CENTRE DE LUTTE CONTRE LE CANCER LEON BERARD

An Introduction to Radiation Biology

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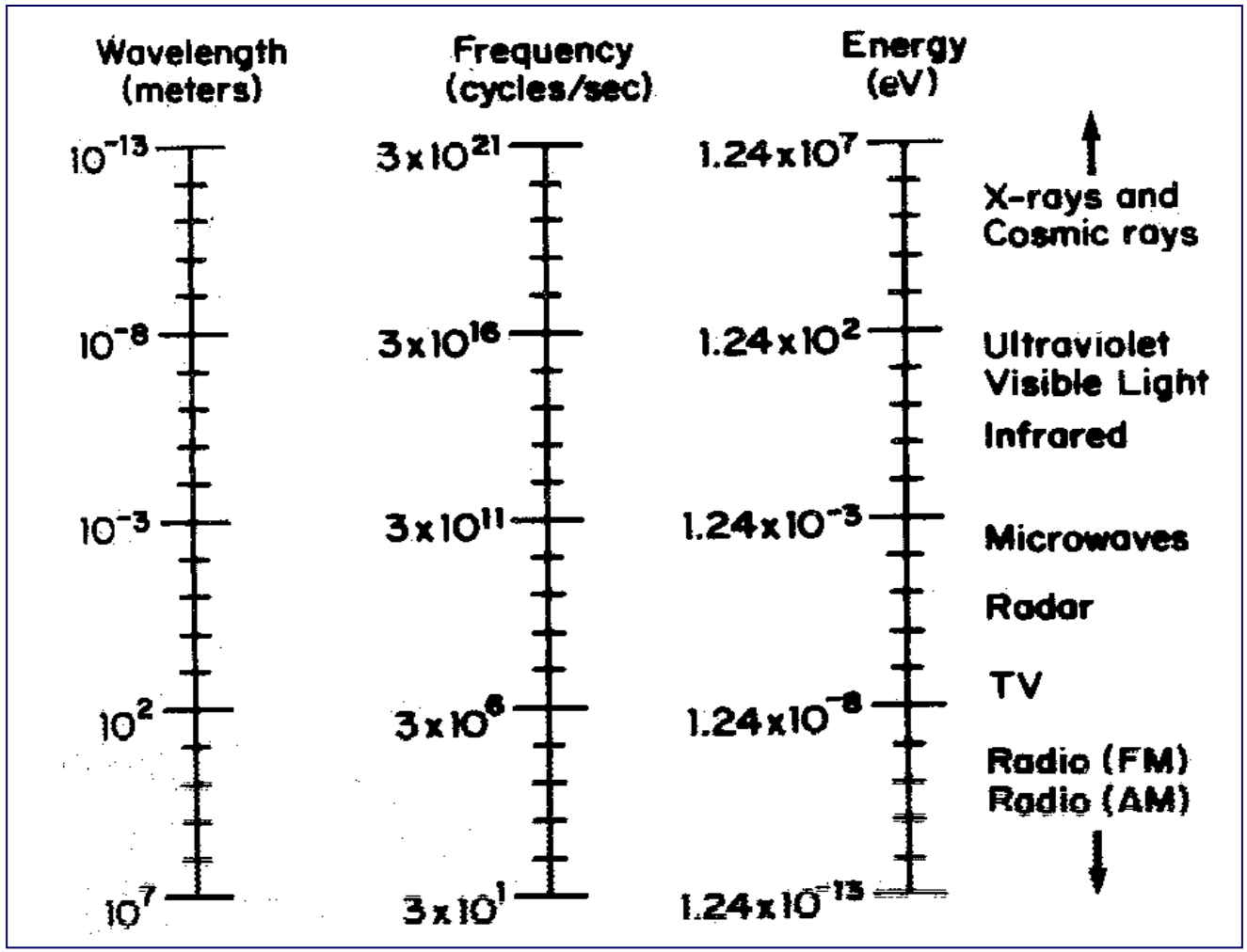
Types of ionizing radiation

- Electromagnetic radiation (low LET): photons, γ-rays, X-rays
- Particulate Radiation(high LET)
  - charged particles: electrons, protons, α particles
  - neutrons
  - heavy charged ions: carbon, neons, argon, …

Electromagnetic radiation

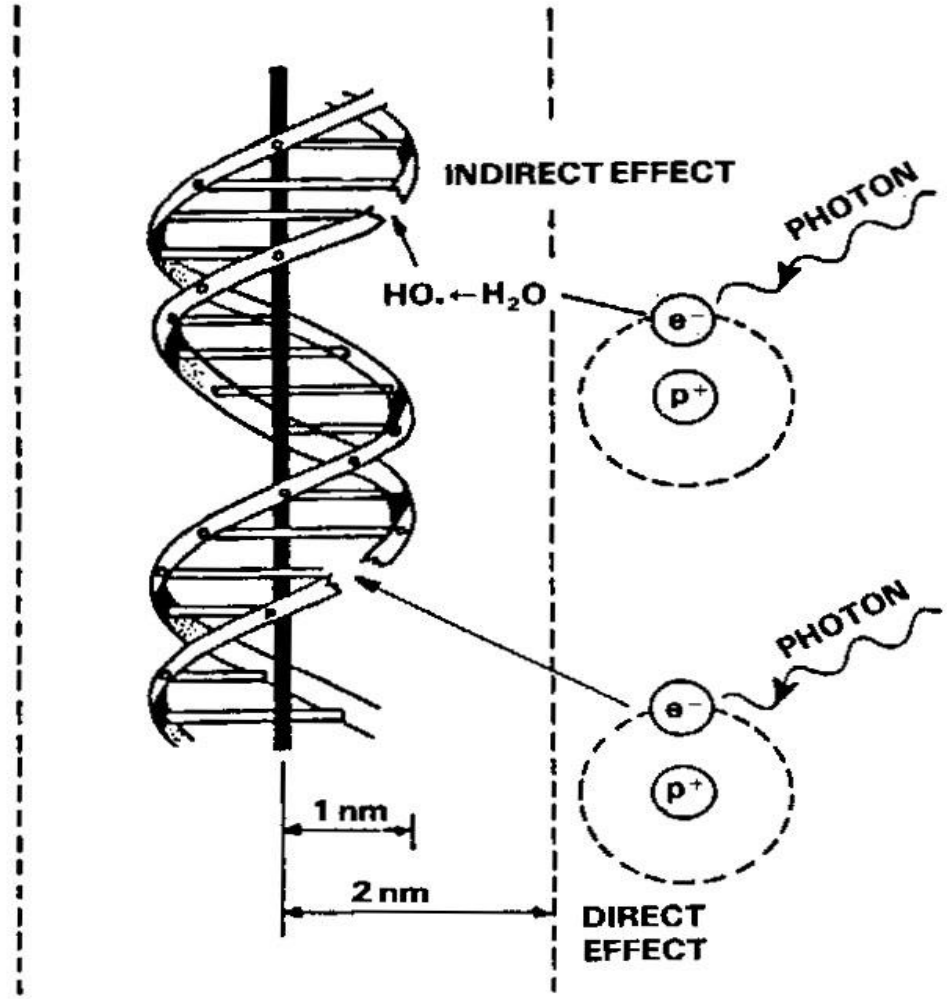


$E = h\nu$

$\nu = c/\lambda$

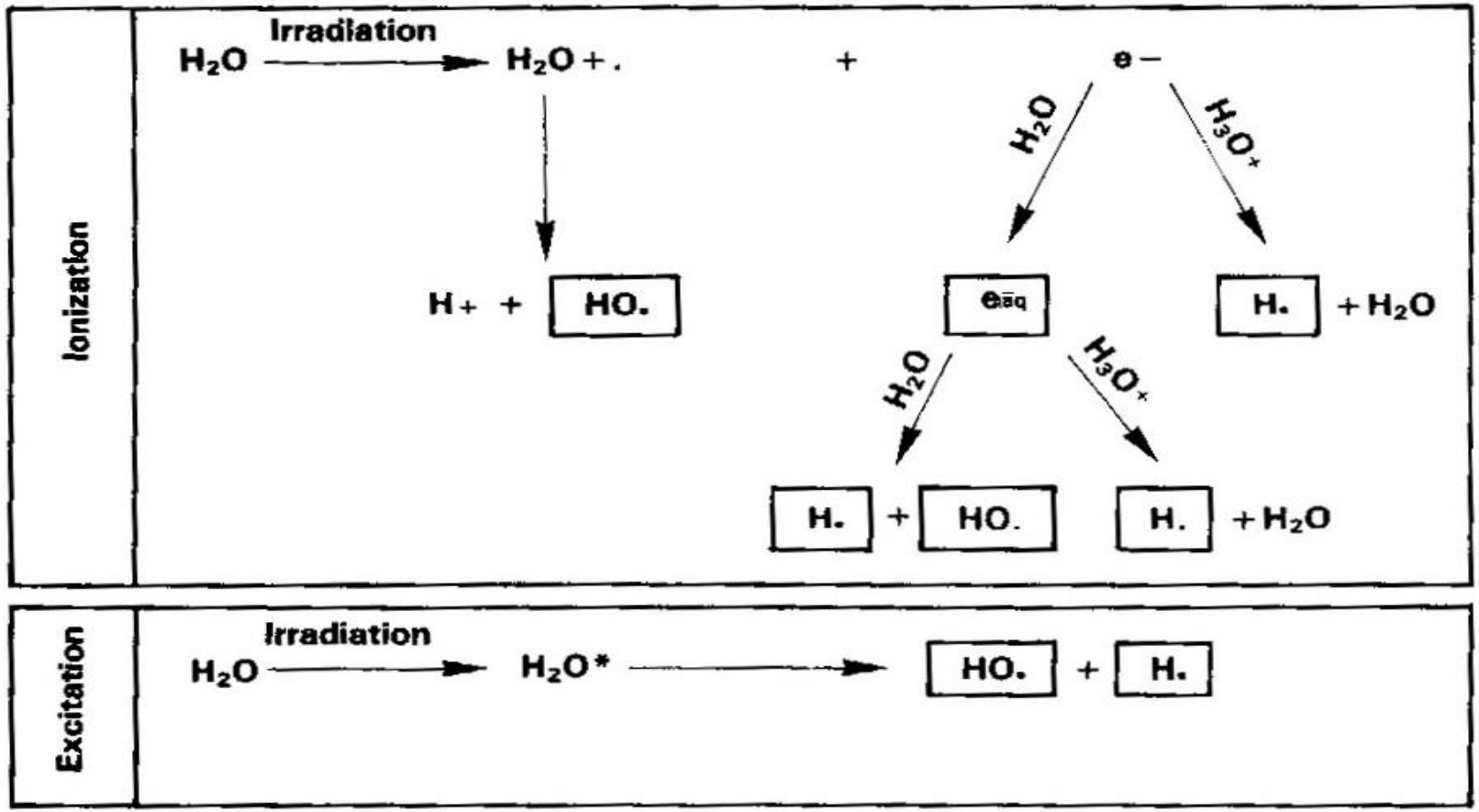
Absorption of X-rays

Direct and indirect action



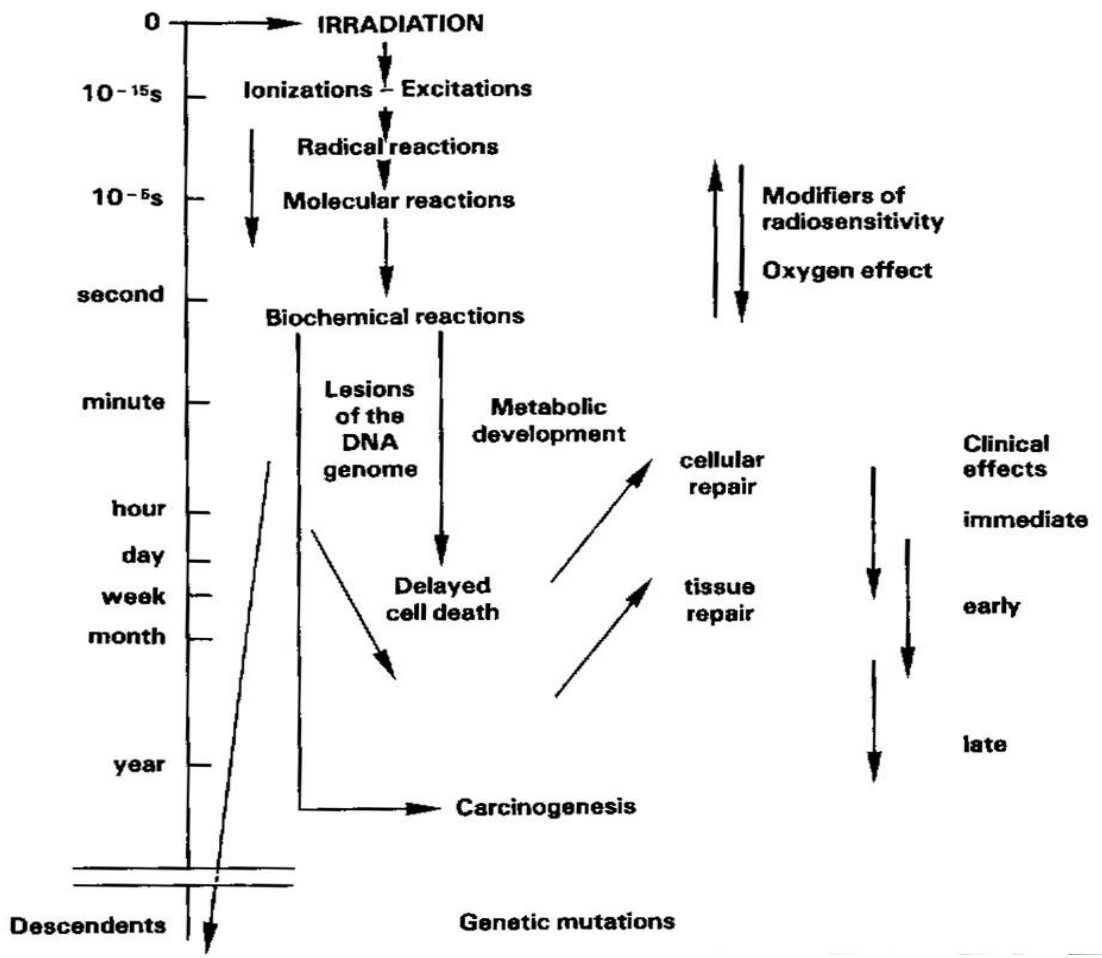
Absorption of X-rays

Radiolysis of water



The physics and chemistry of radiation absorption

Chronology of events



Quantities and units

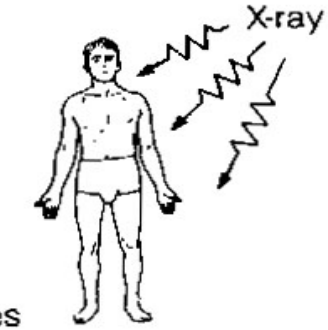
Absorbed dose: 1 Gray (Gy) = 1 joule/kg

= increase of 0.0001 °C per gr water

Total-Body Irradiation

Mass = 70 kg
LD/50/60 = 4 Gy
Energy absorbed =

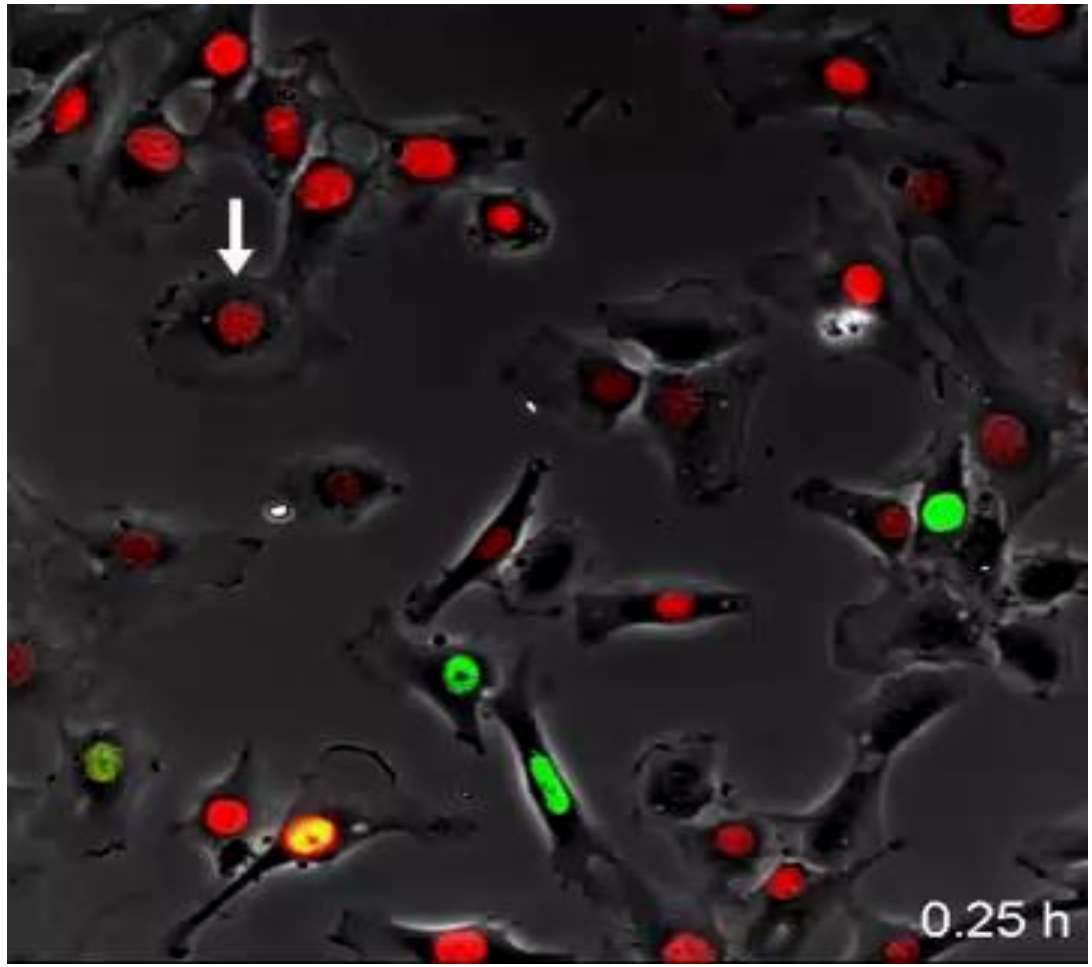
$$70 \times 4 = 280 \text{ joules}$$
$$= \frac{280}{4.18} = 67 \text{ calories}$$



Drinking Hot Coffee

Excess temperature (°C) = 60° − 37° = 23°
Volume of coffee consumed to equal the energy in the LD/50/60 = $\frac{67}{23}$
= 3 mL
= 1 sip

Dynamics of the cell cycle in a growing population

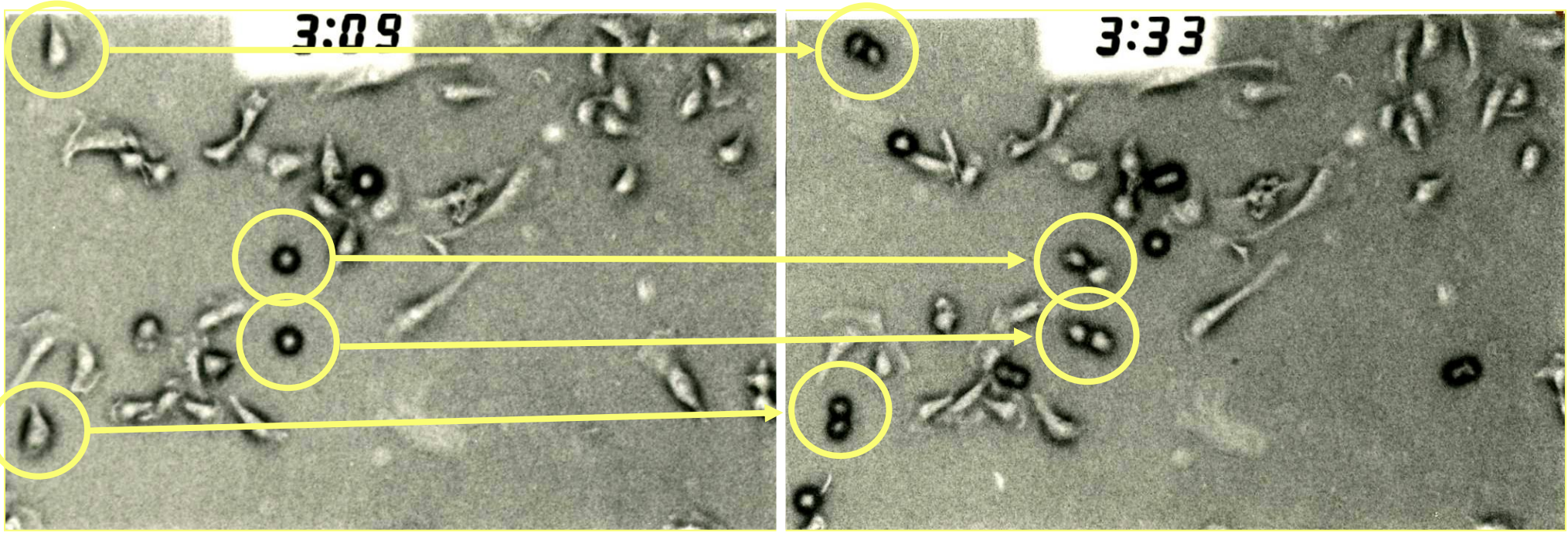


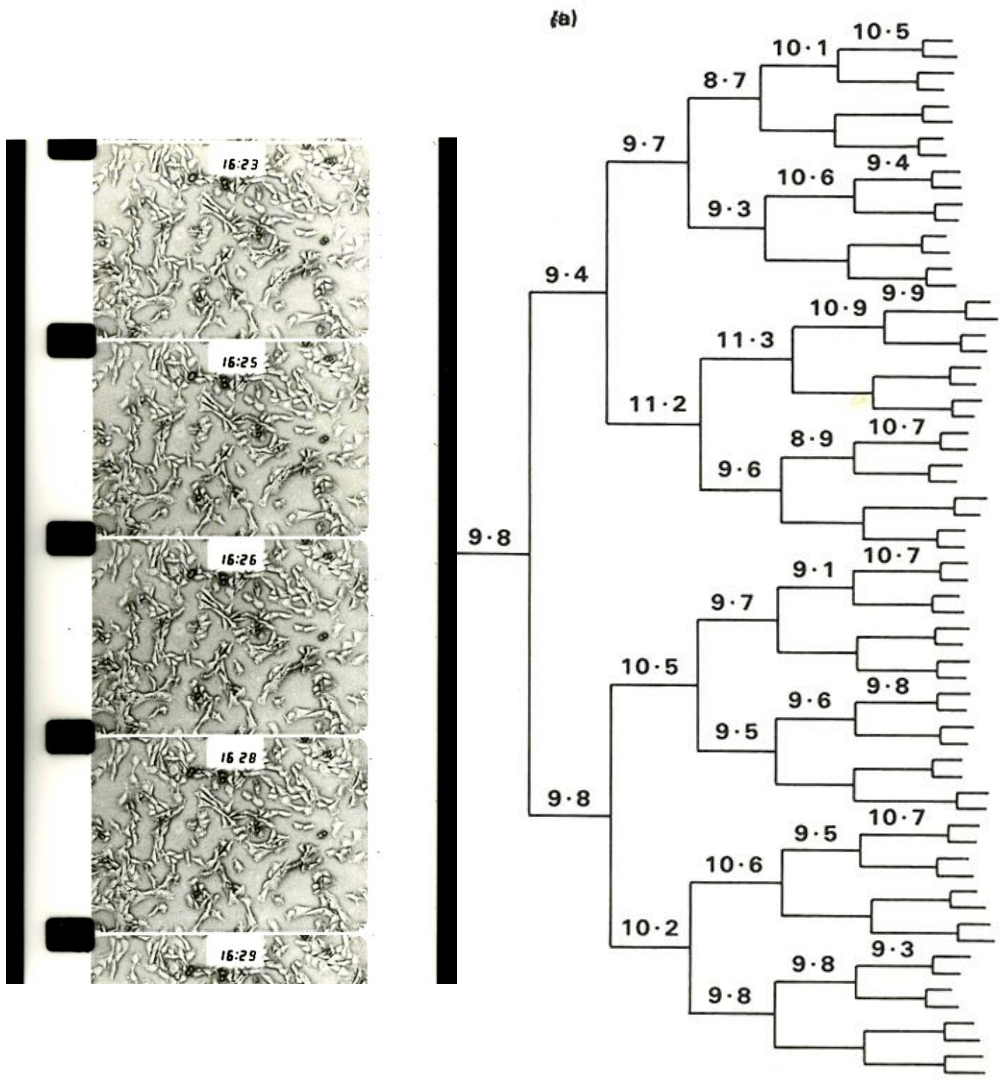
G1 - early S - late S & G2

FUCCI imaging of the cell cycle: two interphase regulators, Cdt1 & Geminin.

Cdt1 (red) only expressed during G1 and early S Geminin (green) only expressed during S/G2.
G1 - red
early S – yellow
late S & G2 - green.

human fibroblasts visualized by time-lapse live-cell imaging


3:09
3:33



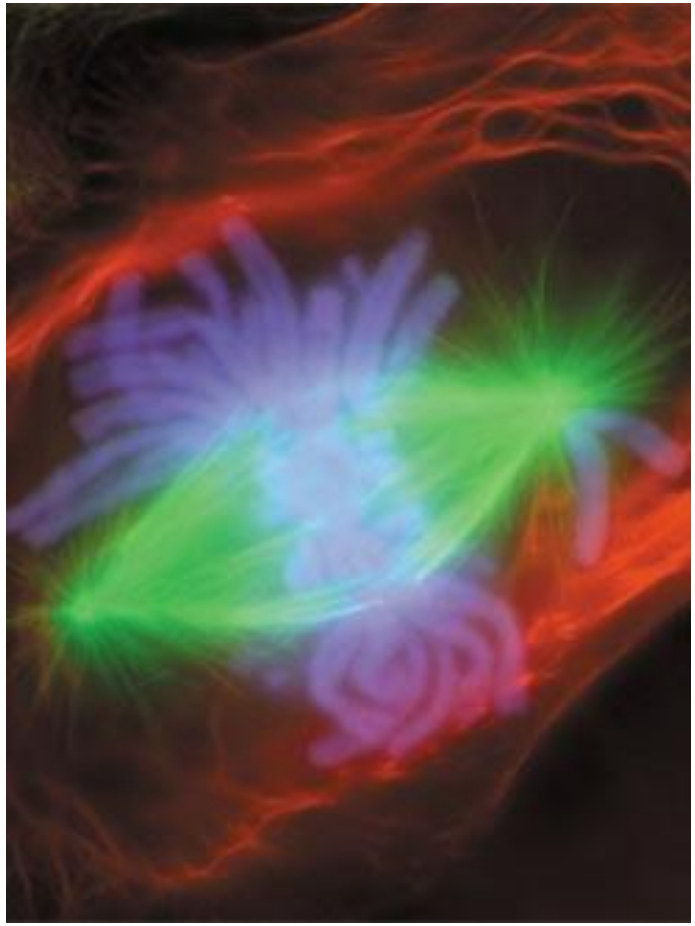
Cellular pedigree of normal (unstressed) cells

(time-laps microcinematography)

Duration of the cell cycle : 8 hours - days

Duration of the phases, but G1, are about the same for all cell types

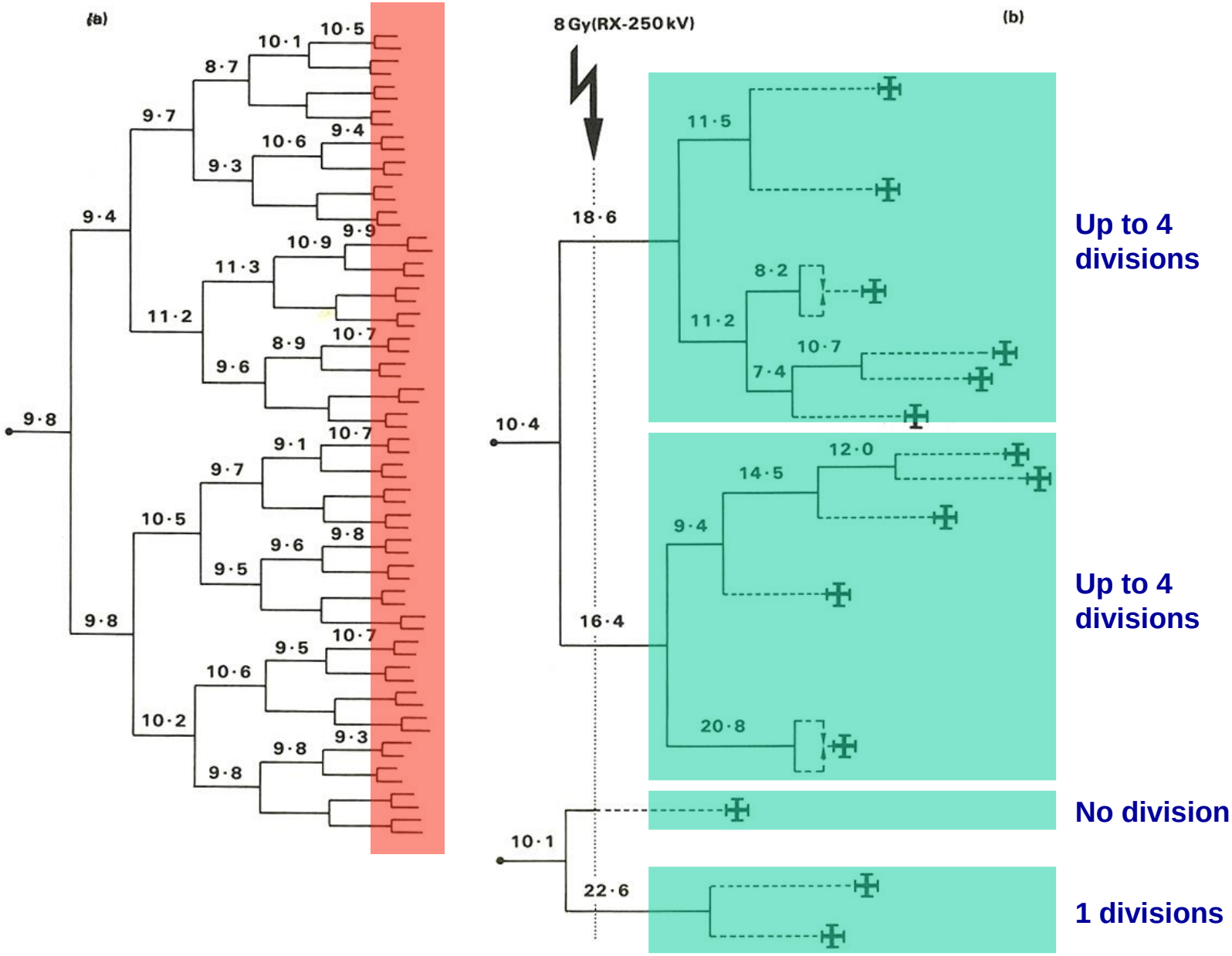
Effects of irradiation on mitosis



Effects on mitosis in plant cells:
endosperm of Haemanthus - time-lapse movie A. Bajer (1962)

Unirradiated cells

Irradiated cells



Mort cellulaire

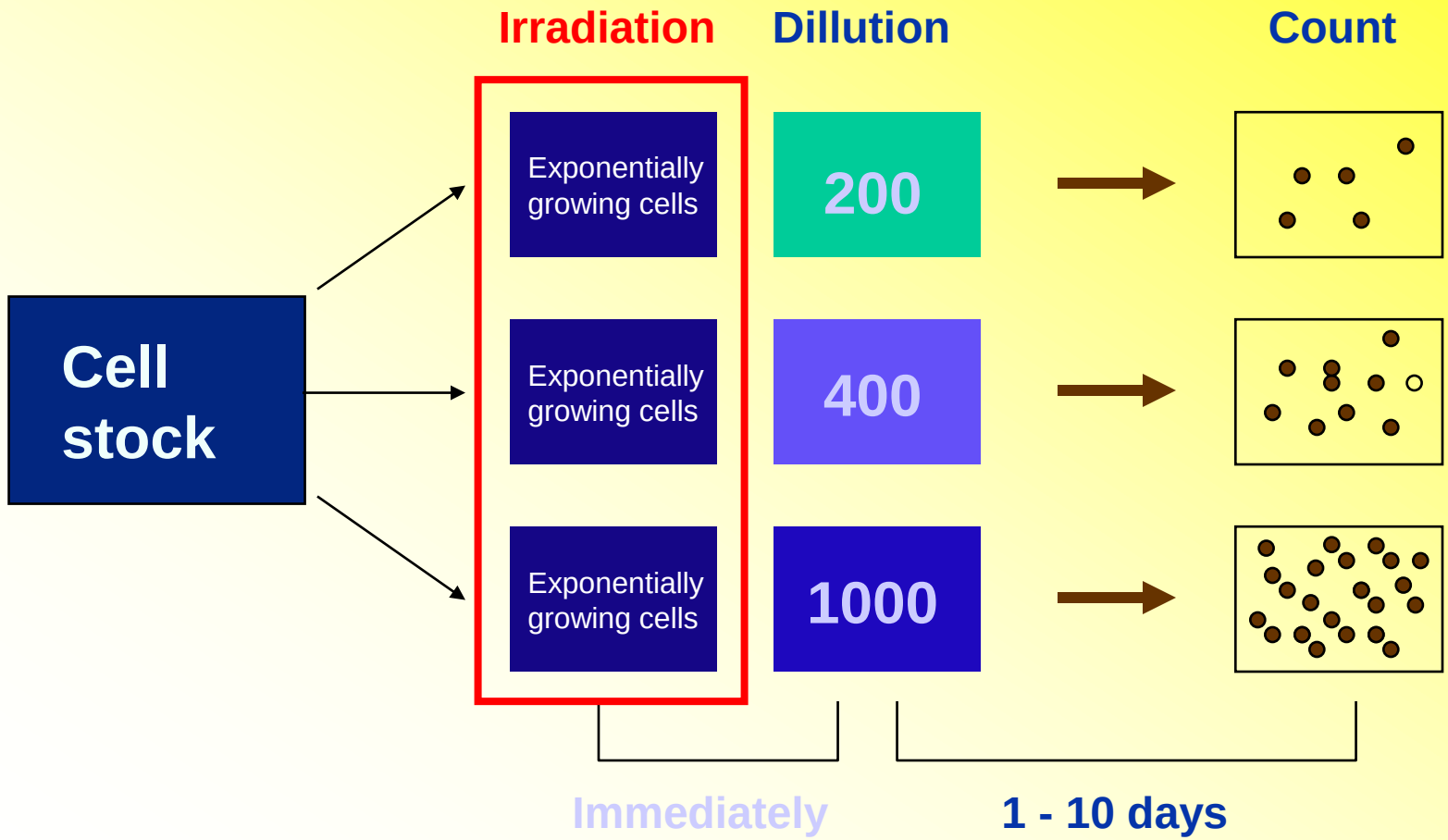
Définition fonctionnelle :

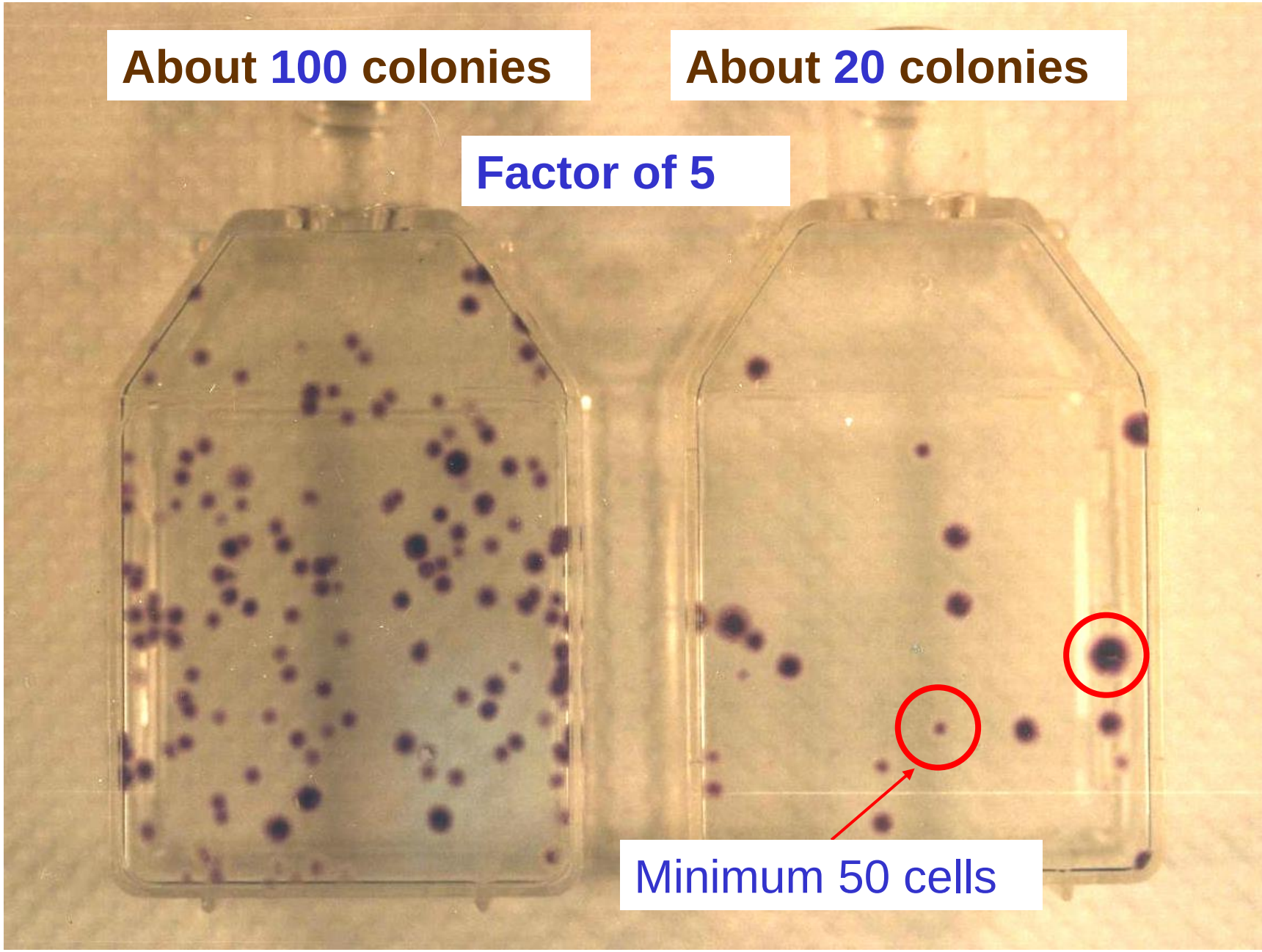
Mort « clonogénique »

Perte de la capacité de donner naissance à une colonie ou *clone*

- *Radiothérapie* (régression tumorale)
- *Radioprotection* (syndromes aigus : e.g. Intestinal, hématopoïétique)

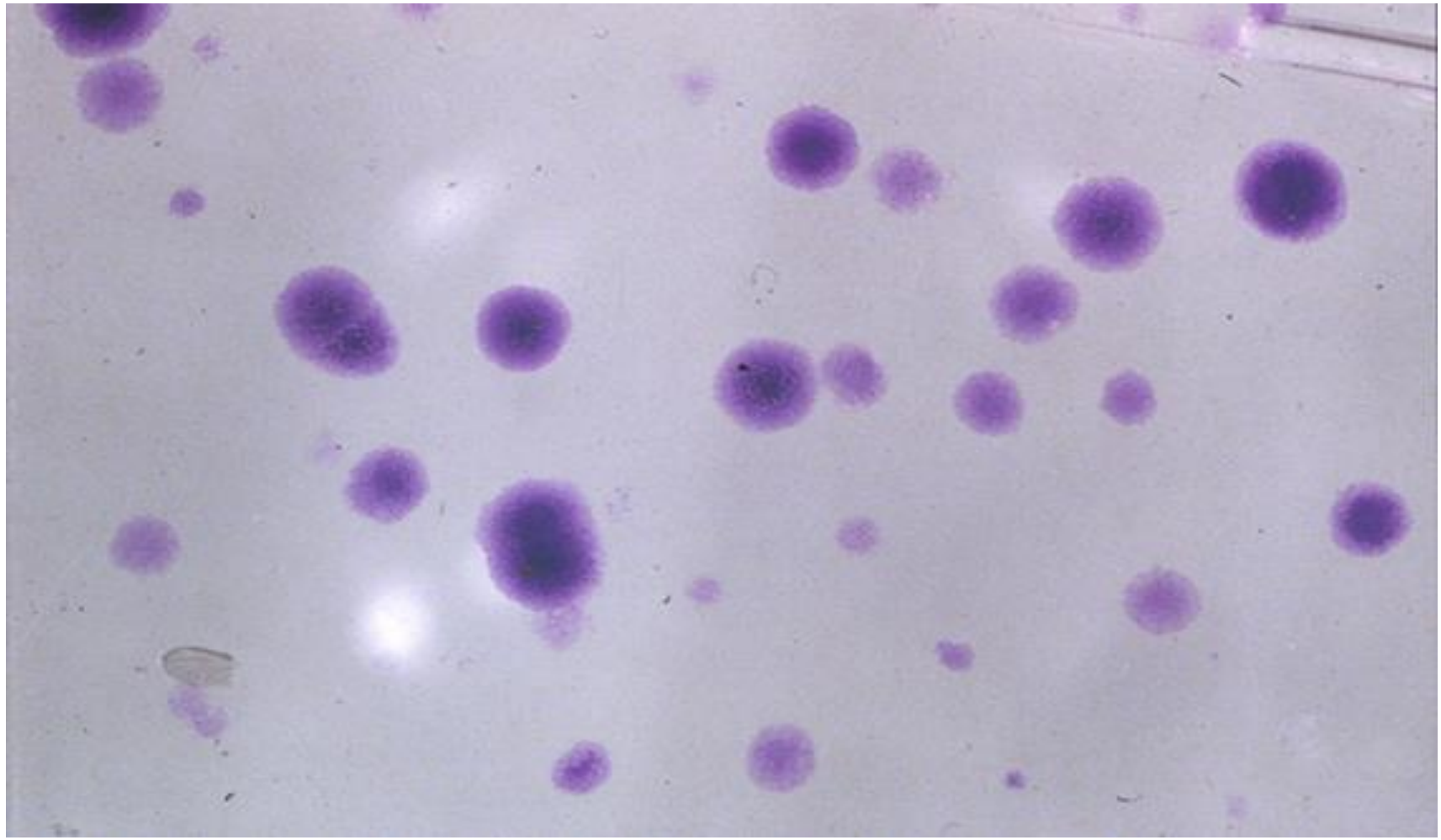
Colony formation *in vitro* (2)



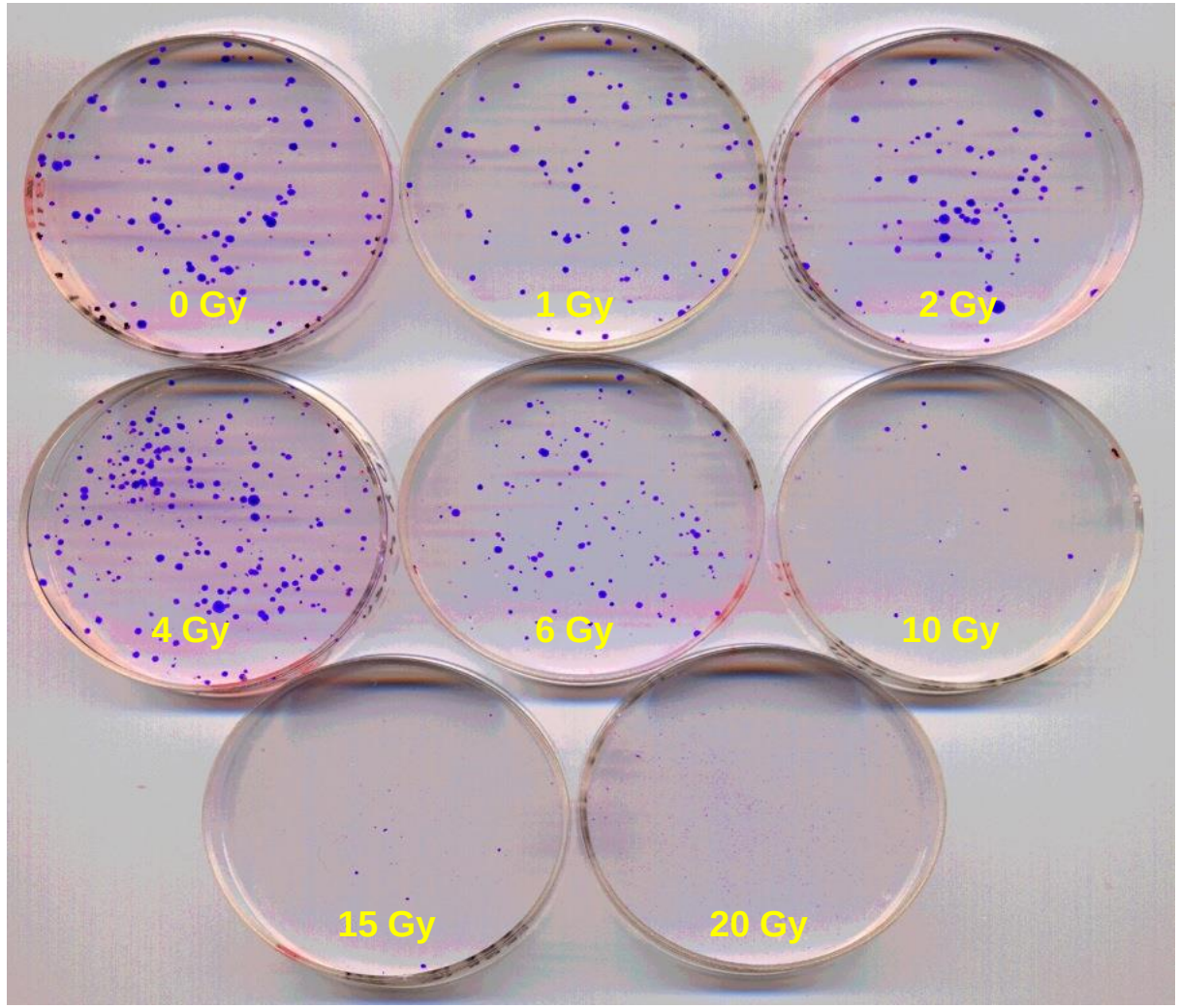

About 100 colonies
About 20 colonies
Factor of 5
Minimum 50 cells

Digest in Radiation Biology

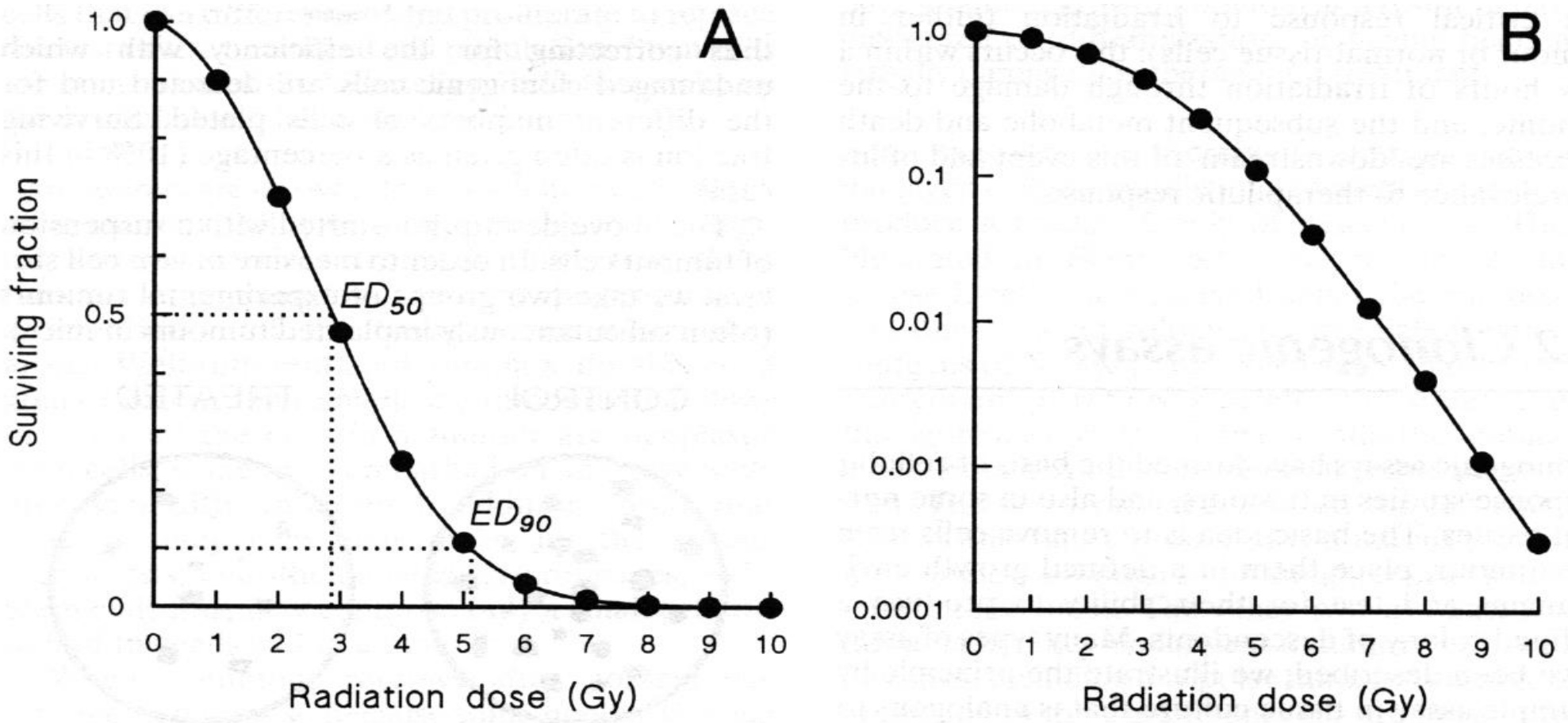
Clonogenic cell survival.

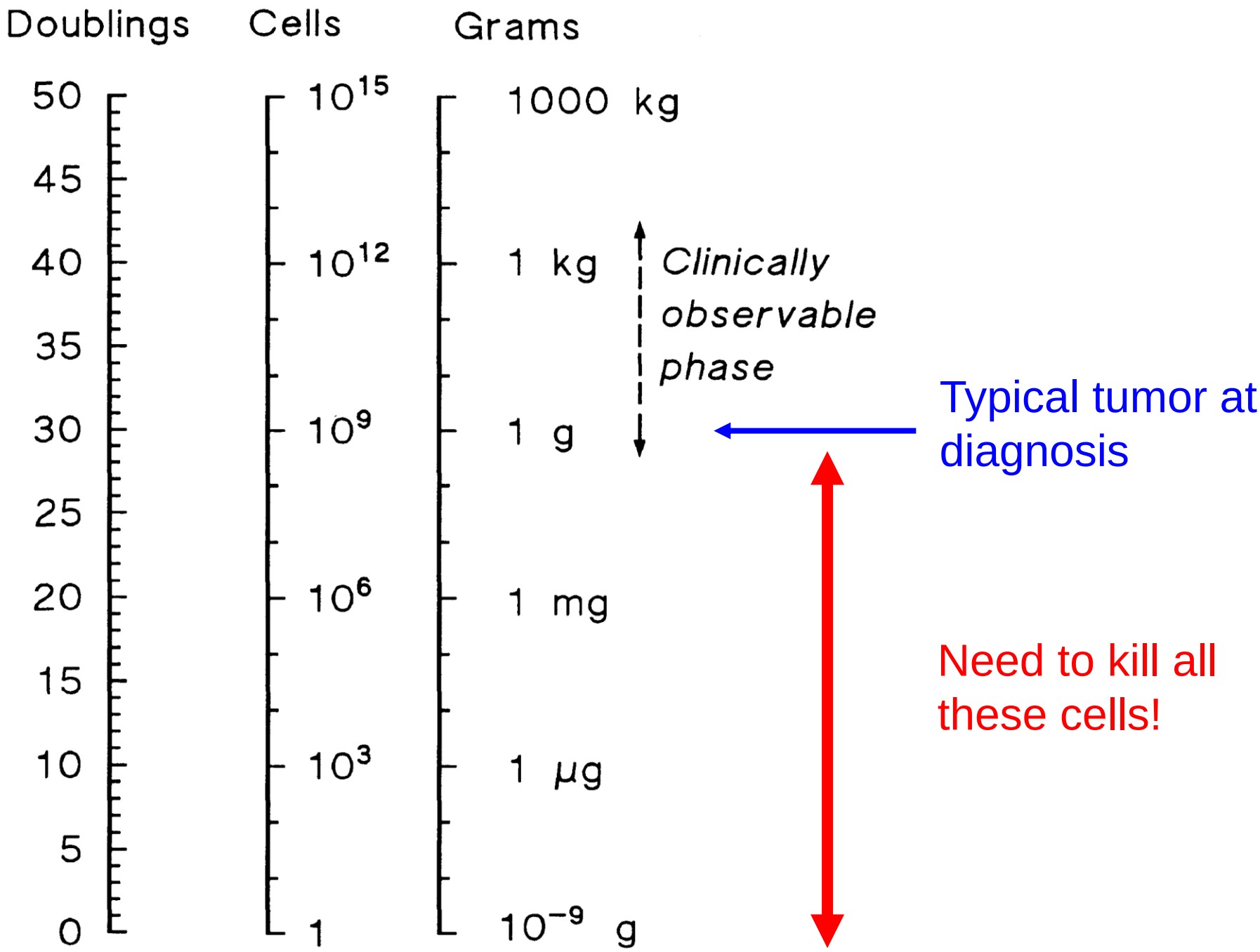


Colony assay: in vitro survival

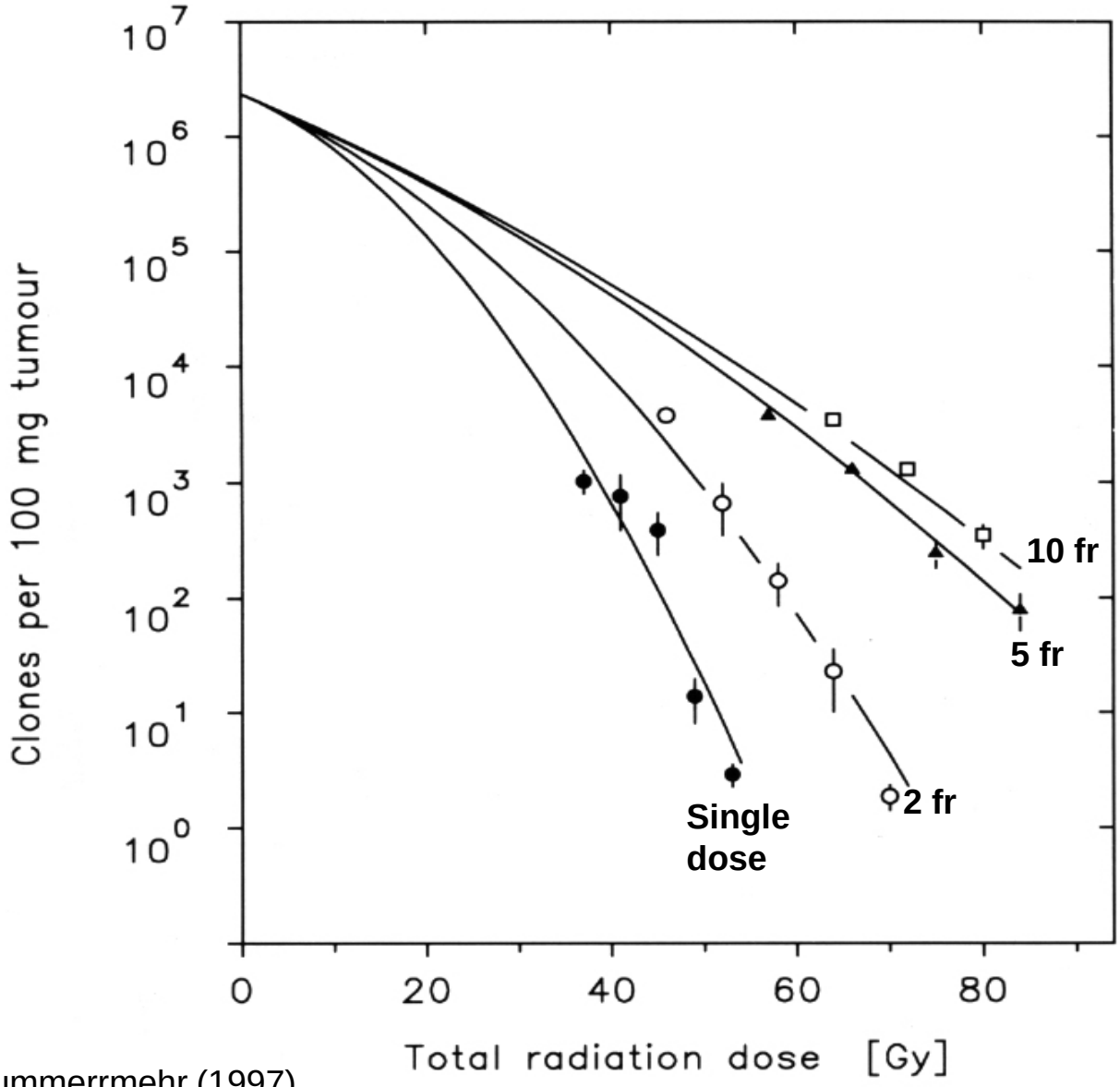


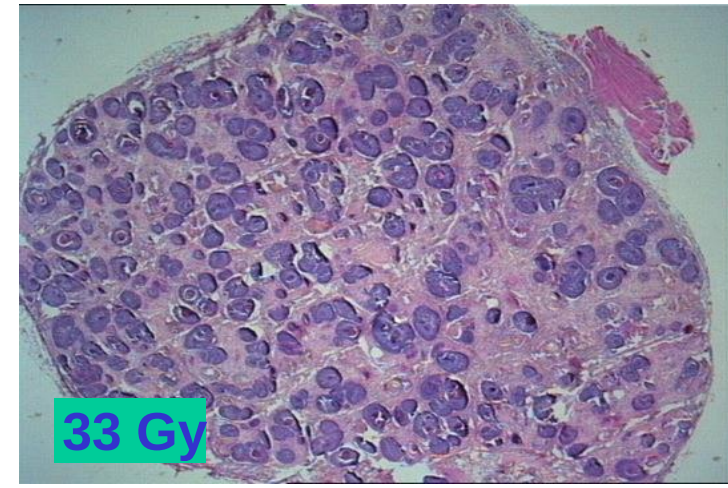
Cell survival curves

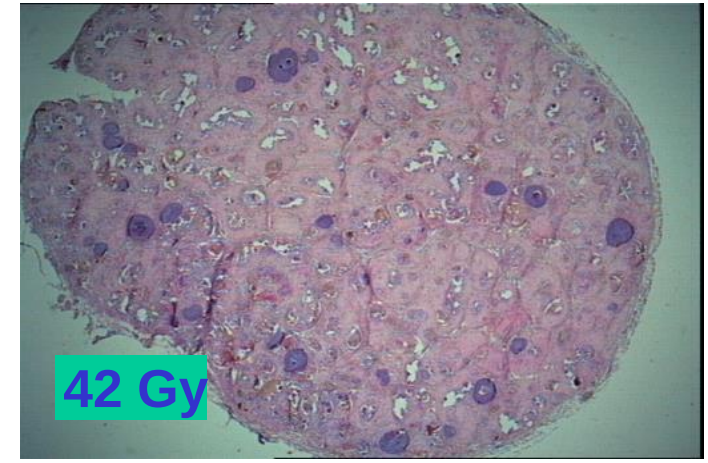


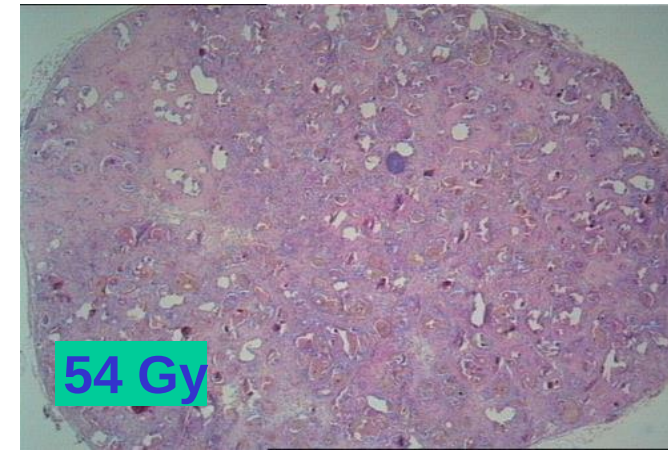

Doublings
Cells
Grams
50
45
40
35
30
25
20
15
10
5
0
10^{15}
10^{12}
10^{9}
10^{6}
10^{3}
1
1000 kg
1 kg
1 g
1 mg
1 μg
10^{-9} g
Clinically observable phase
Typical tumor at diagnosis
Need to kill all these cells!

In situ survival curves of AT17 carcinoma (at 17 d)



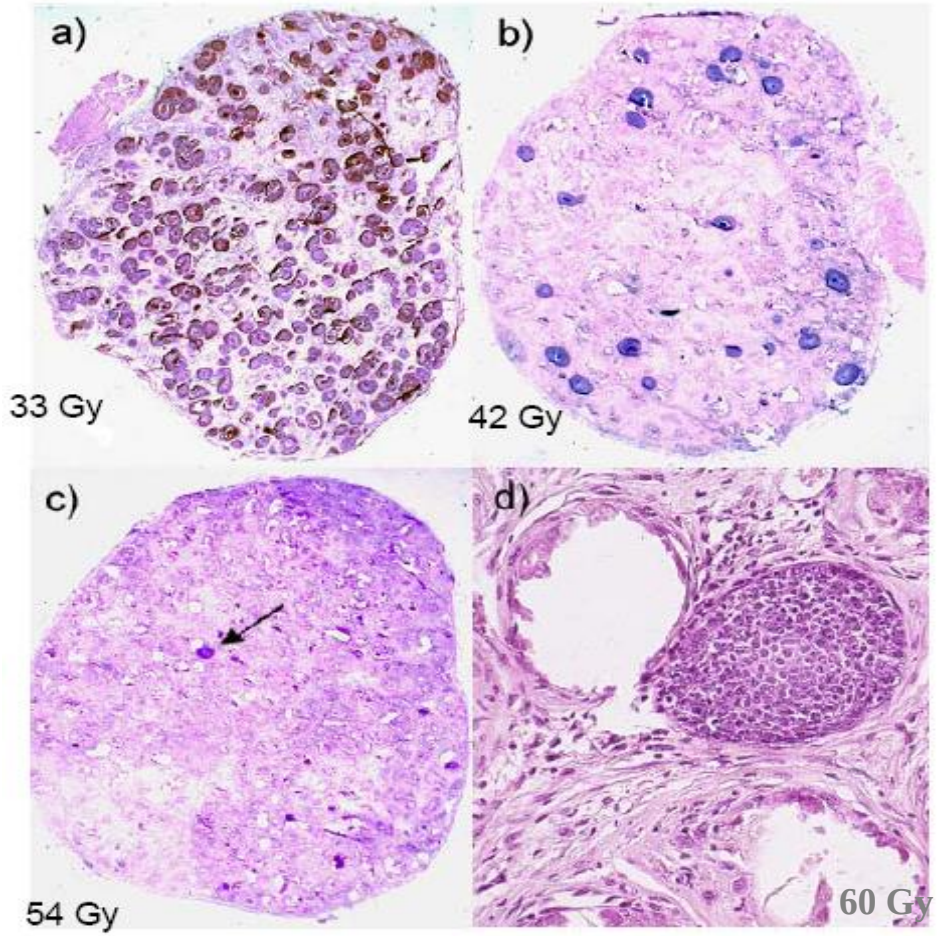


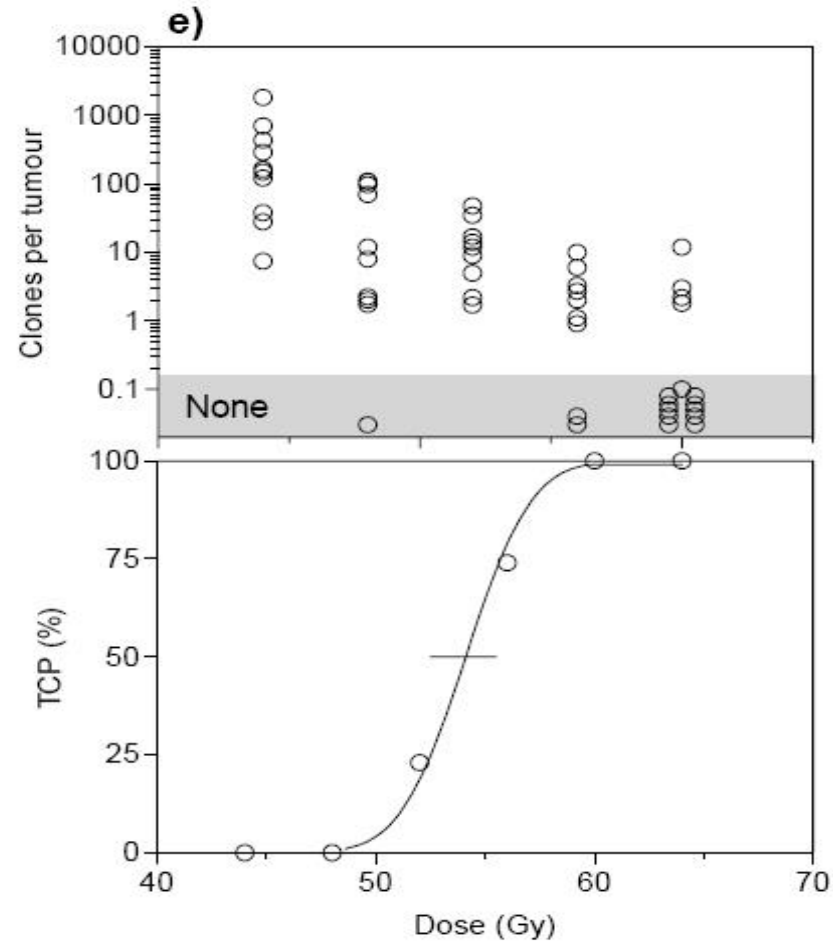




Kummermehr (1997)

Endpoints: local tumour control





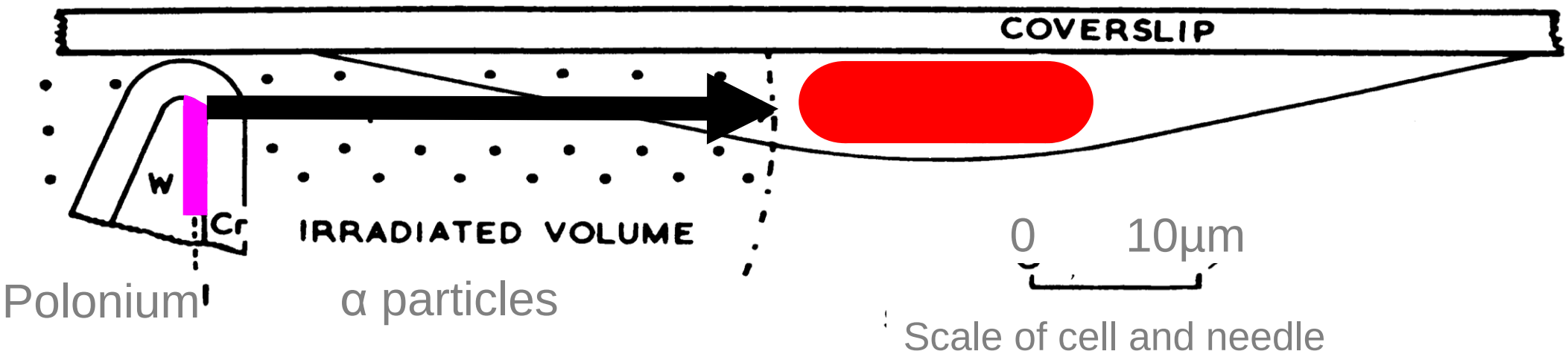
What do we mean by cell death?

- Cell death
  - Loss of reproductive (clonogenic) capacity
  - Cell may or may not appear dead
  - Cells are unable to contribute to tumor growth or metastasis – goal of treatment

- For normal cells, this definition may not be relevant
  - Has no meaning for non-dividing cells
  - Different definitions may be better

DNA is the principal target

Microbeam experiments with α particles from polonium show that the cell nucleus is the sensitive site



Munro TR. *Radiat Res* 1970;42:451

DNA

transcription translation

DNA → RNA → Protein

- Only molecule which is repaired

Endogenous DNA damage

- In every human cell per day:
  - 50,000 SSB
  - 10,000 depurinations
  - 600 deaminations
  - 2000 oxidative base damages
  - 5000 alkylation damage
  - 10 cross links
  - 10 DSB’s

Ionizing Radiation Damage

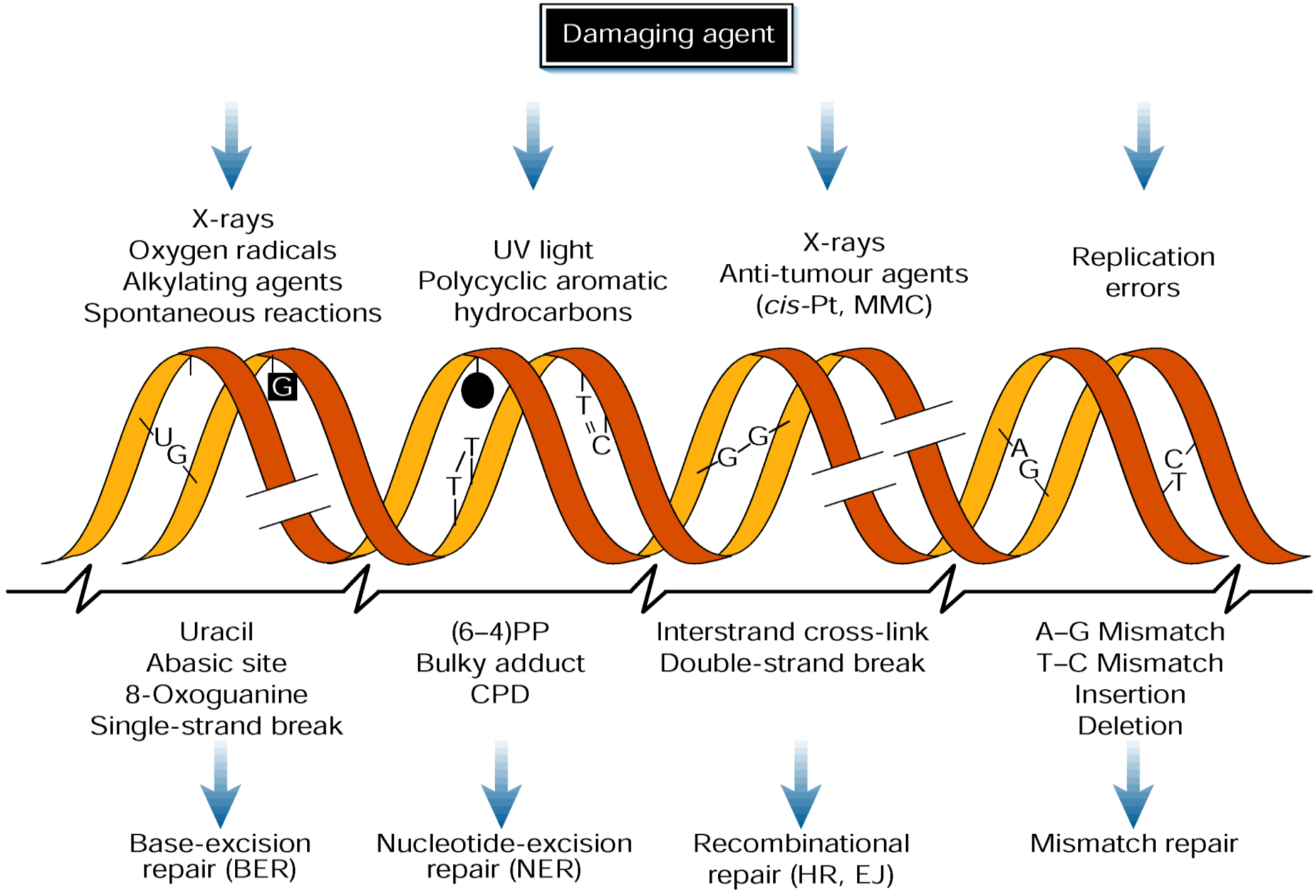
Primary target is the DNA

1Gy of low LET Xrays produces:

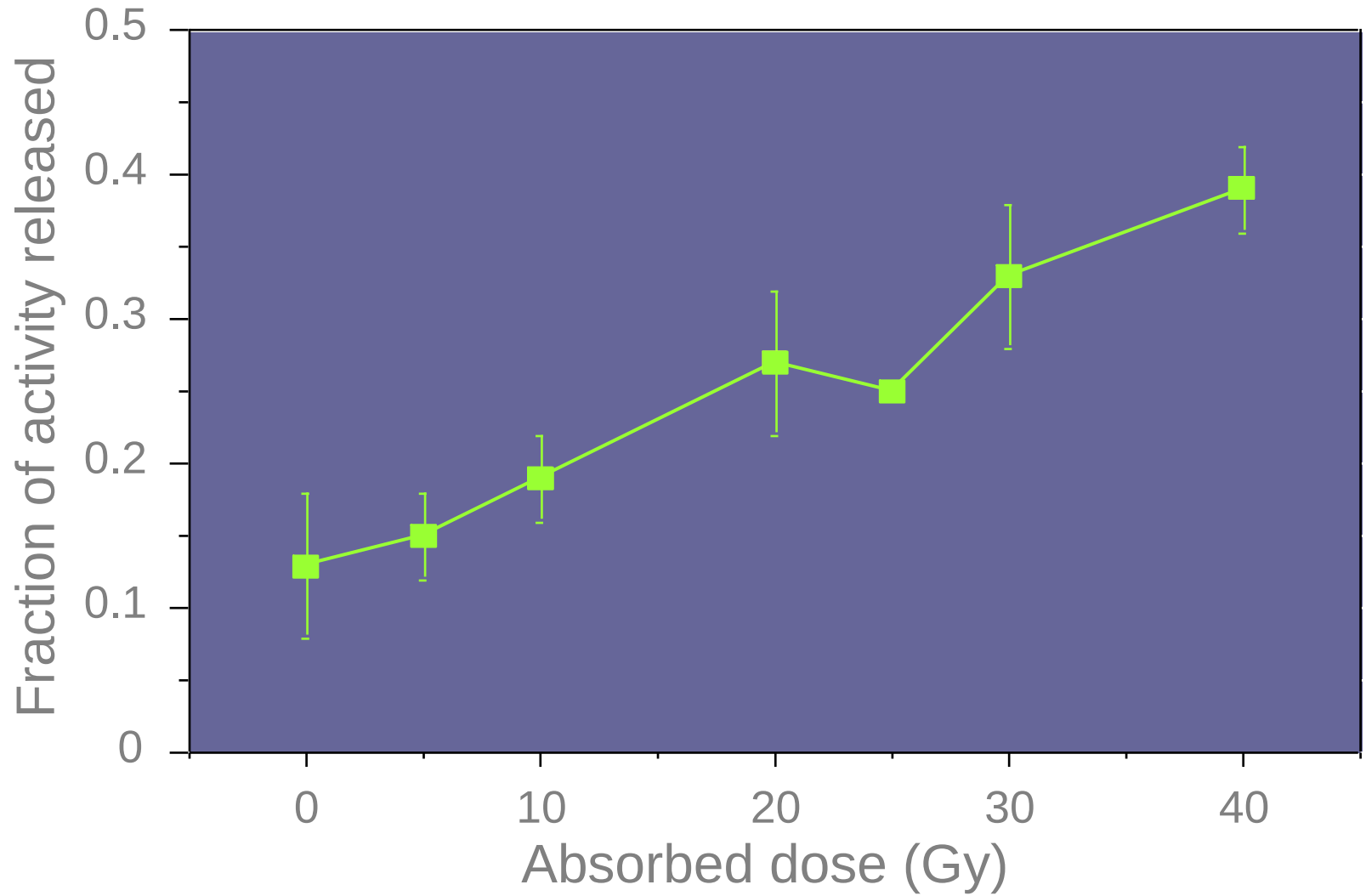
1000	single strand breaks
40	double strand breaks
1000	altered bases

Comparison between IR and UV

1000000 dimers = 40 DSBs


Damaging agent
X-rays
Oxygen radicals
Alkylating agents
Spontaneous reactions
UV light
Polycyclic aromatic
hydrocarbons
X-rays
Anti-tumour agents
(*cis*-Pt, MMC)
Replication
errors
U
G
G
T
T
T
C
G
G
A
G
C
T
Uracil
Abasic site
8-Oxoguanine
Single-strand break
(6–4)PP
Bulky adduct
CPD
Interstrand cross-link
Double-strand break
A–G Mismatch
T–C Mismatch
Insertion
Deletion
Base-excision
repair (BER)
Nucleotide-excision
repair (NER)
Recombinational
repair (HR, EJ)
Mismatch repair
Repair process

Quantification of DNA damages

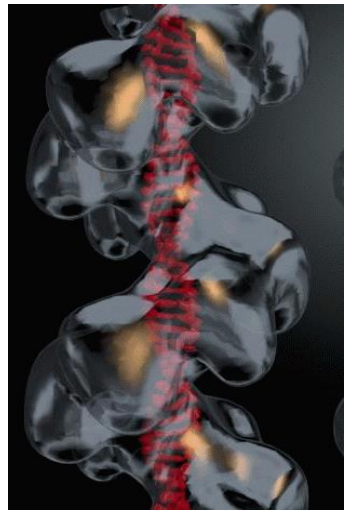


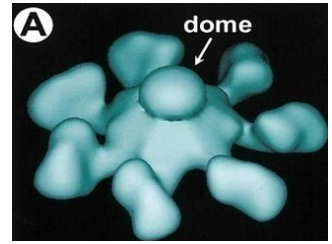
Initial cellular responses to radiation

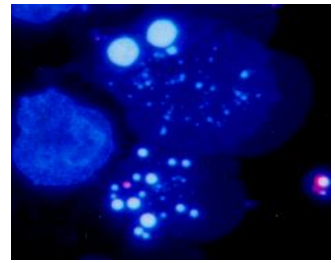
Sensors of damage

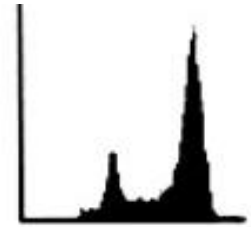
Biological Pathways

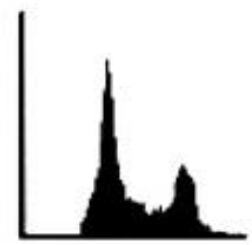
DNA Repair	Early Apoptosis	Cell cycle checkpoints	Gene expression

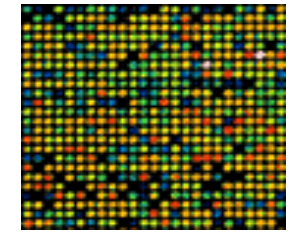


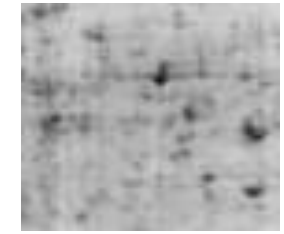




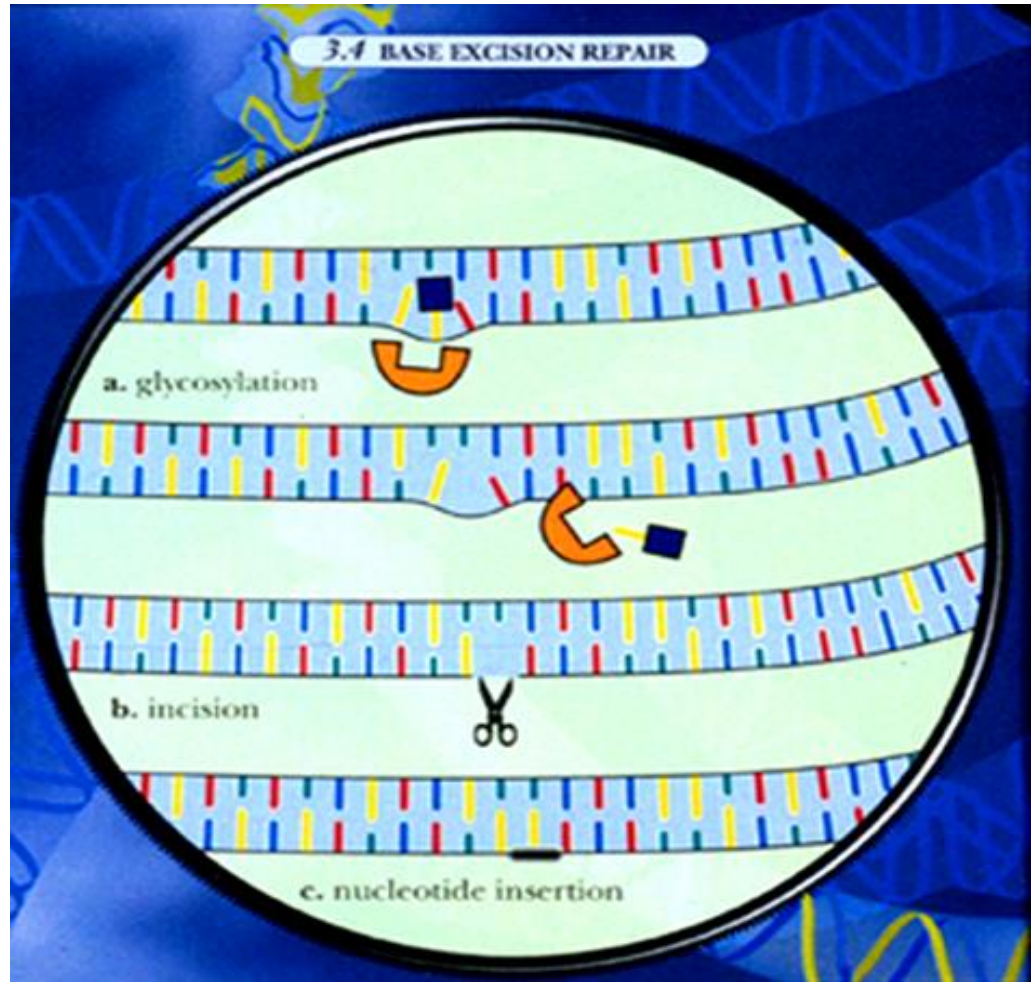








DNA Repair


3.4 BASE EXCISION REPAIR
a. glycosylation
b. incision
c. nucleotide insertion

DSB Repair

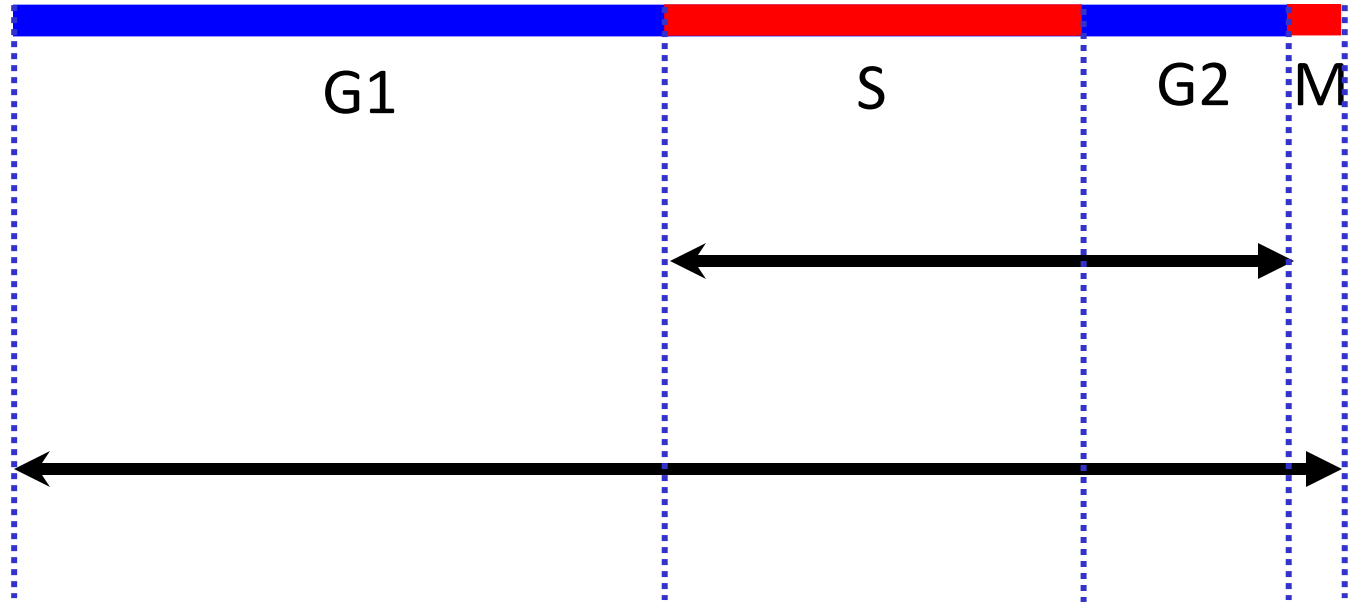
Homologous Recombination (HR)

Non–homologous End-joining (NHEJ)

DNA Repair Through the cell cycle

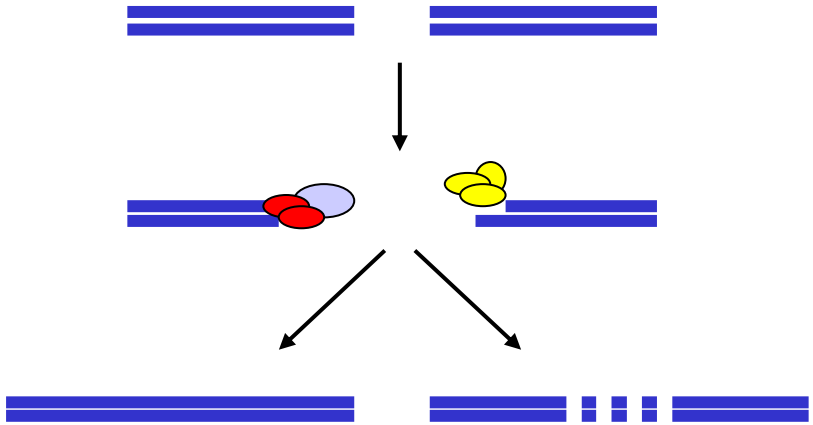
Homologous
Recombination

Non-Homologous
End Joining

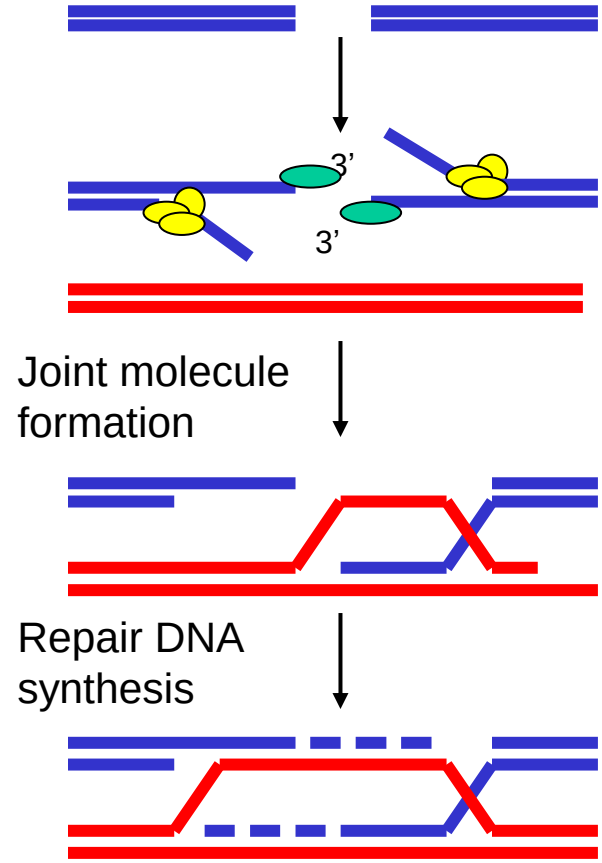


HR and NHEJ

Non-homologous end-joining



Homologous recombination



HR versus NHEJ

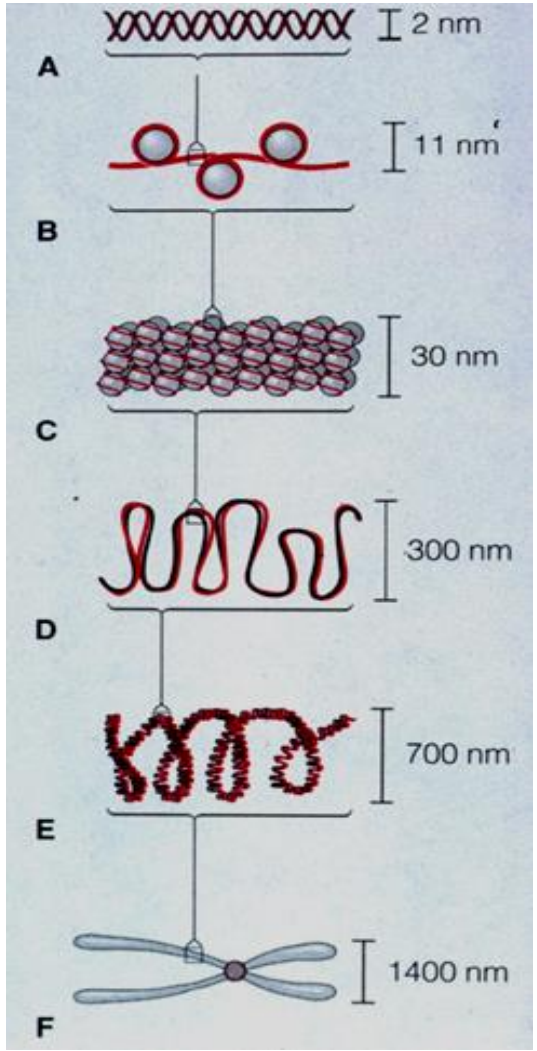
- NHEJ
  - Repairs most DSB - 80%
  - Important for radiosensitivity
  - Error prone
  - All parts of the cell cycle
  - ½ time ~2-4 hours
  - Defects rare in cancer
  - **Non-proliferating tissues**

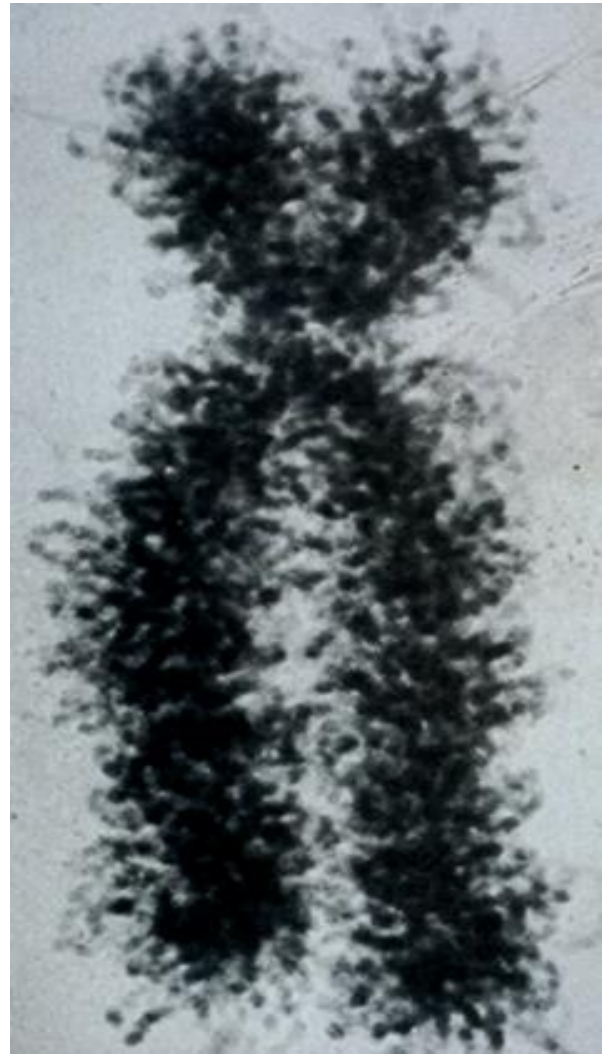
- HR
  - Repairs fewer DSB – 20%
  - Important for radiosensitivity
  - Error free
  - S and G2 phase
  - responsible for change in sensitivity in the cell cycle
  - ½ time long – 24hours?
  - Varies more between cell lines (high in stem cells)
  - Defects common in cancer
  - **Proliferating tissues**

Early versus late responding tissue

Digest in Radiation Biology

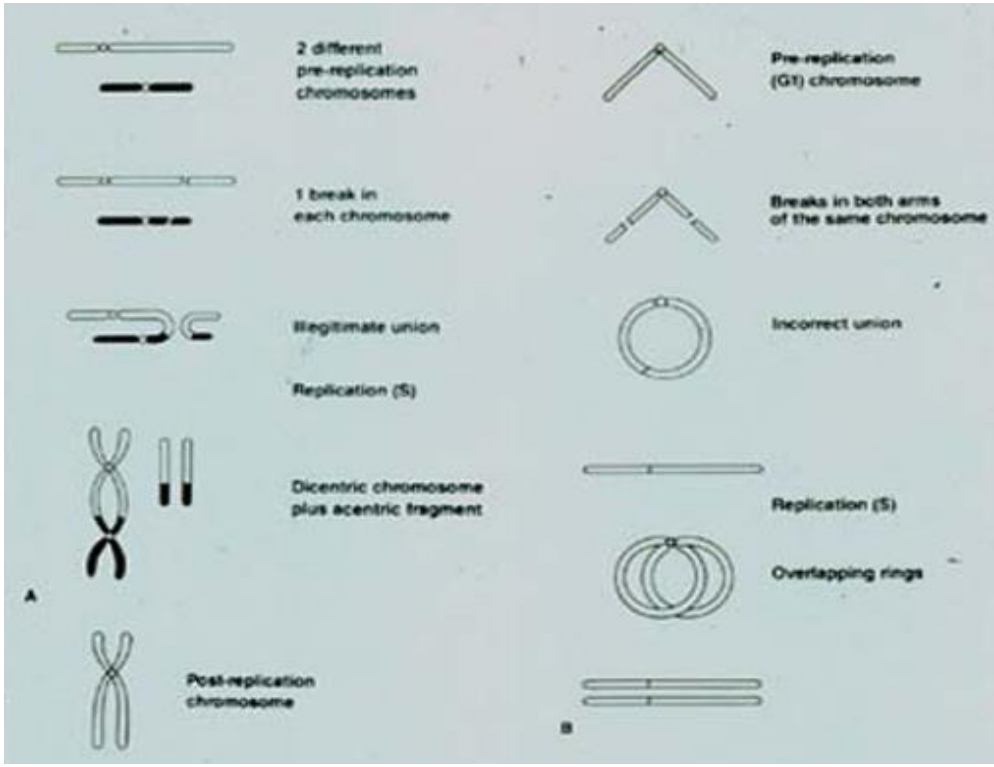
Structure of chromosome

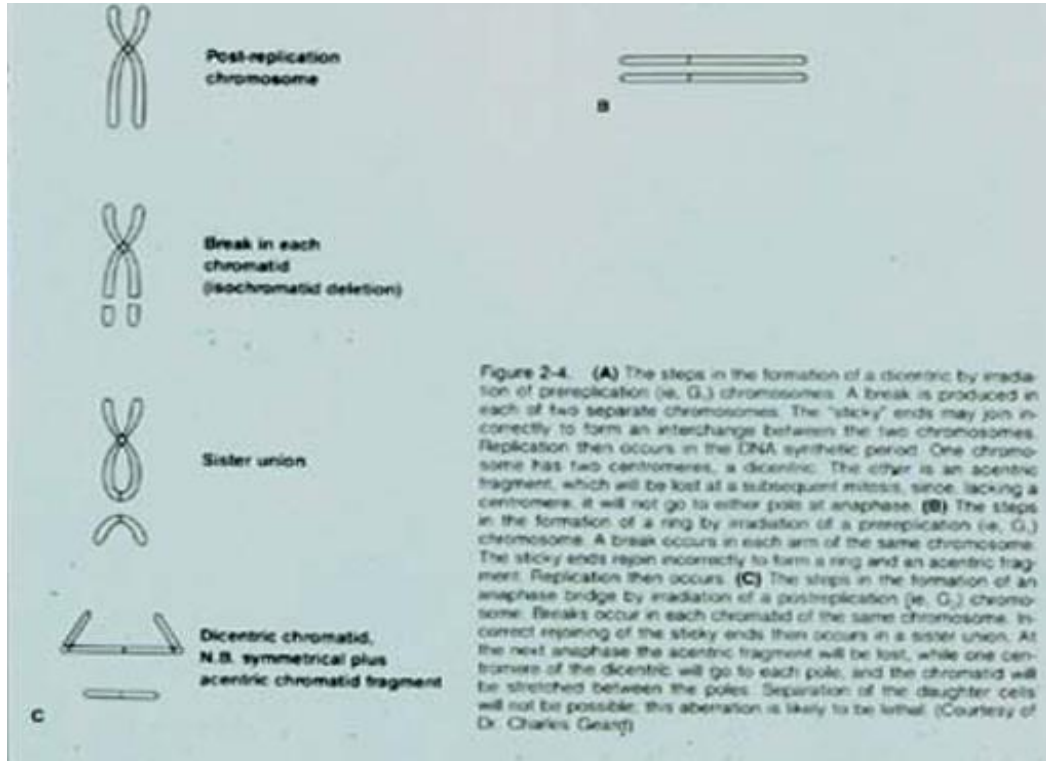




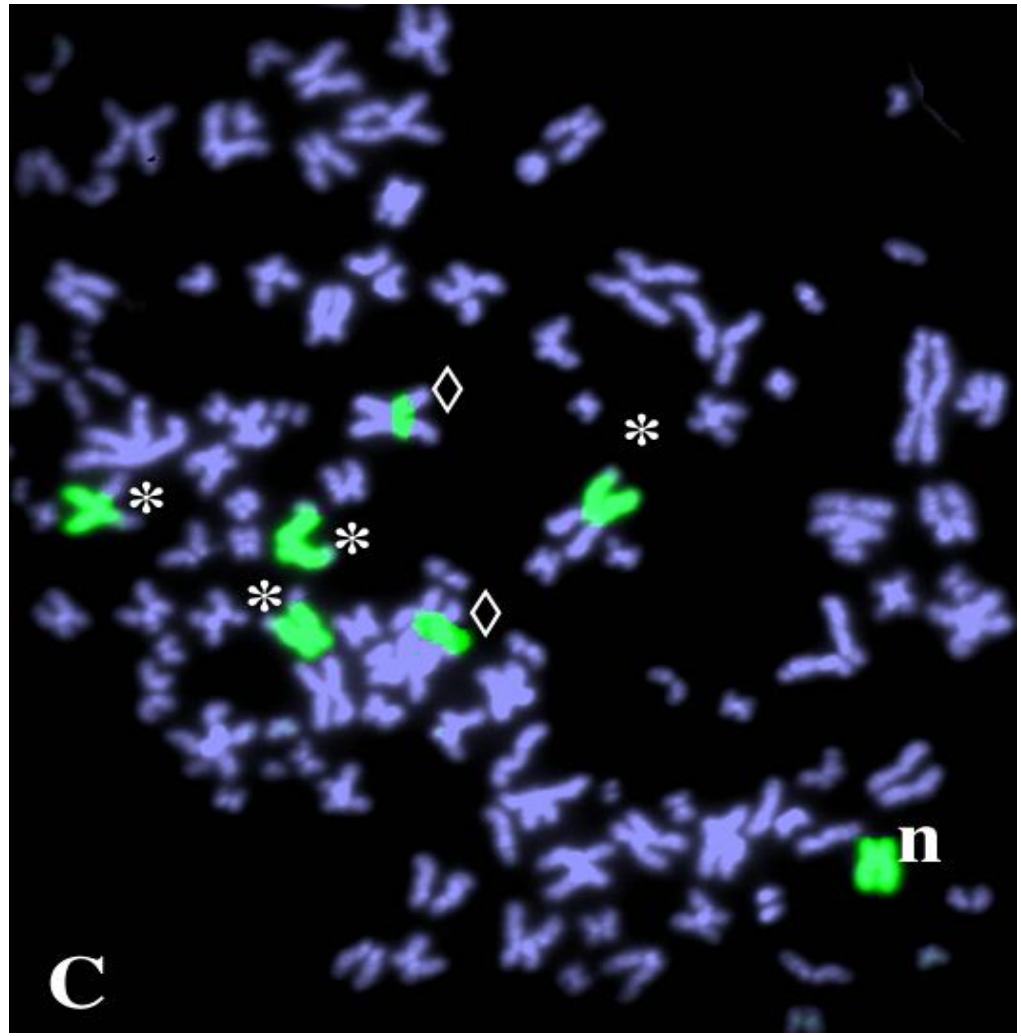
Digest in Radiation Biology

Chromosome and chromatid aberrations

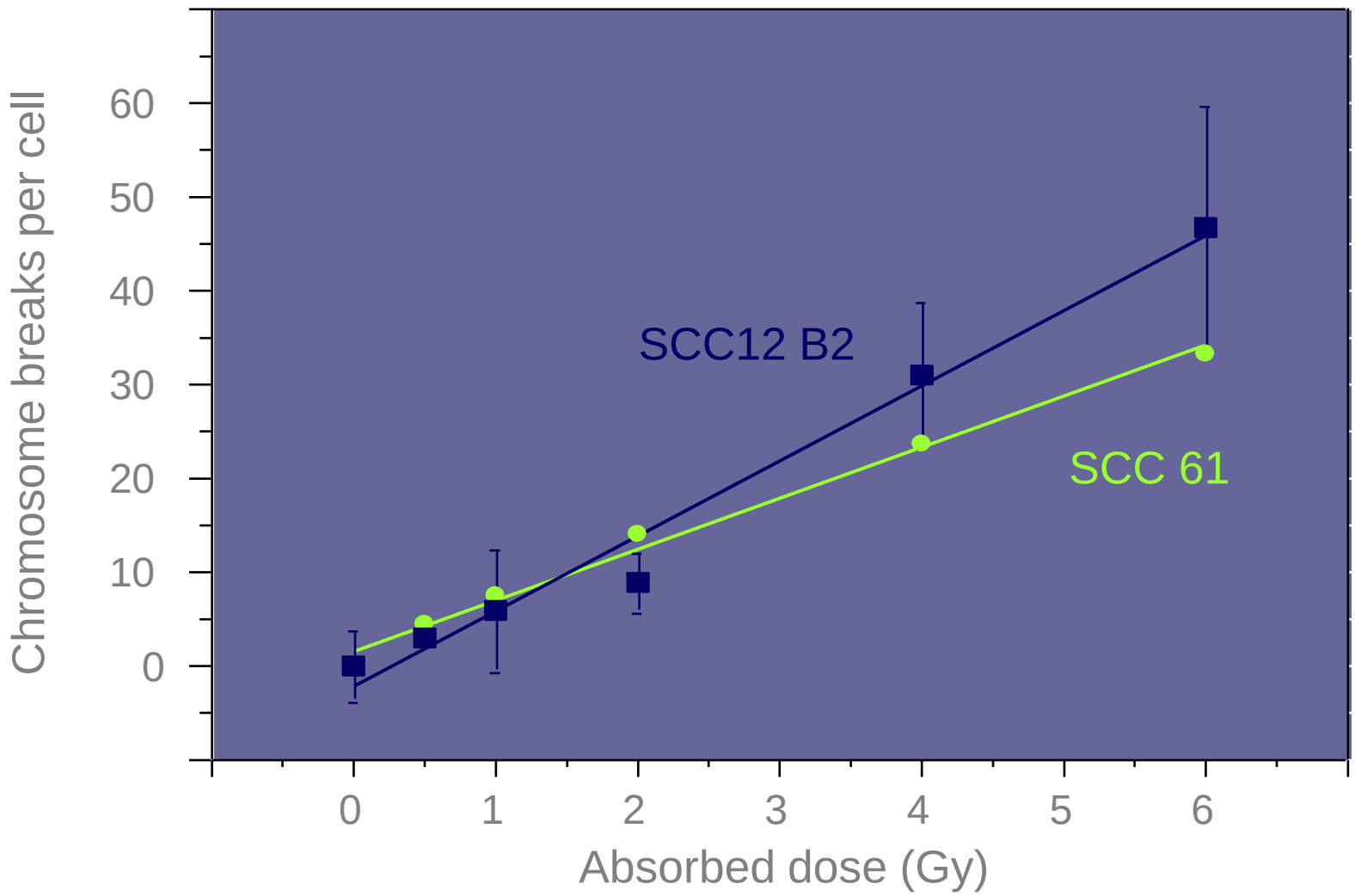




Chromosome aberrations



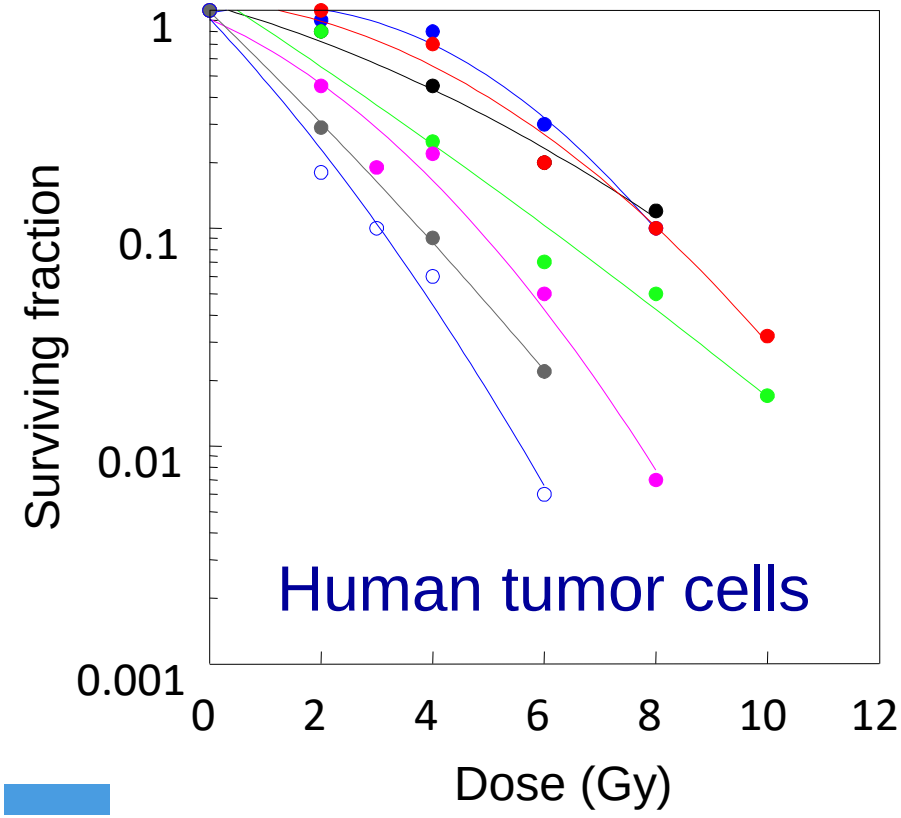
Quantification of chromosome breaks

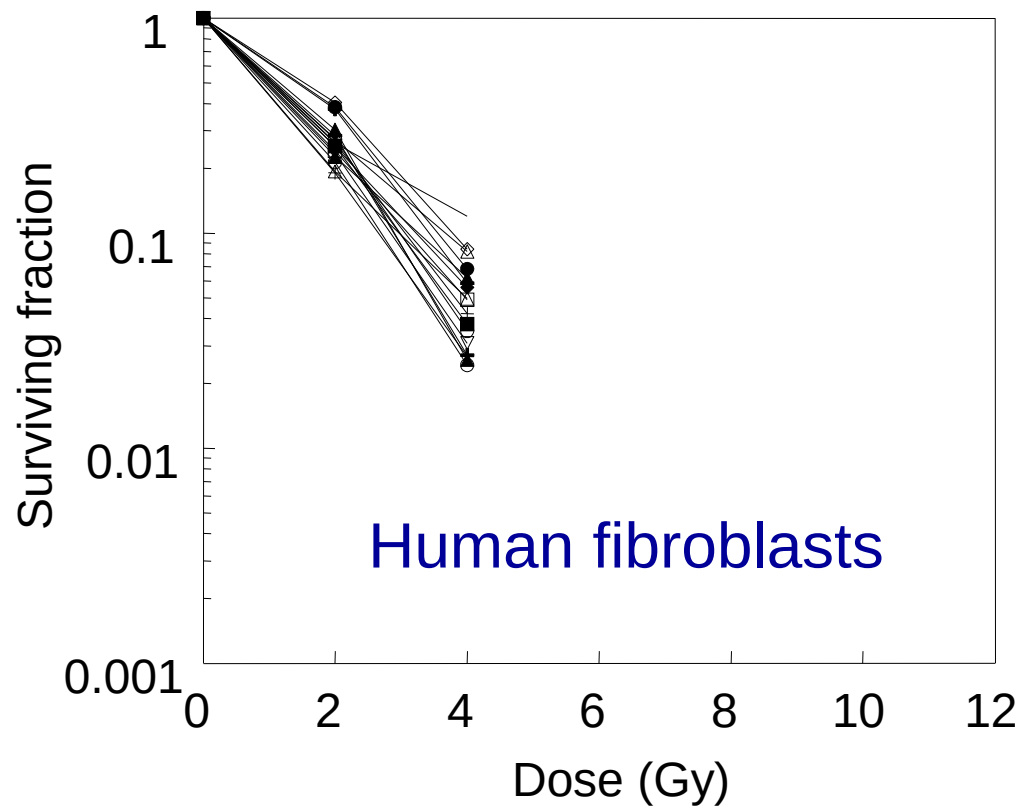


From Hittelman et al.

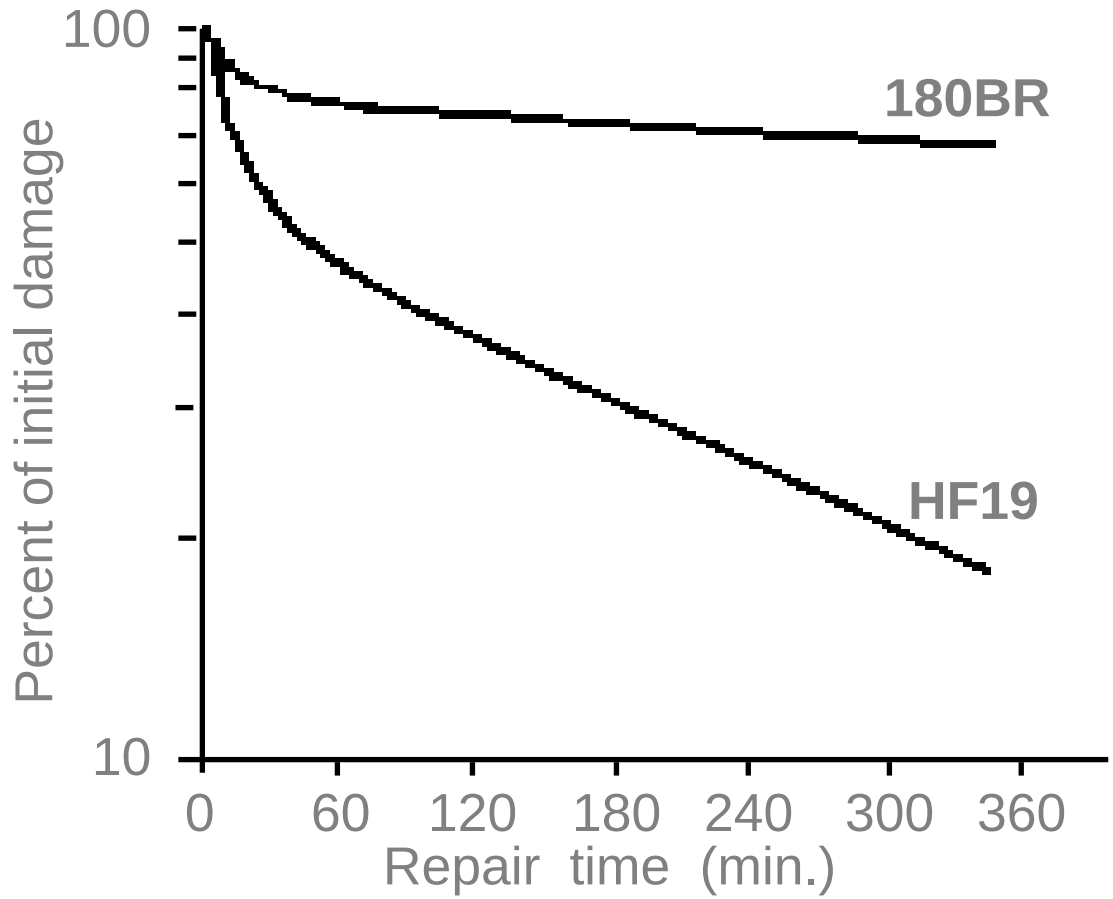
DNA Repair and Cancer

1. Most anticancer agents work by damaging DNA
2. Changes in DNA repair influence radiosensitivity





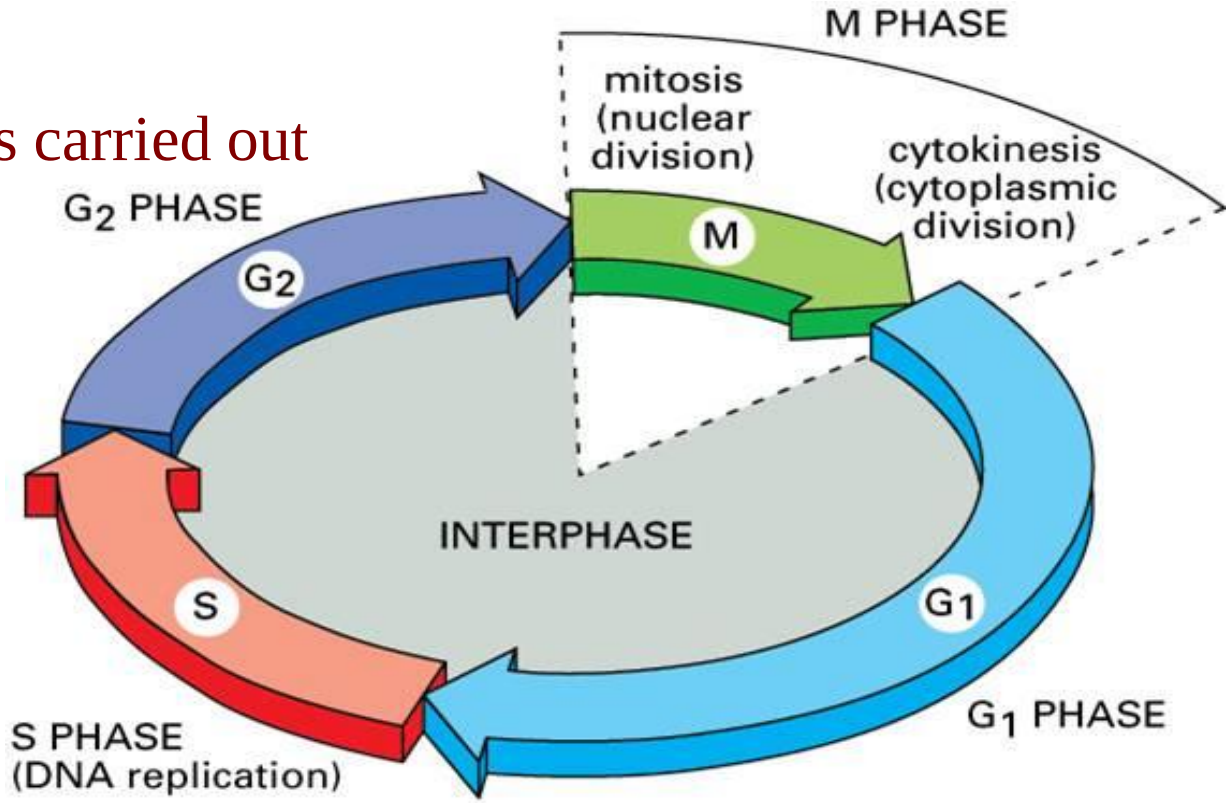
Quantification of DNA Repair



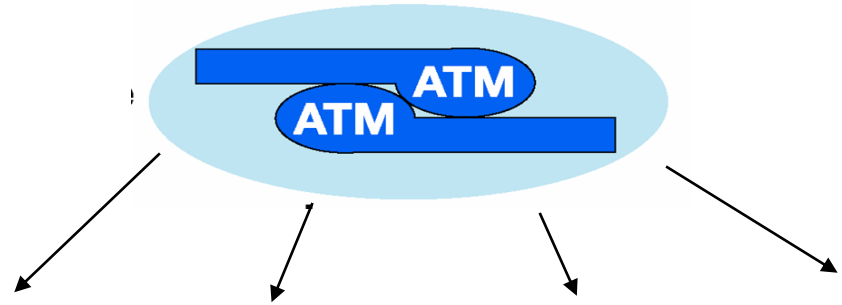
From Badie et al.

The cell cycle multiplies cells

- The cell cycle consists of two major phases:
  - Interphase,
    - chromosomes duplicate and cell parts are made
    - 90% of the cell cycle
    - Normal cell functions carried out
  - The mitotic phase
    - cell division

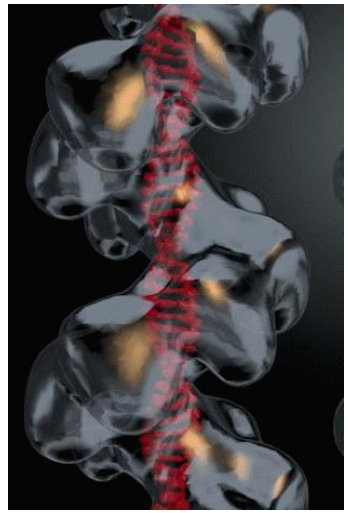


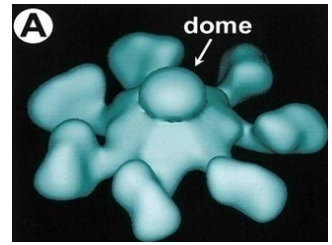
ATM – a key player in DNA damage

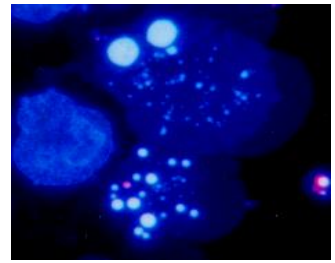


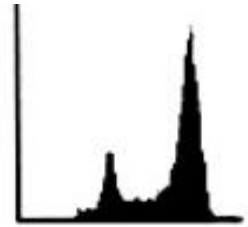
Biological Pathways

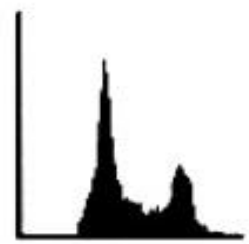
DNA Repair	Early Apoptosis	Cell cycle checkpoints	Gene expression

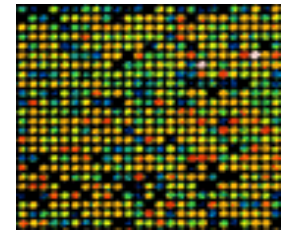


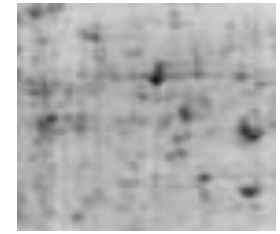




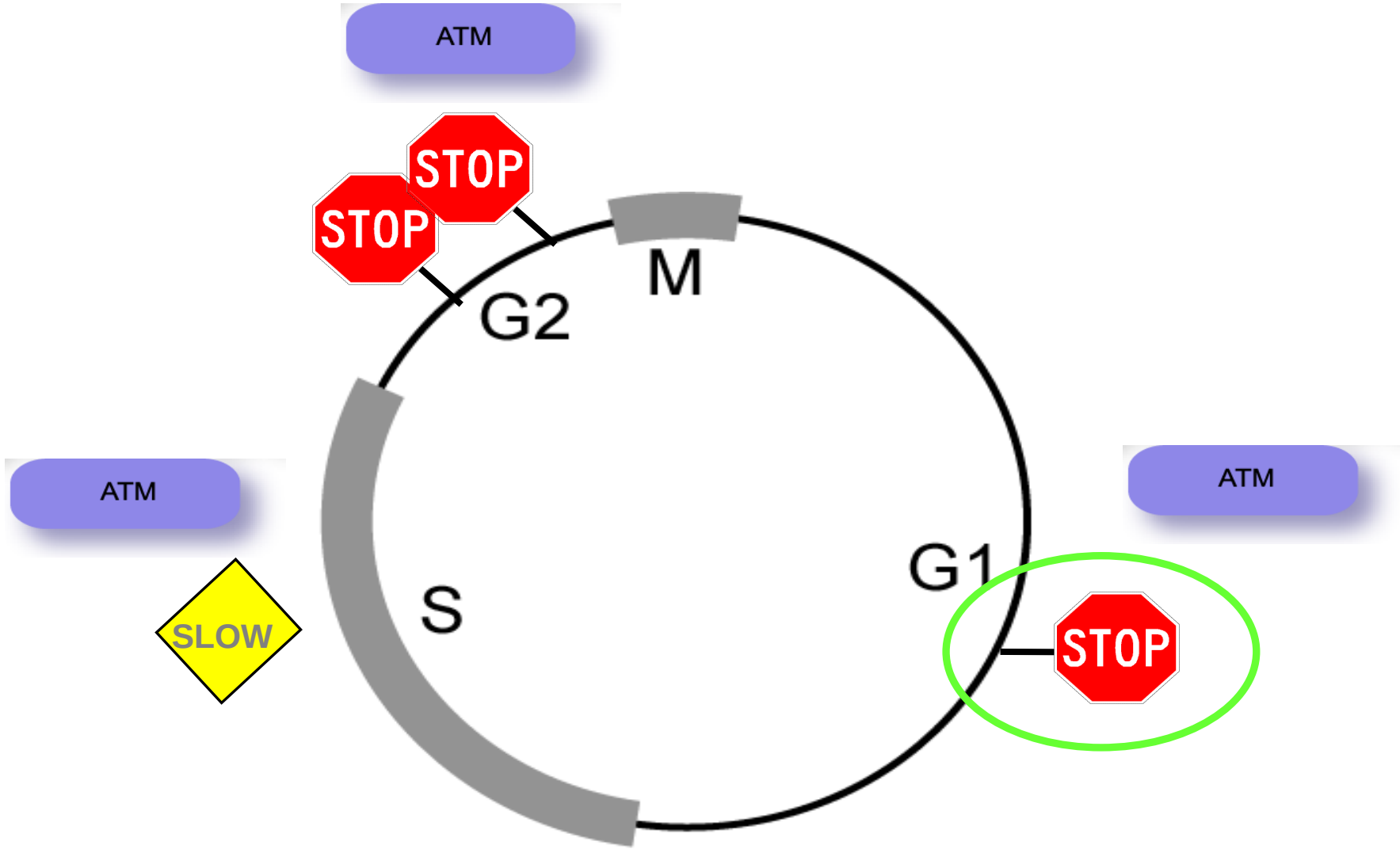




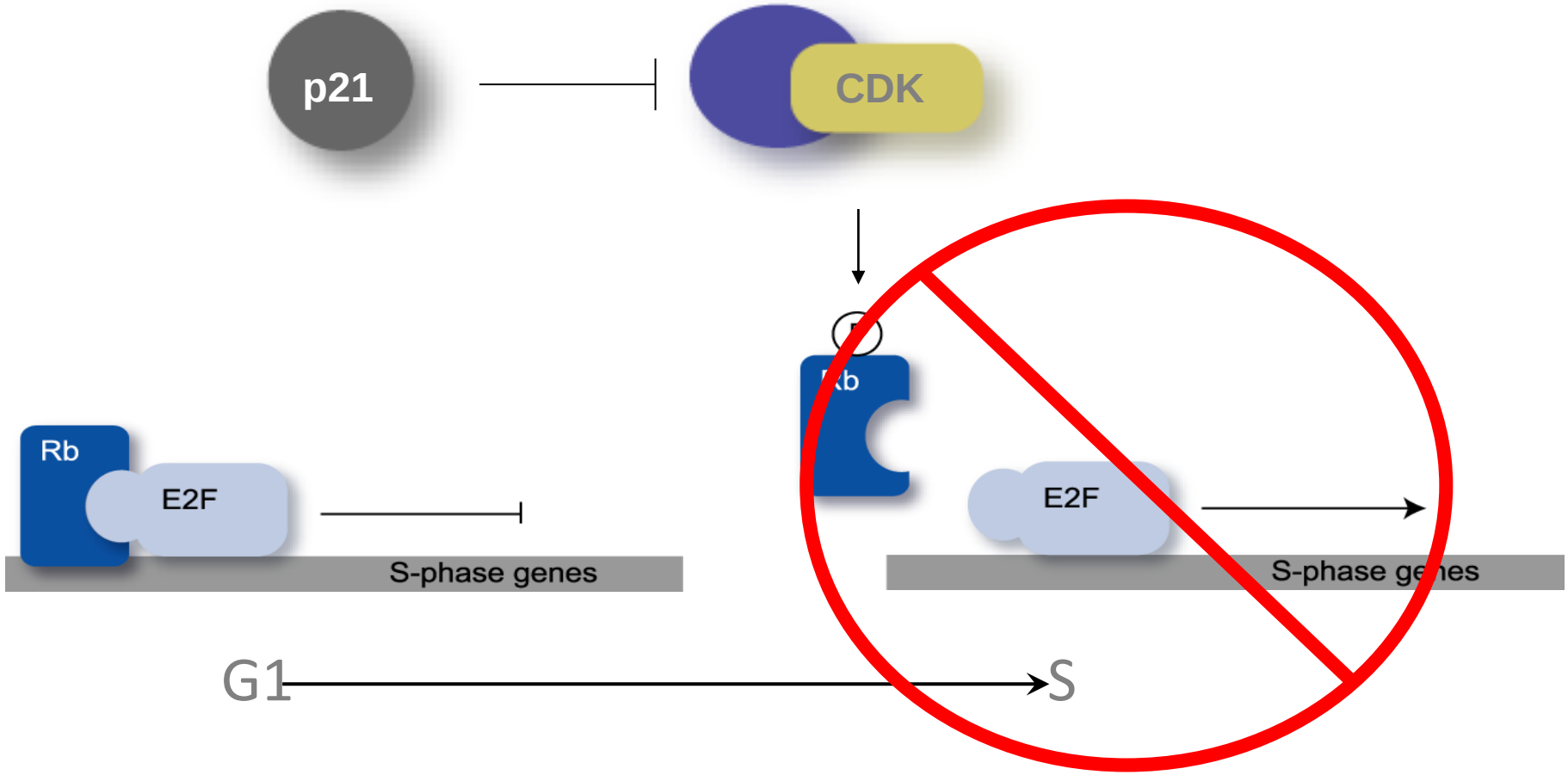




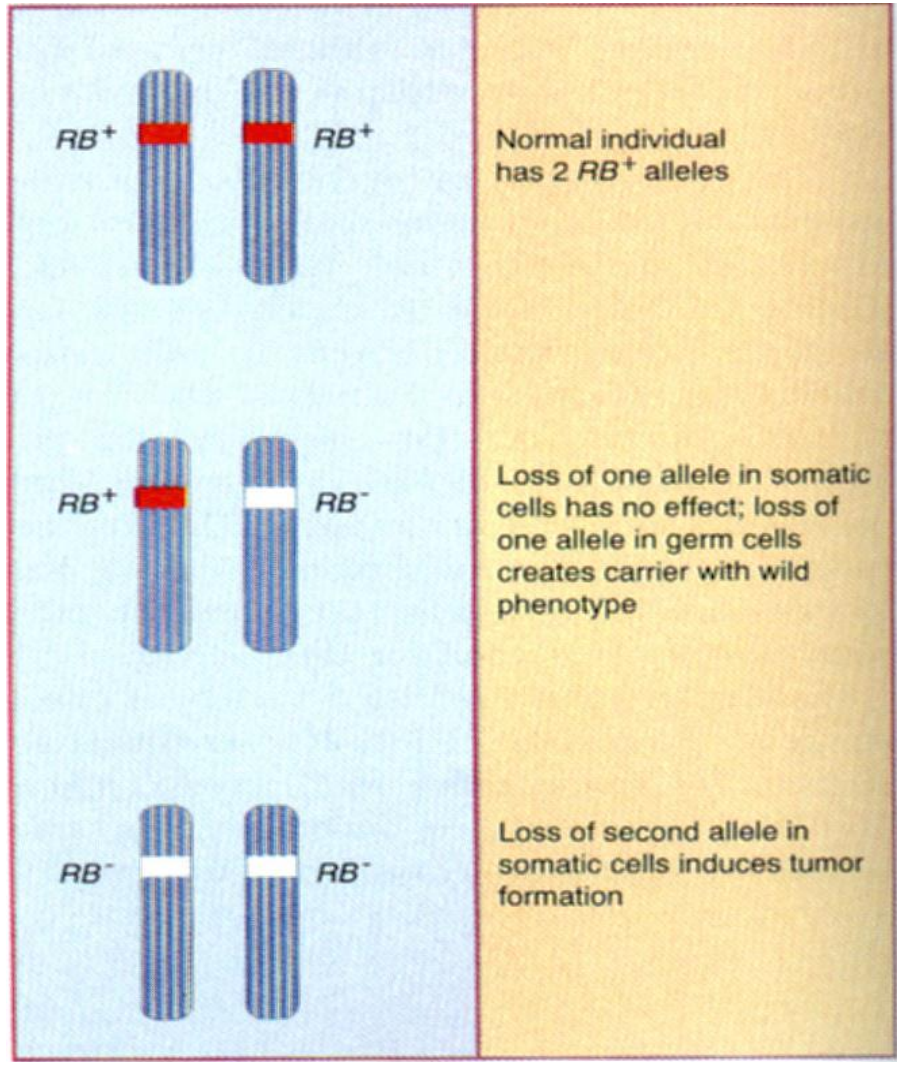
IR induces 4 distinct checkpoints



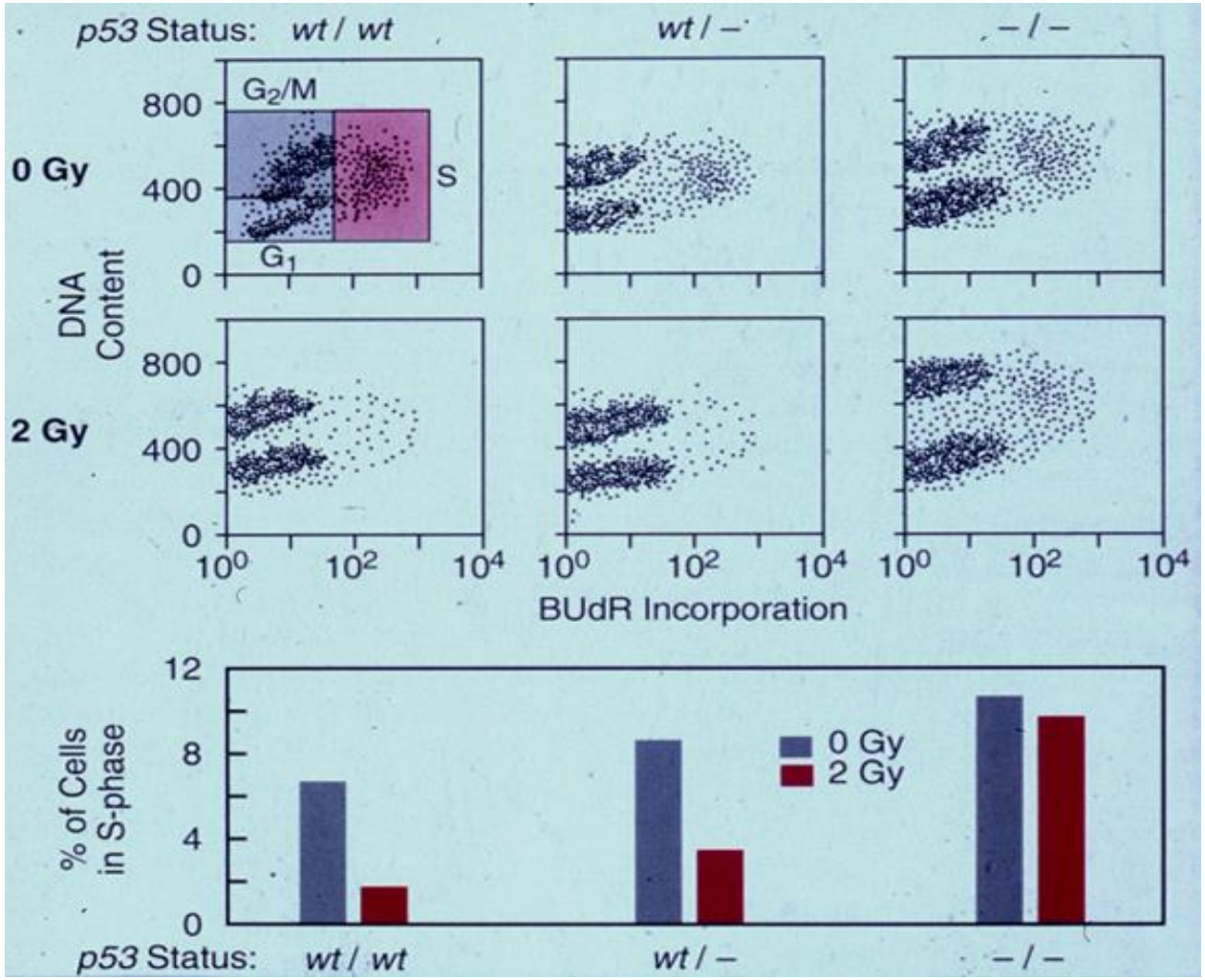
G1/S Transition



Tumor suppressor gene: the retinoblastoma example



The p53-dependant signaling pathways

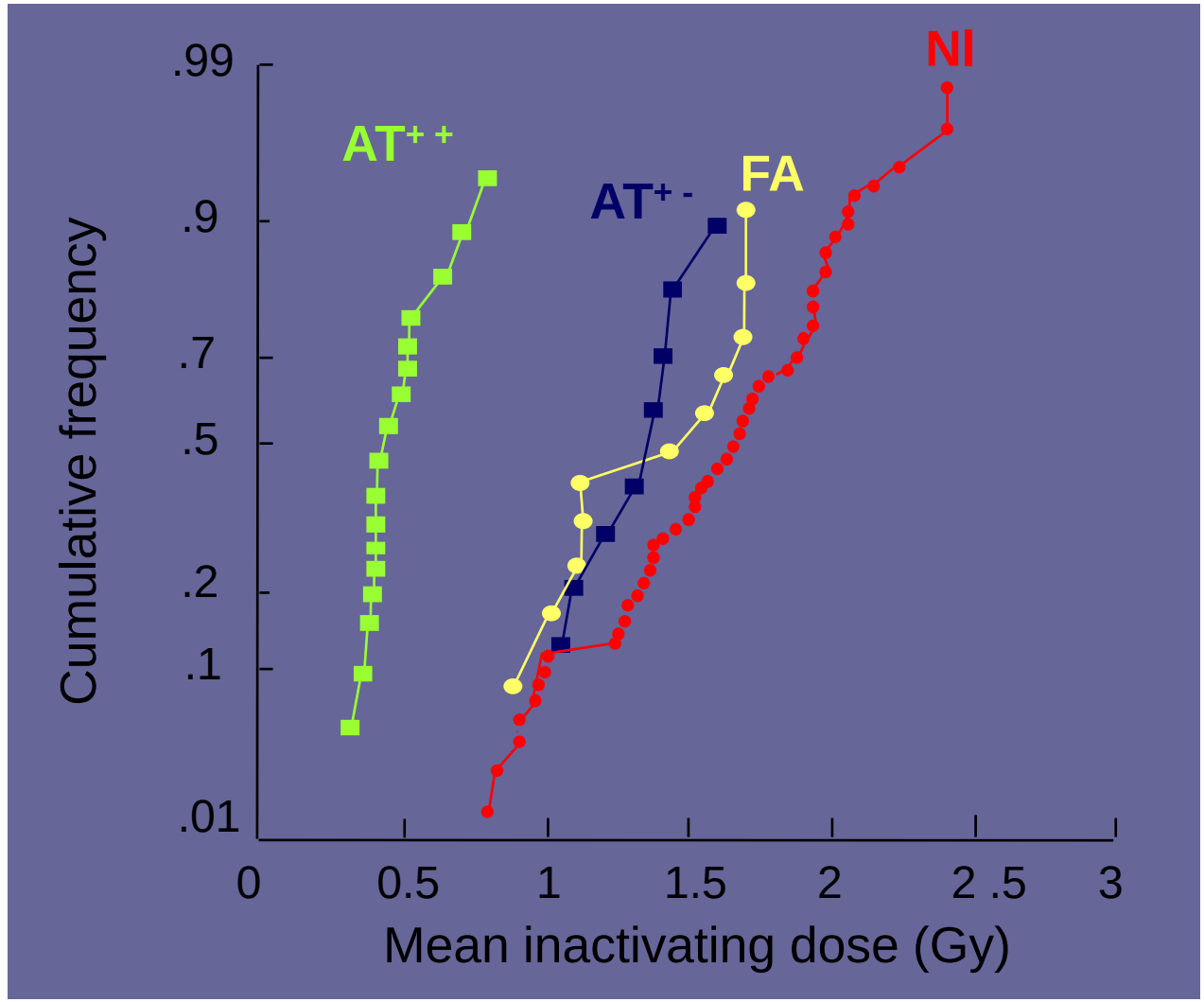


HR and Human Disease

Many diseases associated with the sensors and transducers

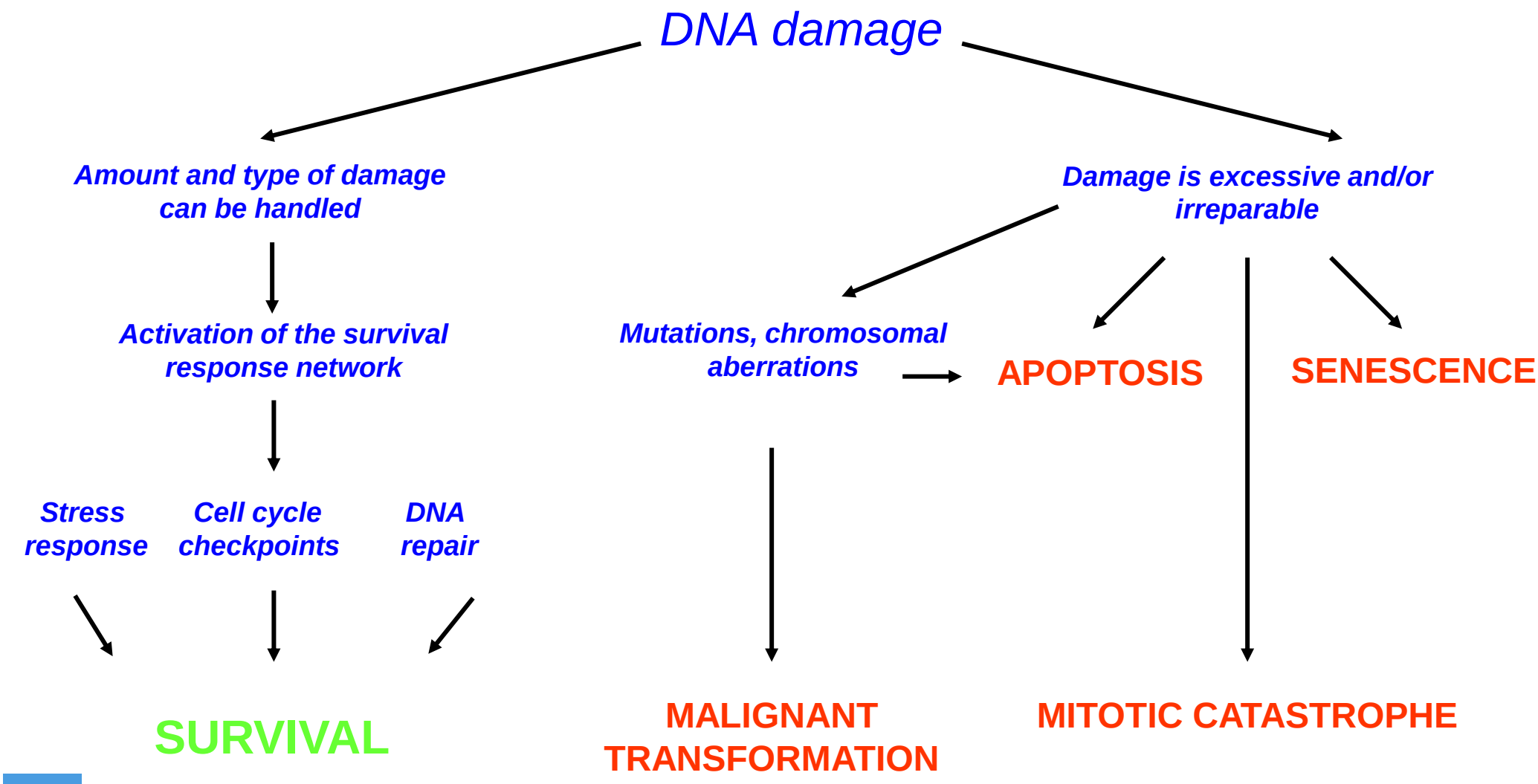
- Ataxia Telangiectasia – mutations in ATM
  - Patients are radiosensitive
  - Elevated risk of cancer
  - Have several developmental and neural abnormalities
- AT like disorder – mutations in MRE11
- Nijmegen breakage syndrome – mutations in NBS
- Familial (inherited) breast cancer - BRCA1, BRCA2
  - Inherited breast and ovarian cancer
- Fanconi's Anemia – FANCA,B,C,D1,D2,E
  - FANCB,D1=BRCA2
  - Sensitive to crosslinking agents
  - Increased risk of cancer

Hypersensitivity syndromes



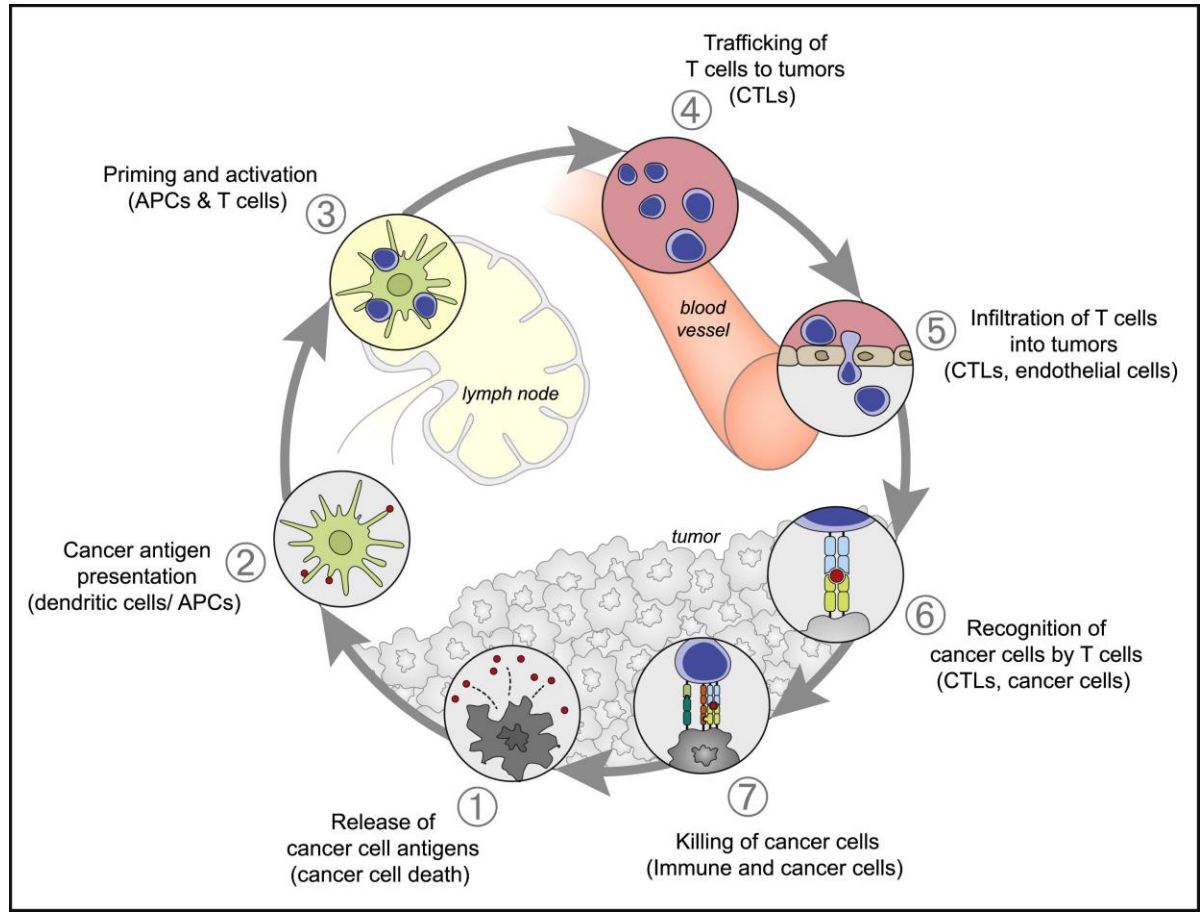
Deschavanne & Malaise, 1986

Cellular response to radiation damage



Adapted from: Shiloh, Nat Rev 2003

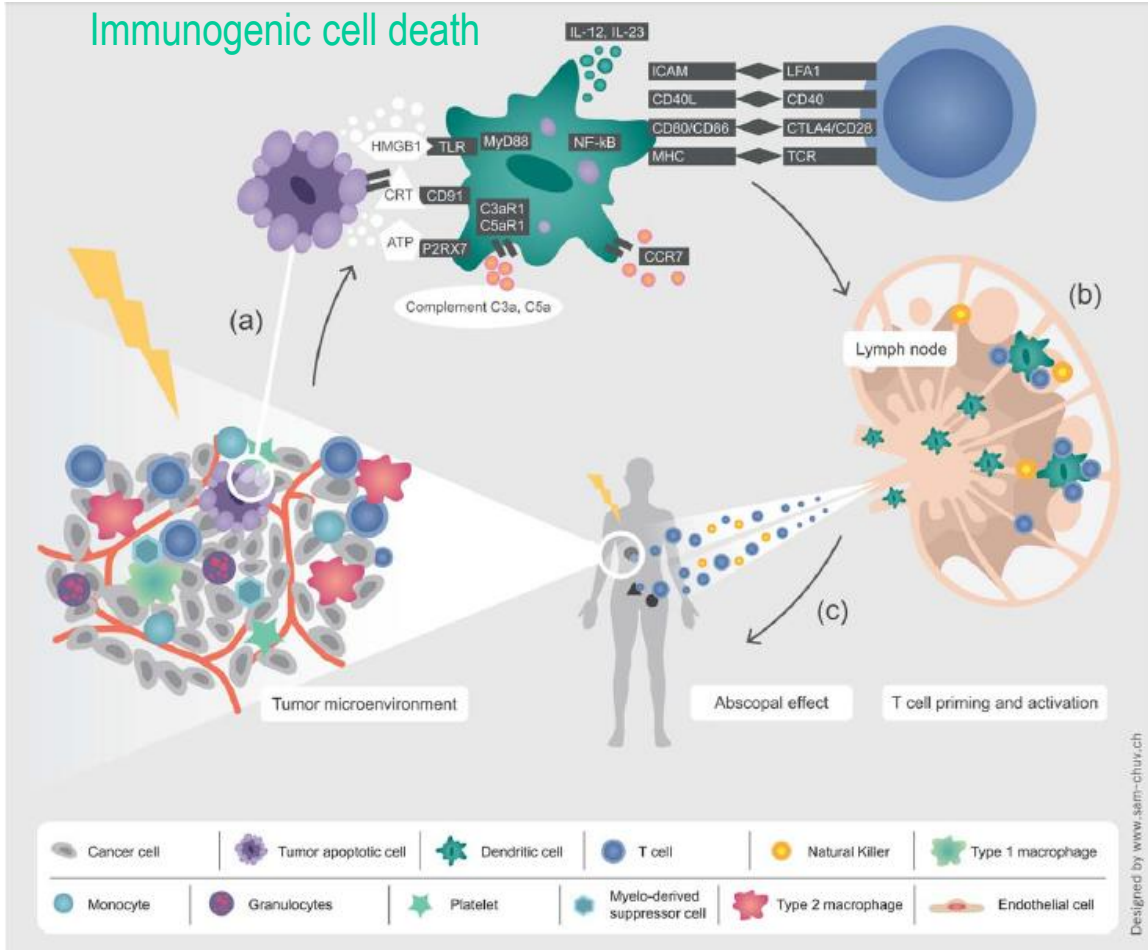
Immune modulation



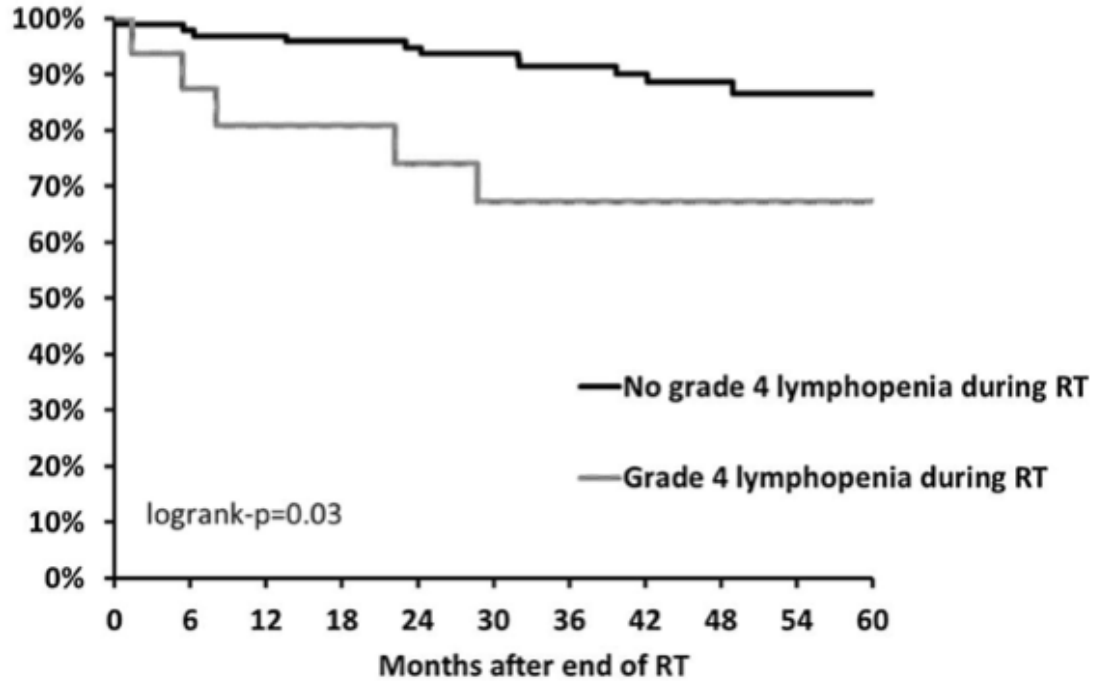
Chen & Mellman, Immunity, 2013

Immune mechanisms triggered by RT

Herrera FG et al CA Cancer J Clin 2017



Prognostic impact of lymphocytopenia on oropharyngeal SCC outcome after radiotherapy



Jensen et al, CTRO, 2017

Summary of DNA damage repair

- DSBs are the most important damage produced by IR
- DSBs are sensed by ATM
  - Apoptosis (rarely)
  - Checkpoint activation
  - DNA repair
- Repair requires large repair factories containing many proteins
  - NHEJ (DNAPKcs, Ku70/80, Artemis, XRCC4, Ligase)
  - HR (BRCA1/2, Rad51/52, FANCD2)
- Impaired DNA repair machinery causes (extreme) radiosensitivity
- IR enhances an immune response