# Radiation Carcinogenesis

November 22, 2016

Dhyan Chandra, Ph.D.
Pharmacology and Therapeutics
Roswell Park Cancer Institute
Email: dhyan.chandra@roswellpark.org

# Overview

- History of radiation and radiation-induced damage
- Bystander effect of radiation
- Methods for DNA damage analysis
- Stages of carcinogenesis and models
- Mechanism of radiation-induced carcinogenesis
- Role of oncogenes and tumor suppressors
- Risk projections and risk estimates
- Importance of dose and age on tumor incidence
- Second malignancy after radiotherapy

# Radiation and cancer

- 1895- Roentgen discovered X-rays
- 1896- Henri Becquerel discovered radioactivity
- 1897- Rutherford discovered α and β rays
- 1898- Curies discovered polonium and radium
- 1902- First report on radiation-induced skin cancer
- 1911- First report of leukemia in 5 radiation workers

Marie Curie and Her Daughter Irene –

Thought to have Died of Leukemia

# Types of radiation

**Ionizing radiation:**

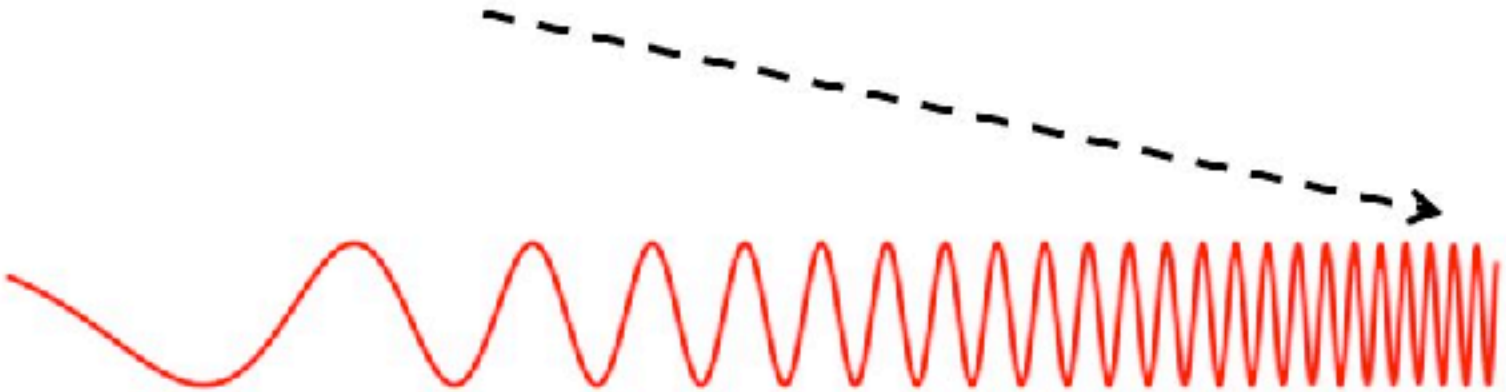
- α particles (2 protons and 2 neutrons)
- β particles (electron equivalent)
- Neutrons
- Gamma rays
- X-rays

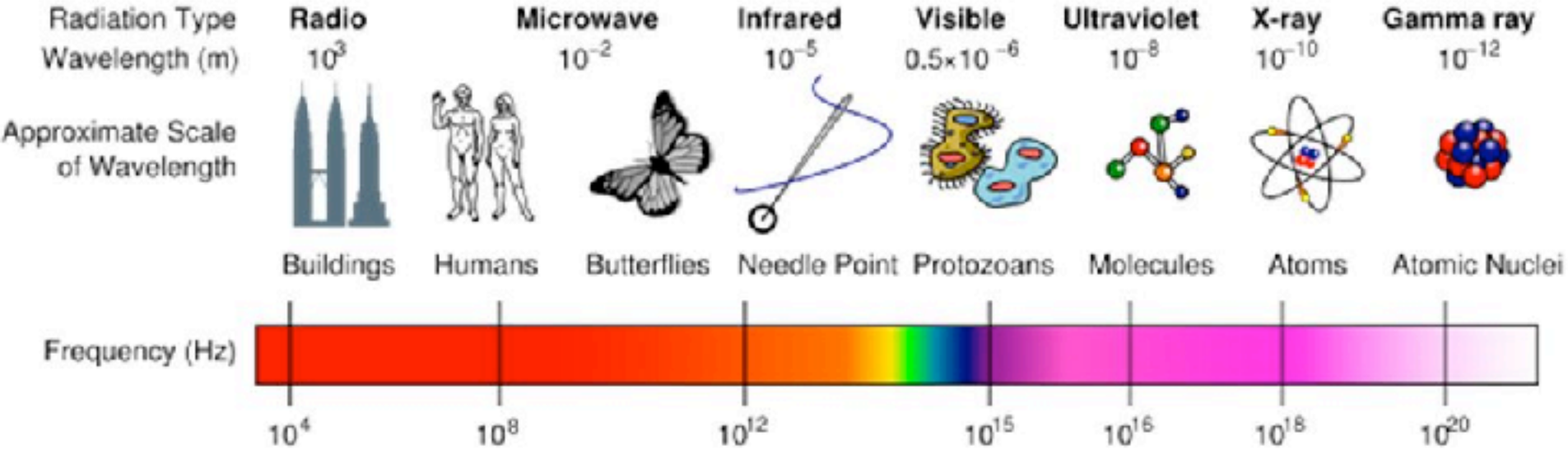
**Non-ionizing radiation:**

- Microwaves
- Visible light
- Radio waves and TV waves
- UV radiation (except shortest wavelengths)

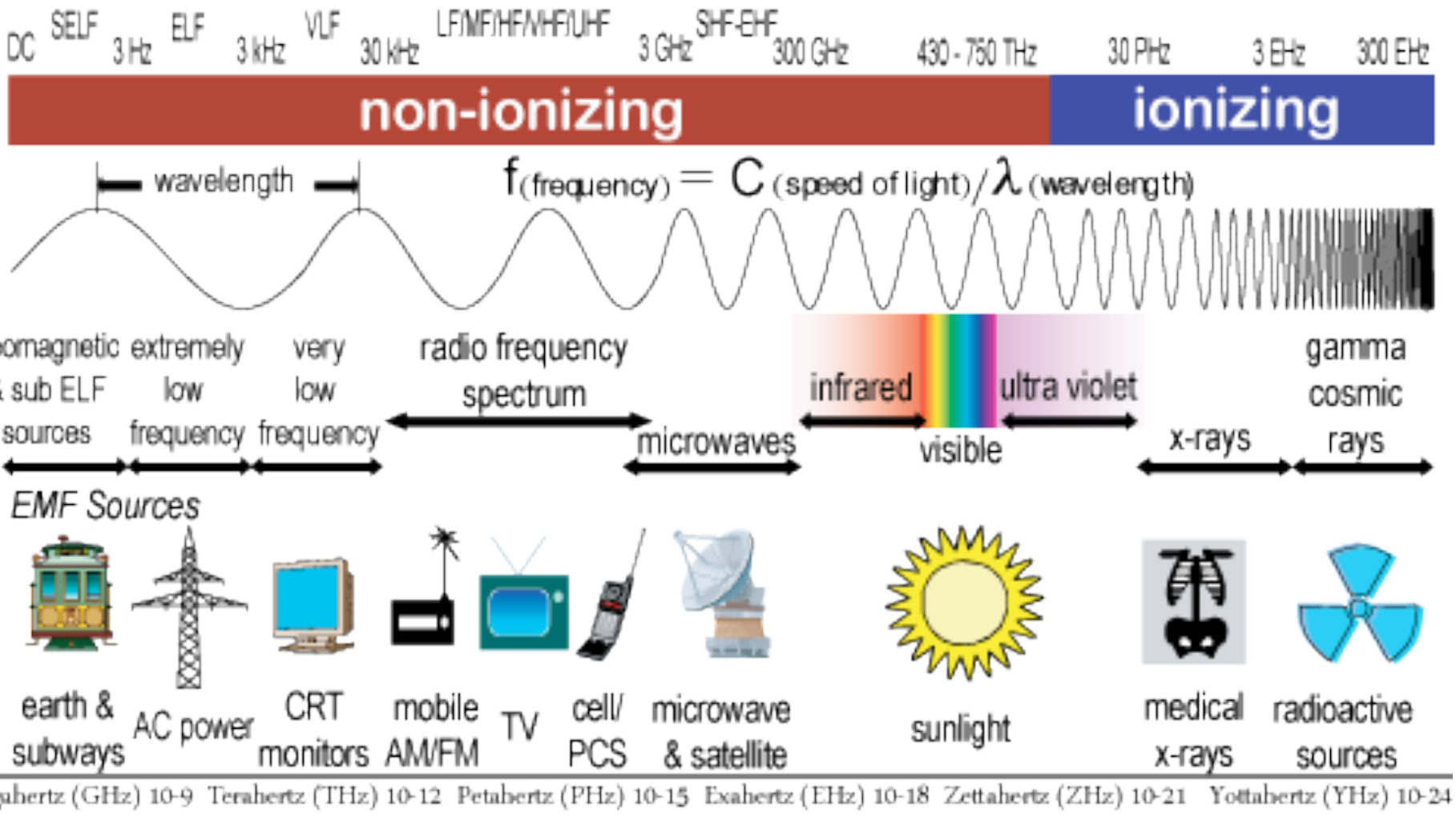
# Gamma Rays and EM Spectrum

Electromagnetic radiation with wavelength of ~$10^{-12}$ m.



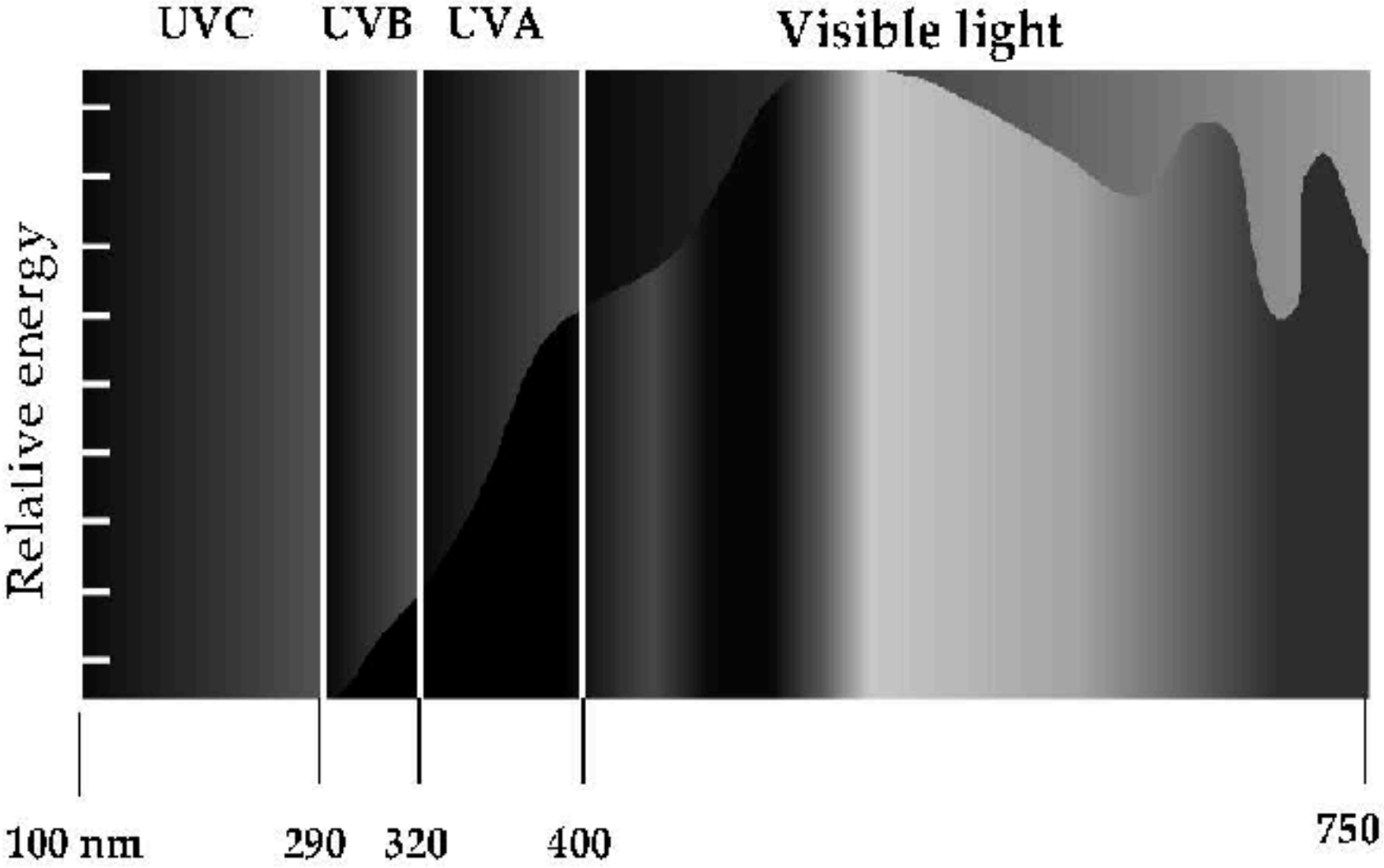


# THE ELECTROMAGNETIC SPECTRUM




Gigahertz (GHz) 10-9 Terahertz (THz) 10-12 Petahertz (PHz) 10-15 Exahertz (EHz) 10-18 Zettahertz (ZHz) 10-21 Yottahertz (YHz) 10-24

# Ultraviolet / Visible Light Spectrum




Adapted from: "Ultraviolet light as a carcinogen", Ananthaswamy, 1997

# Units and doses

**Activity:**
Quantity of a radionuclide which describes the rate at which decays occur in an amount of a radionuclide.

The SI unit of radioactivity is the becquerel (Bq), which replaced the old unit, the curie (Ci).

**Becquerel (Bq)**: One becquerel corresponds to 1 disintegration of a radionuclide per second.

**Curie (Ci)**: Old unit of radioactivity, corresponding to $3.7 \times 10^{10}$ radioactive disintegrations per second

# Units and doses

**Absorbed dose (D):**
The energy imparted per unit mass by ionizing radiation to matter at a specific point.

**Gy:** The SI unit of absorbed dose is joule per kilogram (J kg-1). The special name for this unit is gray (Gy).

**Rad:** The previously used special unit of absorbed dose, the rad, was defined to be an energy absorption of 100 ergs/gram. Therefore, 1 Gy = 100 rad.

# Units and doses

**Relative biological effectiveness (RBE)** - A factor used to compare the biological effectiveness of different types of ionizing radiation. It is the inverse ratio of the amount of absorbed radiation, required to produce a given effect, to a standard (or reference) radiation required to produce the same effect.

**Rem (roentgen equivalent in man) -** Old unit of equivalent or effective dose. It is the product of absorbed dose (in rad) and the radiation weighting factor. 1 rem = .01 Sv.

**Sievert (Sv)** - SI unit of equivalent dose or effective dose. 1 Sv = 100 rem.

# Linear energy transfer (LET)

- The rate of energy loss or deposition along the track of an ionizing particle

- Loss of energy/unit distance traveled in matter

- Units = KeV/μm

- Varies depending of quality of radiation

# Linear energy transfer (LET)

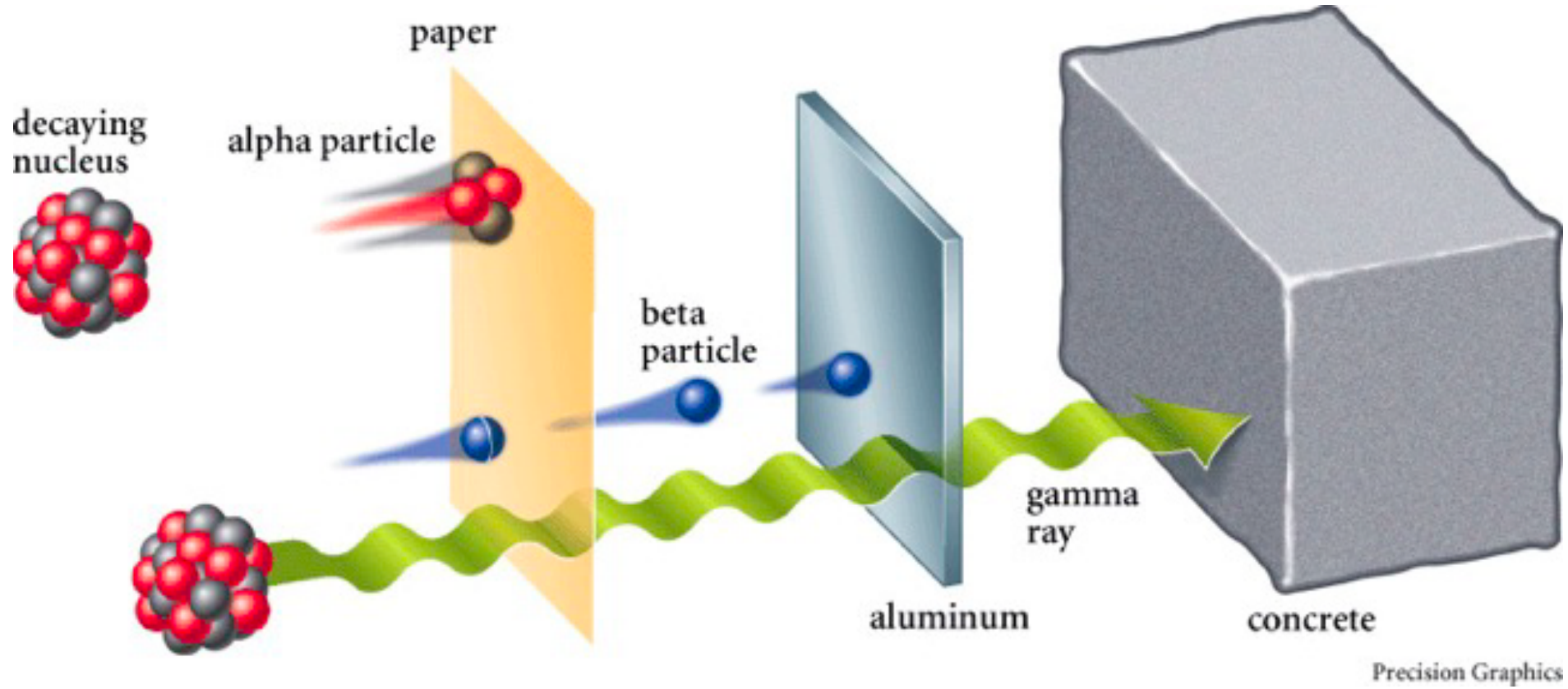
**x-ray or γ-ray**: x x Sparsely Ionizing

**β particle:** x x x

**Neutron**: x x x x x x x Densely Ionizing

**α particle** xxxxxxxx

The more sparsely ionizing, the more penetrating


paper
decaying nucleus
alpha particle
beta particle
gamma ray
aluminum
concrete
Precision Graphics

# Radiation-induced cancer in human

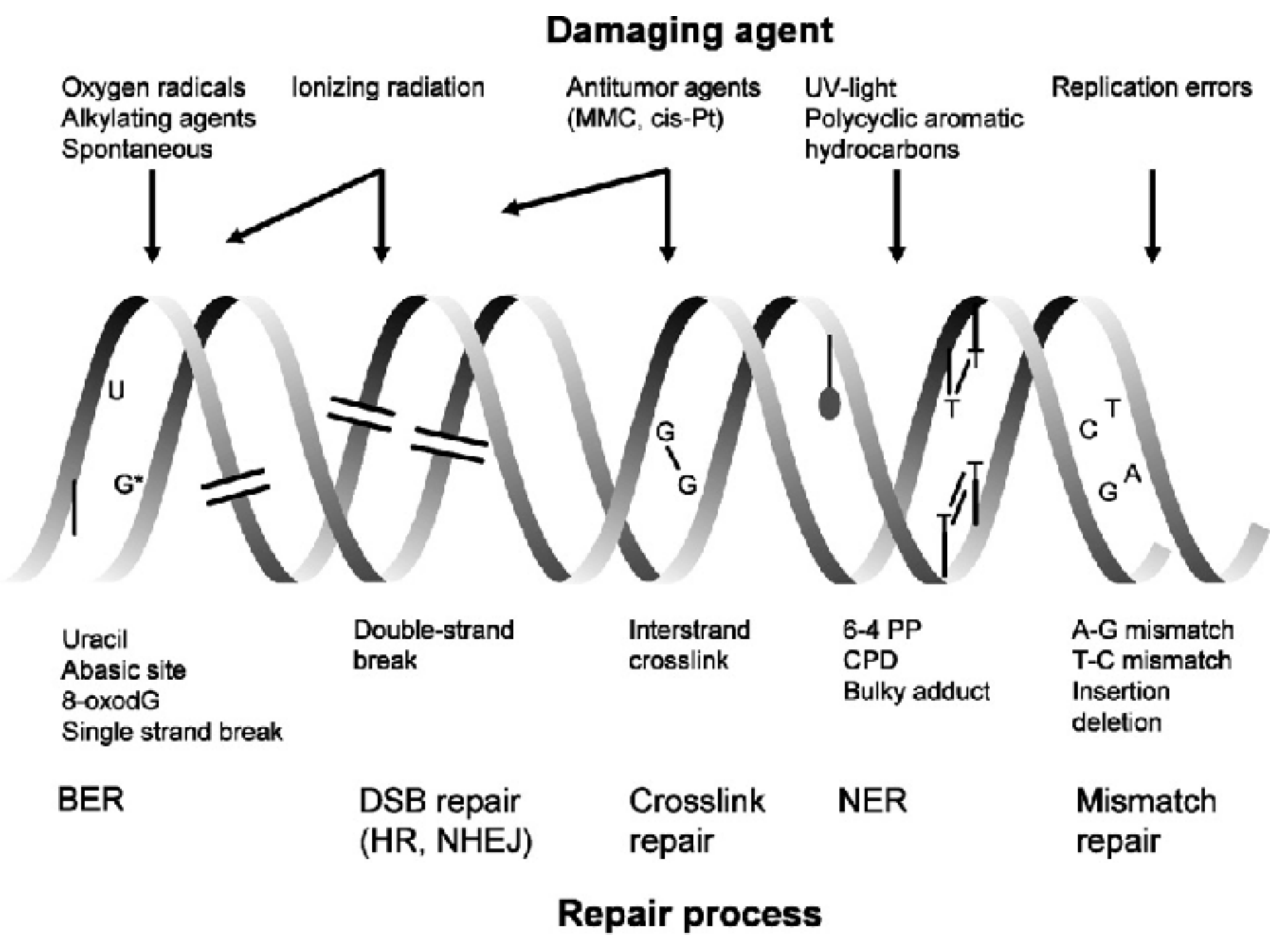
- Atomic bomb survivors
- Accidents
- Medically exposed individuals including cancer patients undergoing radiation therapy

# Early cases of human experience

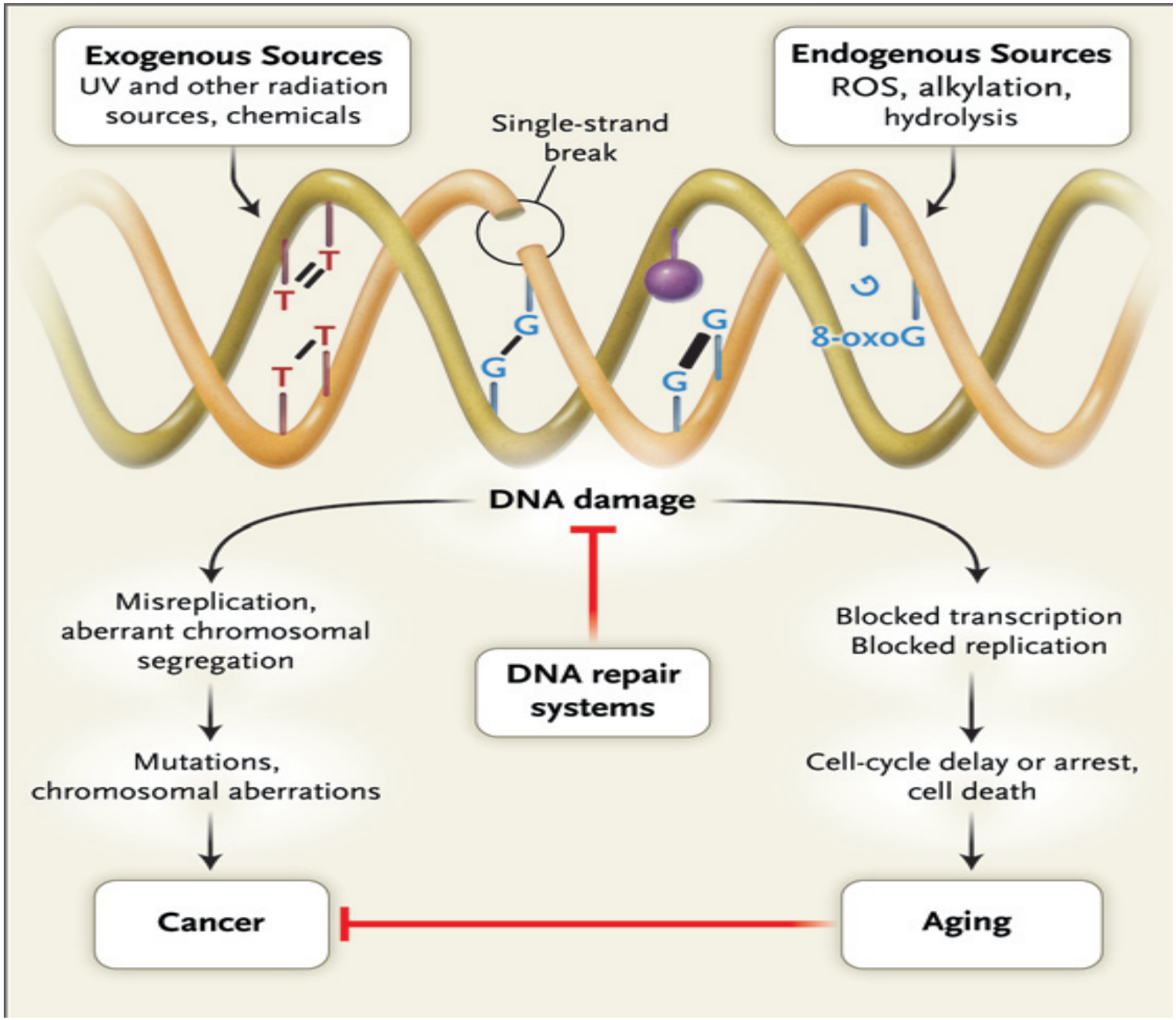
- Skin cancer in early x-ray workers
- Lung cancer in underground uranium miners in Saxony and Colorado
- Bone cancer in radium dial painters
- Liver cancer in thorotrast patients (use of thorium dioxide as radiocontrast agent in medical radiography in 30s-40s

# Later cases of human experience

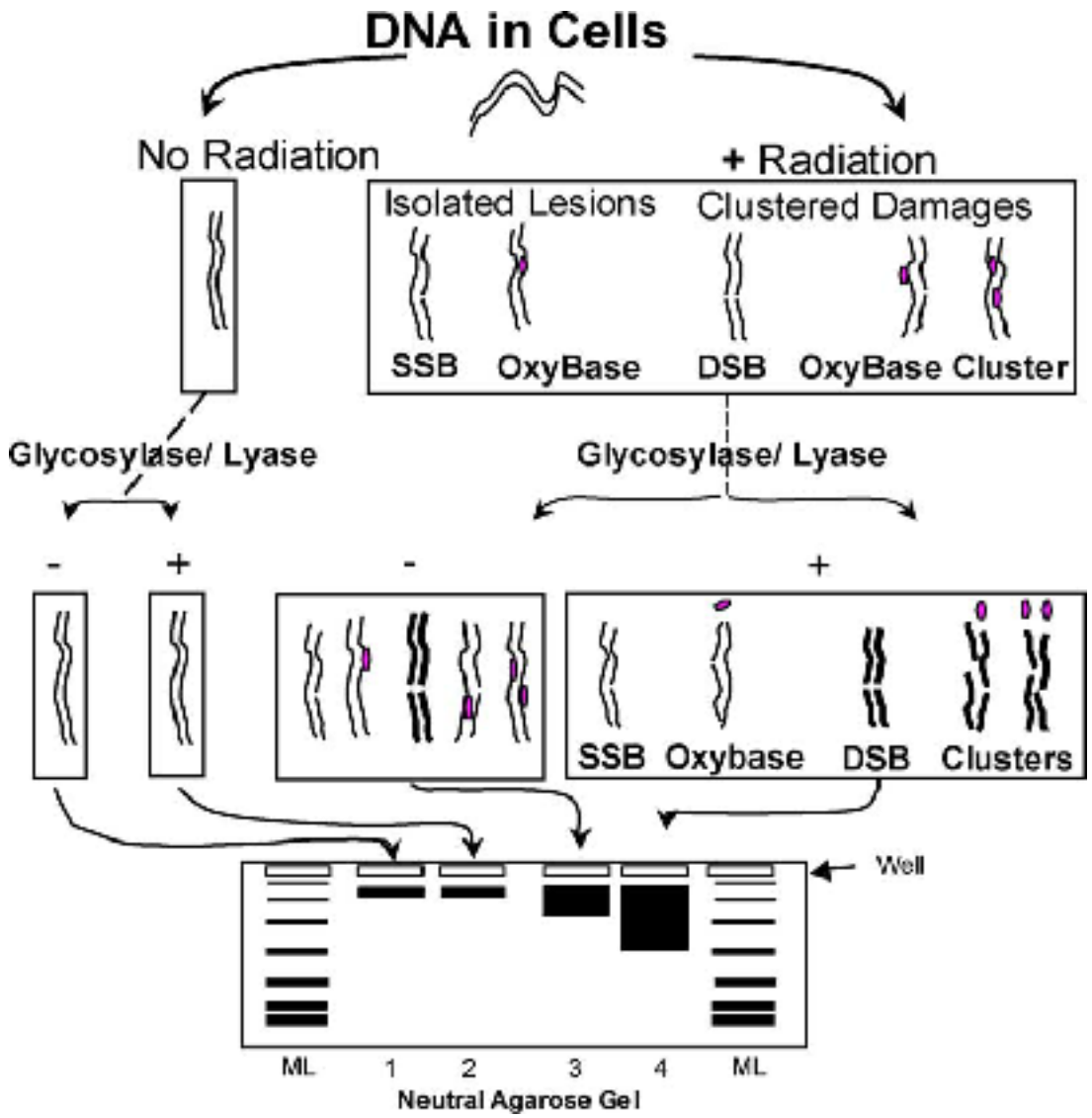
- Hiroshima/Nagasaki survivors
- Radiation treatment of Anklyosing Spondylitis patientsn (arthritis of spine)
- Elevated incidence of leukemia in early radiologists ca 1922
- Thyroid cancer from treatment for enlarged thymus
- Thyroid and other cancers for treatment of tinea capitis by radiation
- Breast cancer due to frequent chest X-Ray fluoroscopy in tuberculosispatients between 1925 to 1954


Damaging agent
Oxygen radicals
Alkylating agents
Spontaneous
Ionizing radiation
Antitumor agents
(MMC, cis-Pt)
UV-light
Polycyclic aromatic
hydrocarbons
Replication errors
U
G*
G
G
T
T
T
T
C
T
G
A
Uracil
Abasic site
8-oxodG
Single strand break
Double-strand
break
Interstrand
crosslink
6-4 PP
CPD
Bulky adduct
A-G mismatch
T-C mismatch
Insertion
deletion
BER
DSB repair
(HR, NHEJ)
Crosslink
repair
NER
Mismatch
repair
Repair process

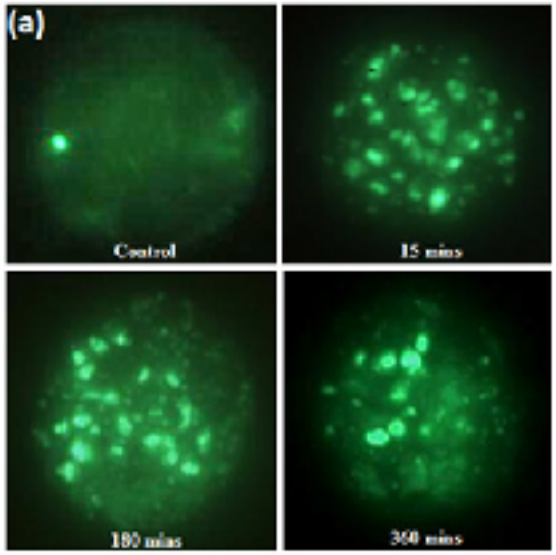
# Sources and consequences of DNA damage

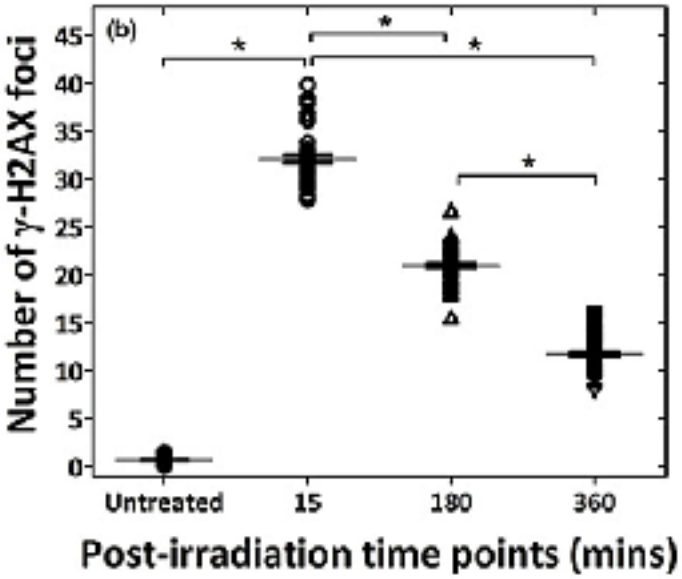


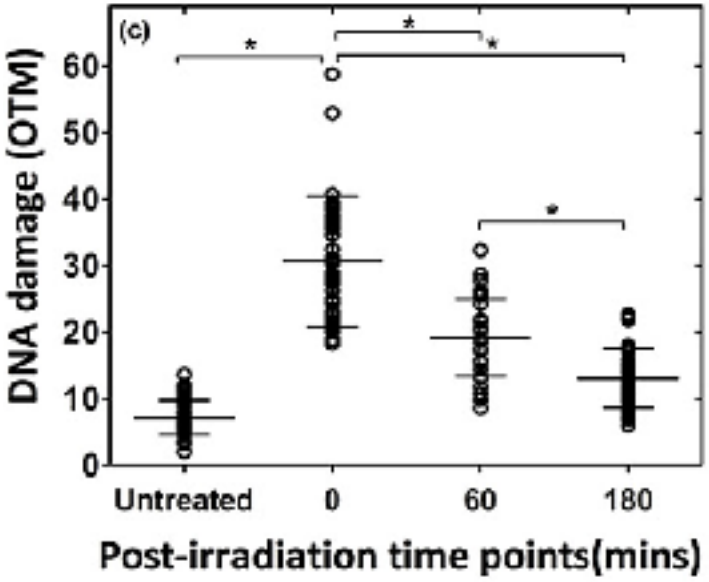
# Measurements of DNA damage

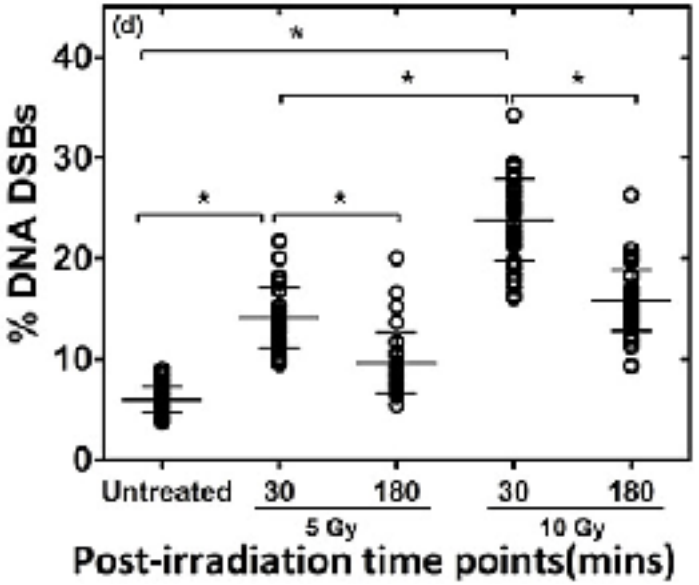


# Measurements of DNA damage



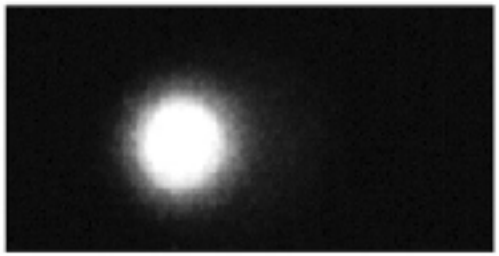



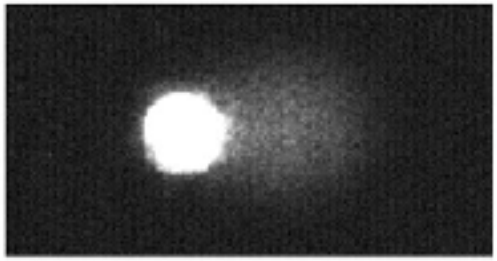

Goutam et al., Int J Radiat Biol Phys. 2012, July 24

# Measurements of DNA damage

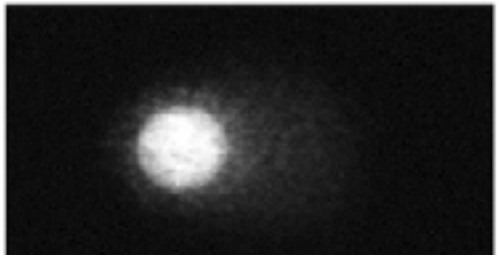
Undamaged cell



Cell irradiated with 12.5 Gy X-rays

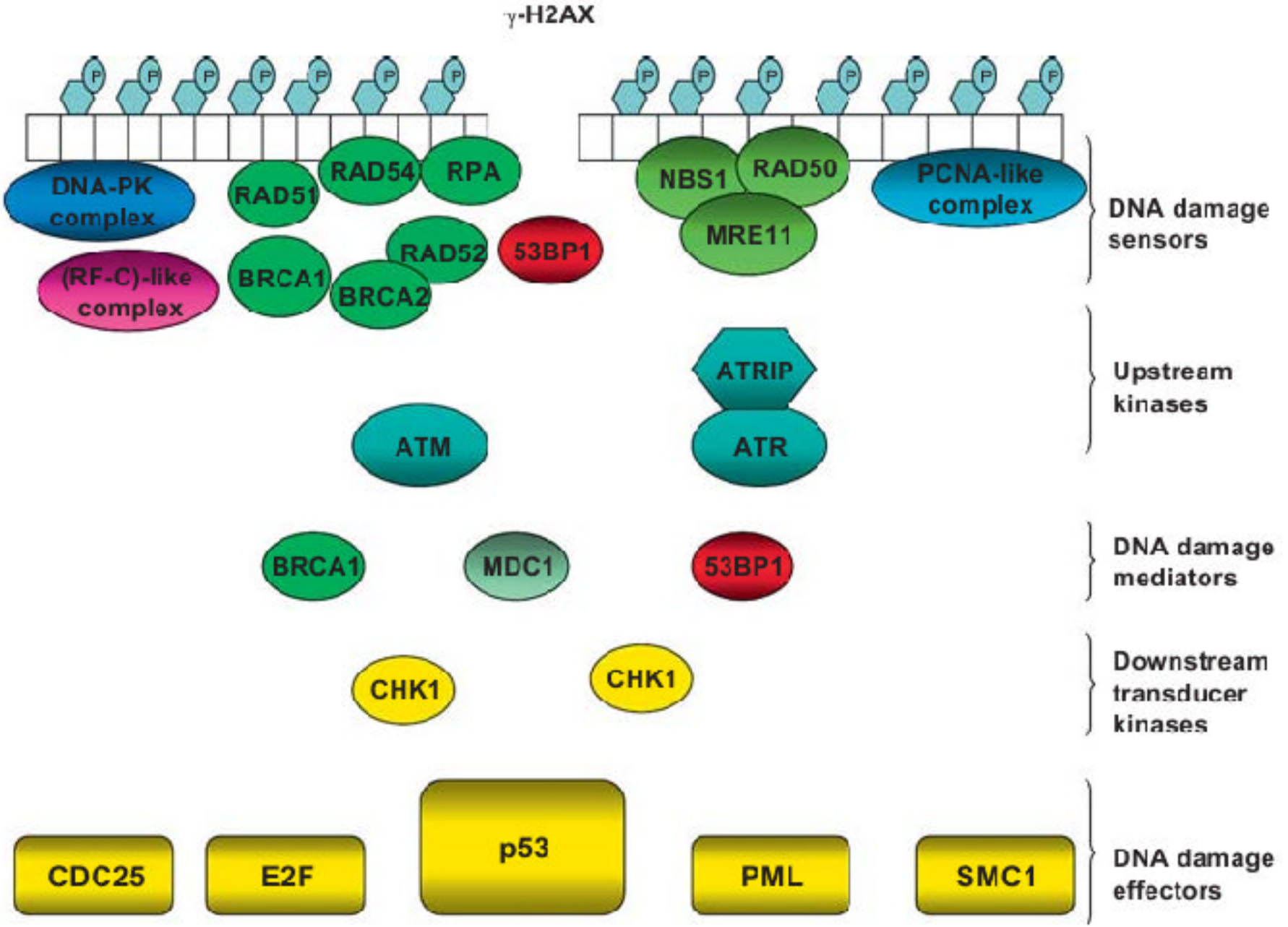


Cell treated with a DNA cross-linking agent and irradiated with 12.5 Gy X-rays

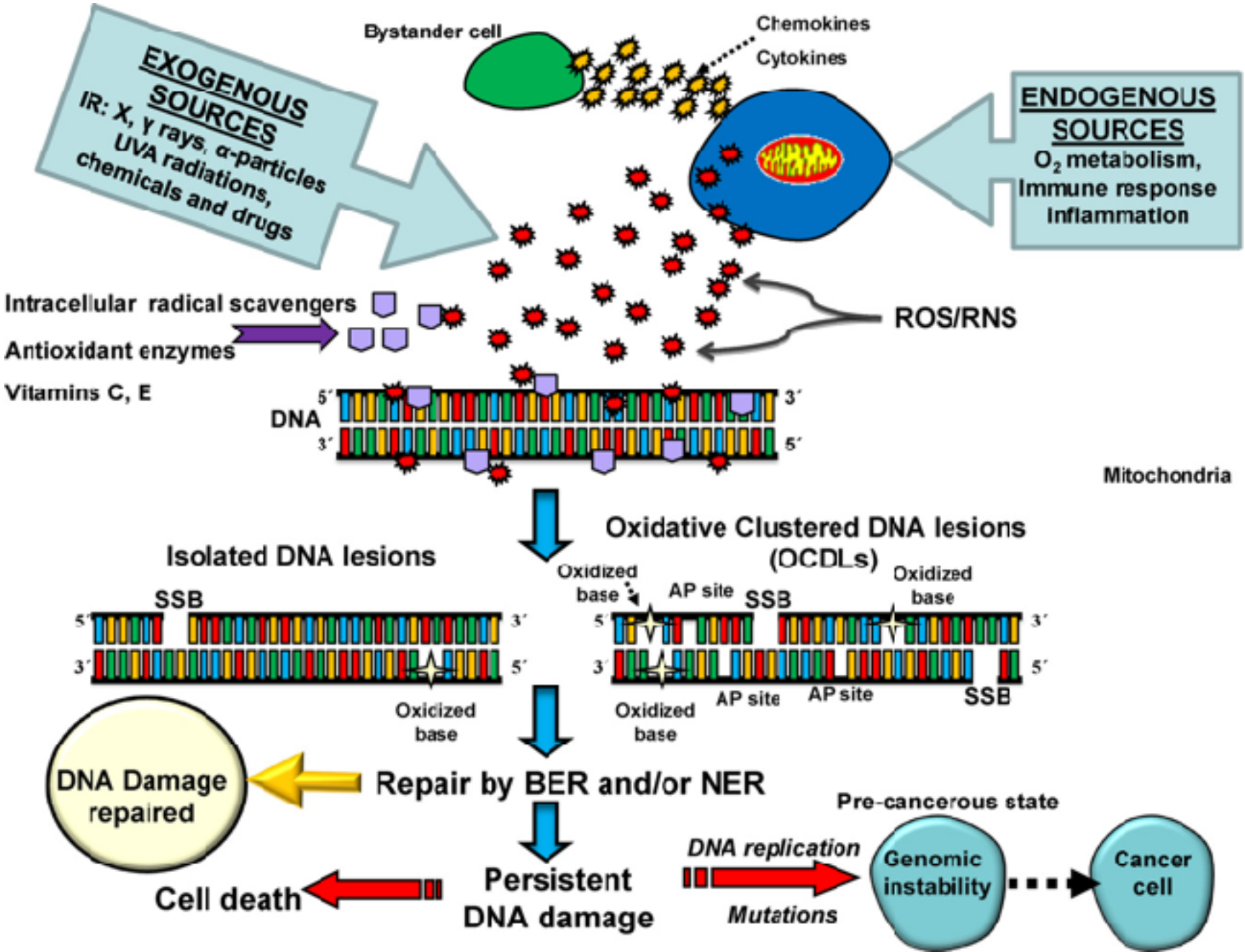


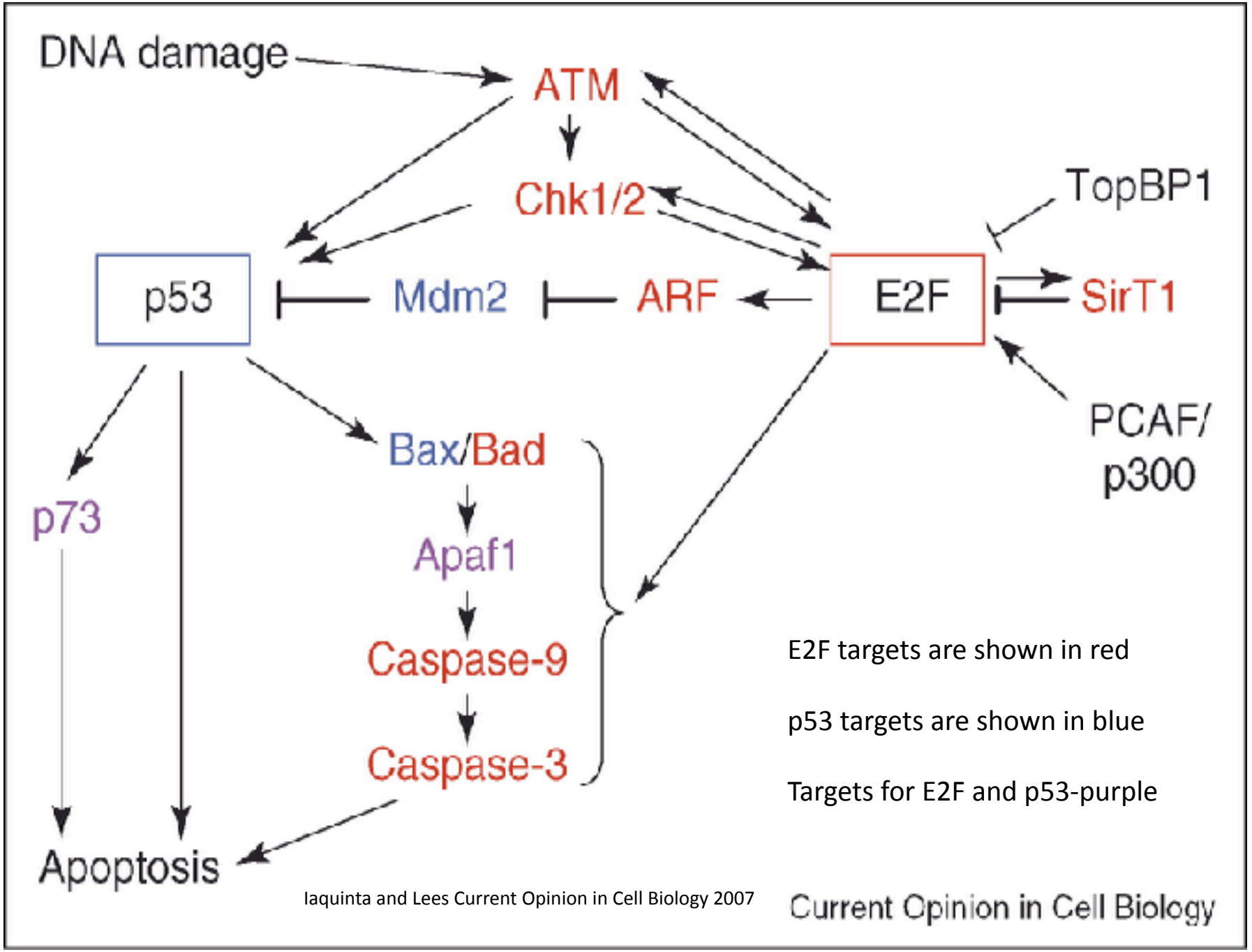
Hartley et al., Cancer Cell Culture: Methods and Protocol Vol 731 Chapter 25

# Major regulatory steps in the process of DNA damage response

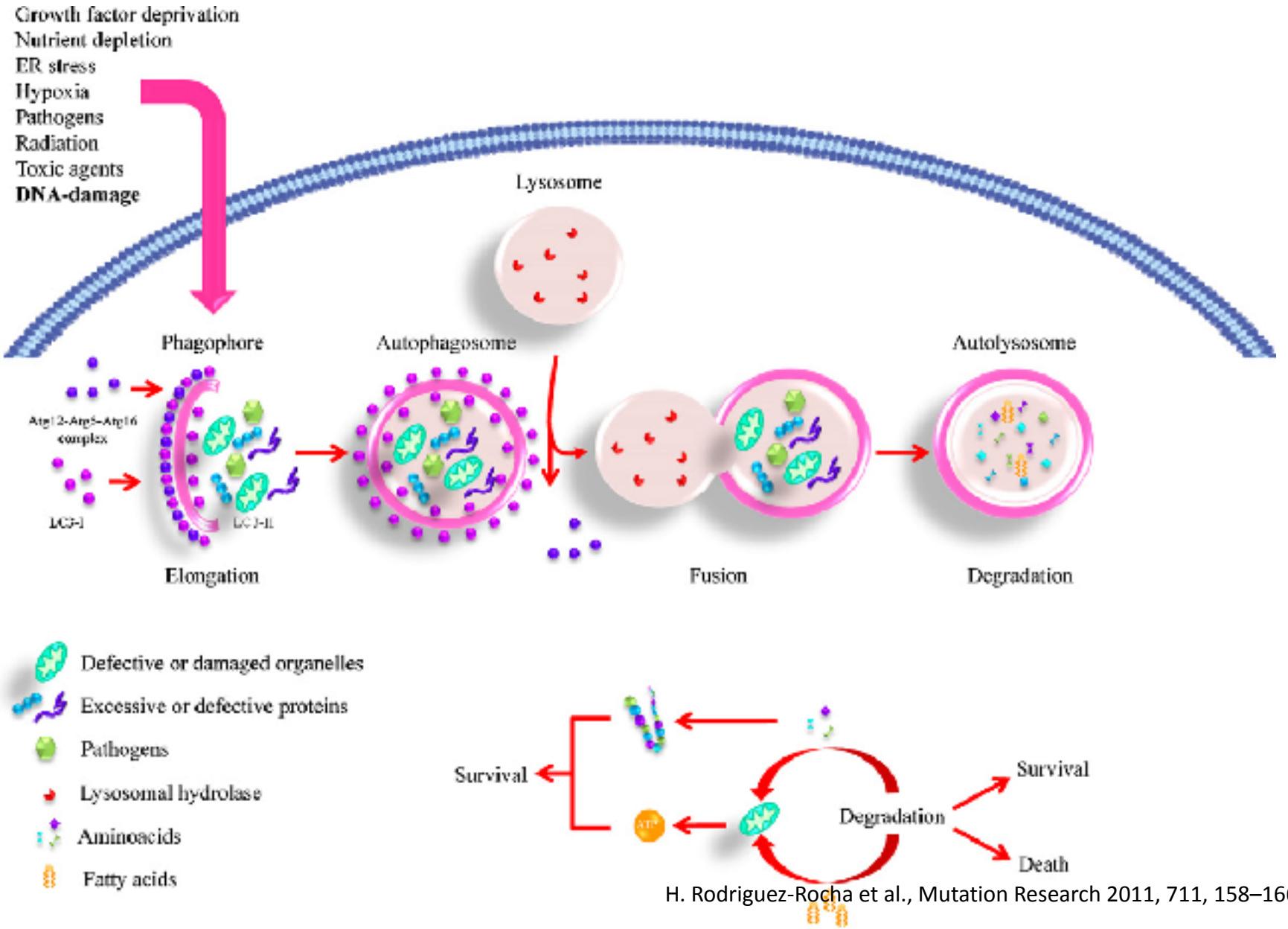


# DNA damage and human cancer



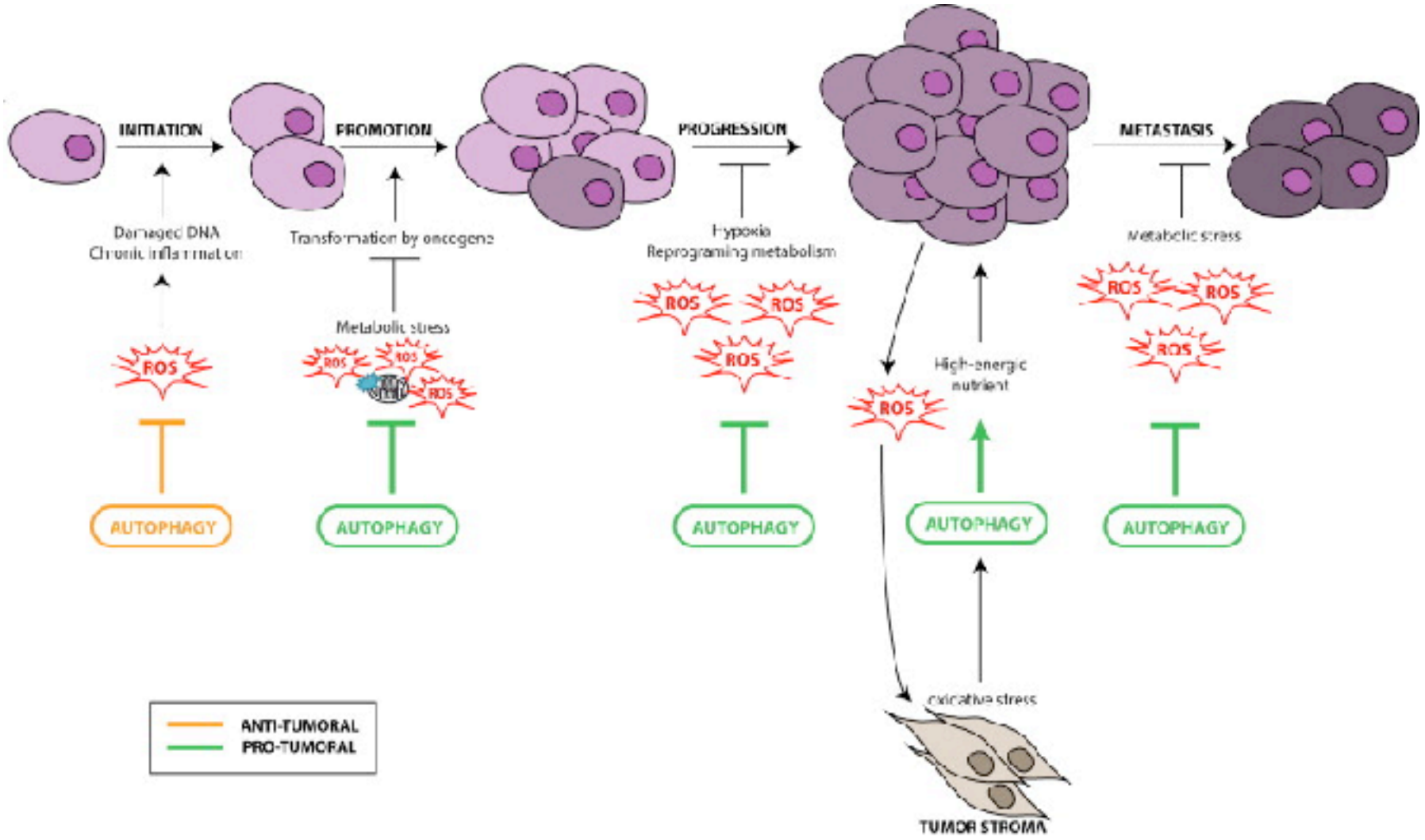

DNA damage
ATM
Chk1/2
TopBP1
p53
Mdm2
ARF
E2F
SirT1
PCAF/
p300
Bax/Bad
p73
Apaf1
Caspase-9
Caspase-3
Apoptosis
E2F targets are shown in red
p53 targets are shown in blue
Targets for E2F and p53-purple
Iaquinta and Lees Current Opinion in Cell Biology 2007
Current Opinion in Cell Biology

# DNA damage and autophagy




H. Rodriguez-Rocha et al., Mutation Research 2011, 711, 158–166

# DNA damage, autophagy, and Cancer



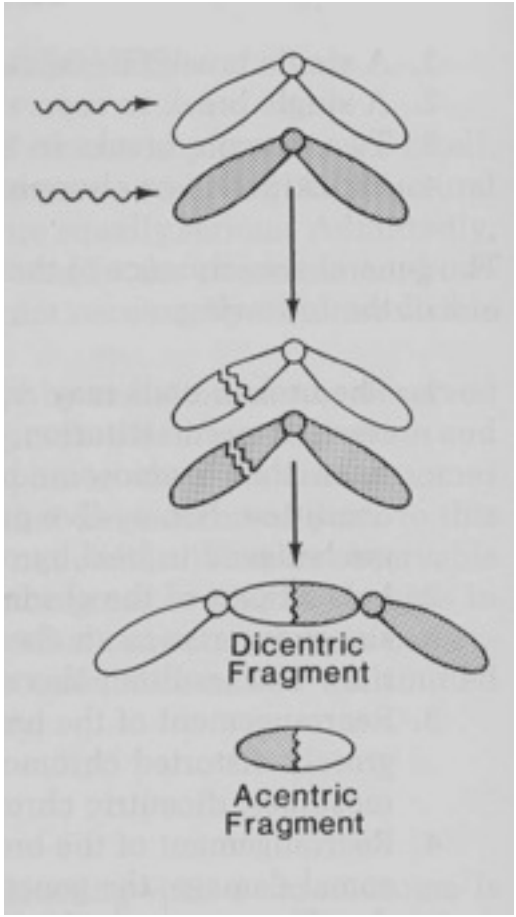

http://dx.doi.org/10.1016/j.redox.2014.12.003

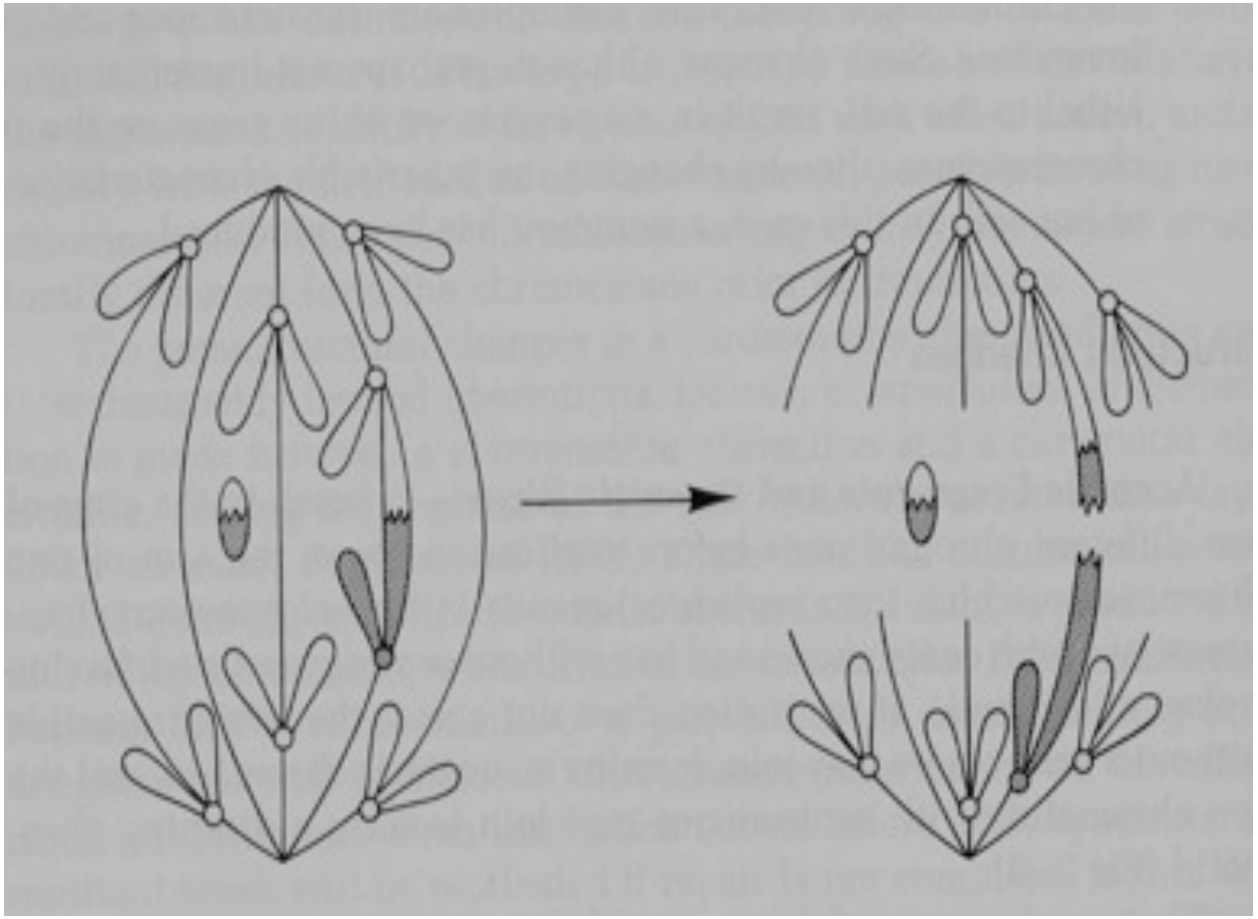
# Radiation-induced chromosomal aberrations

X-rays or ionizing radiation induces DSBs in the chromosomes. DSBs causes sticky ends, which can join with any other sticky ends.

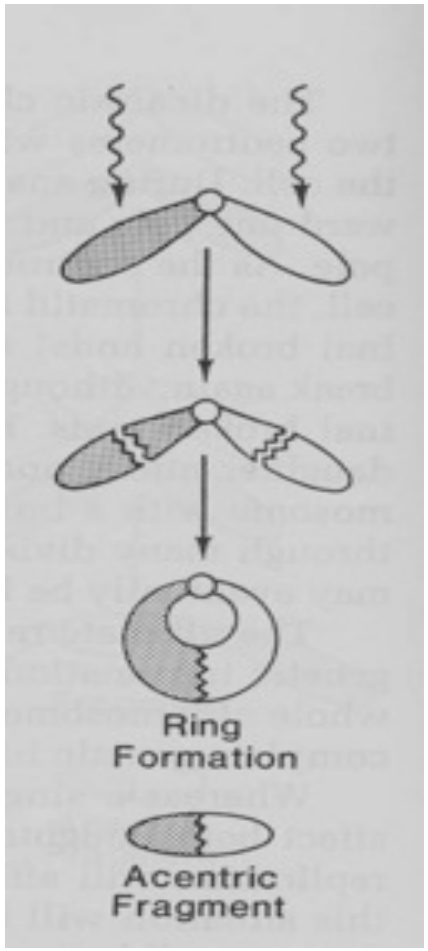
1) Rejoin to original configurations
2) The breaks fails to rejoin causing deletion
3) Broken ends may join other sticky ends

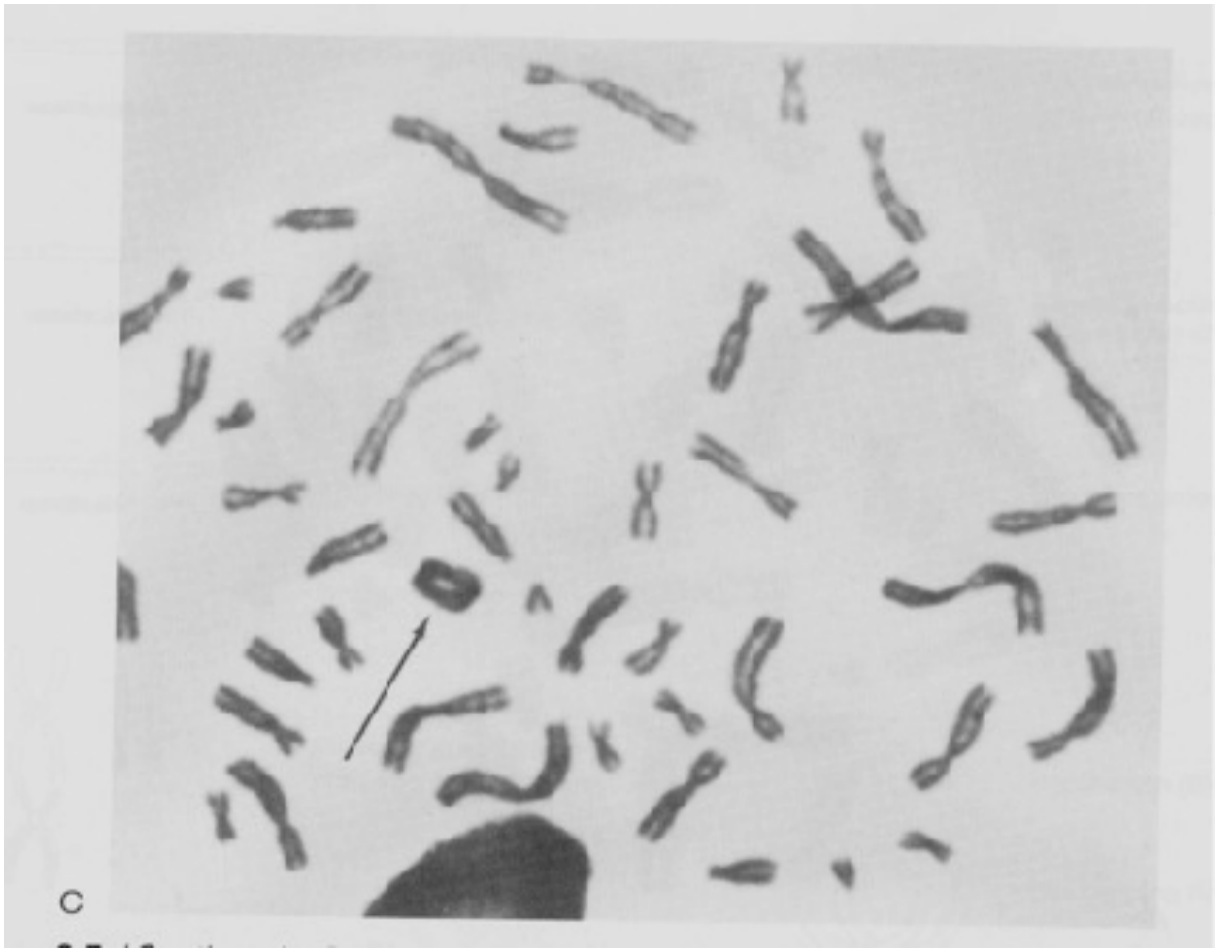
# Acentric and dicentric chromosomes



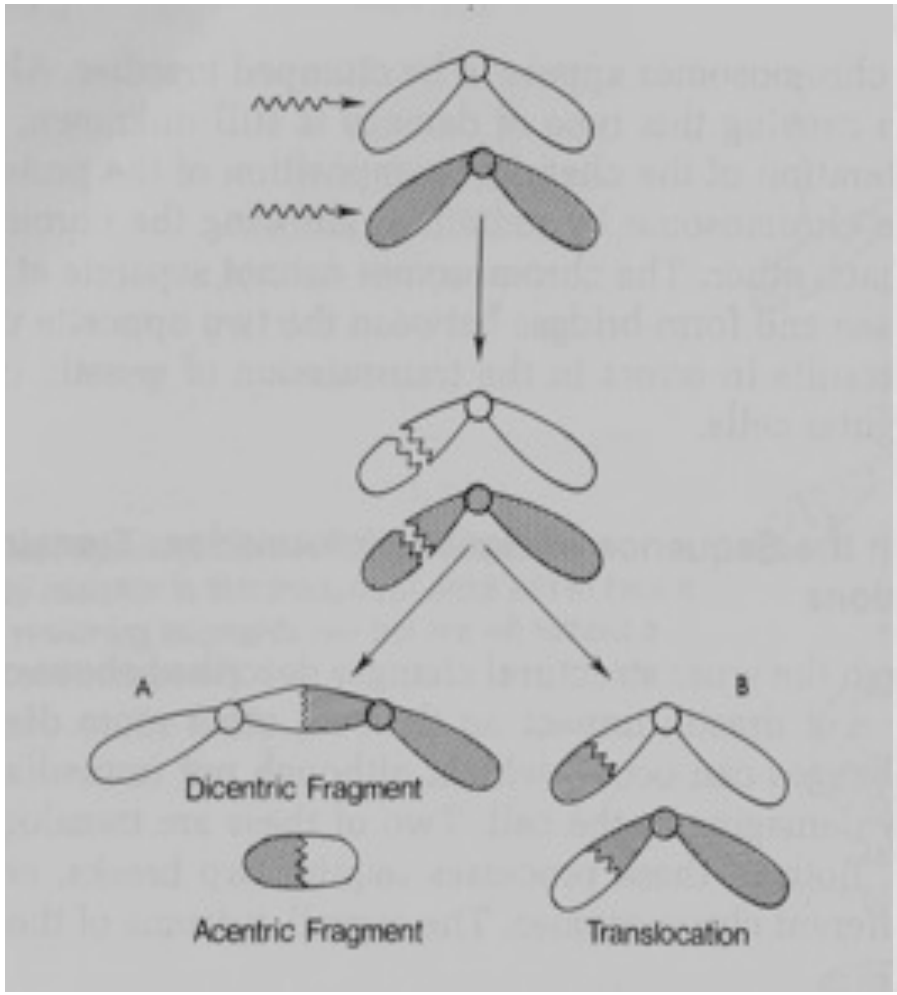



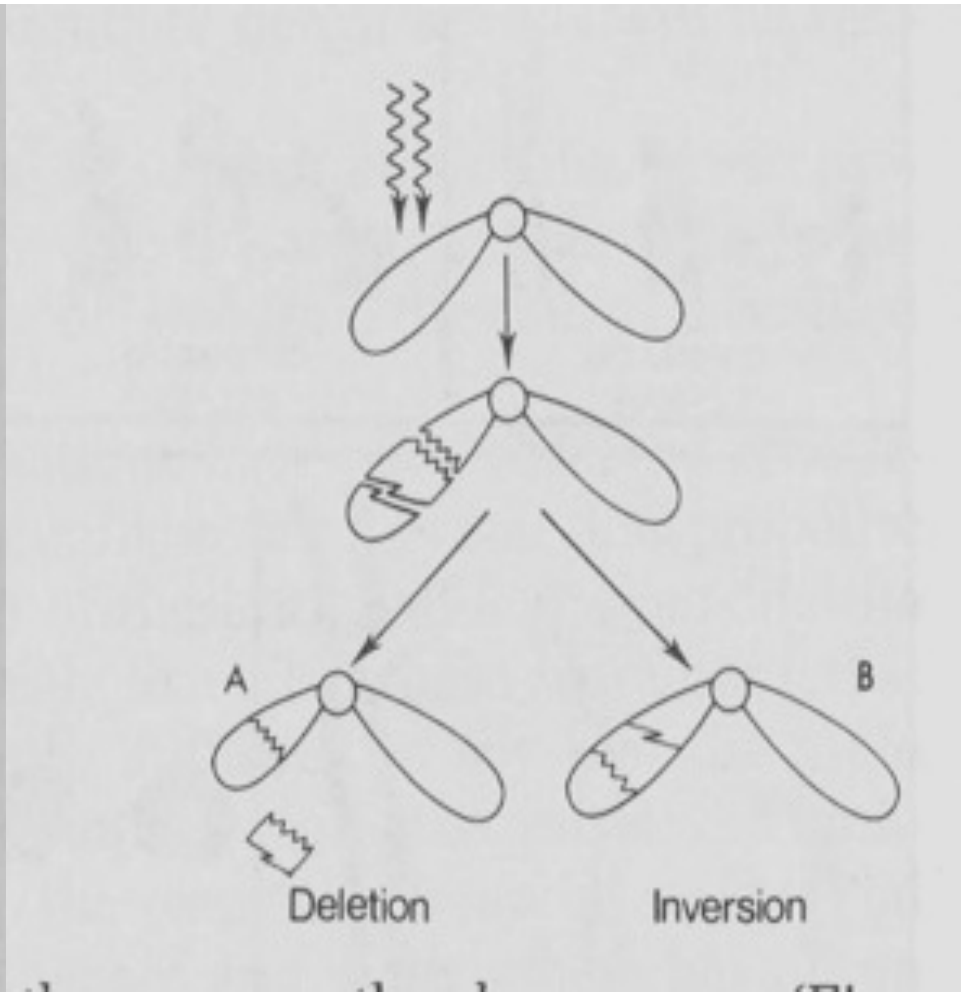
# Ring chromosome



# Translocation, deletion, and inversion



# Bystander effect

- Genetic alterations can occur in cells that receive no direct radiation exposure
- Damage signals transmitted from neighboring irradiated cells

# Bystander effect

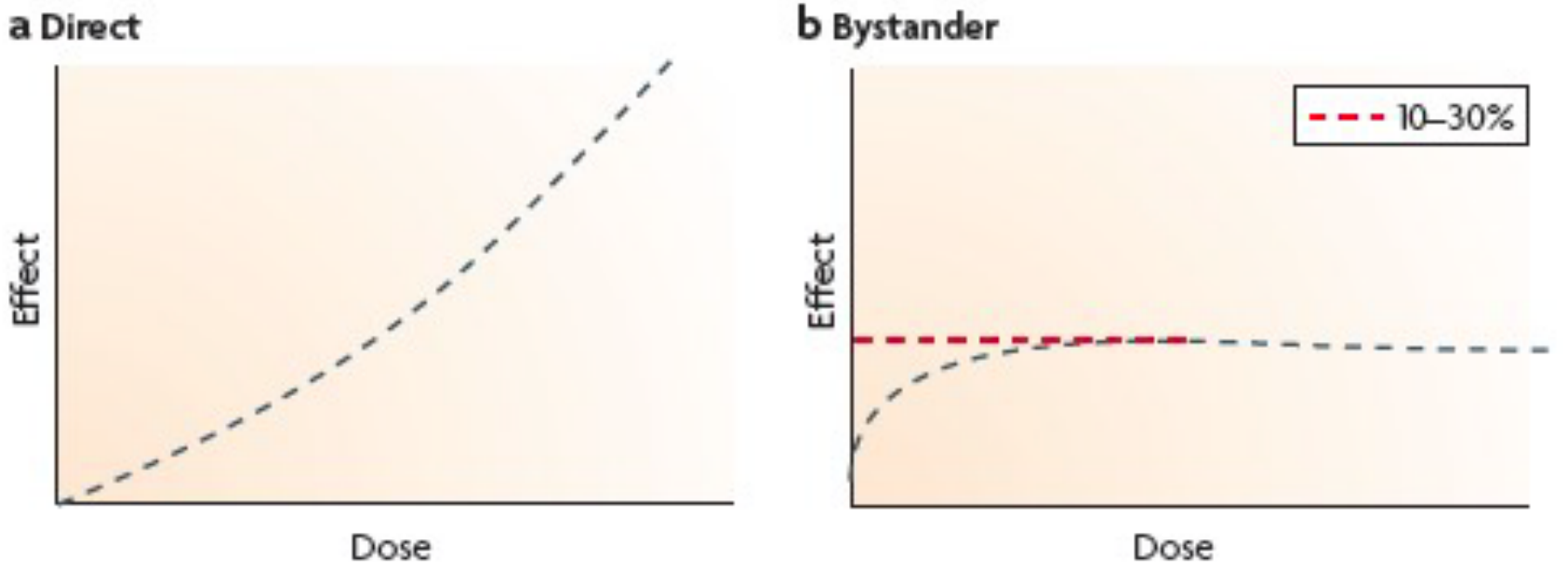
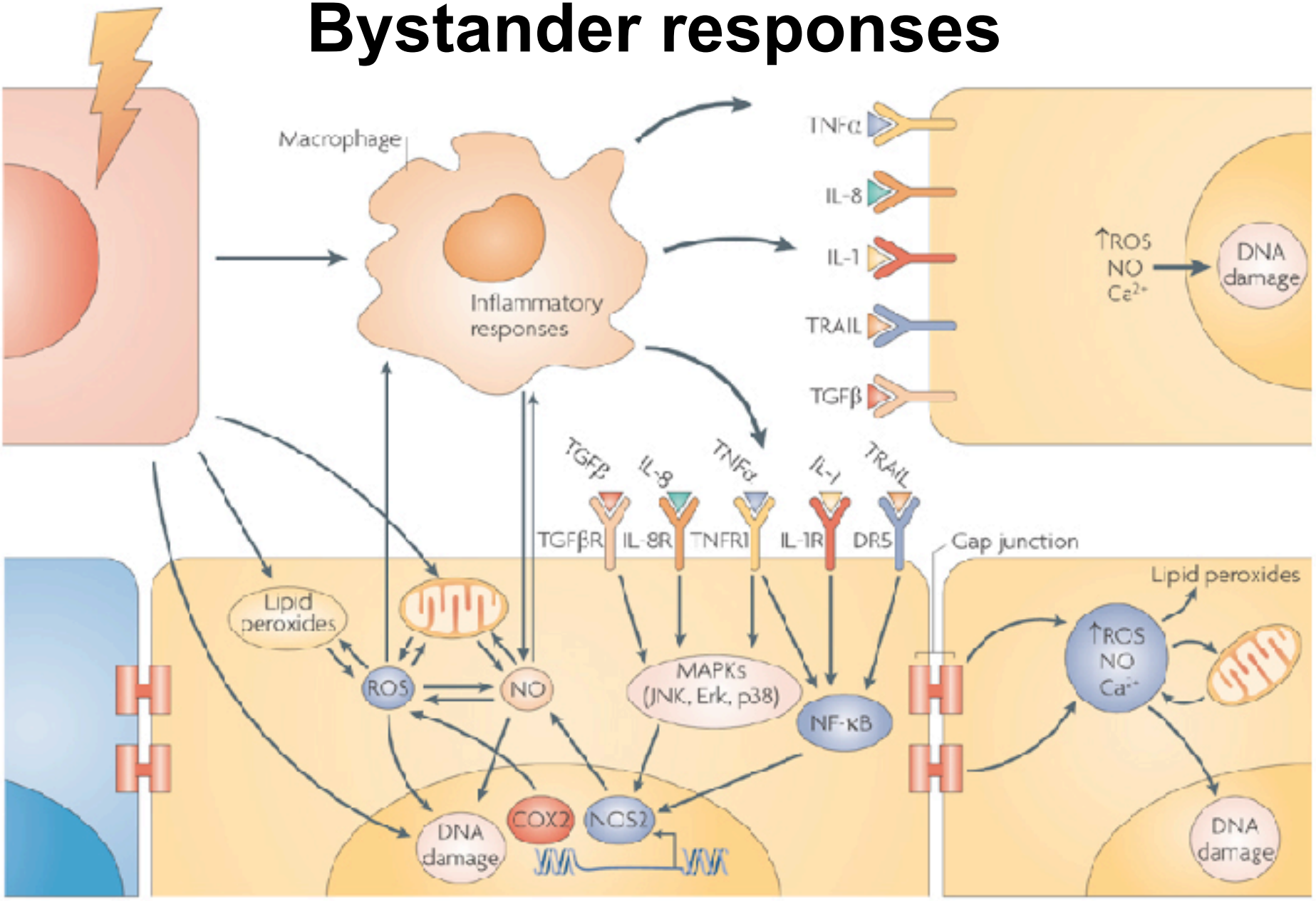



Figure 2 | **Key aspects of radiation-induced bystander responses.** Typical dose response curves for direct (**a**) and bystander (**b**) responses are shown, highlighting the commonly observed saturation of response for bystander effects.

Prise and Sullivan 2009

www.nature.com/reviews/cancer

# Bystander responses




Prise and Sullivan 2009

# Cancer incidence at various ages

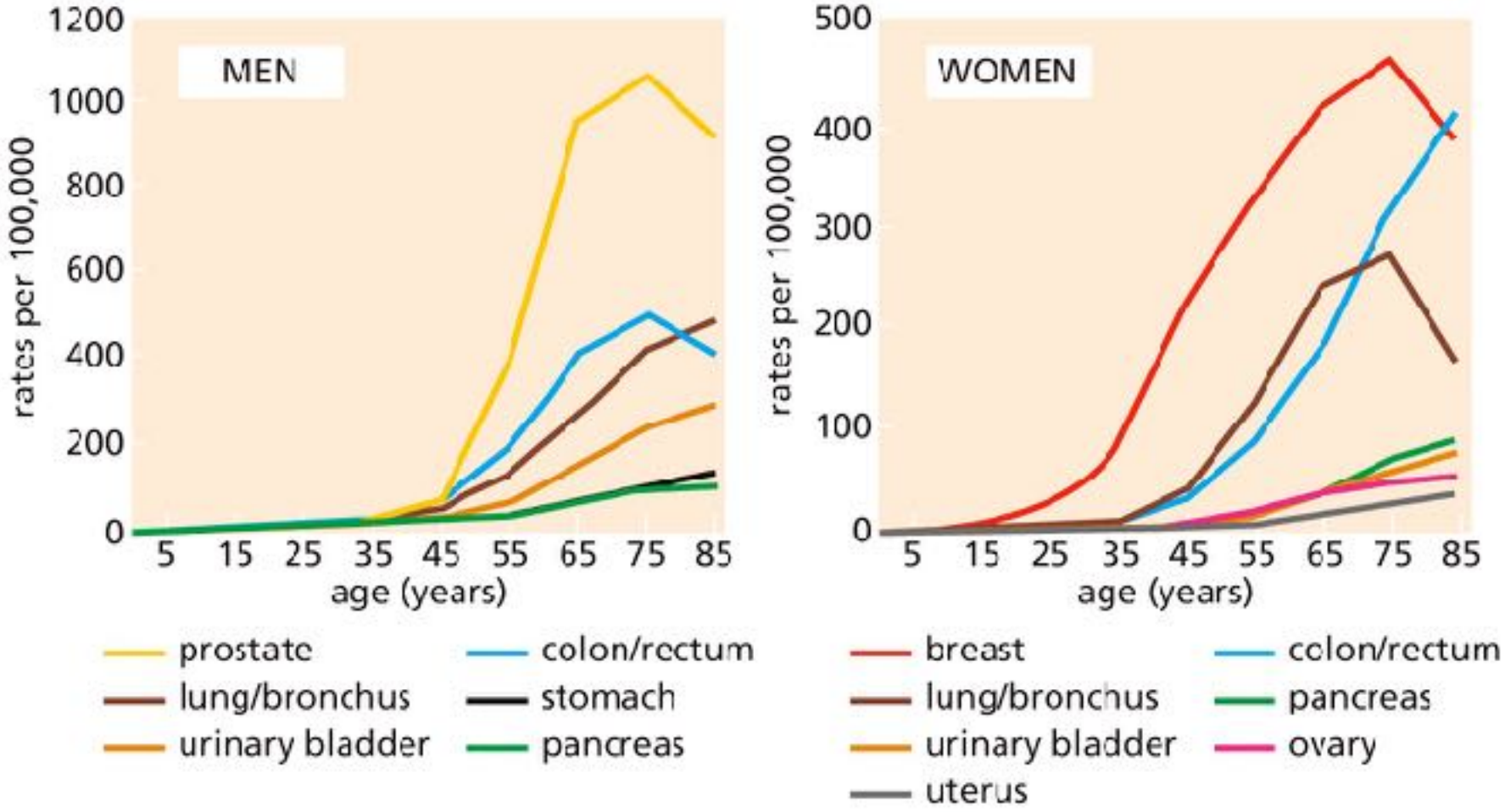



Figure 11.1 The Biology of Cancer (© Garland Science 2014)

# Multistep tumorigenesis in variety of organ sites

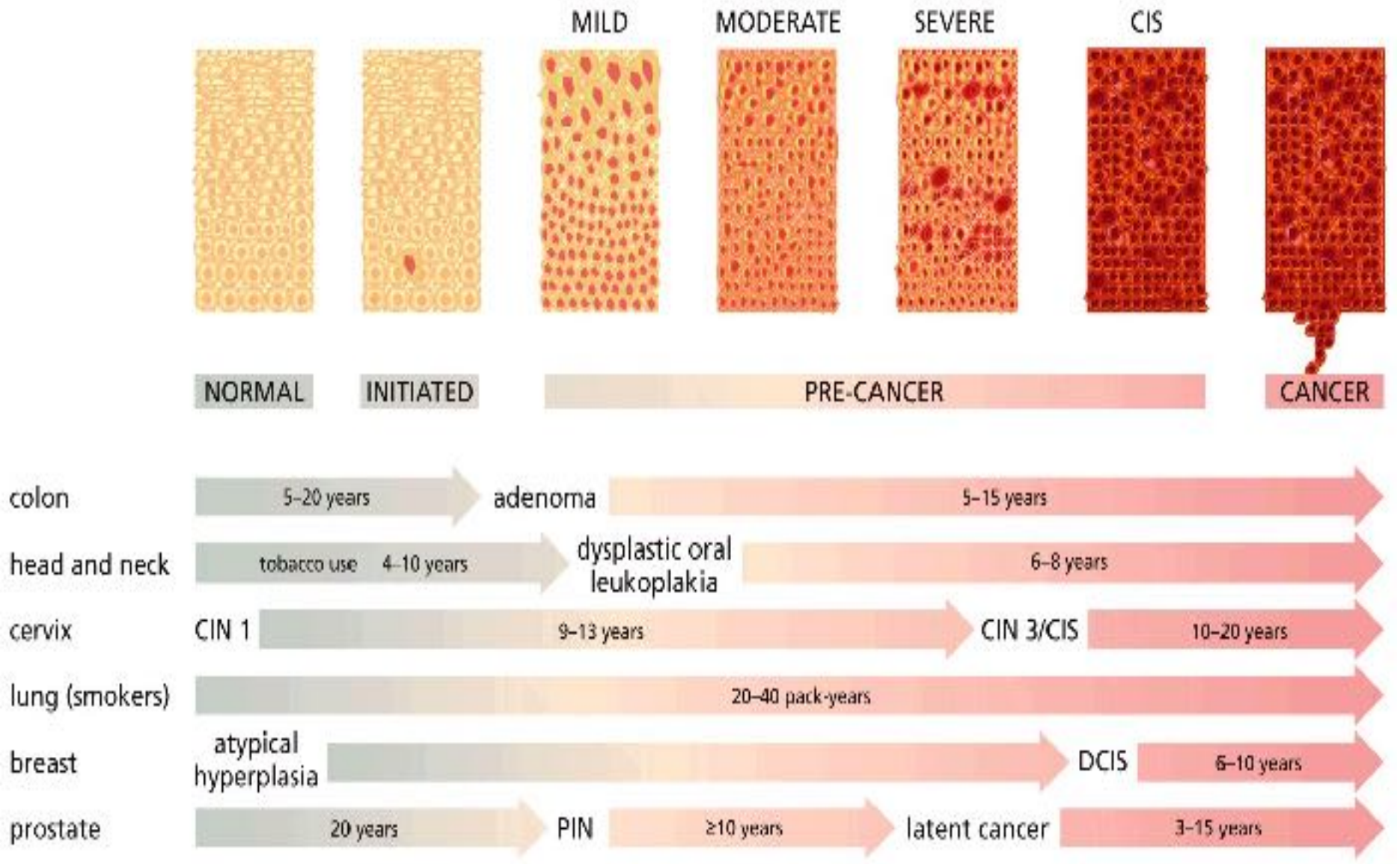



Figure 11.8a The Biology of Cancer (© Garland Science 2014)

# Mouse skin model

INITIATION | PROMOTION | PROGRESSION

1. Covalent binding of carcinogen to DNA, cell replication, and fixation of mutation.
2. Mutation induction in critical target genes of stem cells, e.g. H-ras
3. Phenotypically "normal" epidermis

1. Expansion of initiated stem cells through epigenetic mechanisms
2. Altered gene expression/ enzyme activities
3. Angiogenesis

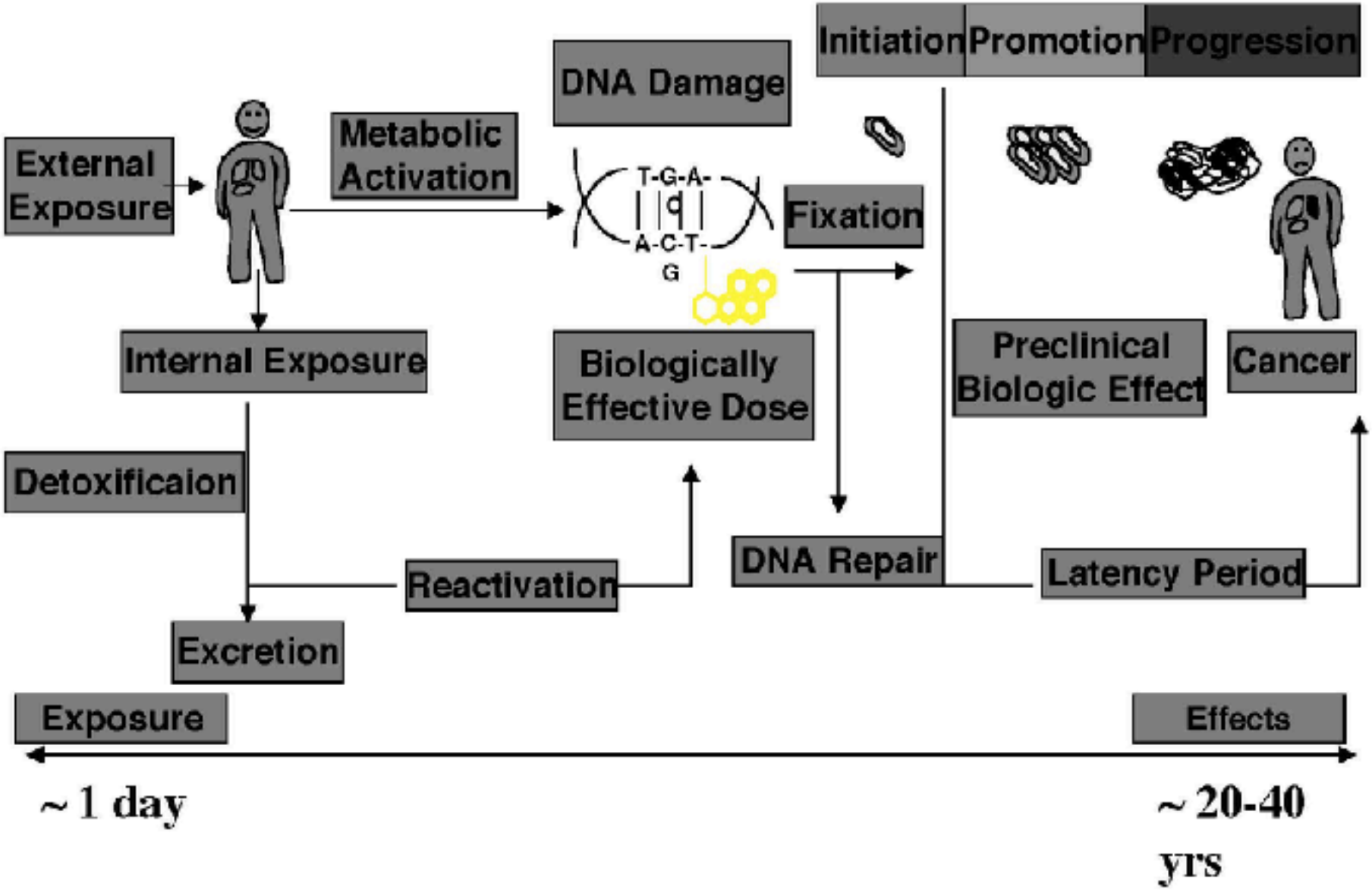
1. Production and maintenance of chronic cell proliferation
2. Development of clonal outgrowths; benign papillomas
3. Altered differentiation
4. Diploid stem line

1. Additional genetic events occurring stochastically
2. Aneuploidy e.g. nonrandom trisomies of chromosomes 6 & 7
3. LOH
4. Further alteration in differentiation
5. Dysplasia

1. Invasion
2. Metastasis
3. Loss of tumor suppressor activity e.g. p53 mutation
4. Gene amplification e.g. mutated Hras allele

GENETIC SUSCEPTIBILITY

# Overview of carcinogenesis



# Oncogene activation and inactivation of tumor suppression genes

- Activation of proto-oncogenes
- Loss of function of tumor suppressors
- Infection with certain viruses
- Substitution of normal promoters of proto-oncogenes with strong promoters of viruses
- Chromosomal aberrations

# Oncogene activation and inactivation of tumor suppression genes

- Mutational event in initiation of radiation carcinogenesis most likely involves LOH of a tumor suppressor gene

- Deletion of RB tumor suppressor gene on 13q14

- Hypersensitivity of retinoblastoma patients to the induction of secondary cancers

# Oncogene activation and inactivation of tumor suppression genes

- Knockout mice heterozygous for p53 tumor suppressor gene more susceptible to radiation induced tumors

- Expression of p53 mutations occur late in radiation-induced malignant transformation

- Activation of oncogene RAS family reported in mouse lymphomas

# Oncogene activation and inactivation of tumor suppression genes

- Radiation may induce papillary thyroid carcinomas in children as a result of oncogene activation

- Amplification/overexpression of MDM2 found in X-ray transformed foci and expression of mutant p53

- Multiple pathways for transformation

# Four-stage hypothesis

- Chromosomal damage in normal dividing cells
- Defect in differentiation genes
- Gene defect in hyperplastic cells
- Gene defect in cancer cells

# Chromosomal damage in normal cells

- **Low or high dose radiation exposure can lead to chromosomal damage in normal cells.**
- **These cells may undergo cell death, divide, or differentiate.**

# Defect in differentiation genes

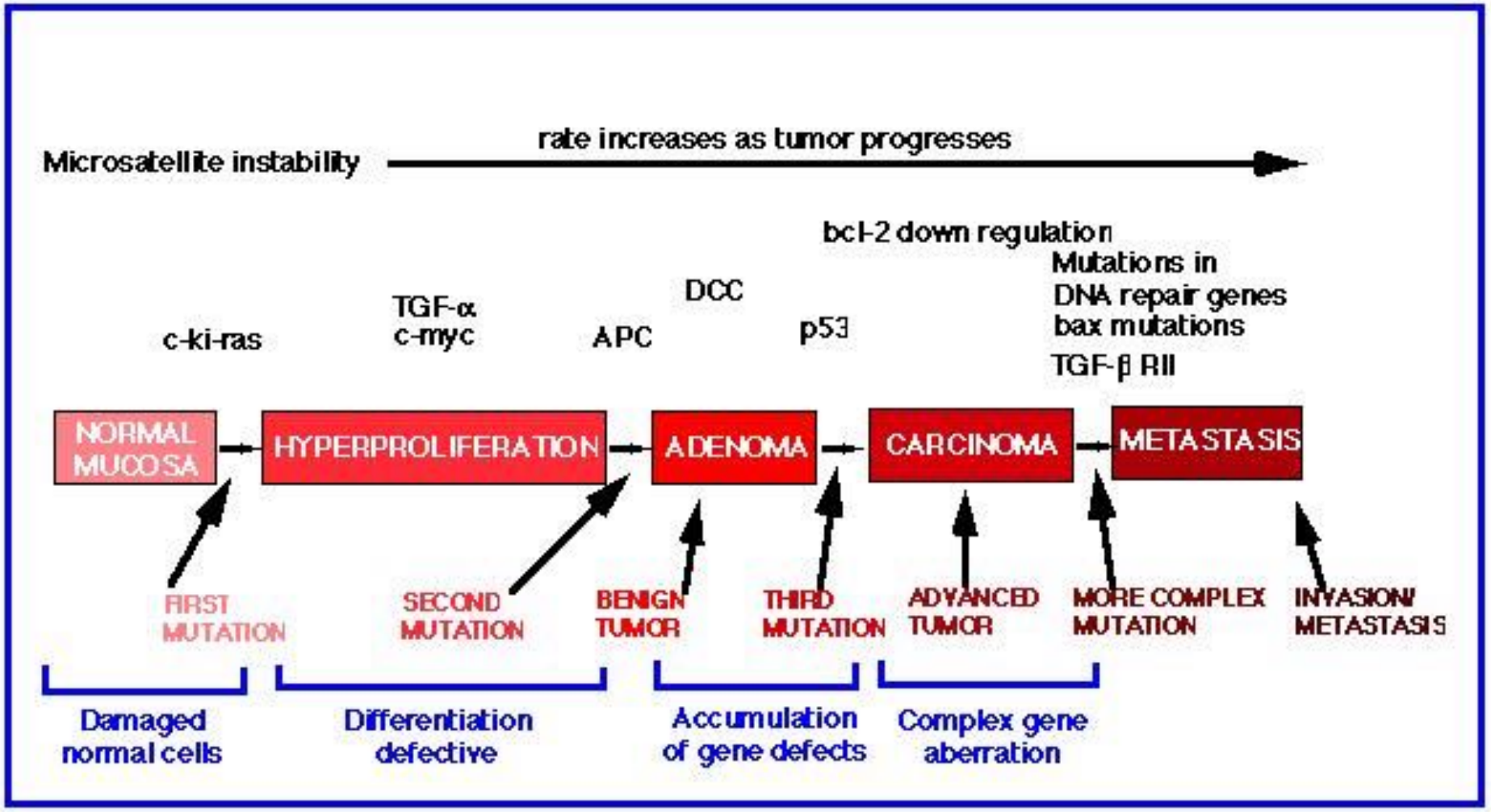
- **One or two normal damaged cells develop a defect in differentiation genes, which prevent them from a normal pattern of differentiation and death.**

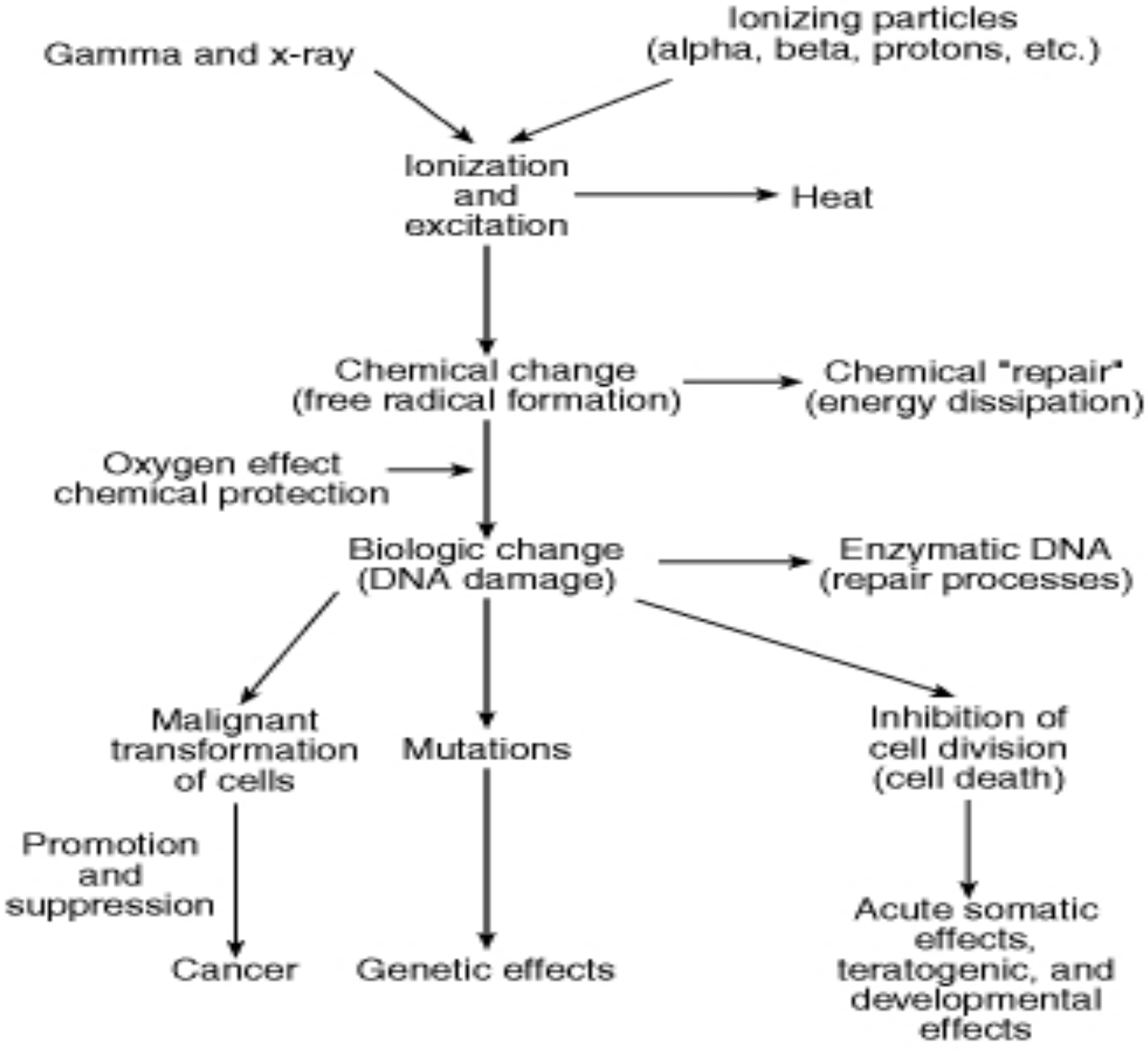
- **Continuing division of these cells leads to hyperplasia and develop in adenoma.**

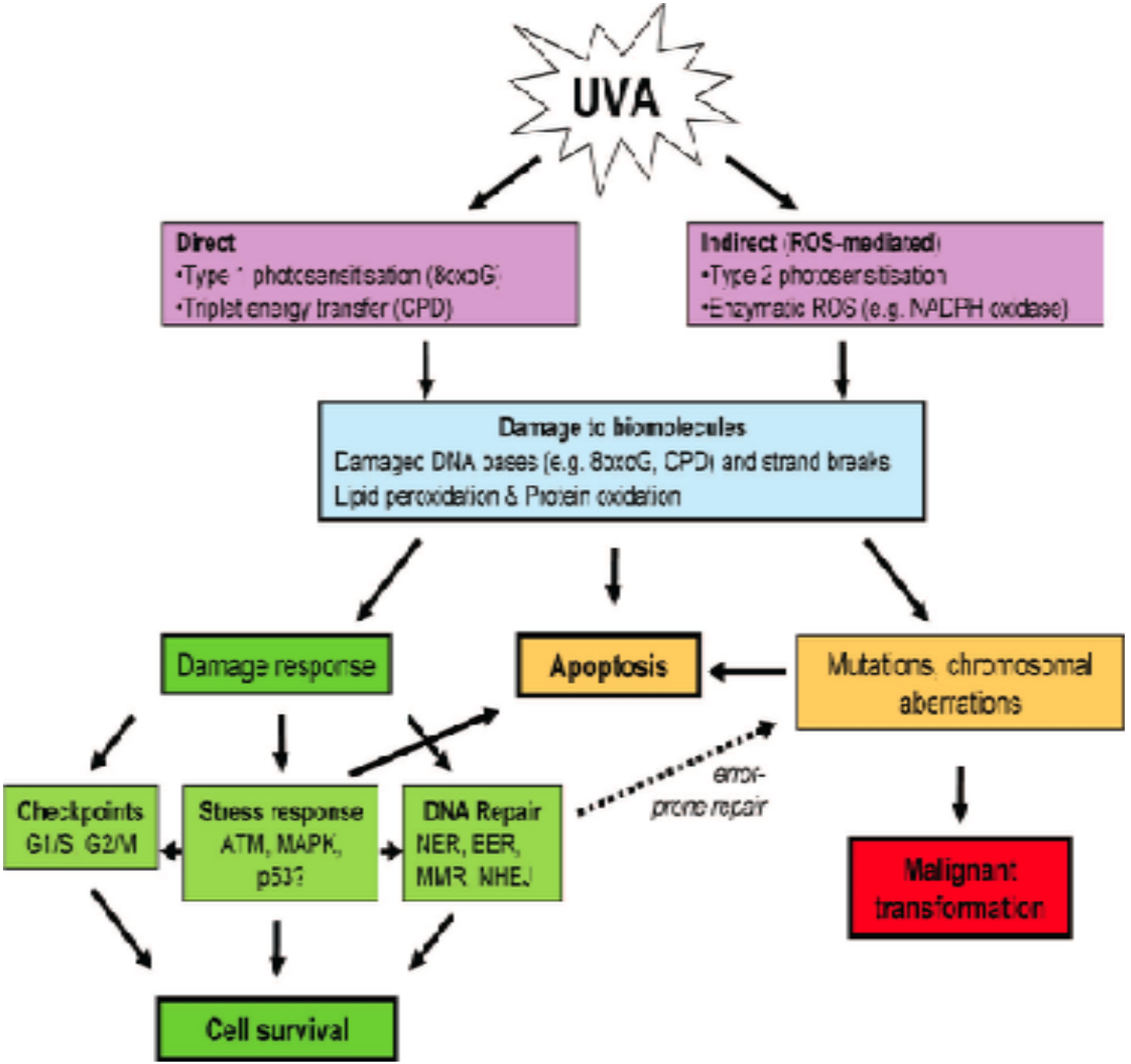
# Accumulated gene defects in cells causes cancer

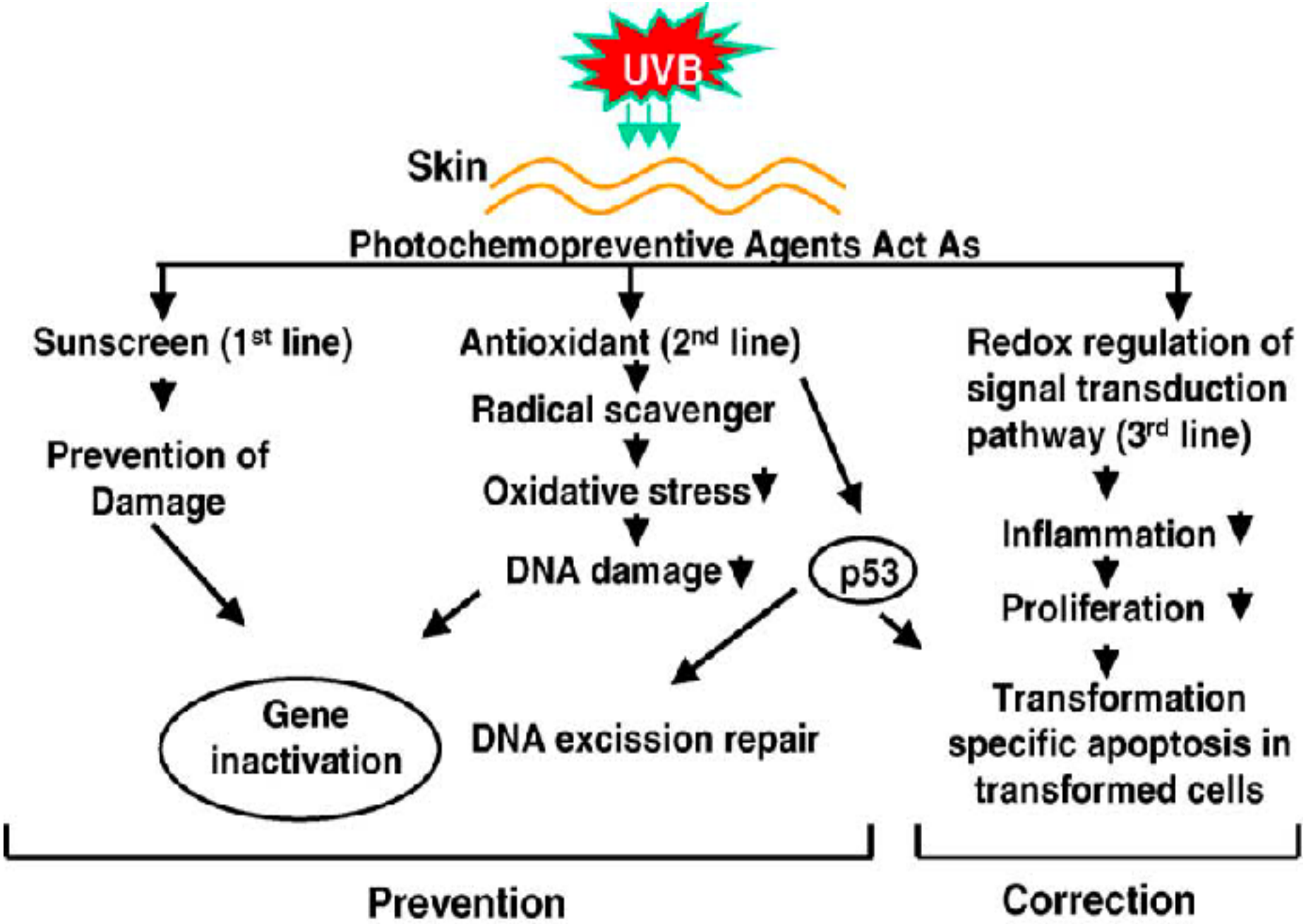
- **One or two hyperplastic cells in any adenoma can accumulate additional gene defects due to mutations or chromosomal damage, which can make them cancerous.**

# Colon tumor model




Gamma and x-ray
Ionizing particles
(alpha, beta, protons, etc.)
Ionization
and
excitation
Heat
Chemical change
(free radical formation)
Chemical "repair"
(energy dissipation)
Oxygen effect
chemical protection
Biologic change
(DNA damage)
Enzymatic DNA
(repair processes)
Malignant
transformation
of cells
Mutations
Inhibition of
cell division
(cell death)
Promotion
and
suppression
Cancer
Genetic effects
Acute somatic
effects,
teratogenic, and
developmental
effects


UVA
Direct
•Type I photosensitisation (8oxoG)
•Triplet energy transfer (CPD)
Indirect (ROS-mediated)
•Type 2 photosensitisation
•Enzymatic ROS (e.g. NADPH oxidase)
Damage to biomolecules
Damaged DNA bases (e.g. 8oxoG, CPD) and strand breaks
Lipid peroxidation & Protein oxidation
Damage response
Apoptosis
Mutations, chromosomal aberrations
Checkpoints G1/S G2/M
Stress response ATM, MAPK, p53
DNA Repair NER, BER, MMR, NHEJ
error-prone repair
Malignant transformation
Cell survival


UVB
Skin
Photochemopreventive Agents Act As
Sunscreen (1st line)
Prevention of Damage
Antioxidant (2nd line)
Radical scavenger
Oxidative stress
DNA damage
Redox regulation of signal transduction pathway (3rd line)
Inflammation
Proliferation
Transformation specific apoptosis in transformed cells
p53
Gene inactivation
DNA excission repair
Prevention
Correction

# Types of risk model

- **Absolute Risk Model** – radiation induces cancers over and above the natural incidence.

  -leukemia follows an absolute risk model

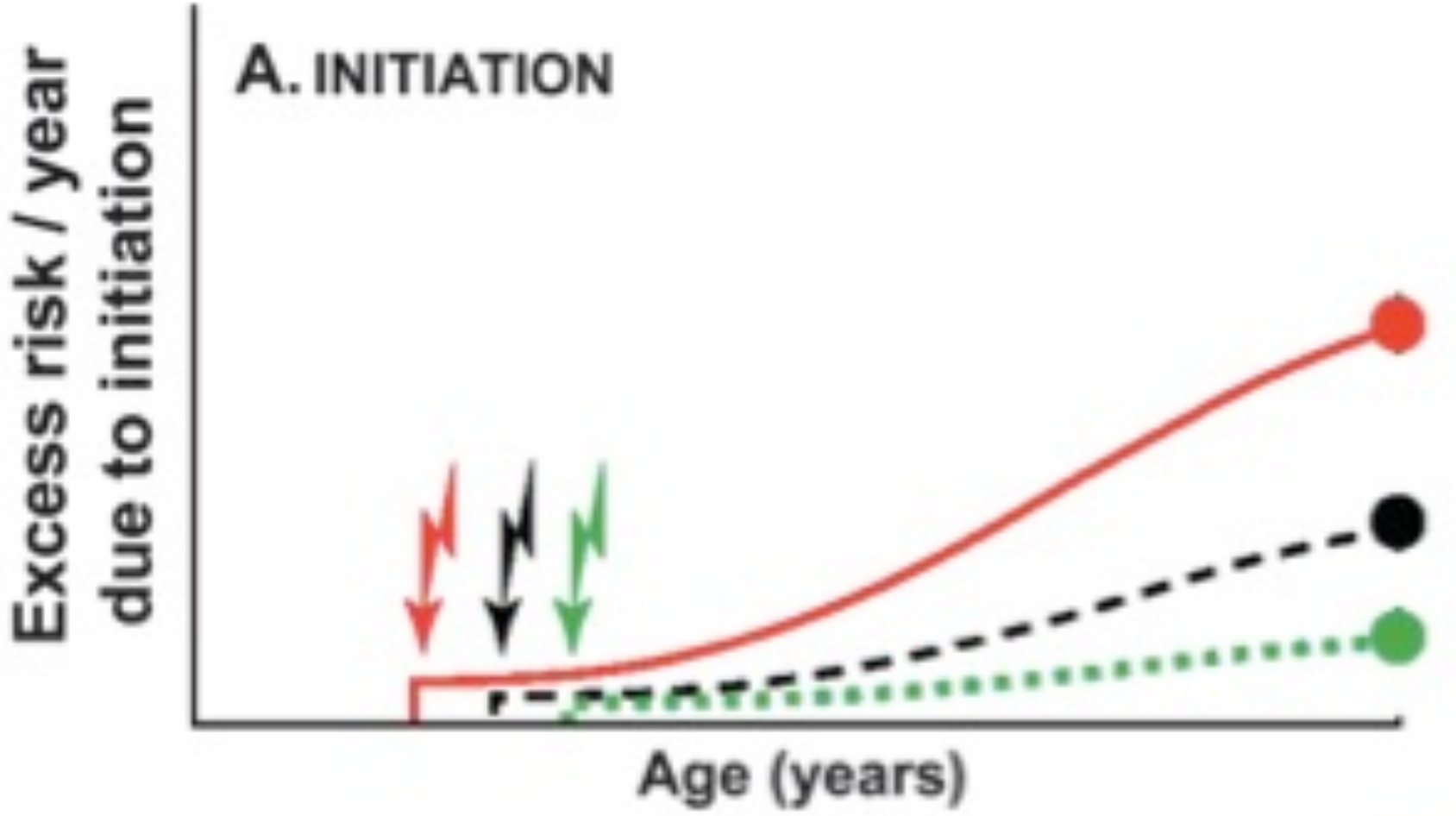
- **Relative Risk Model –** radiation increases the natural incidence at all ages proportional to spontaneous background rates (predicts a larger number of induced cancers in old age following radiation)

- **Time-dependent relative risk –** function of dose, age at exposure, time since exposure, gender, etc.

# Cancer latency

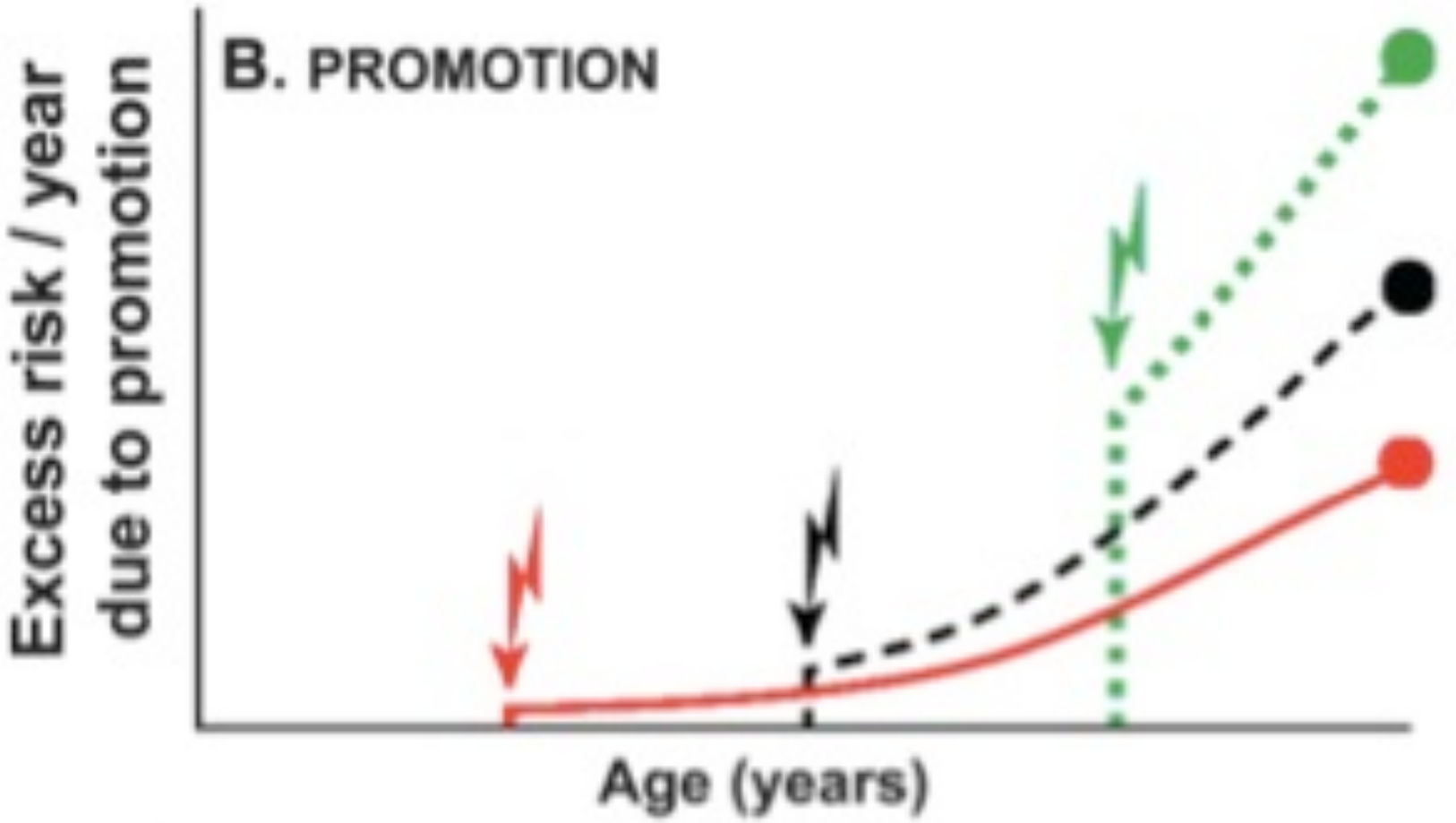
- Leukemia has the sortest latency of about 5 years
- Whereas, solid cancers have a latency of 20 or more years following radiation

# Risk related to initiation upon radiation exposure




Shuryak et. al., JNCI 2010

# Risk related to promotion upon radiation exposure

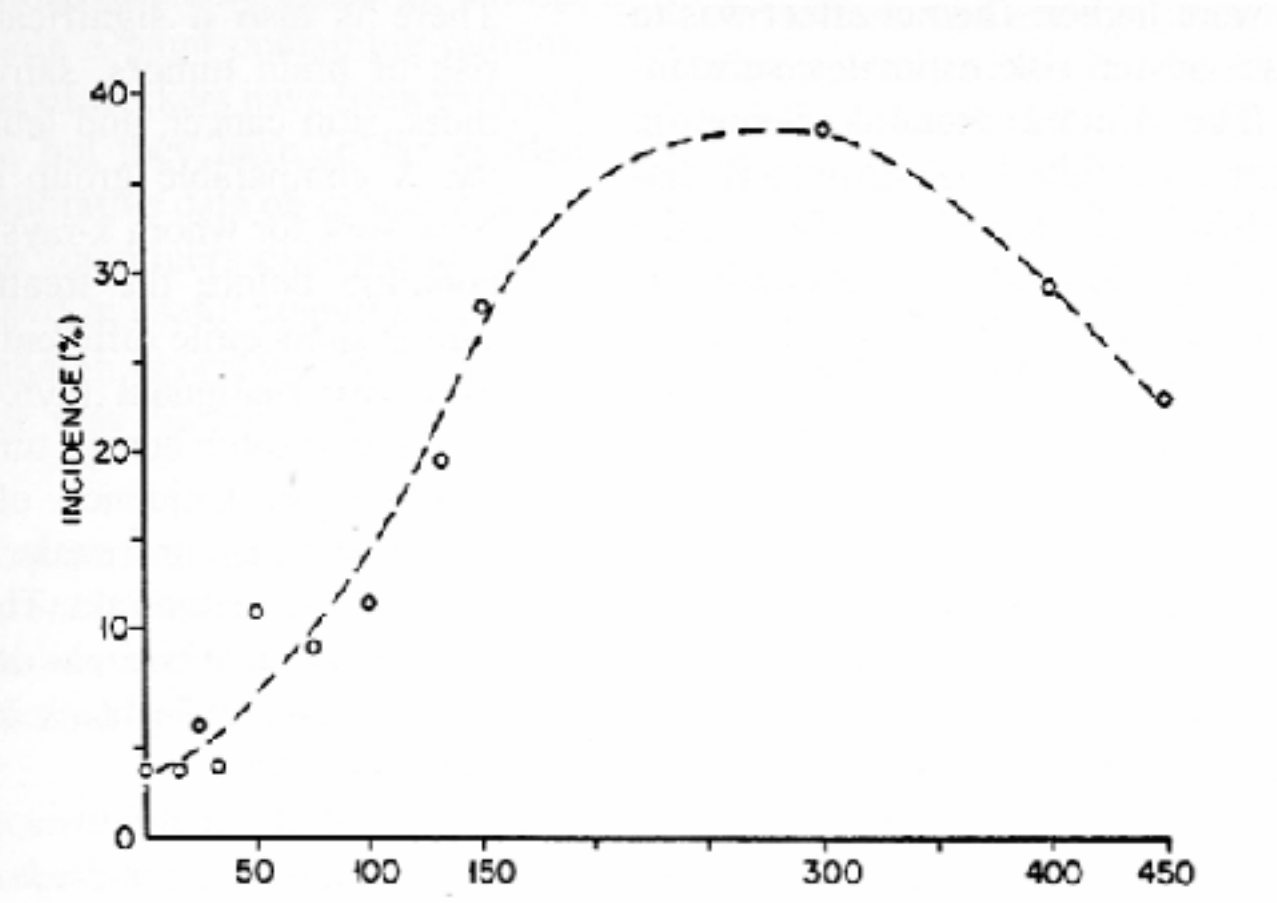



Shuryak et. al., JNCI 2010

# Risk related to initiation and promotion upon radiation exposure

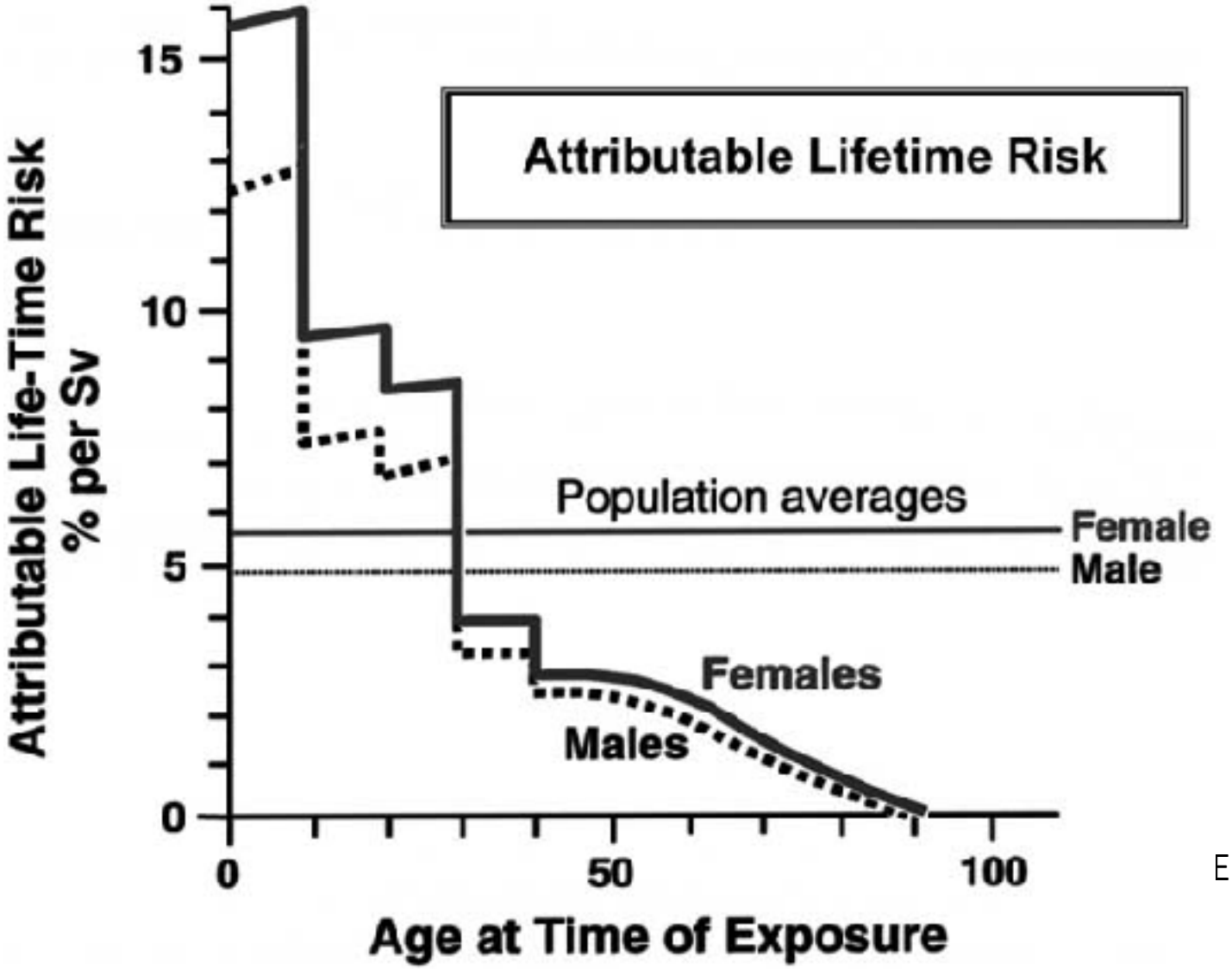

Shuryak et. al., JNCI 2010

# Dose-response relationships




Example of myeloid leukemia in male mice given total body x-irradiation

# Age plays a critical role for cancer risk



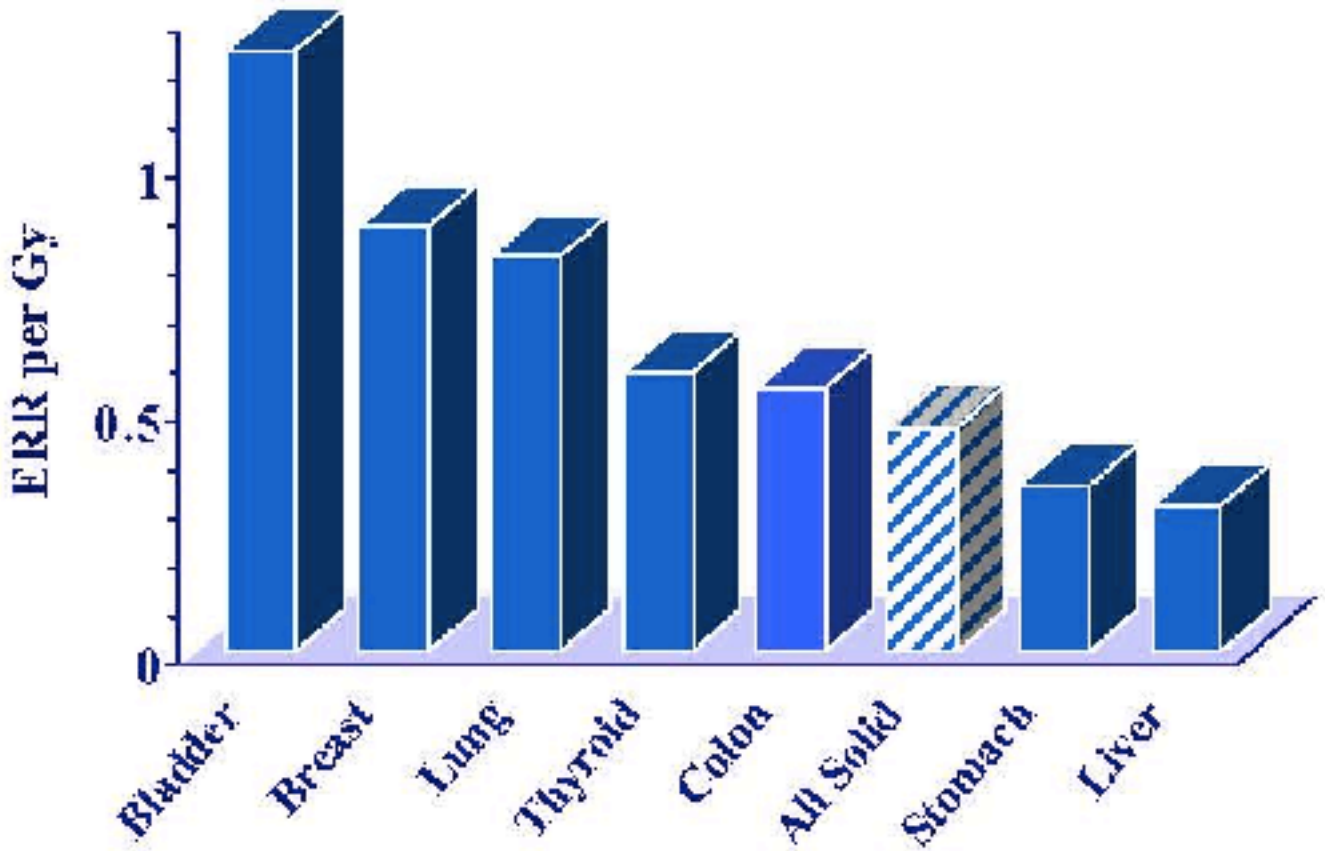

Eric Hall, Ph.D.,

**The data suggest that children and young adults are much more susceptible to radiation-induced cancer than the older aged populations.**

***FIGURE 10.8*** ● The attributable lifetime risk from a single small dose of radiation at various ages at the time of exposure. Note the dramatic decrease in radiosensitivity with age. The higher risk for the younger age-groups is not expressed until late in life. These estimates are based on a relative risk model and on a dose and dose-rate effectiveness factor (DDREF) of 2. (Adapted from ICRP: Recommendations. *Annals of the ICRP Publication 60*, Oxford, England, Pergamon Press, 1990.)

# Site–Specific Risk Estimates

ERR, Excess Relative Risk




*For person age 70 exposed at age 30*

Eric Hall, Ph.D.,

# Lowest dose category with significant increase in cancer risk in Atomic-bomb survivors

- Cancer incidence: 5-100 mSv. Mean: 29 mSv

  (Pierce et al 2000)

- Cancer mortality: 5-125 mSv. Mean: 34 mSv

  (Preston et al., 2003)

# Summary

- Data suggest linear dose response with no thresold
  Increased risk: 0-100 mSv

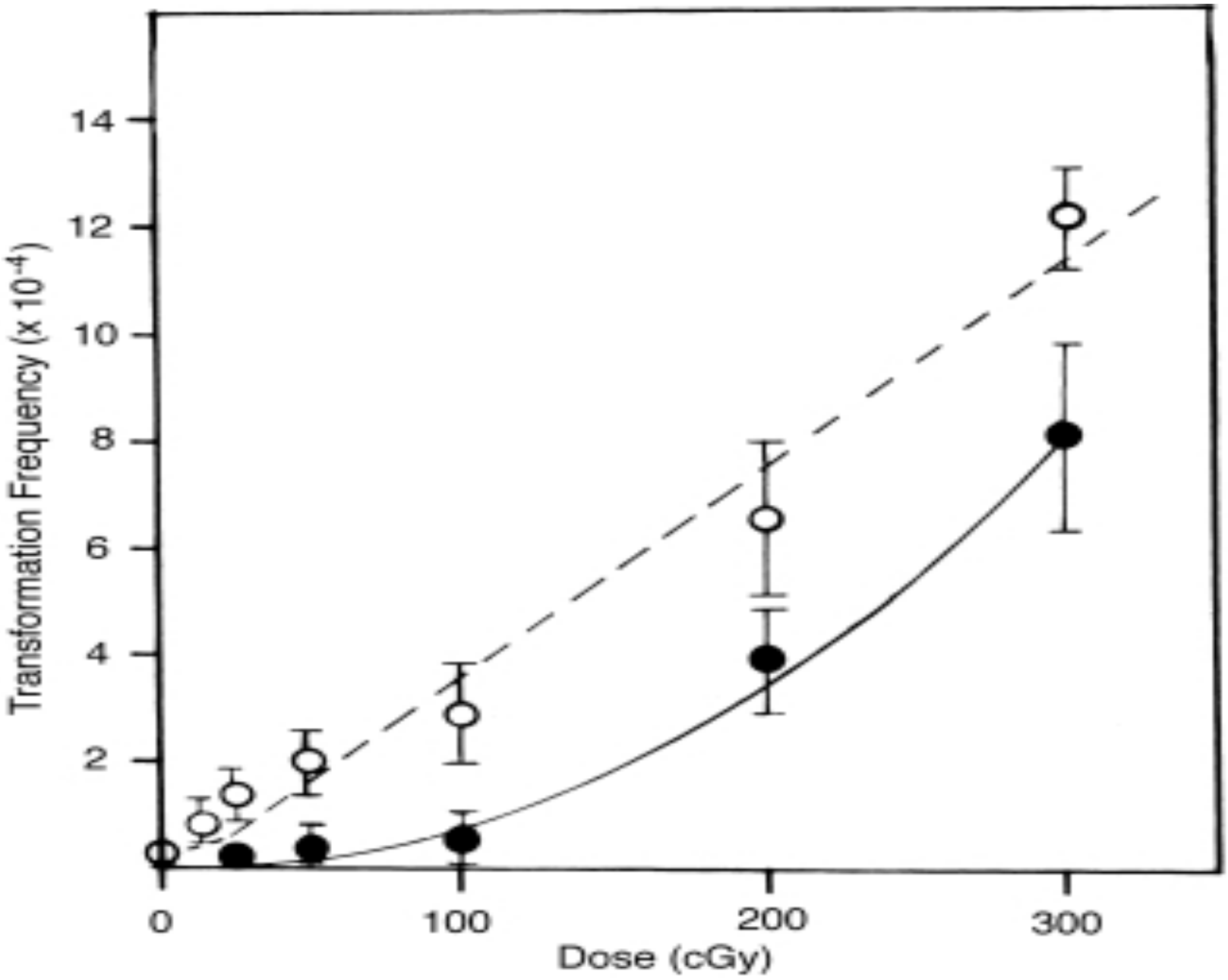
- Women have higher risk than men

- Excess risk cintinues throughout life

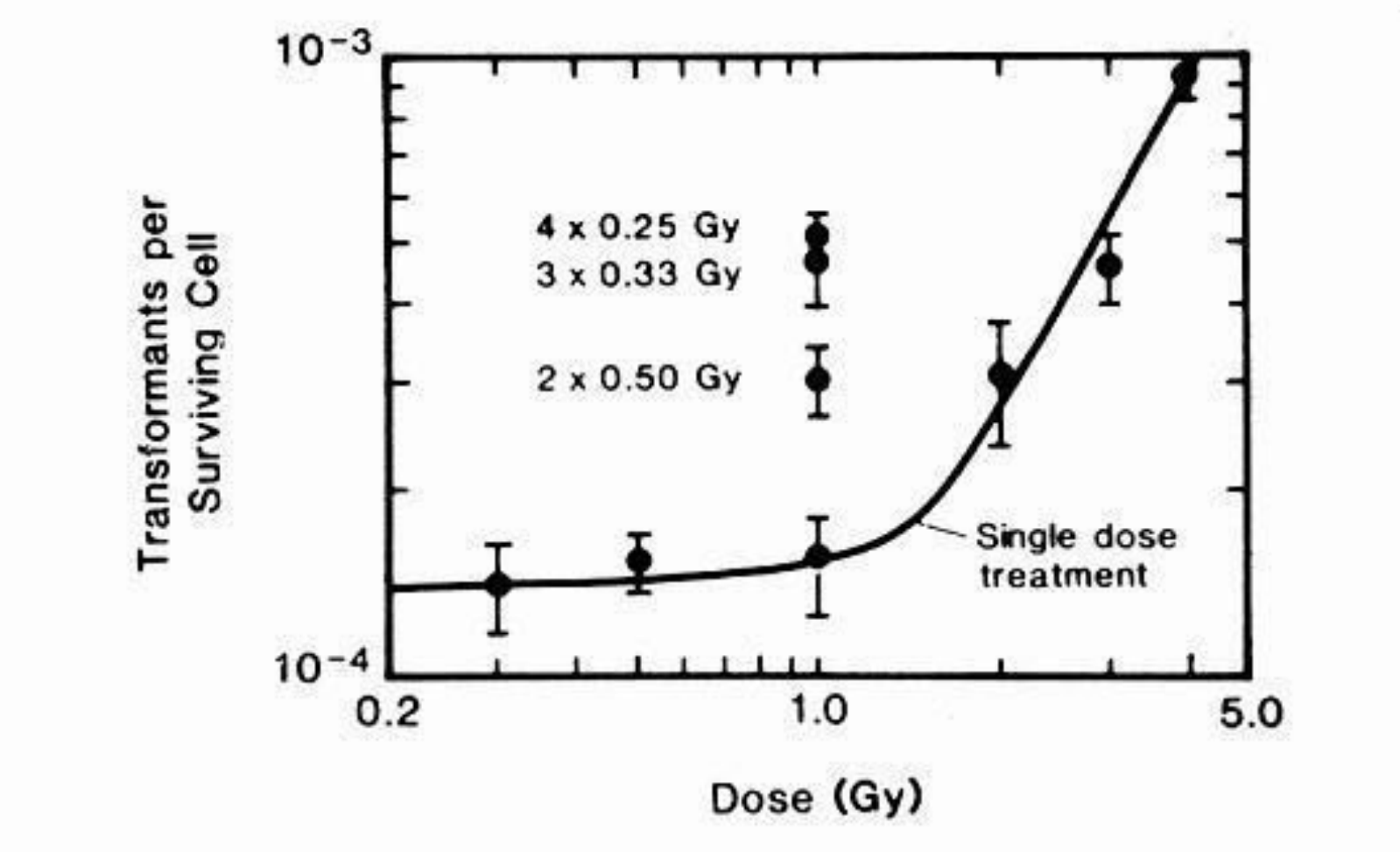
# Tissue culture model

- Above 100 rads: the transformation frequency may exhibit a quadratic dependence on doses.
- Between 30 and 100 rads: the transformation frequency may not vary with dose
- Below 30 rads: the transformation frequency may be directly proportional to dose.

**Dose-response curves for the induction of neoplastic transformation in mouse cells by x-irradiation. The upper curve is for BALB/3T3 cells; the bottom curve for C3H/10T 1/2 cells.**

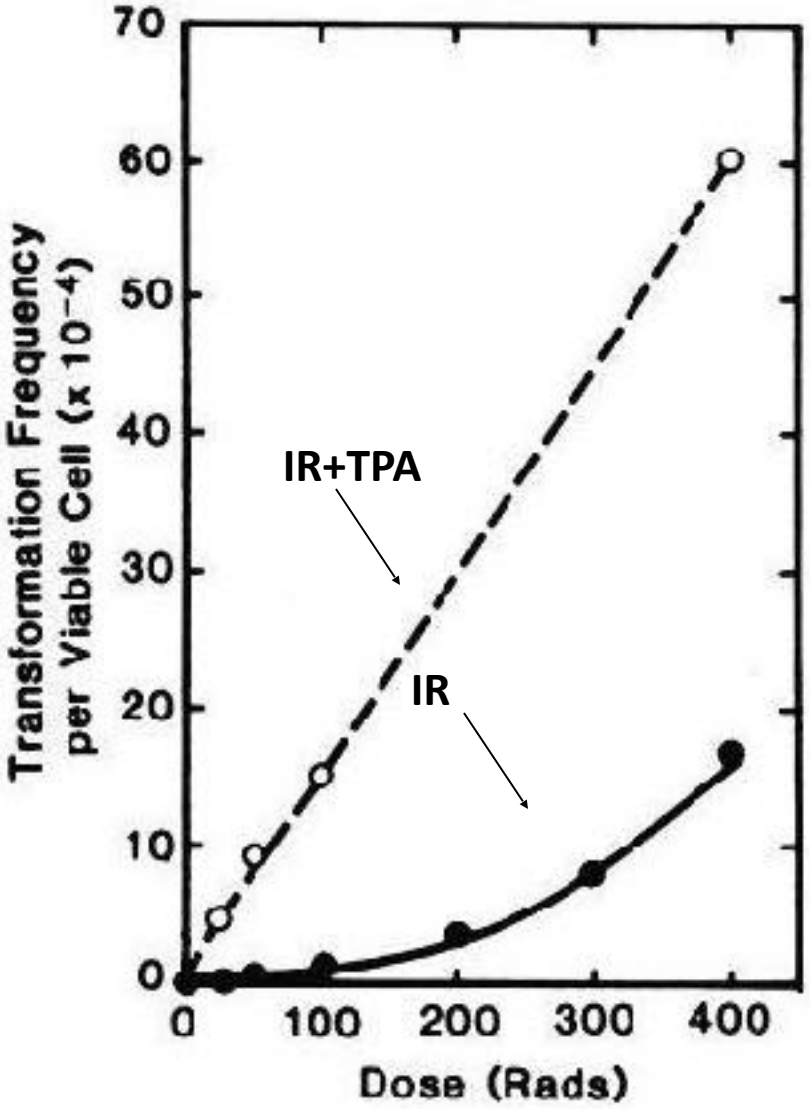


# Transformation incidence of irradiated cells



# Radiation + promoter

C3H 10T1/2 cells



# Occurrence of secondary cancers following radiotherapy

- Current advances in cancer therapy has increased survival of patients

- The occurrence of radiation-induced secondary cancers is serious concern

- Accurate dosing and dosimetry are critical during radiation therapy

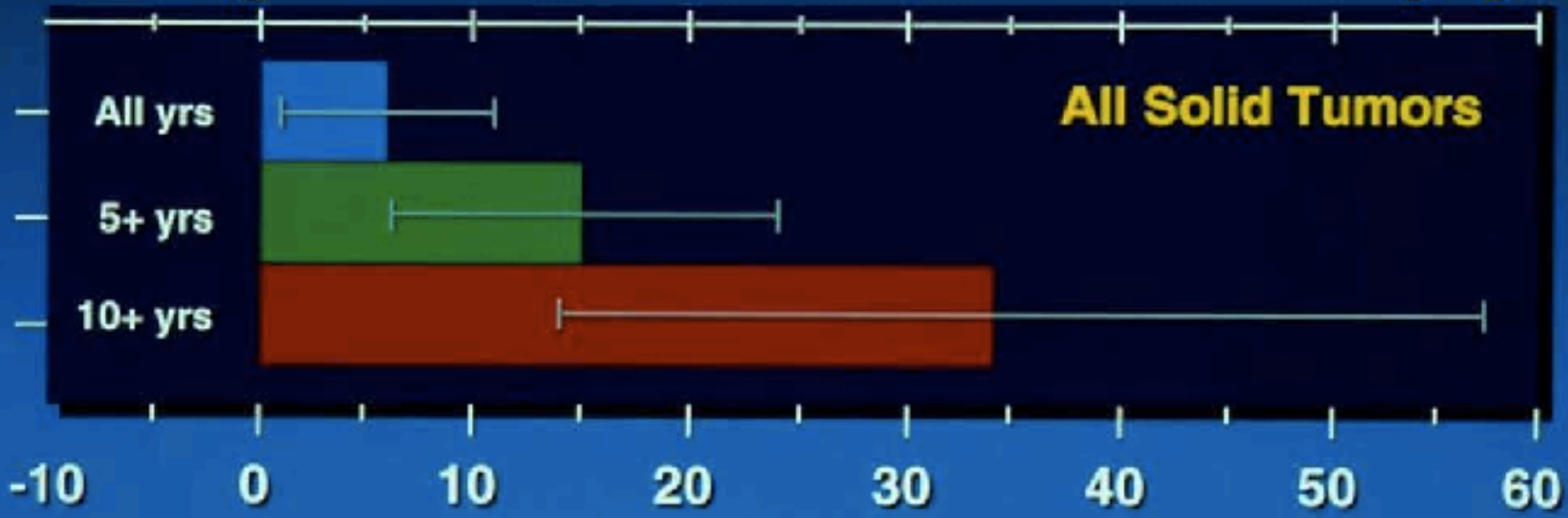
# Occurrence of secondary cancers following radiotherapy

- Risk of secondary cancers is hard to assess due to lack of proper control

- In prostate and cervix cancer, surgery is an option

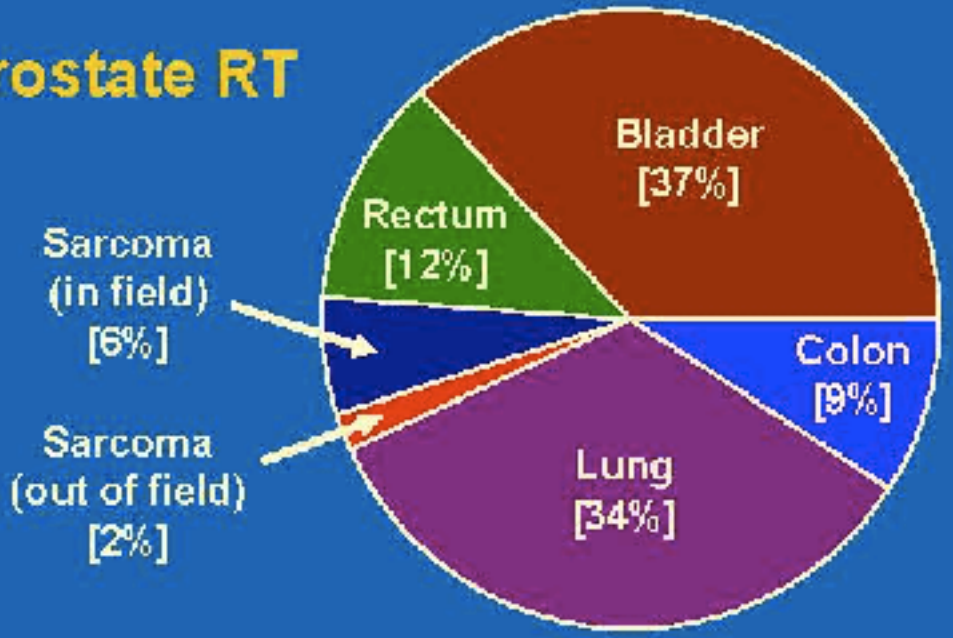
- Higher risk of breast cancer in young patients with Hodgkin lymphoma

## Percentage Increase in Relative Risk for RT vs. Surgery %




## Second Cancers After Prostate RT

% contribution to total number of radiation-induced second cancers (5+ yrs)




Eric Hall, Ph.D.,

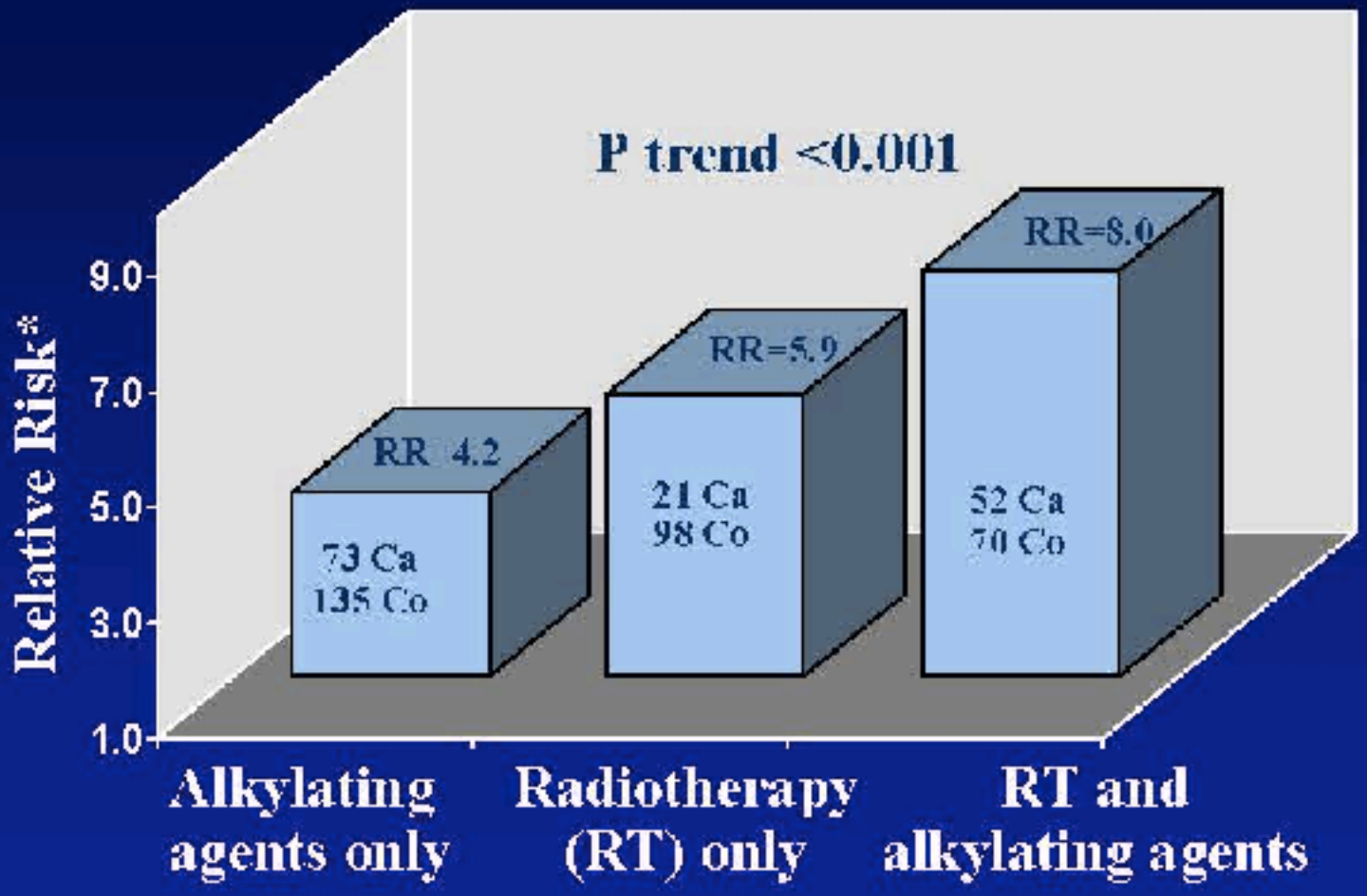
# Risk of *Radiation-Associated* Second Malignancy After Prostate-Cancer Radiotherapy

| | |
|---|---|
| All survivors | 1 in 290 |
| 5+ yrs survivors | 1 in 125 |
| 10+ yrs survivors | 1 in 70 |

*Brenner et al 1999*

Eric Hall, Ph.D.,

# Lung Cancer after Hodgkin's Disease by Type of Treatment *




*Travis LB, et al. JNCI, 2002*

*Adjusted for tobacco use

Eric Hall, Ph.D.,

# Summary

- History of radiation and radiation-induced damage
- Bystander effect of radiation
- Methods for DNA damage analysis
- Stages of carcinogenesis and models
- Mechanism of radiation-induced carcinogenesis
- Role of oncogenes and tumor suppressors
- Risk projections and risk estimates
- Importance of dose and age on tumor incidence
- Second malignancy after radiotherapy