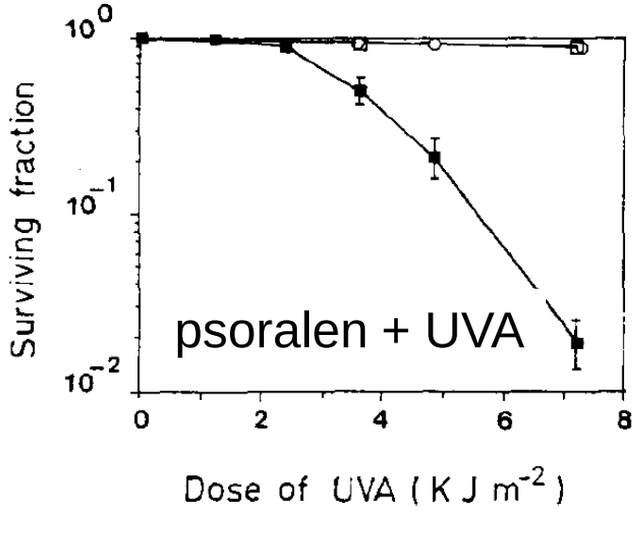






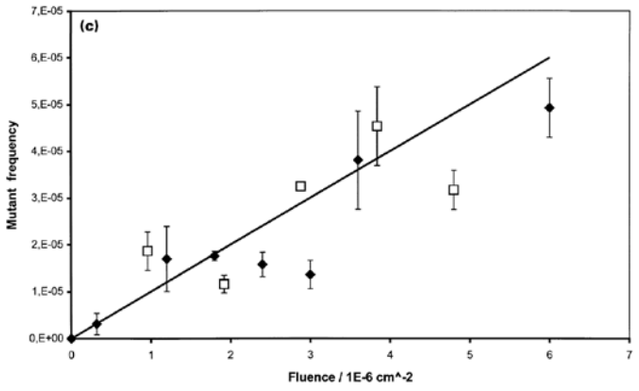




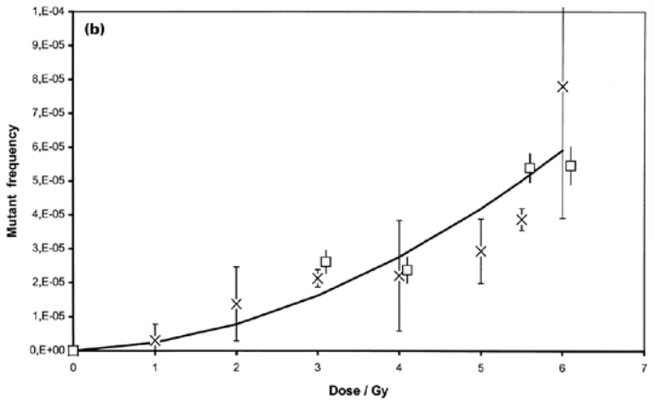


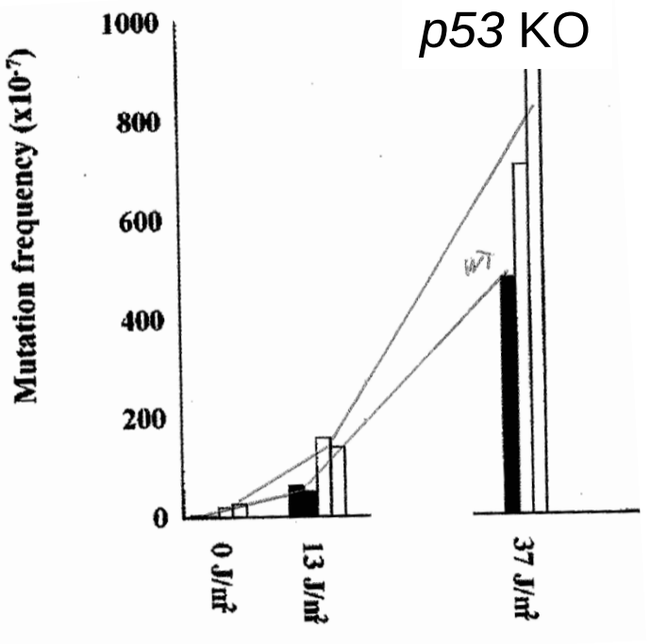
Mutation dose-responses: *Hprt* (6-Thioguanine^{R})

alpha rays (He^{++})

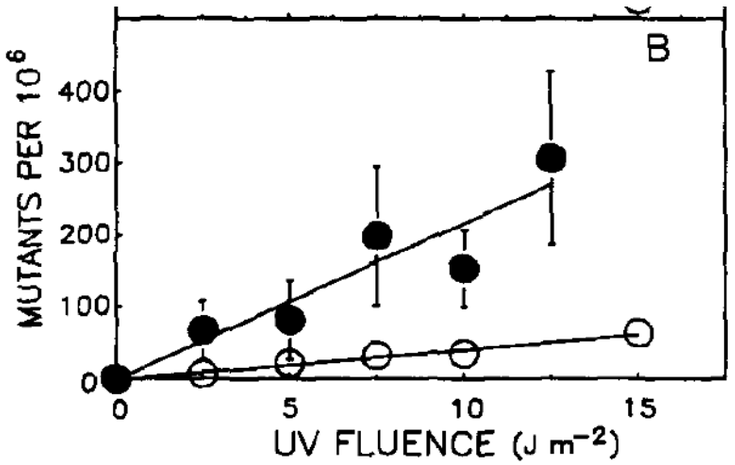


x-ray

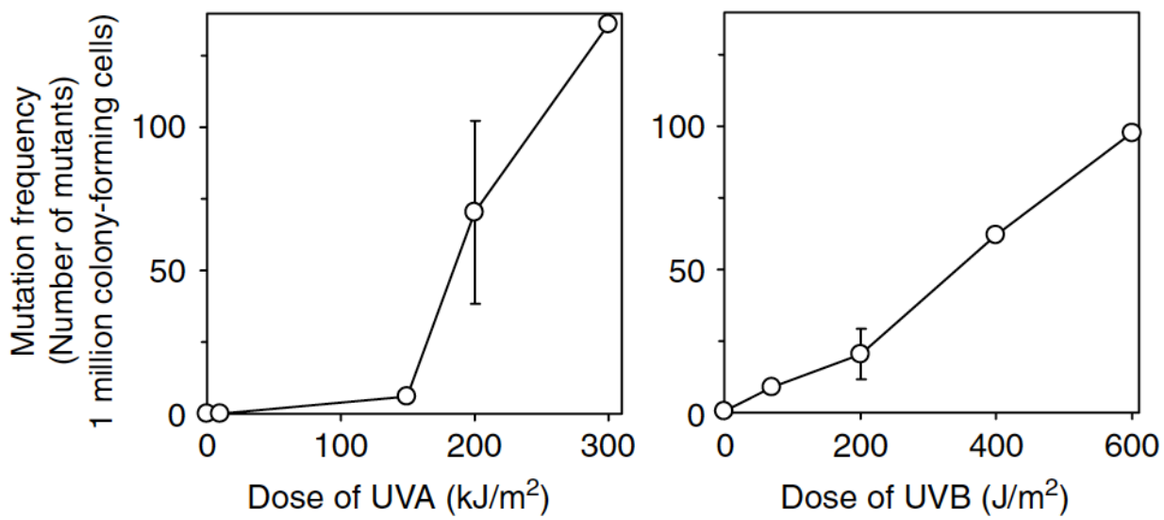


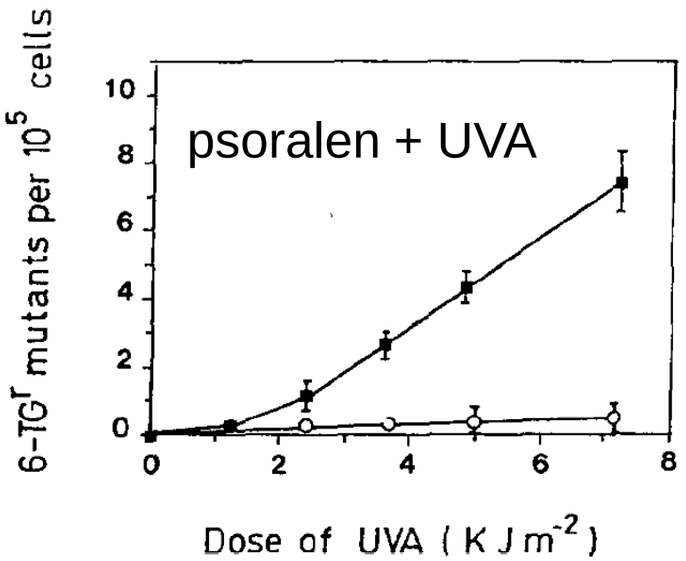


UVC

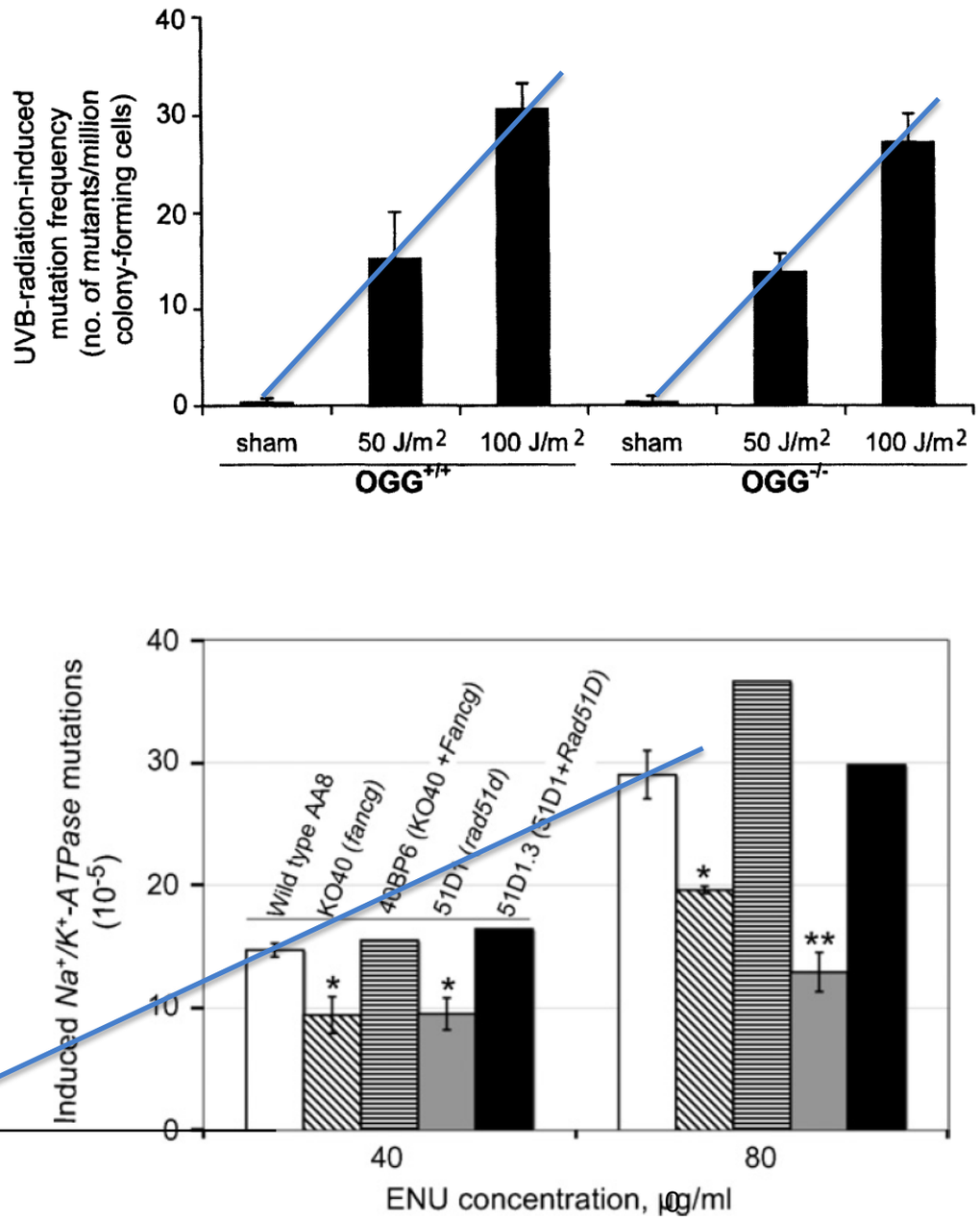


UVC melanoma & HeLa

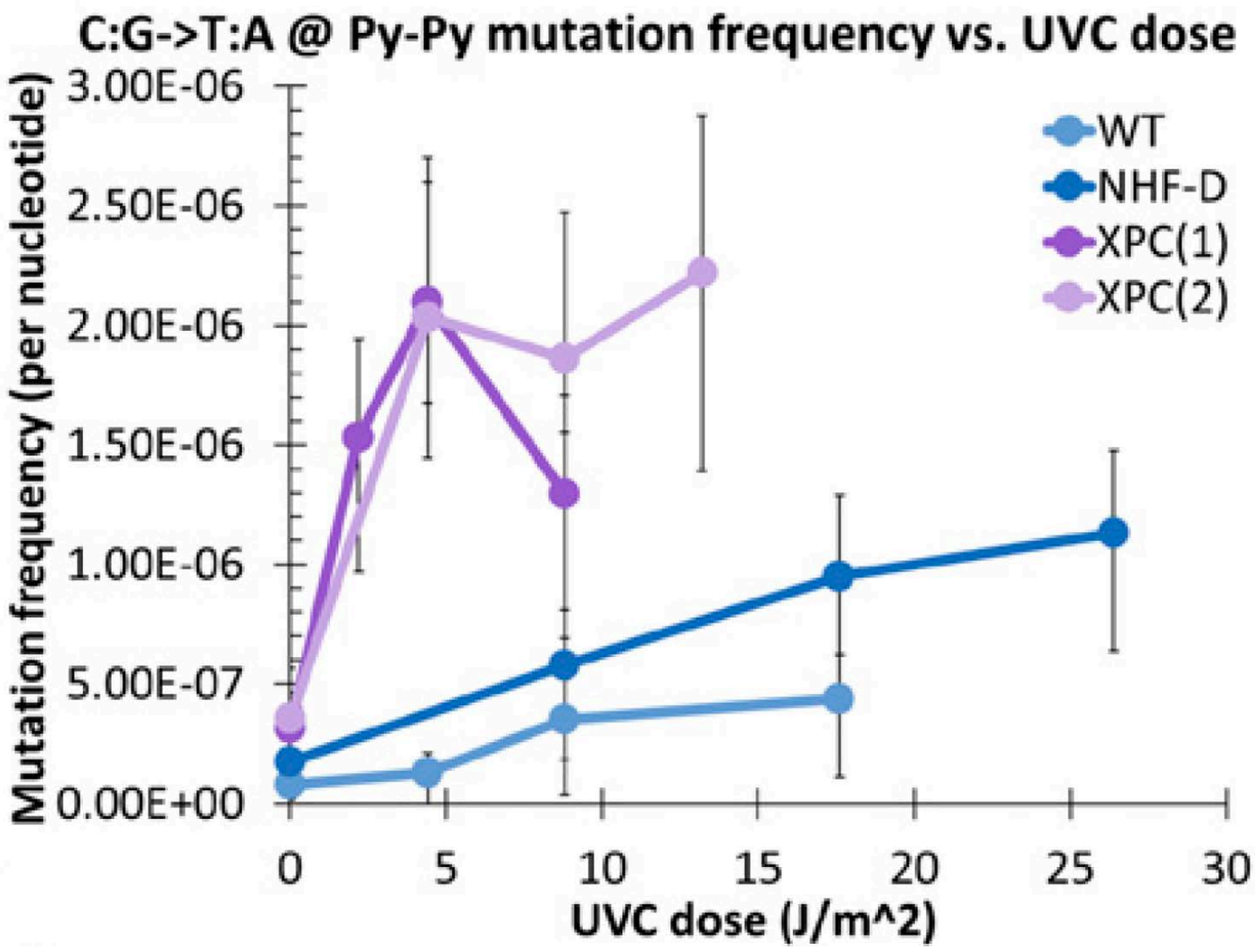




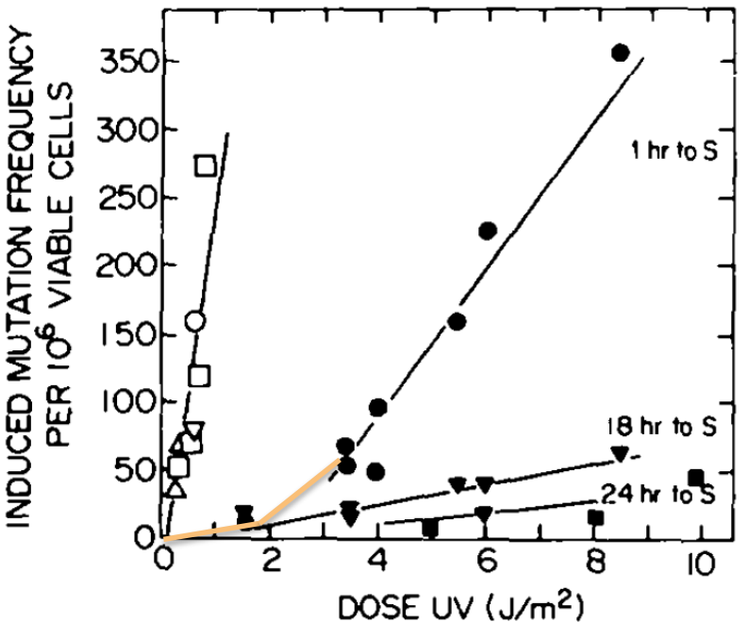
Mutation dose-responses: Ouabain^R



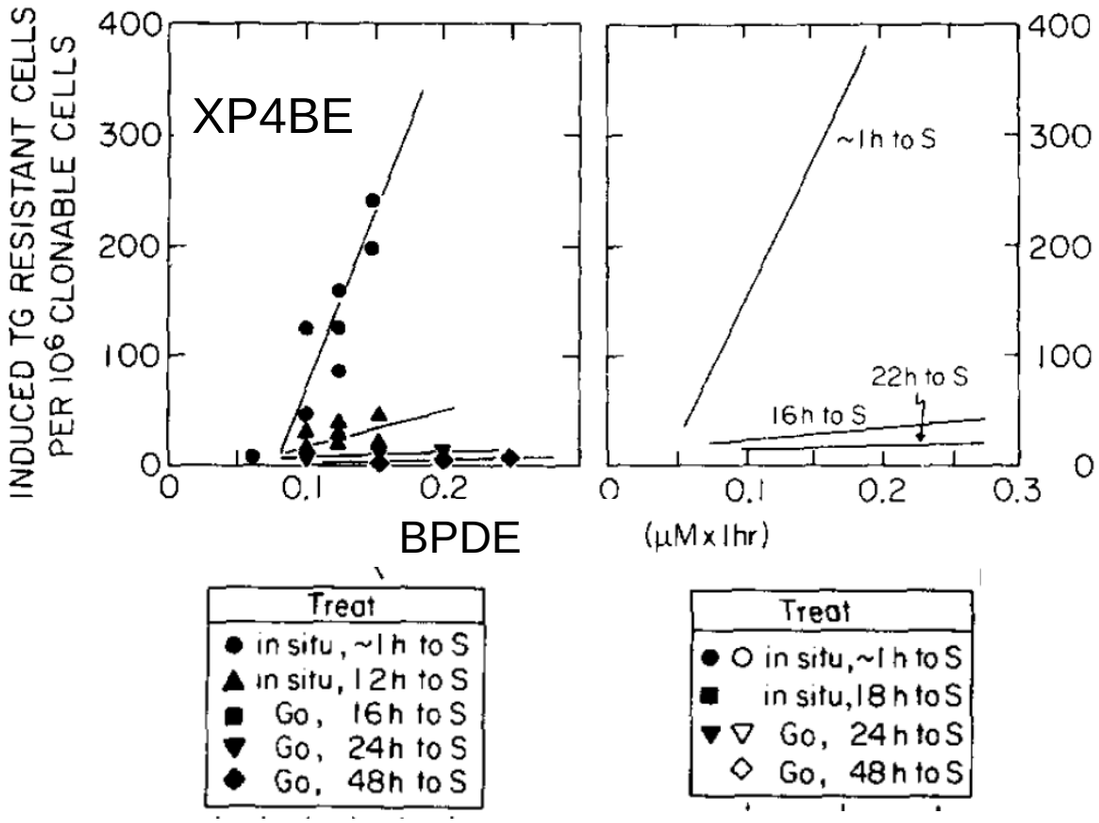
Mutation dose-responses: Unselected deep sequencing



Mutation threshold subtleties #1



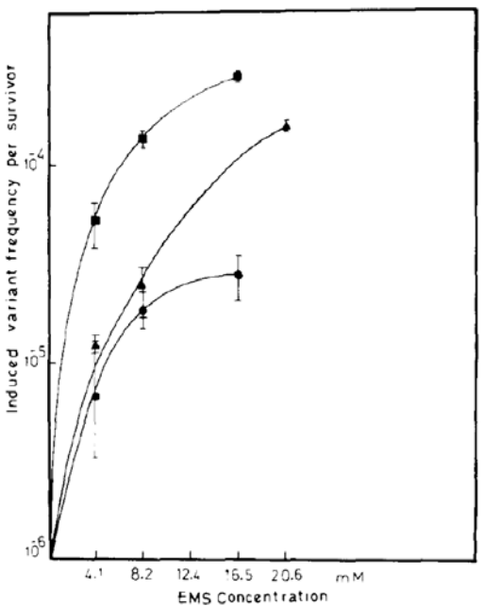
This ”threshold dose” is just the absence of a “catastrophic dose” when there is little time for repair before DNA replication.



Mutation threshold subtleties #2

Hprt (6-Thioguanine^R)

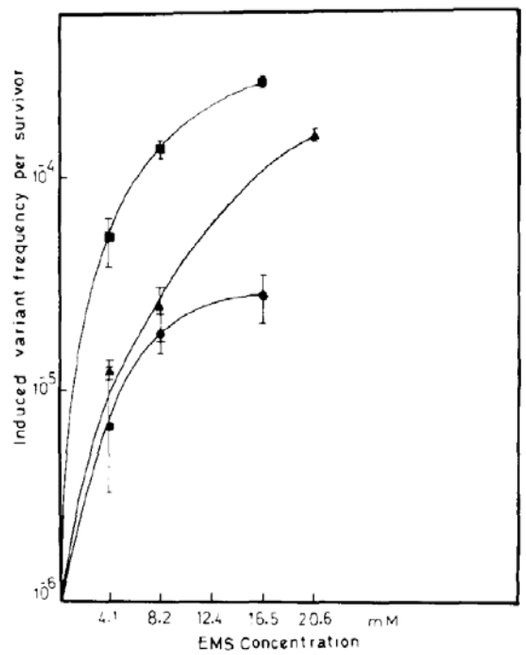
log



EMS

(Ouabain^R)

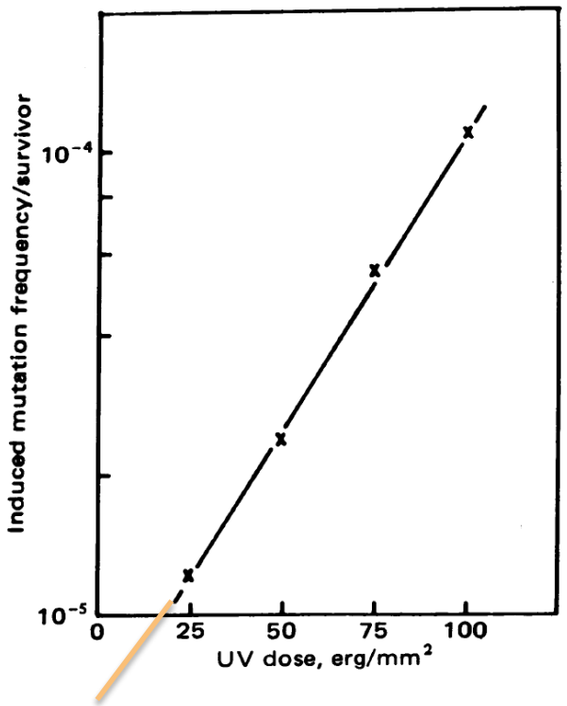
log



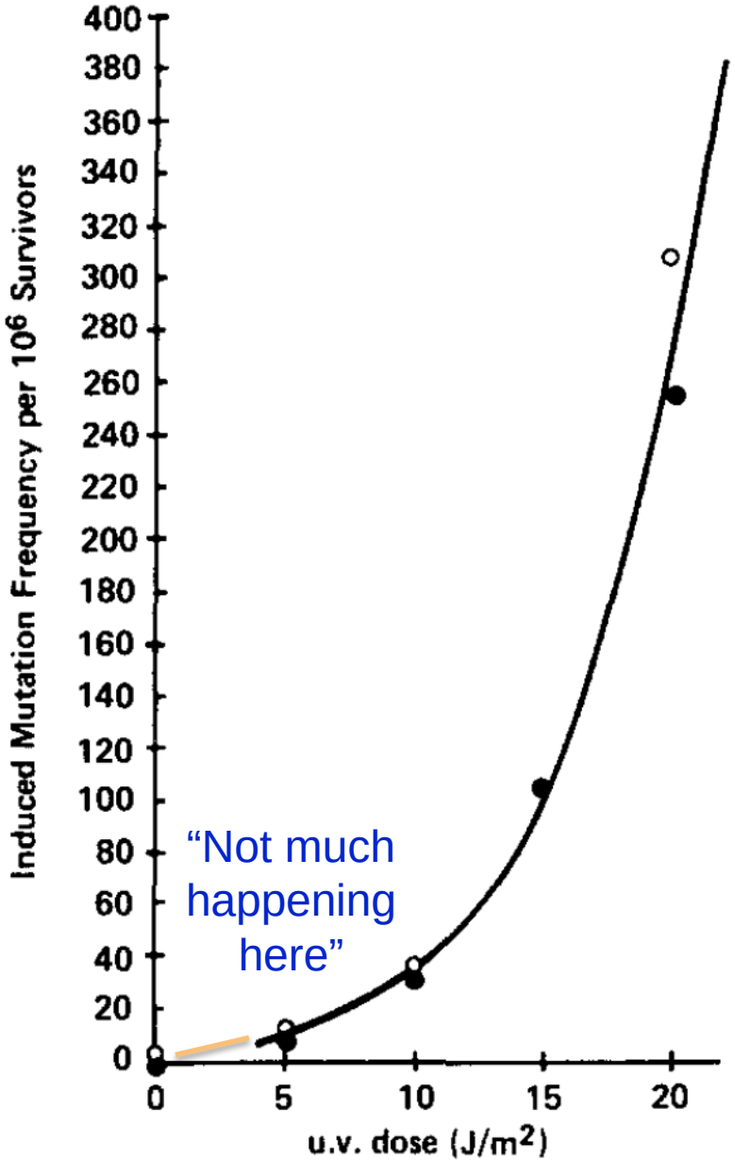
If we plot:
log (mutations/survivor)

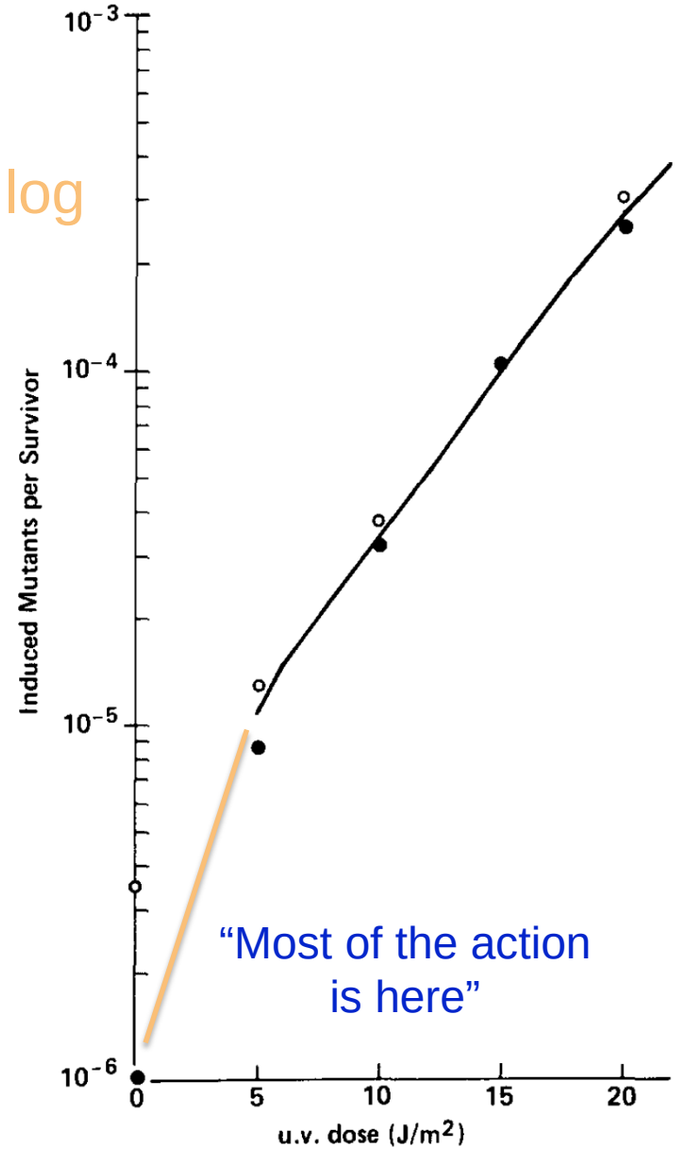
Then:
No threshold
Slope is highest at *low* doses

log



Oua^R direct comparison





Do we care about mutation frequency or mutation frequency change per dose change?
Dollars or the compound interest rate?

Chang, Trosko, Akera, Mutat Res 51:85,1978

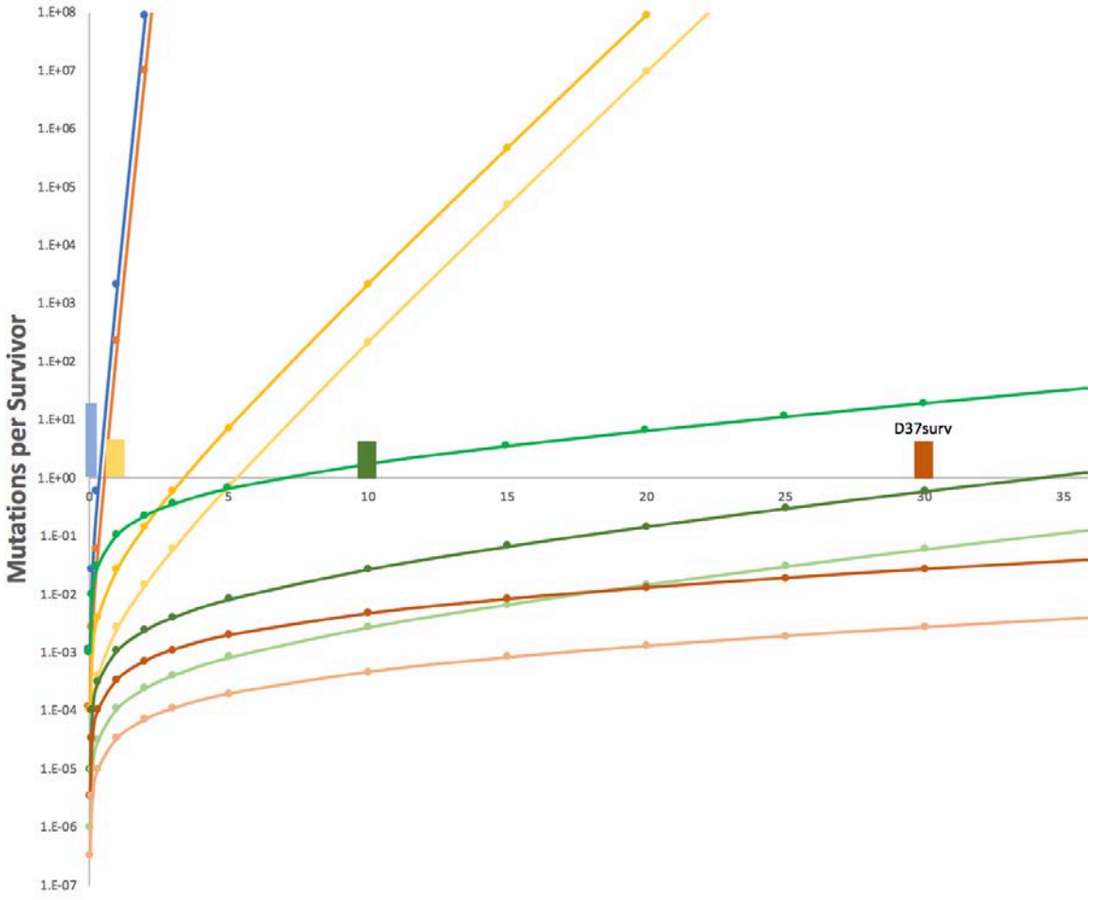
Algebra of the mutation dose-response

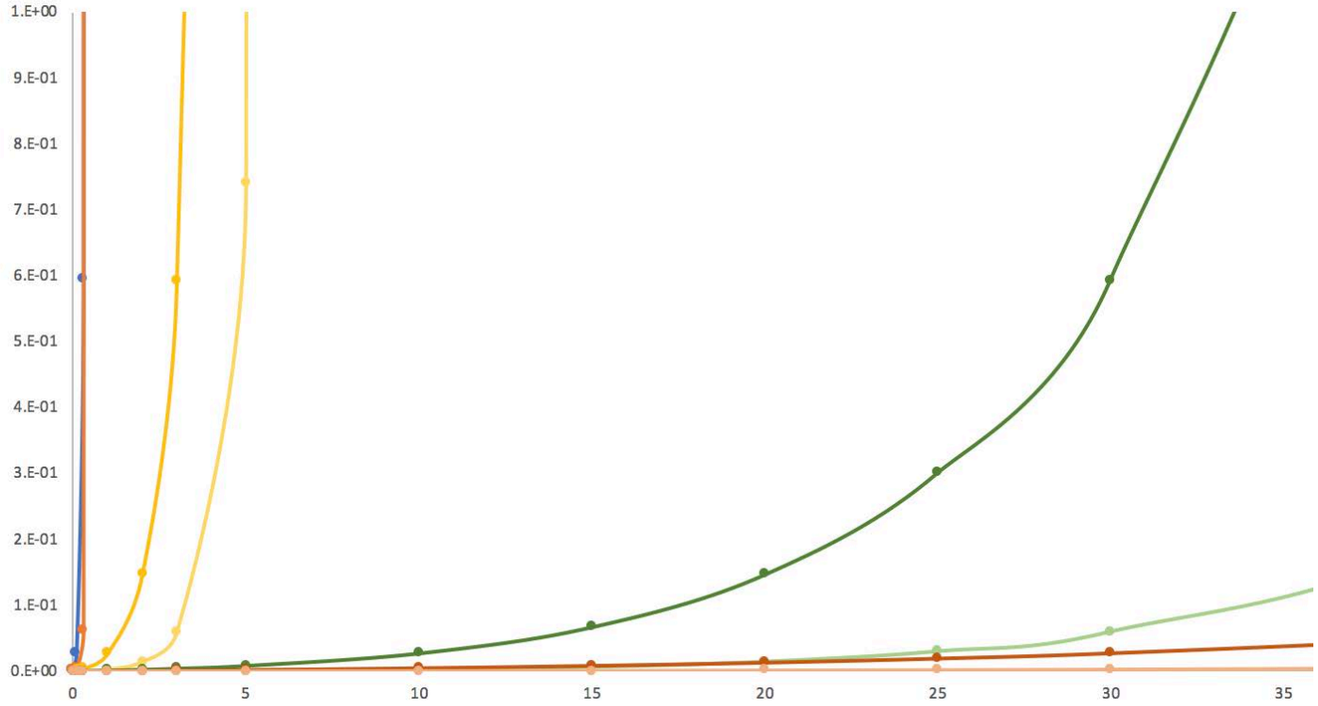
Mutation frequency = mutations per survivor

= mutant cells per initial cell / surviving cells per initial cell

$= (1 - e^{-D/D_{37mut}}) / e^{-D/D_{37surv}}$

$= e^{+D/D_{37surv}} - e^{-D(1/D_{37mut} - 1/D_{37surv})}$





Conclusions 2

- Survival shows a threshold – unless cells are repair-deficient.
- Mutation can show a pseudo-threshold when not plotted as log:
  - Absence of a ”catastrophe curve” when short time for repair before DNA replication.
  - Algebraic consequence of opposing dose dependencies for surviving cells & (1 – nonmutant cells).
    Plotted as log, low doses show *steeper* mutation frequency increase per dose increase.

III. Melanoma: Thresholds & Single Exposures

Melanoma tumors are repair-deficient, so no threshold?

1. Melanoma cells are deficient in post-replication repair. (Gabrielli)

2. Recurrent promoter mutations in melanoma are 100% C →T at PyPy, the UV signature. But should be 80% unless no repair. (Brash)

The patient was repair-deficient?
The founder *cell* was repair-deficient?

Volunteers with a repair deficiency may have no threshold dose.

Single UV exposures in melanoma

Human

Sunburns in childhood are a risk factor for melanoma as an adult.

Caveat: Sunburns also matter when older.
Sunburn could be a marker for Celtic skin type.

Mice

Single neonatal UVB exposure induces melanomas having UV signature mutations.

Caveat: Transgenic mice – *Hgf*, *Braf*, *Cdk4:Nras*

(is hitting these genes the duration-dependent step?)

Four UVB+A exposures generates melanomas *sooner* than chronic exposure. (Marais)

Caveat: Transgenic mice – *Braf*

Mechanisms for carcinogenic single UV exposures in melanoma

1. Single UV exposure makes melanocytes proliferate. (De Gruijl)

2. Alters physiology?

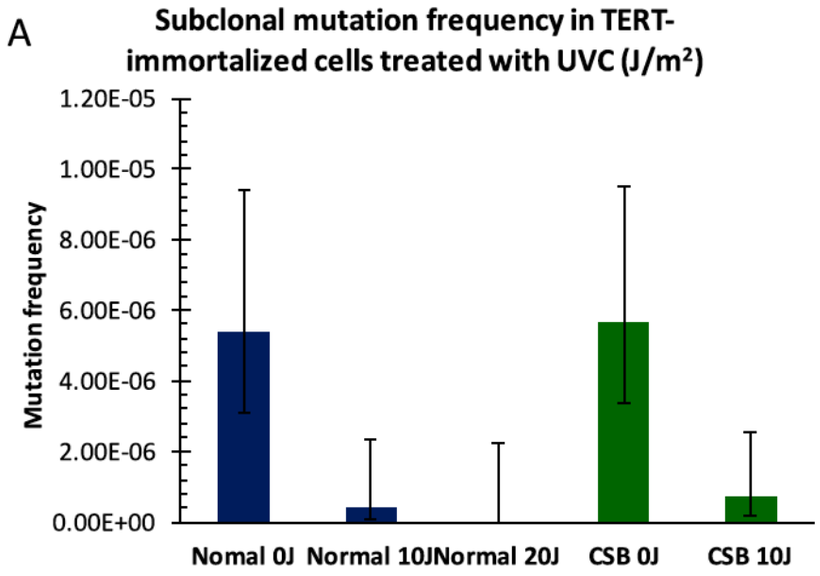
a) Melanomas can be induced by growth factors, w/o mutagens. (Herlyn)

b) Genetic polymorphisms that accelerate neonatal single-dose UV melanomas are in ribosome-related genes & are UV-inducible. (Walker)

c) Melanocytes have 100x CPD hyperhotspots. (Brash)
typically in regulatory regions of ribosome-related genes
many hits, so allows weak drivers to collaborate

3. Single UV exposures: purifying selection

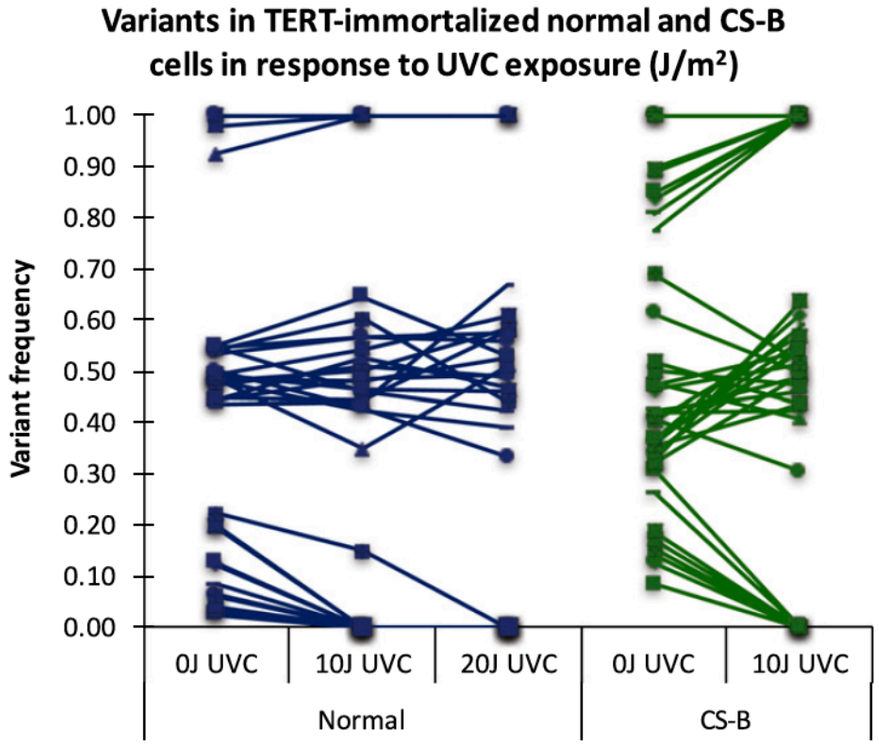
A



1. *TERT*-immortalized cells have 10-100x spontaneous mutation frequency.

2. UV *reduces* this mutation frequency.

B



3. UV eliminates the small subclones; large and medium subclones are untouched.
   If repair-defective (CSB), UV expands some large and medium clones.

Reid...Loeb...Cleaver PNAS 113:10151, 2016

Conclusions 3

- Melanoma may involve no-threshold & single-exposure phenomena.

IV. Linearity, thresholds, & biphasic responses in toxicology

Definitions

- Degree of rigor?

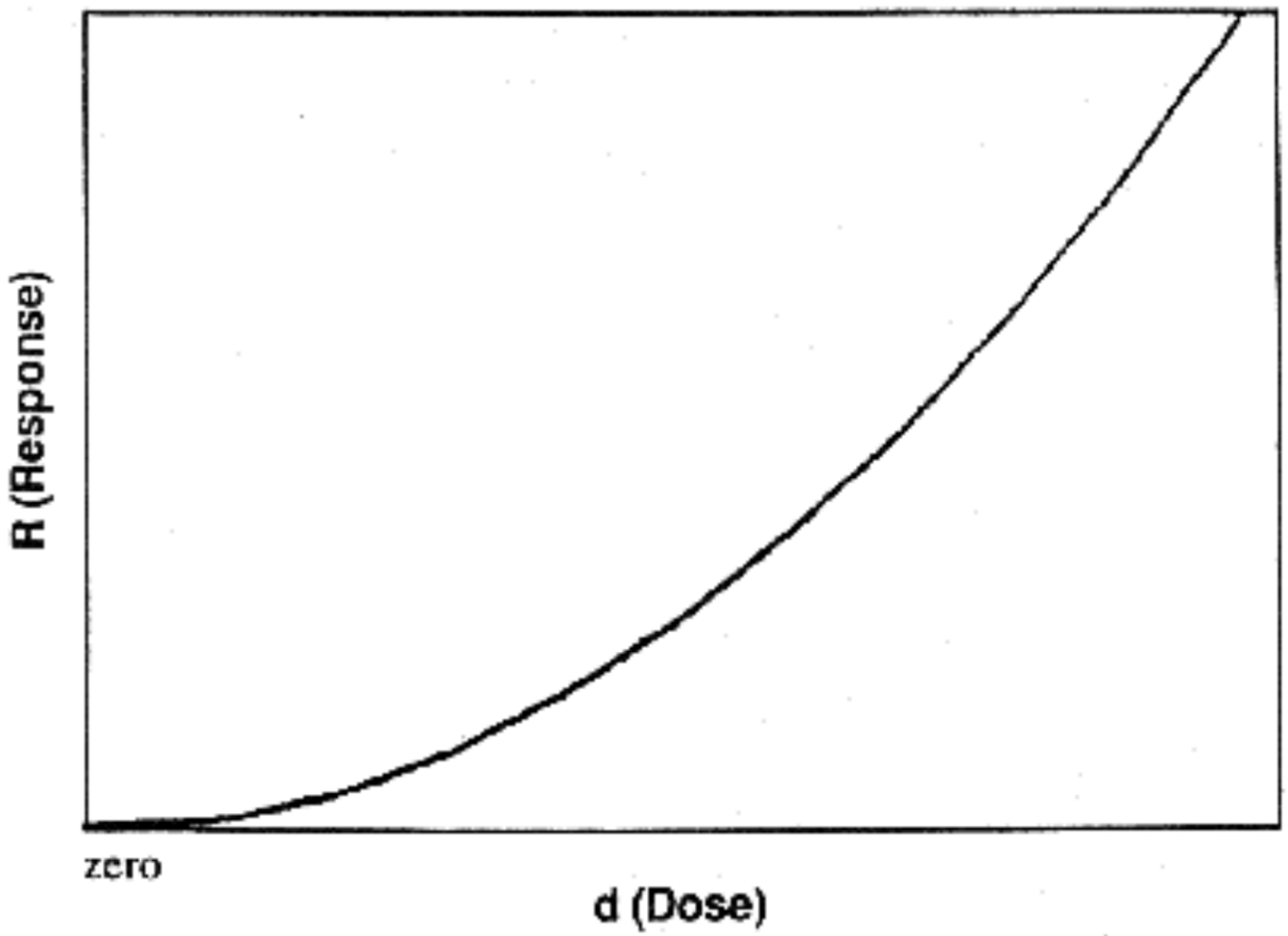


Figure 1. Typical nonlinear, "threshold", dose-response relationship ($R = Ad^3$).

The linear model had sketchy origins

Linearity was inherited from the ionizing radiation field:

1950s **Target theory**
Based on physicists' x-ray scattering from atoms.
Assumed 1 or n irreversible hits, before biology was known.
Calculations used for policy never published;
varied >1000x so were cherry-picked to 100x.

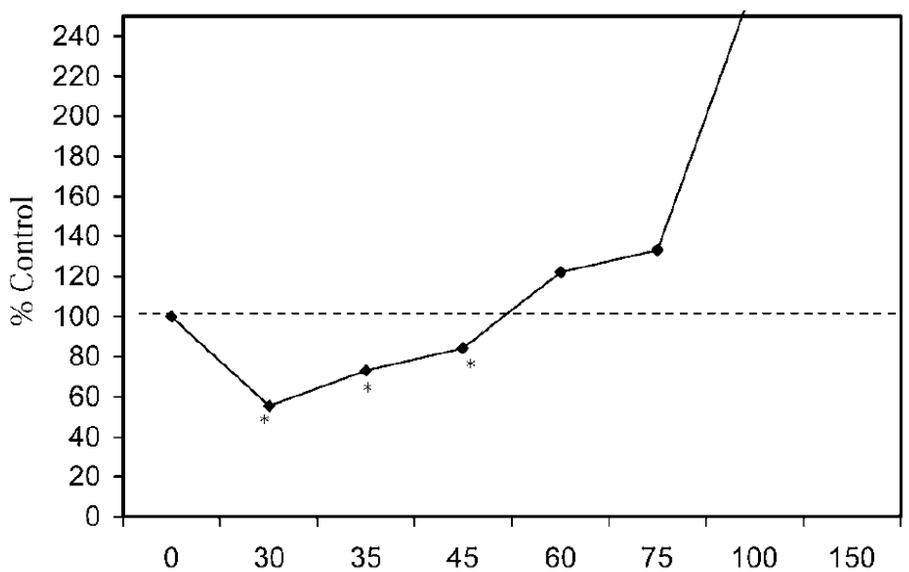
1960s **Mega-mouse experiments**
Statistical errors in control populations.

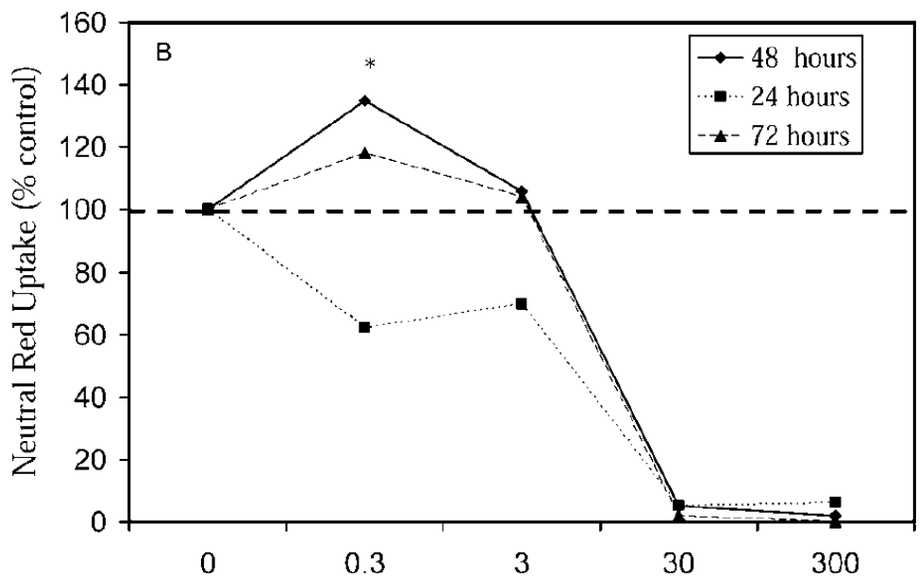
1970s **Assumed mechanism same for spontaneous & genotoxin CA**
So any threshold must have been exceeded before the treatment.
Mutations now known to differ.
Other assumptions:

Chronic exposure	CA increases monotonically w dose
Tumors are monoclonal	No repair or cell death
No growth advantage until all hits occur	

Calabrese et al, Toxicolog Sci 62:330, 2001; Toxicol Appl Pharm 202:289, 2005; Env Toxicol Chem 27:1451; Food Chem Toxicol 81:137, 2015

Biphasic responses ("hormesis")





1. 20% of studies containing doses below a no-adverse-effect range show a "stimulatory" effect that reverses at higher doses.
   0.6% show the reverse.

2. Typically 1.6x; some are 2-100x

3. All 12,000 NCI anti-CA drug screen compounds fit biphasic better than linear.

Calabrese et al, Toxicolog Sci 62:330, 2001; Toxicol Appl Pharm 202:289, 2005; Env Toxicol Chem 27:1451; Food Chem Toxicol 81:137, 2015

Overall Conclusions

1. Biology offers no reason to *expect* a linear or monotonic dose-response, or a threshold.

2. Homeostasis *implies* that:
   - Within the system's operating range, a genotoxin will have smaller impact.
   - Yet having a fire sprinkler in your office doesn't mean that it's a good idea to keep it turned on.
   - Toxicity indicates the dose is outside the operating range.
   - The mechanisms of homeostasis and breakage differ, so low dose responses can't be extrapolated from high doses.
   - Replace "hormesis" with "biphasic"?

Overall Conclusions

3. *Survival*: A threshold dose exists if killing is due to a repairable lesion and the cell is repair-proficient. Repair can keep up.

4. *Mutation*: There is no obvious threshold dose.
   Low doses show a *larger* mutation frequency increase per dose.
   At low doses, repair reduces mutations but also gives more survivors to have mutations.

Dilemmas

1. Thresholds:
   Do we bet on a threshold if we can't measure it?
   Do we bet that a volunteer has no deficiency in repair or other homeostasis mechanism?

2. Single exposures:
   Do we bet that single exposures are harmless if we haven't done the experiment?

3. How close to the railroad tracks do we want kids to play?

4. Who decides – us, an IRB, or the volunteer?