Model Tumor Systems (Chap. 20)

Models (methods) to study tumor responses to the treatment (radiotherapy)

- *In situ*
- *Ex vivo (in vivo – in vivo)*
- *In vitro*

In situ tumor response model

- Tumor Growth Measurement
  - Growth delay
  - Tumor cure (TCD_{50})

Growth Delay

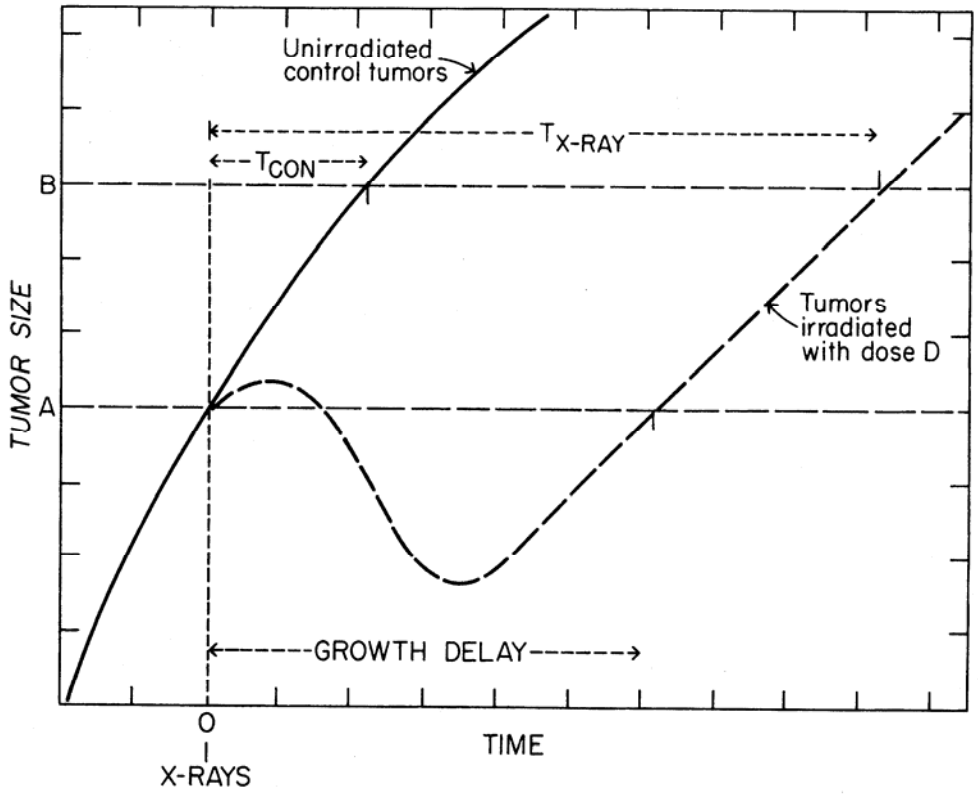


Figure 20.1. The pattern of response of a tumor to a dose of x-rays. The size of the tumor, either the mean diameter or the volume, is plotted as a function of time after irradiation. Two different indices of tumor responses have been used by different investigators. Growth delay represents the time after irradiation that it takes for the tumor to regrow to the size at the time of irradiation. Alternatively, the index of radiation damage may be the time taken for the tumor to grow from a specified size A at the time of irradiation to some specified larger size B. Typically, this may be from 9 to 25 mm in diameter for rat tumors. This quantity is shown as TCON for unirradiated control animals and TX-RAY for tumors irradiated with a dose (D) of x-rays. Either index of tumor response may be plotted as a function of radiation dose.

Growth Delay (ii)

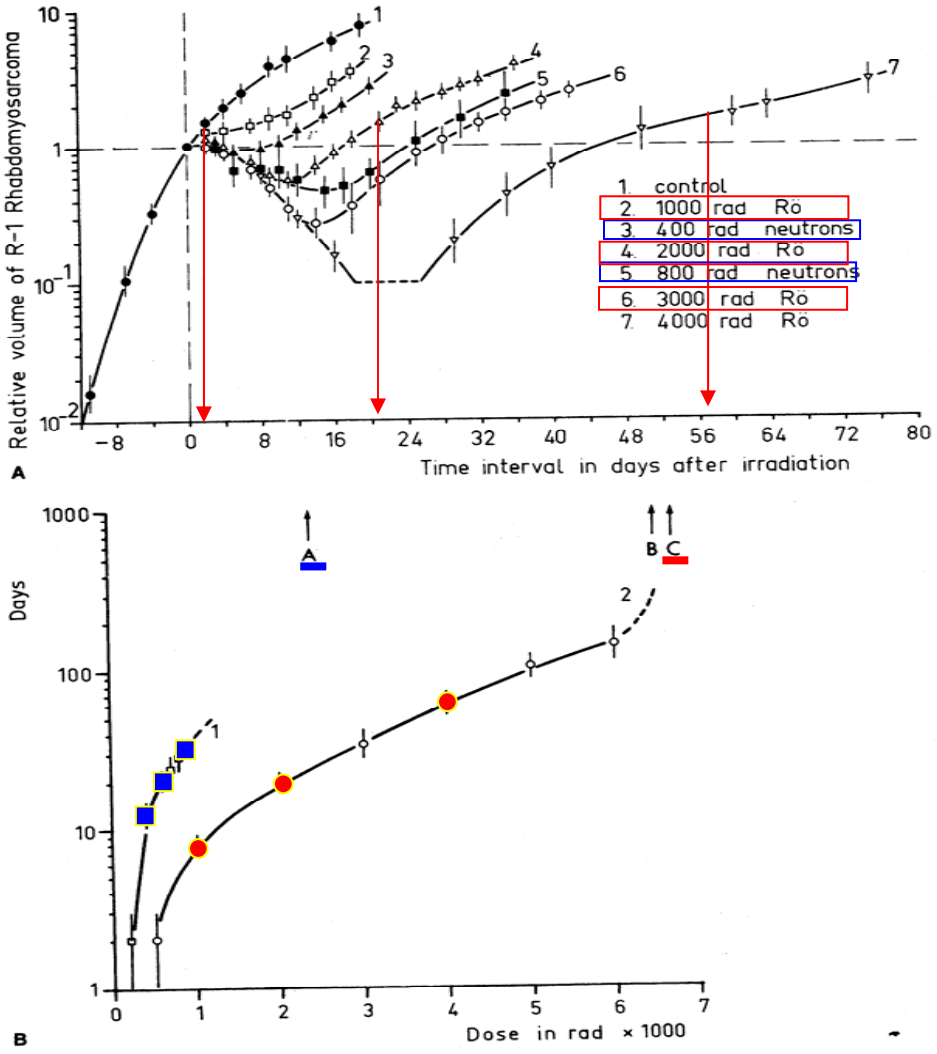
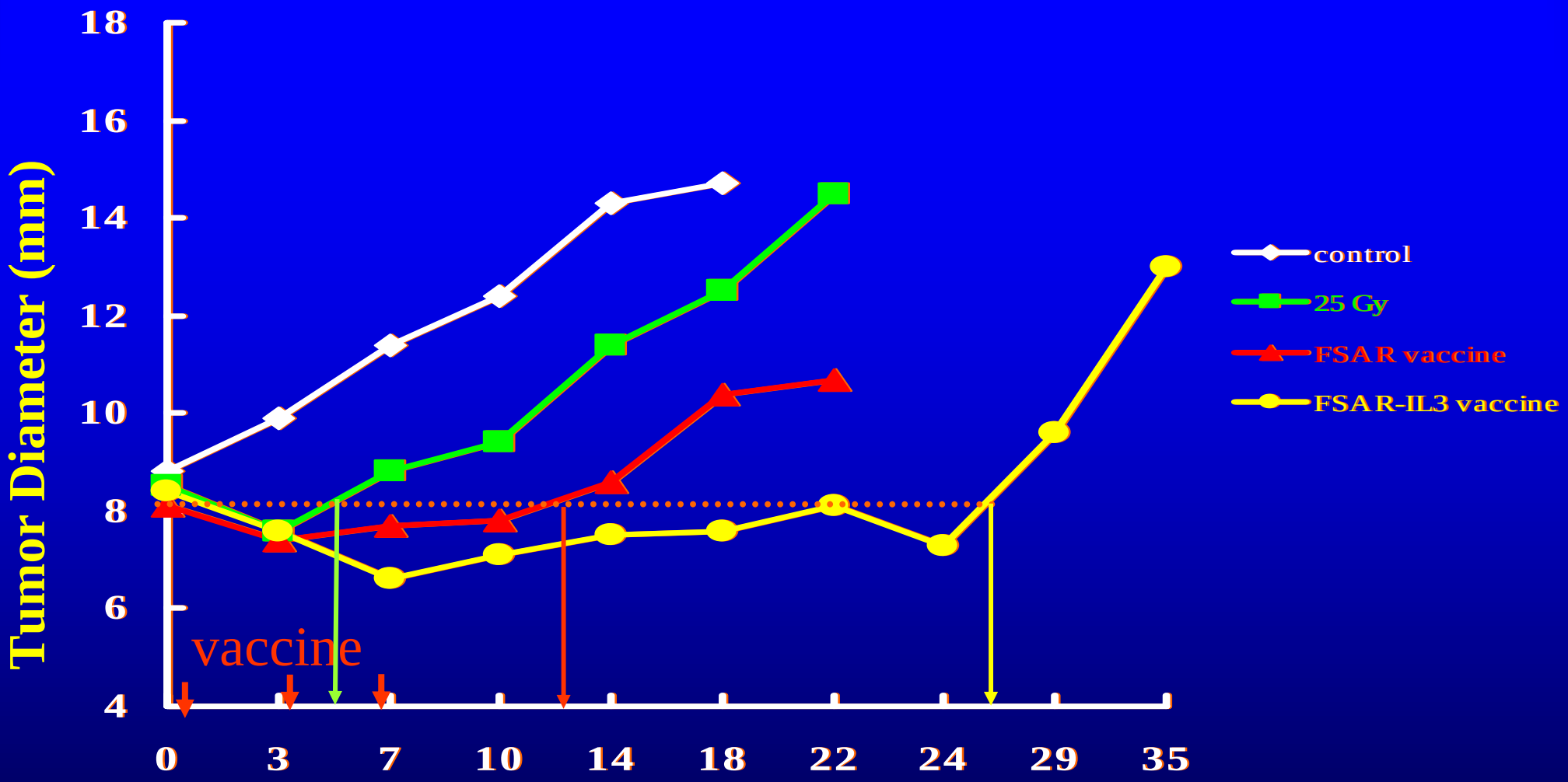


Figure 20.2. **A:** Volume changes of rhabdomyosarcomas in rats after irradiation. Curve 1 represents the growth of the unirradiated control tumors. Curves 2, 4, 6, and 7 refer to tumors irradiated with 10 to 40 Gy (1,000–4,000 rad) of 300-kV x-rays. Curves 3 and 5 refer to tumors irradiated with 4 and 8 Gy (400 and 800 rad) of 15-MeV $d_+ \rightarrow T$ fast neutrons. **B:** Growth delay of rhabdomyosarcomas in rats as a function of dose of x-rays (curve 2) or fast neutrons (curve 1). *A* and *C* indicate the doses of neutrons and x-rays required to "cure" 90% of the tumors, calculated on the basis of cell-survival curves. *B* indicates the observed TCD_{90} for x-rays. Note the good agreement between calculated and observed values of the TCD_{90} for x-rays. (From Barendsen GW, Broerse JJ: Experimental radiotherapy of a rat rhabdomyosarcoma with 15 MeV neutrons and 300 kV x-rays: I. Effects of single exposures. Eur J Cancer 5:373–391, 1969, with permission.)

FSAR Response to Irradiation/Vaccine in C3H/HeN mice



Time after 25 Gy Irradiation (day)

Cancer Gene Therapy, 2000

Combining Radiation Therapy with IL-3 Gene Immunotherapy

	Tumor Growth Delay (day)	
	FSAN	FSAR
XRT	6	6
Tumor Vaccine	7	13
IL-3 Tumor Vaccine	21	27

Tumor Cure (TCD_{50})

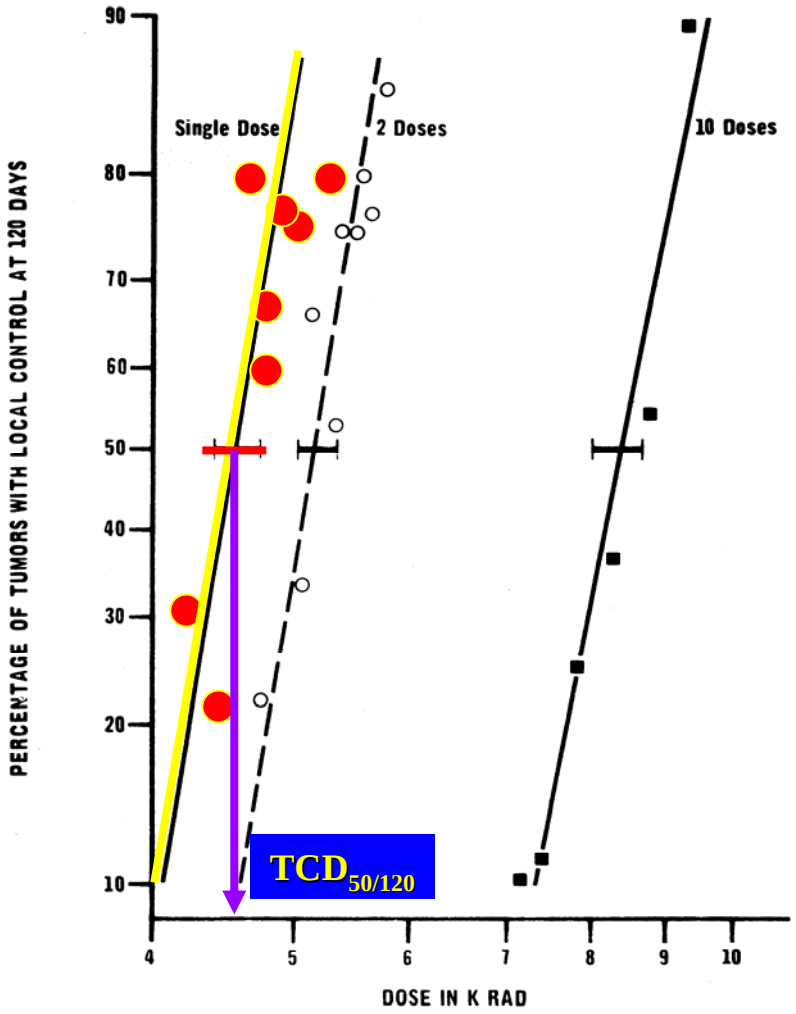


Figure 20.3. Percentage of mouse mammary tumors locally controlled as a function of x-ray dose, for single exposures and for two different fractionation patterns. The tumors were isotransplants derived from a spontaneous mammary carcinoma in a C_3H mouse. The transplantation was made into the outer portion of the ear with 4×10^4 viable cells. The tumors were treated when they reached a diameter of 2 mm (*i.e.*, a volume of about 4 mm^3). (From Suit H, Wette R: Radiation dose fractionation and tumor control probability. Radiat Res 29:267–281, 1966, with permission.)

Summary of *in situ* tumor response model

- **Advantage:**
  - **Similar to clinical situation**
- **Disadvantage:**
  - **Lack of radiobiological parameters**
    - **eg. Do, n, Dq, α/β**

Ex vivo cell survival assays

1. **Tumor cell survival assays:**
   - **Dilution assay (TD_{50})**
   - **Lung colony assay (TD_{50})**
   - ***In vivo/in vitro* assay**
2. **Xenograft system:**
3. **Spheroids:**

Dilution Assay *(in vivo / in vivo)*

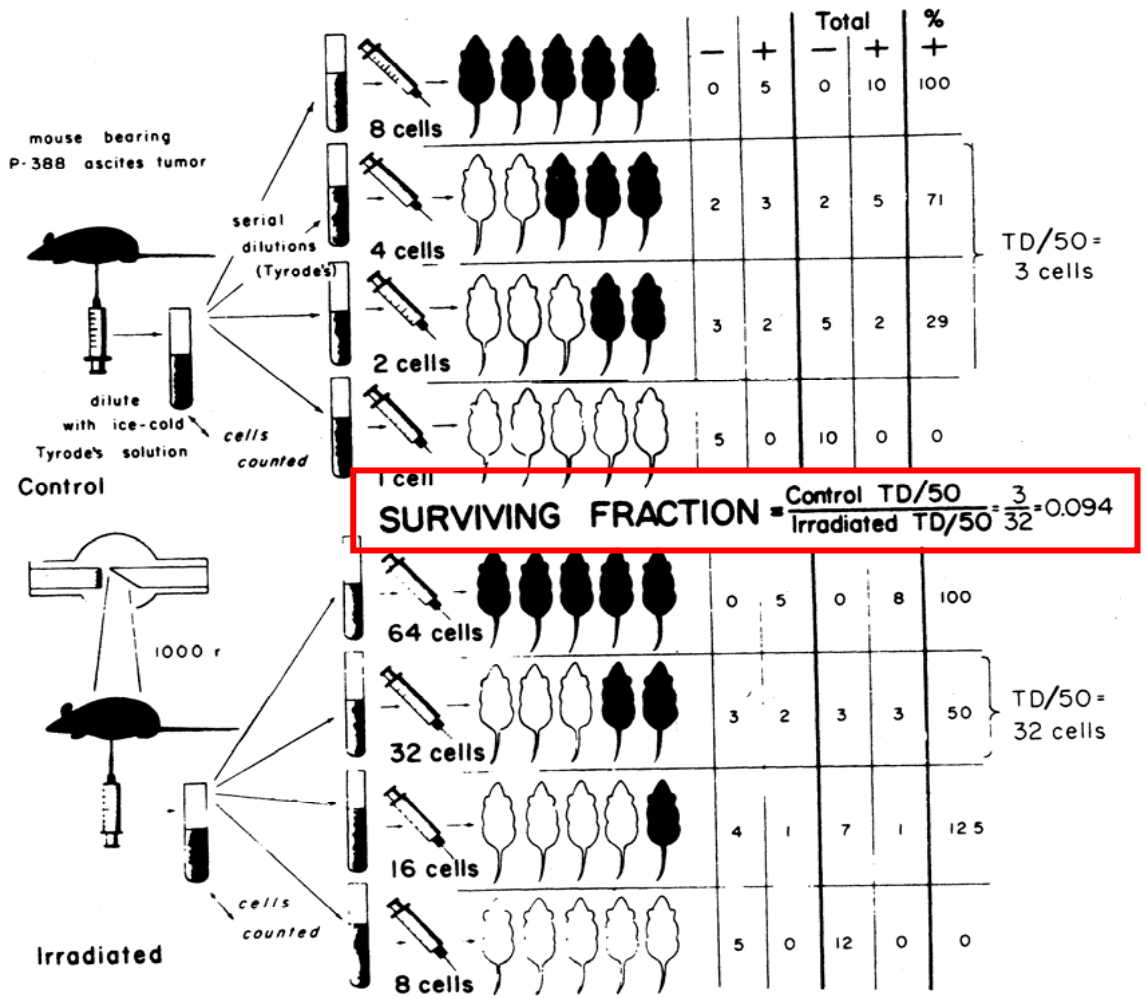


Figure 20.4. Schematic representation to show the general features of the dilution assay technique. Various numbers of tumor cells from the donor animal are injected into groups of recipients, and a determination is made of the number of cells required for a tumor to take in half of the animals of the group (TD_{50}). The ratio of this quantity for control and irradiated donors is the surviving fraction. (From Andrew S Jr, Berry RJ: Radiat Res 16:76, 1962, with permission.)

Dilution Assay (ii)

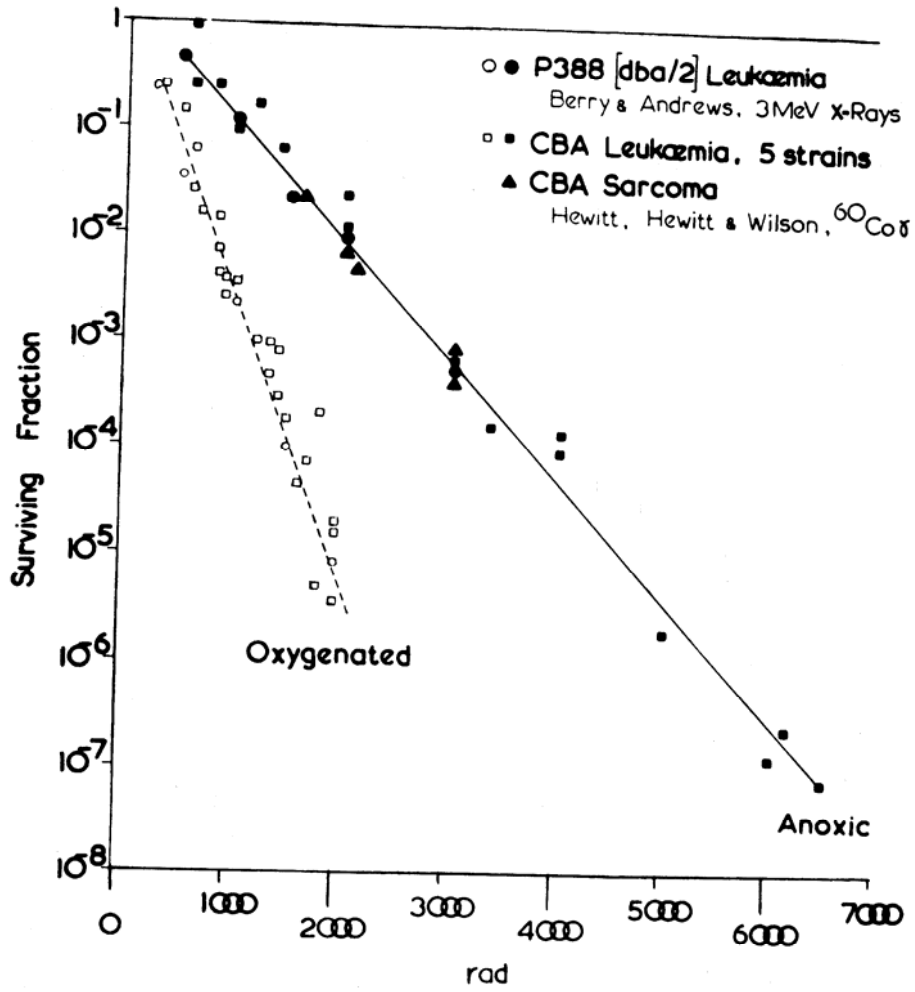


Figure 20.5. Dose–response curves *in vivo*, using the dilution assay technique, for various murine tumors under oxygenated and hypoxic conditions. (From Berry RJ: Br J Radiol 37:948, 1964, with permission.)

Lung Colony Assay *(in vivo / in vivo)*

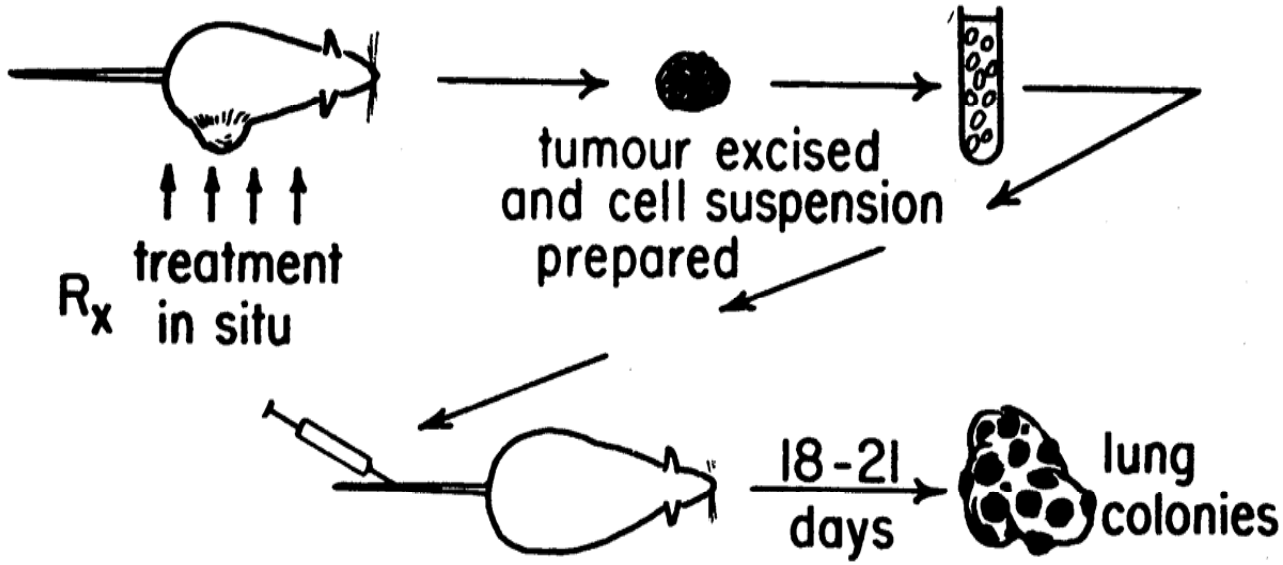
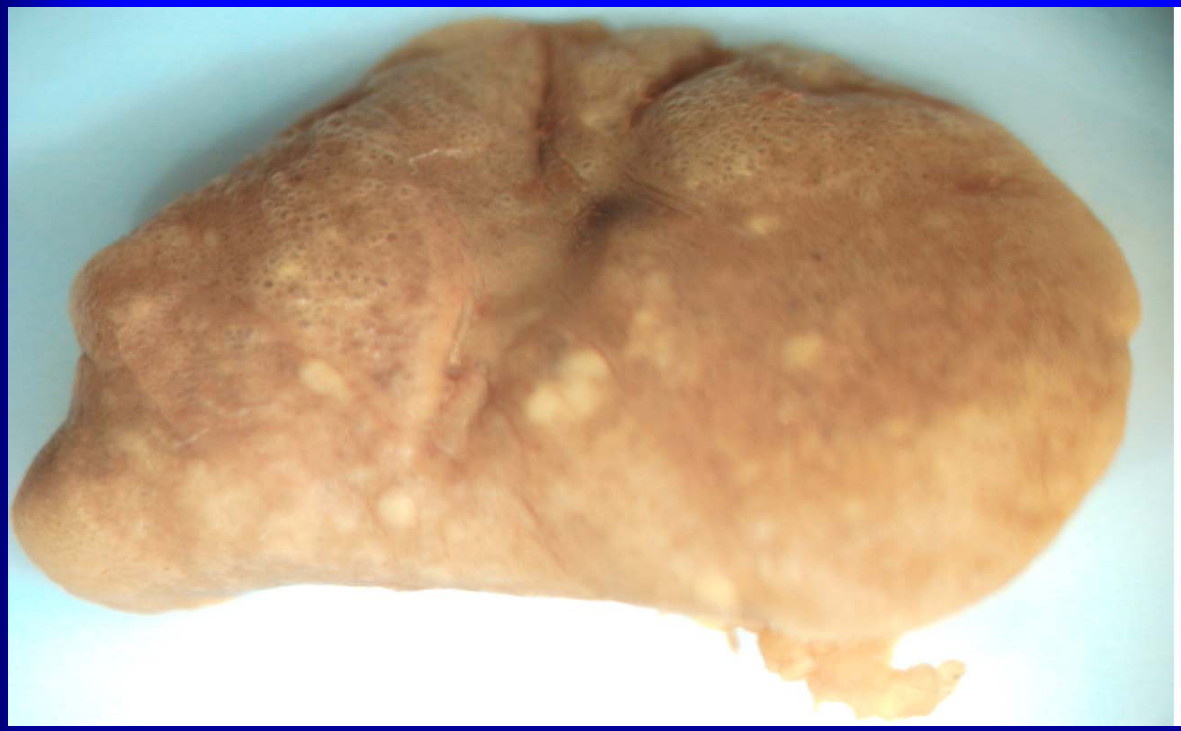
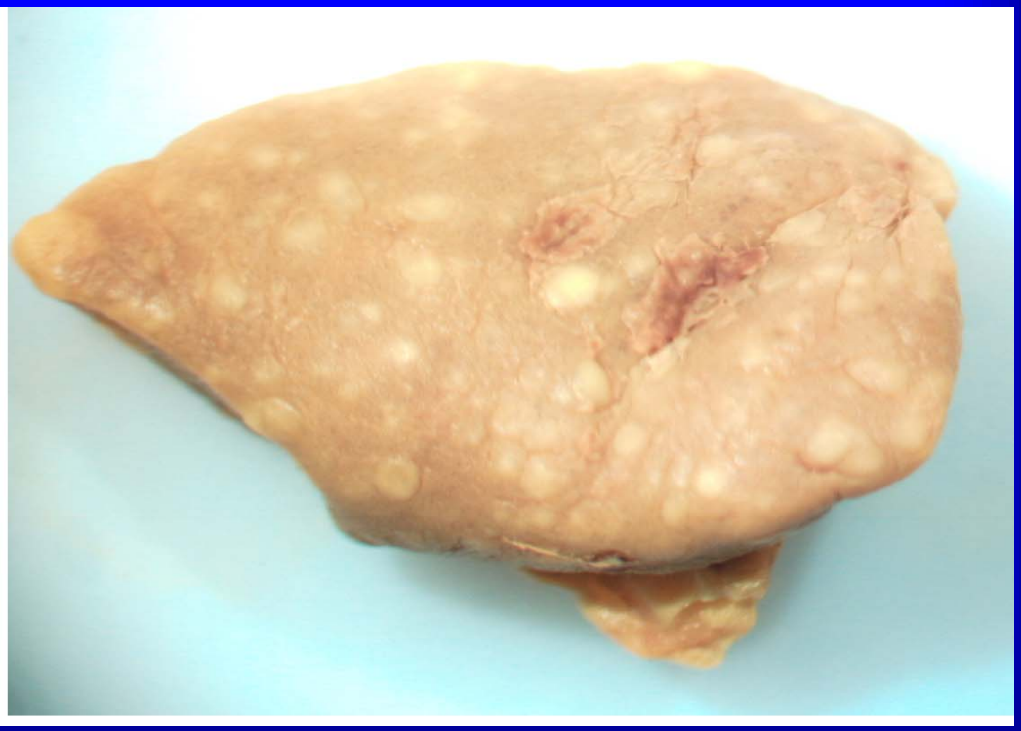


Figure 20.6. The lung colony assay system. The tumor is irradiated *in situ*, after which it is excised and made into a single-cell suspension. A known number of cells then is injected intravenously into recipient animals. About 3 weeks later the recipient animals are sacrificed and the colonies that have formed in the lungs are counted. The number of lung colonies is a measure of the number of surviving clonogenic cells in the injected suspension. (From Hill RP, Bush RS: Br J Radiol 46:167–174, 1973, with permission.)

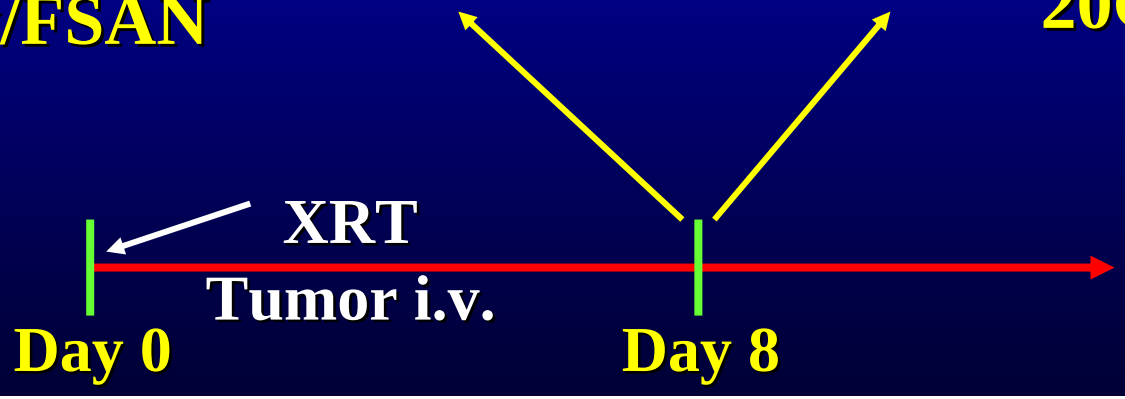
Lung Colony of FSAN Tumors





0Gy/FSAN

20Gy/FSAN

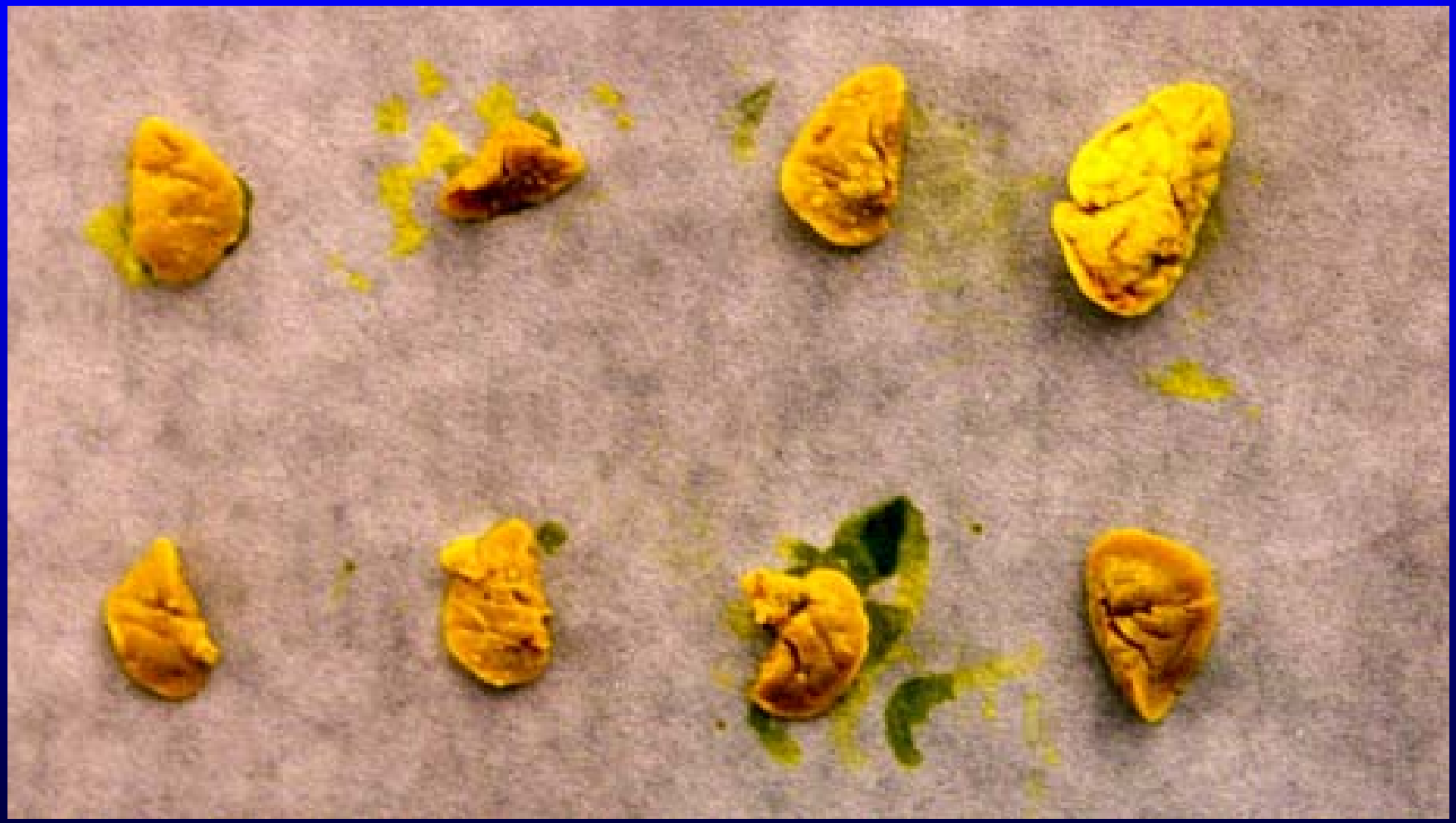


Tumorigenicity of IL-3 Gene-Transduced Cells

Day 5 Day 8 Day 12 Day 14

FSAN

FSAN-JmIL3



Summary of Tumor Cell Survival Assay

- **Advantage:**
  - **Obtain Survival Curve and Radiobiological Parameters**
- **Disadvantage:**
  - **Need a lot of animals**
  - **Animal variation**

In vivo/in vitro Assay

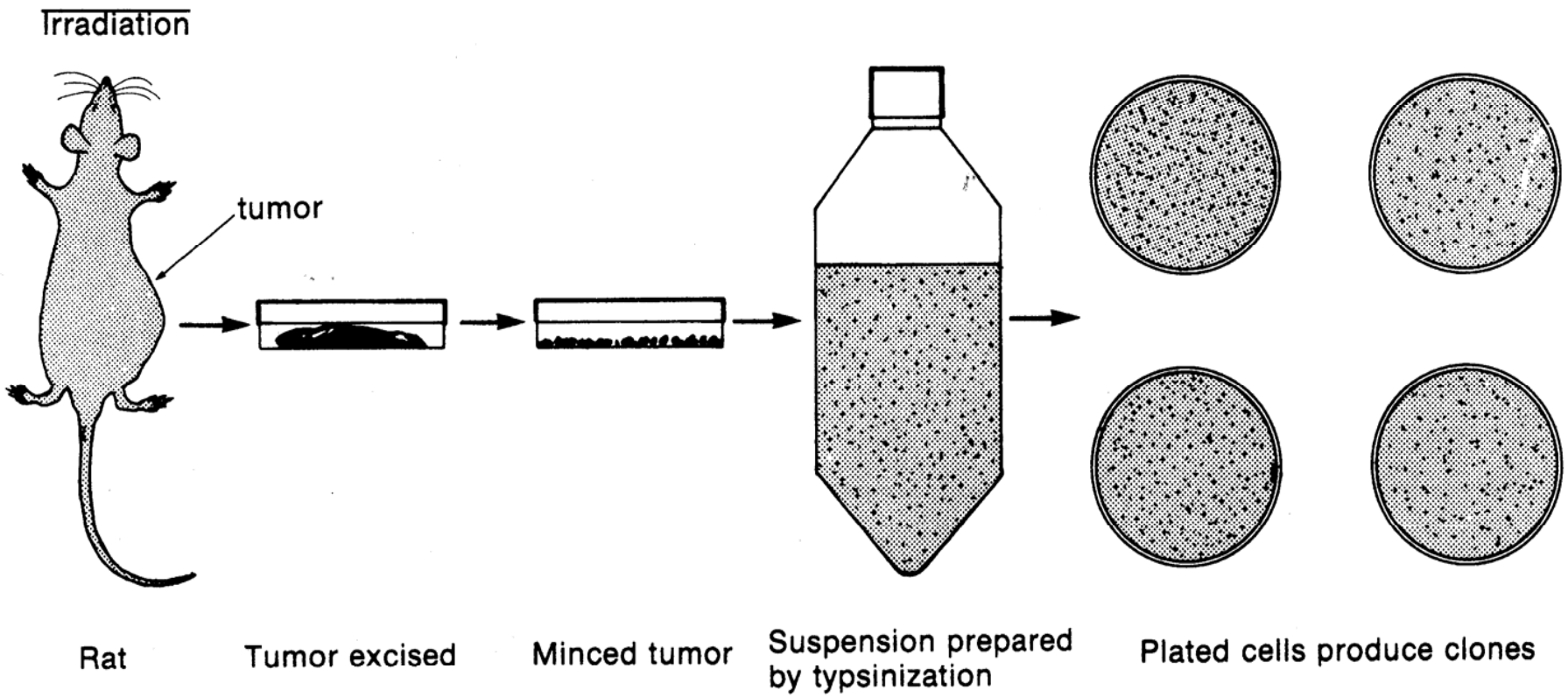
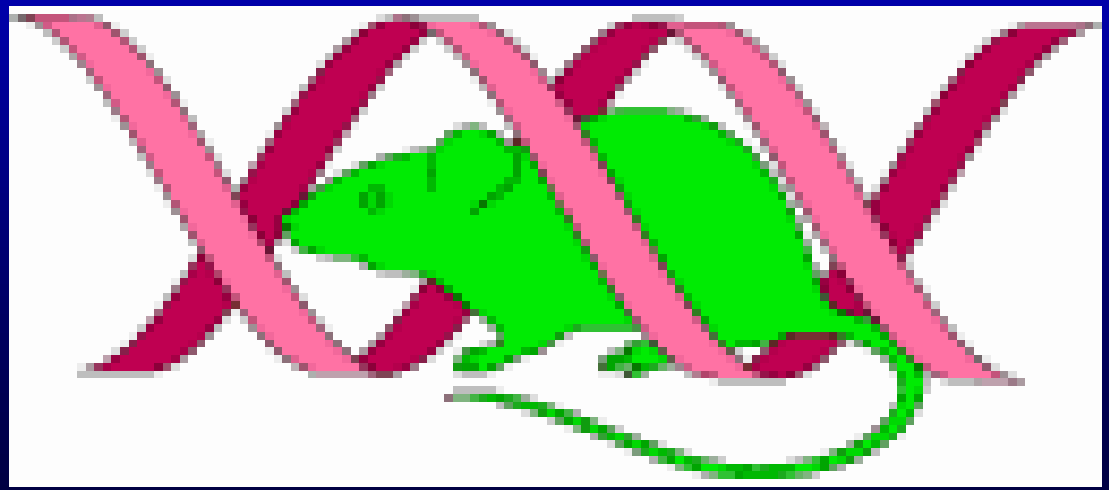


Figure 20.7. The principle of the *in vivo/in vitro* assay system using the rhabdomyosarcoma in the rat. The solid tumor in the animal can be removed and the tumor cells assayed for colony formation in petri dishes. This cell line can be transferred to and fro between the animal and the petri dish. (Courtesy of Drs. G. W. Barendsen and J. J. Broerse.)

Xenograft System

- Nude mice
- SCID mice
- Transgenic animals (humanized animals)



Xenograft vs Clinical Response

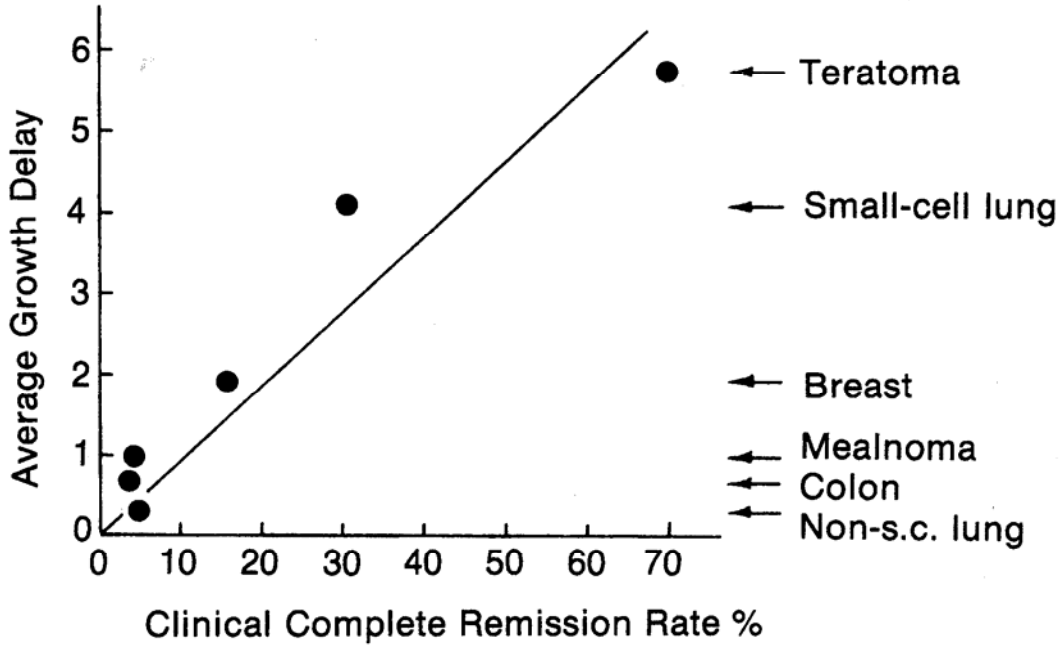
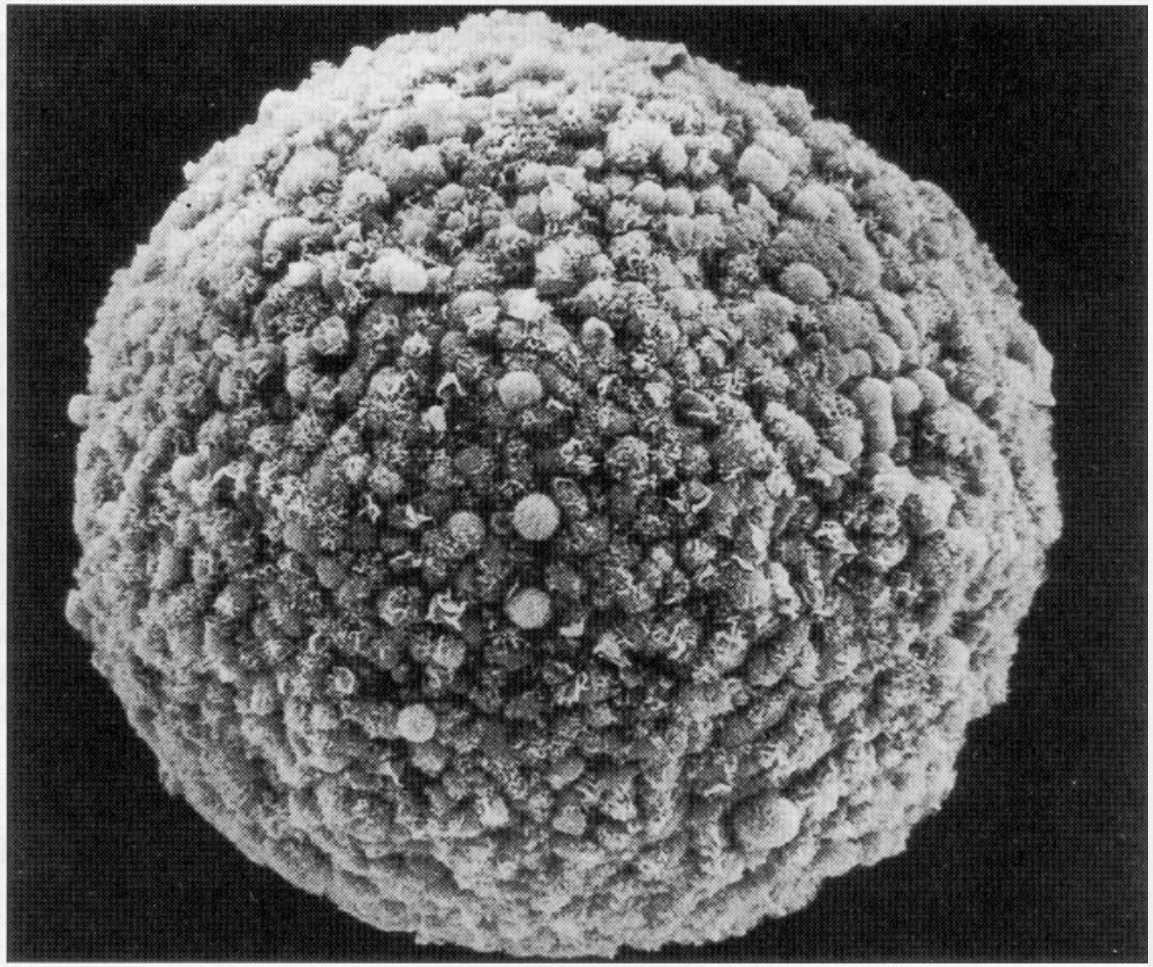


Figure 20.8. Correlation between response of human tumor xenografts and clinical complete remission rates to chemotherapy. Ordinate is growth delay observed in 3 to 10 xenograft lines treated with the clinically used drugs that proved most effective in the xenografts. (Steel GG: How well do xenografts maintain the therapeutic response characteristics of the source tumor in the donor patient? In Kallman RF [ed]: Rodent Tumors in Experimental Cancer Therapy. New York, Pergamon, 1987, with permission.)

Summary of Xenograft

- **Advantage:**
  - ***In vivo* model for Human Cancer**
- **Disadvantage:**
  - **Host immunity ?**
  - **Stroma effects (microenvironment) ?**

Spheroid



gure 20.9. Photograph of an 800-μm spheroid containing about 8×10^4 cells. (Courtesy of . Sutherland.)

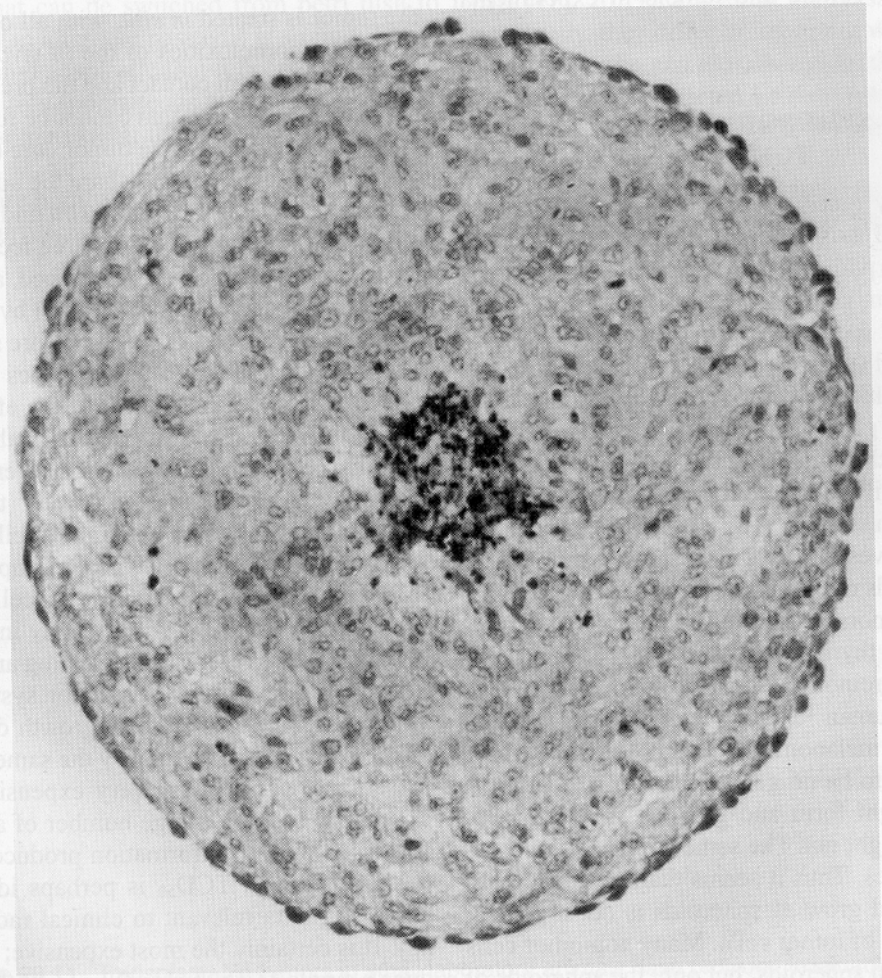
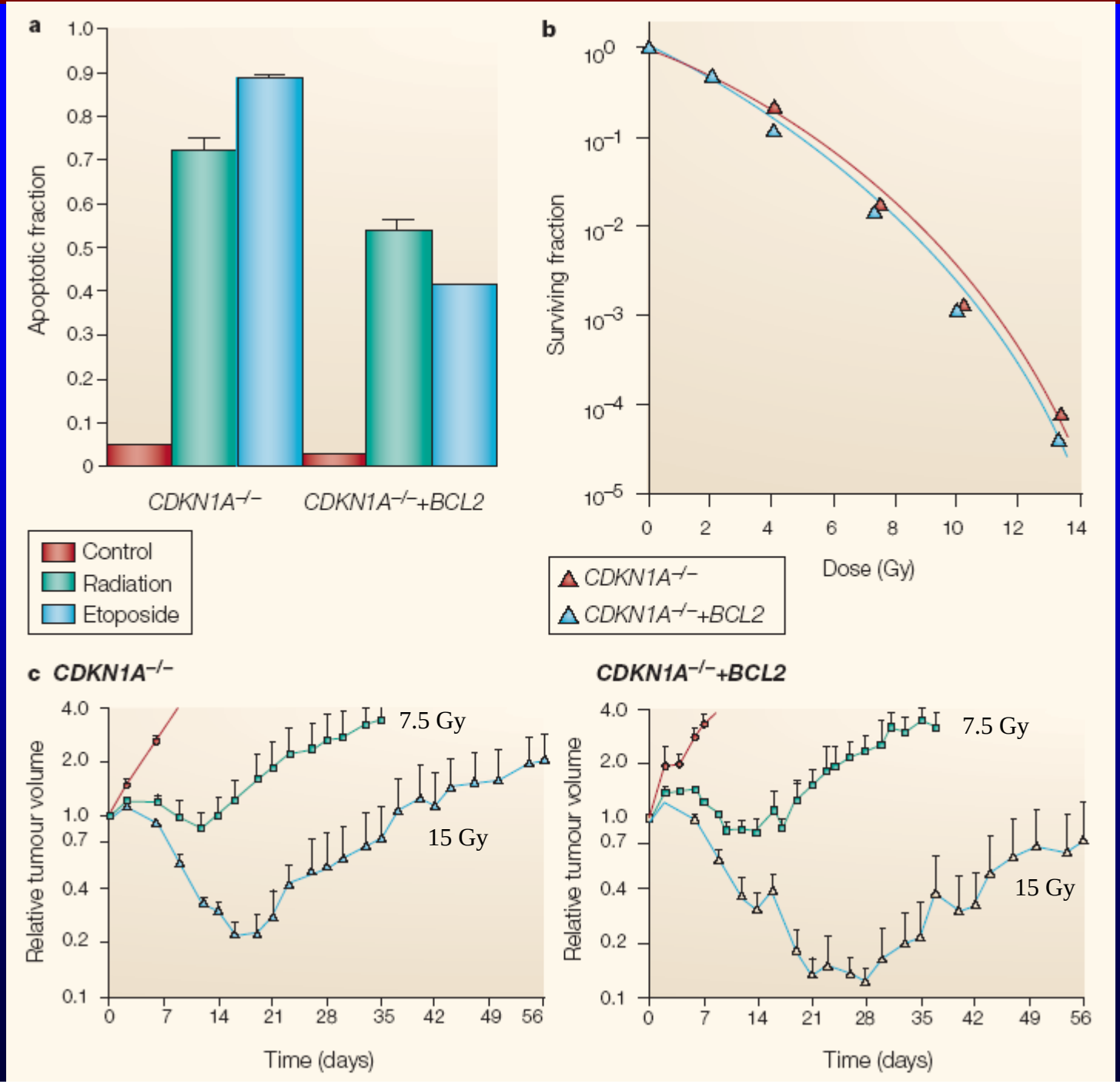
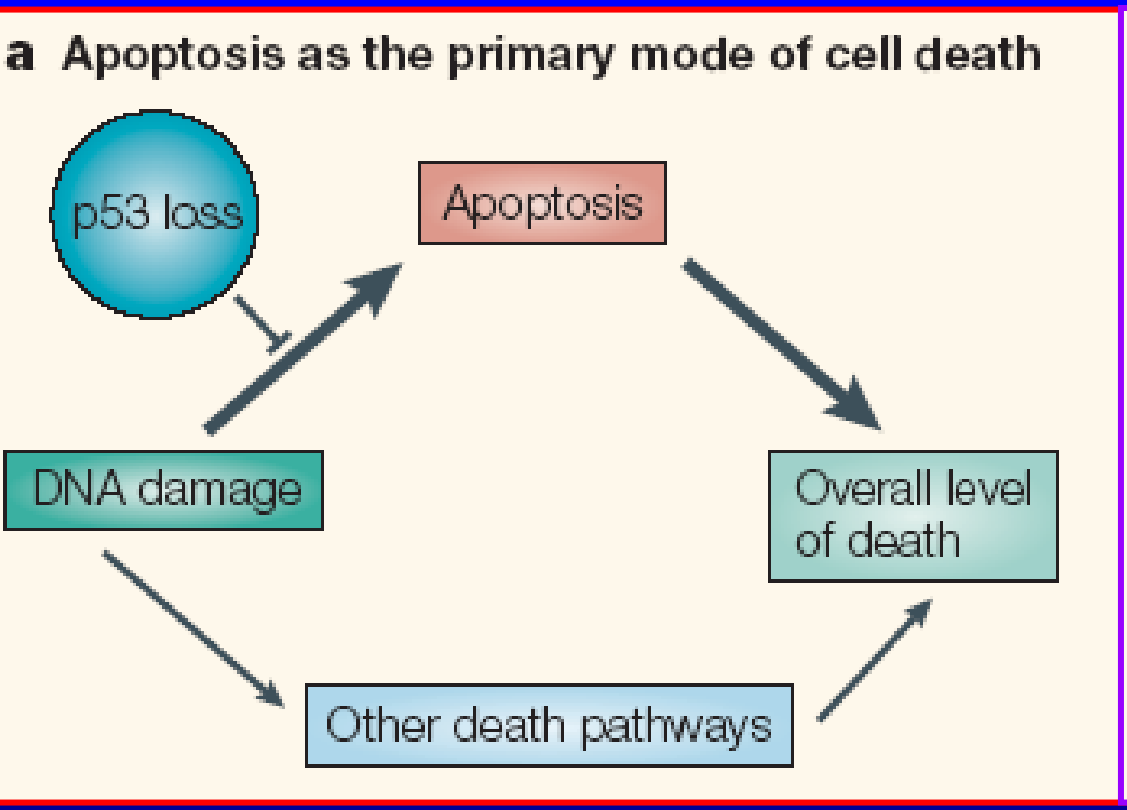


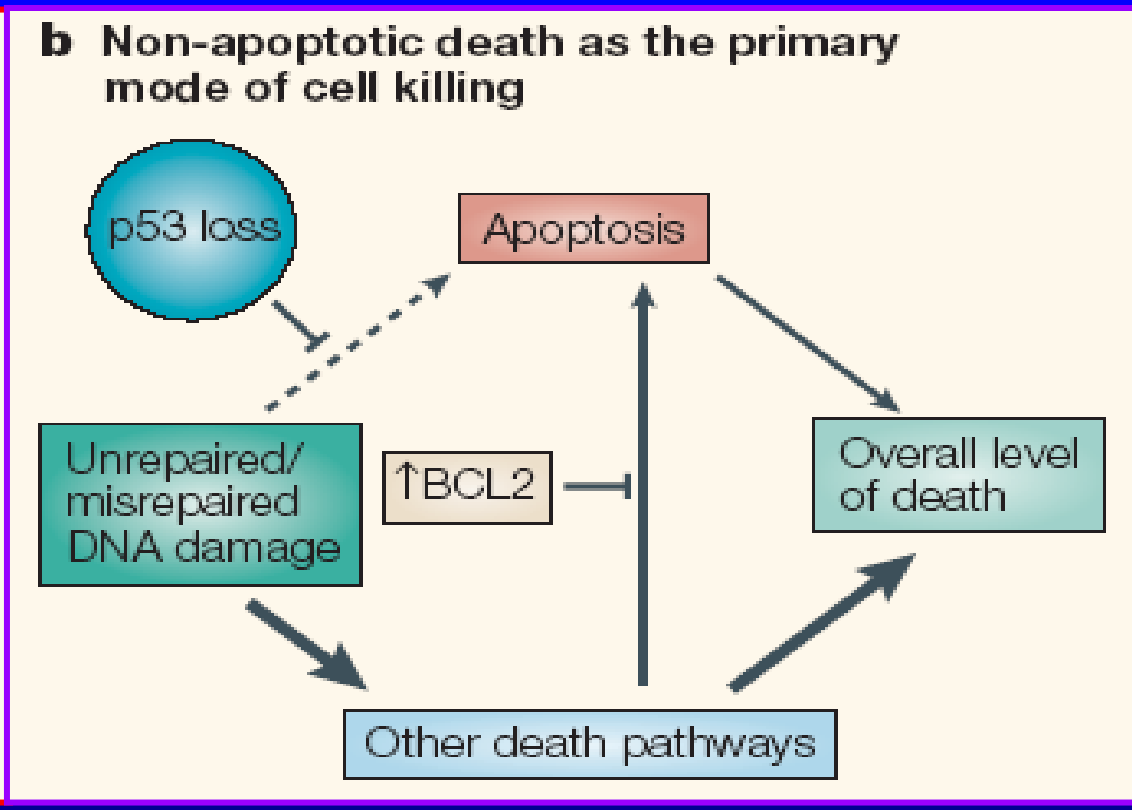
Figure 20.10. Photomicrograph of a spheroid. Note the area of central necrosis. The spheroid w grown for 15 days and was 520 μm in diameter; the viable rim had an average thickness of ab 200 μm. (Courtesy of Dr R. M. Sutherland.)

Apoptosis in tumors



Apoptotic versus non-apoptotic modes of cell death





eg. lymphoma

eg. epithelial tumors

Types of cell death observed following treatment of cells with DNA-damaging agents

Mode of death	General characteristics of death	Detection methods
Apoptosis	Cells visibly shrink and have condensed chromatin with nuclear margination and DNA fragmentation. Blebbing of cell membrane is often seen.	TUNEL staining; annexin-V staining; DNA laddering; caspase activation; electron microscopy; flow cytometry to detect cells with sub-G1 content.
Necrosis	Cells visibly swell and there is an early breakdown of the cell membrane. Cells have an atypical nuclear shape with vacuolization, non-condensed chromatin and disintegrated cellular organelles along with mitochondrial swelling. Typically not genetically determined.	Early permeability to vital dyes such as trypan blue; electron microscopy; flow cytometry for vital dye staining.
Mitotic catastrophe	Typically occurs after or during mitosis and is probably caused by mis-segregation of chromosomes and/or cell fusion. Cells often have micronuclei and it is common to see giant-cell formation or multinucleate cells. This can lead to apoptosis and is typically p53-independent.	Presence of micronuclei after mitosis; multinucleated cells detected by light or electron microscopy.
Senescence	Senescent cells are metabolically active but non-dividing and show an increase in cell size. These cells express senescence-associated β-galactosidase and this process is generally p53-dependent.	Staining for senescence-associated β-galactosidase.
Autophagy	This is a genetically regulated form of programmed cell death in which the cell digests itself. It is characterized by the formation of double-membrane vacuoles in the cytoplasm, which sequester organelles such as mitochondria and ribosomes. Autophagy is caspase and p53 independent.	Exclusion of vital dyes until late stages; prominent cytoplasmic vacuoles detected with monodansylcadaverine; lack of marginated condensed nuclear chromatin by electron microscopy.


http://mx.nthu.edu.tw/~cschiang/

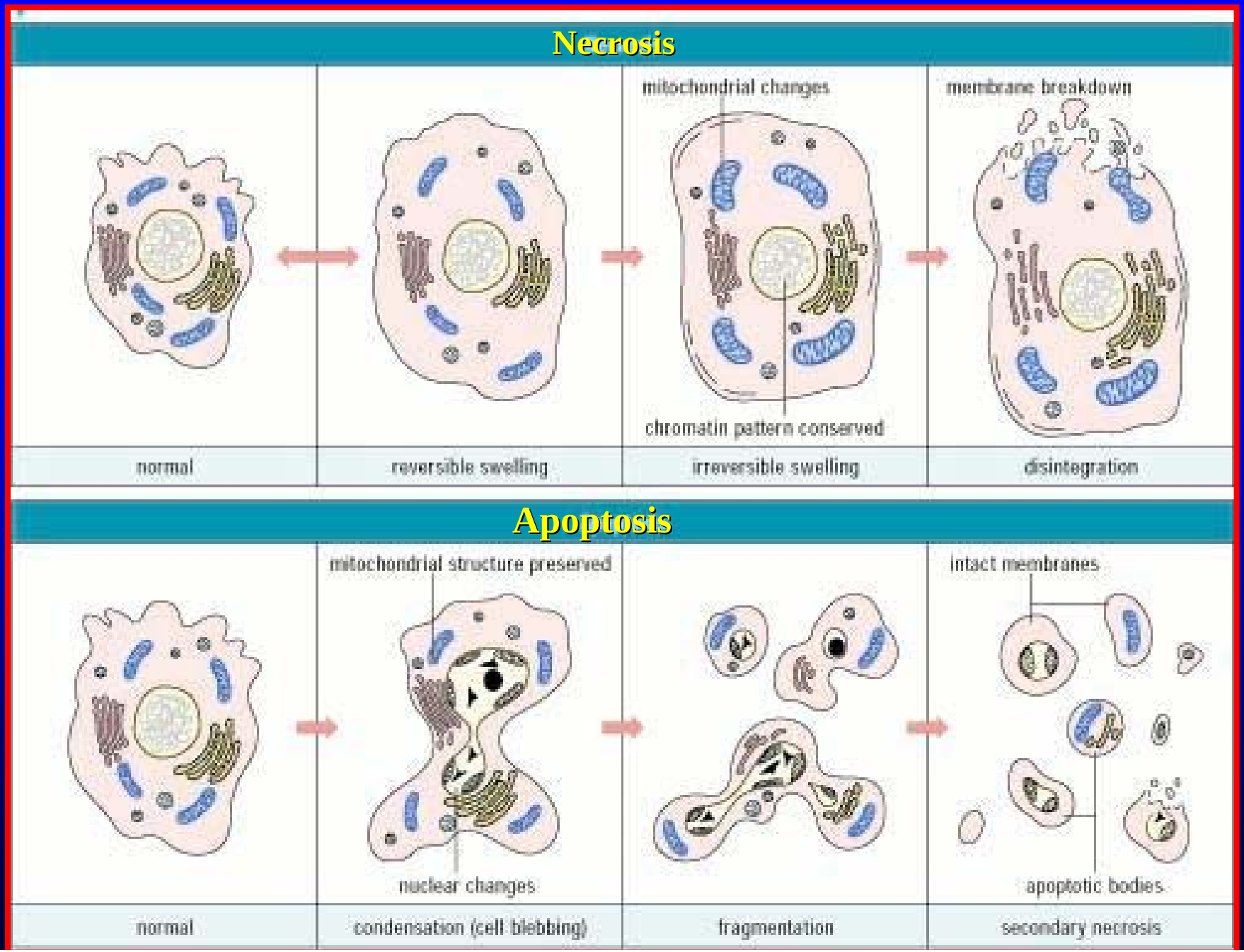
Tumor Cell Death Following Irradiation

Mitotic Cell Death

vs

Apoptotic Cell Death
(interphase cell death)
(programmed cell death)

Cell Death

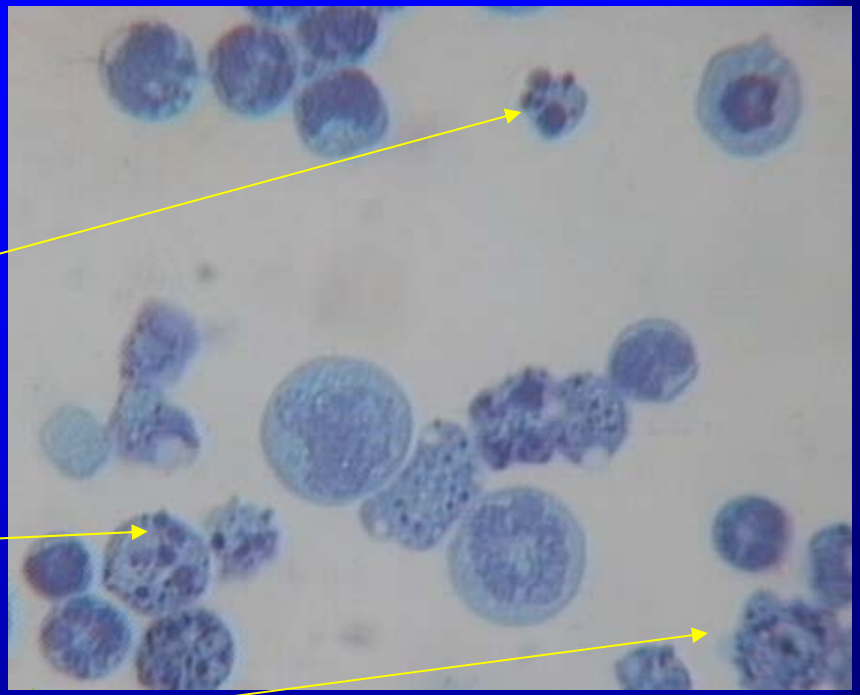

Necrosis
mitochondrial changes
membrane breakdown
chromatin pattern conserved
normal
reversible swelling
irreversible swelling
disintegration
Apoptosis
mitochondrial structure preserved
intact membranes
nuclear changes
apoptotic bodies
normal
condensation (cell blebbing)
fragmentation
secondary necrosis

Morphological Features of Apoptosis

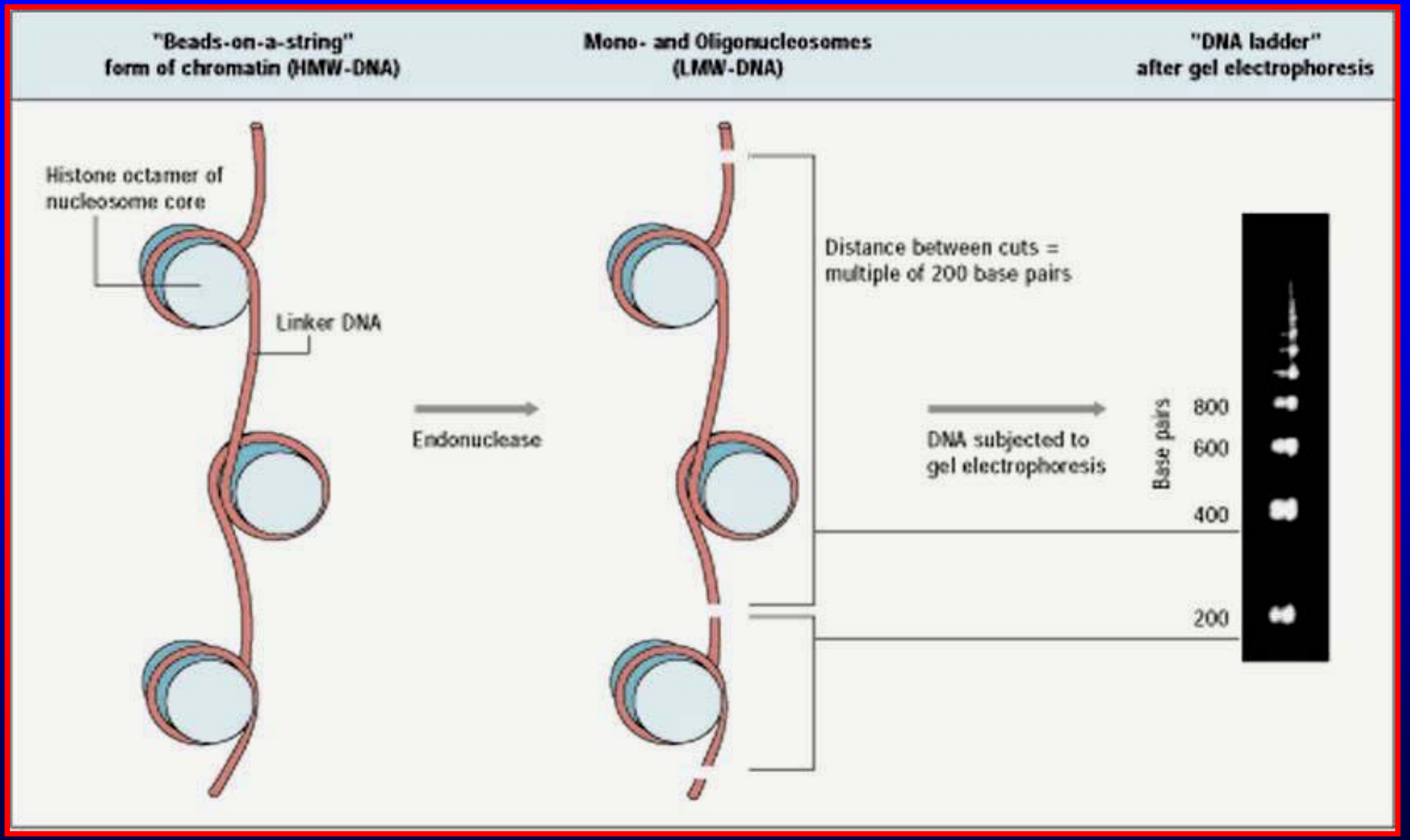
Apoptosis is a tightly regulated "active" process

that is associated with

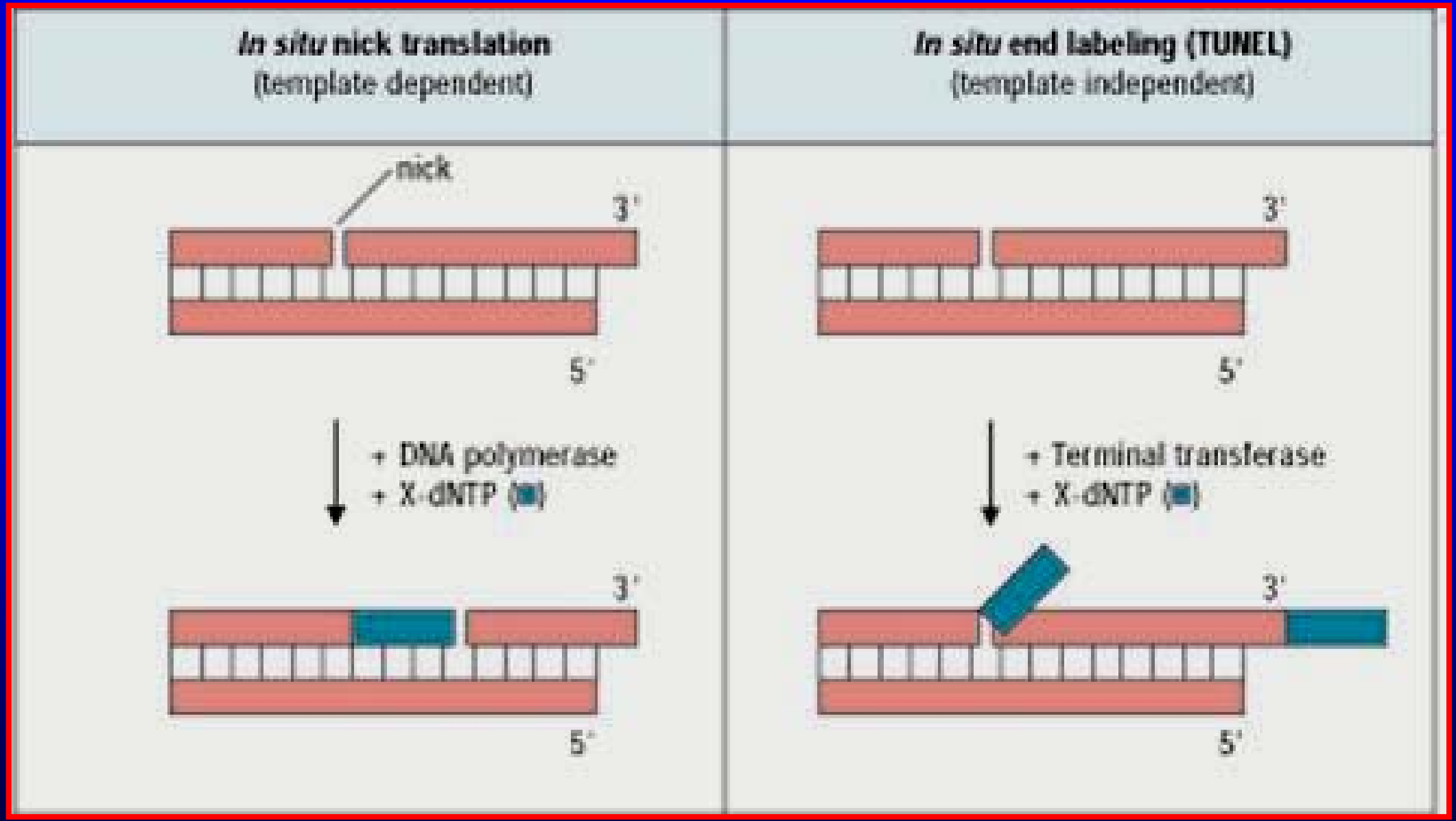
- **Cell and nuclear shrinkage**
- **Nuclear fragmentation with formation of apoptotic bodies**
- **Blebbing of cell membrane, but no early loss of membrane integrity**
- **Deletion of single cells in isolation**
- **Lack of an inflammatory response, phagocytosis of debris by adjacent cells**



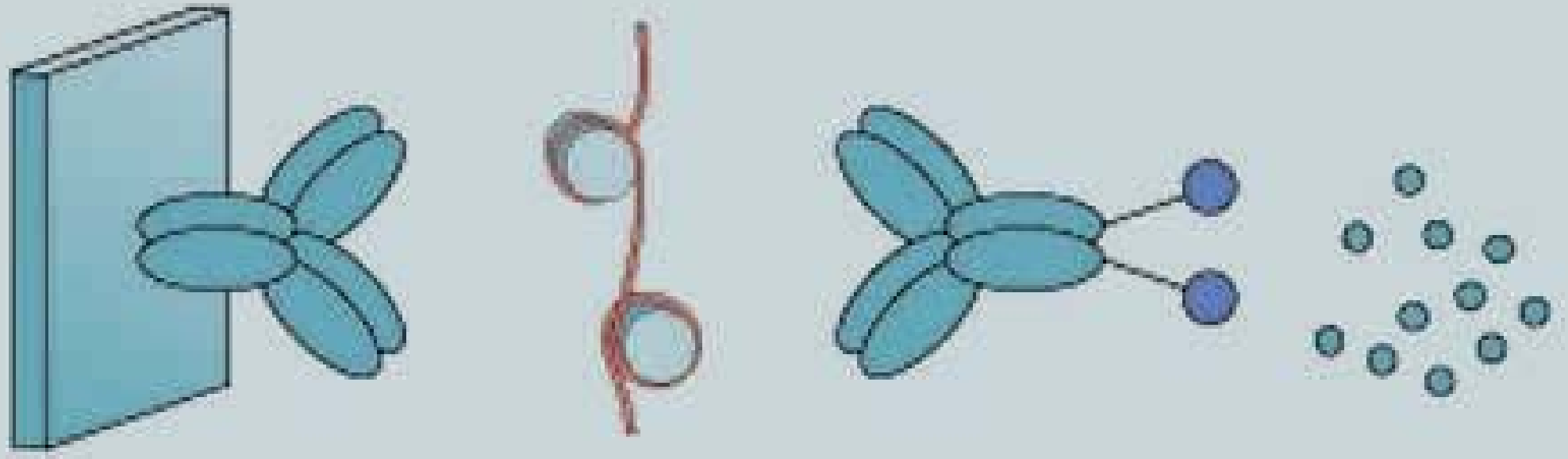
Assay of Apoptosis : DNA ladder



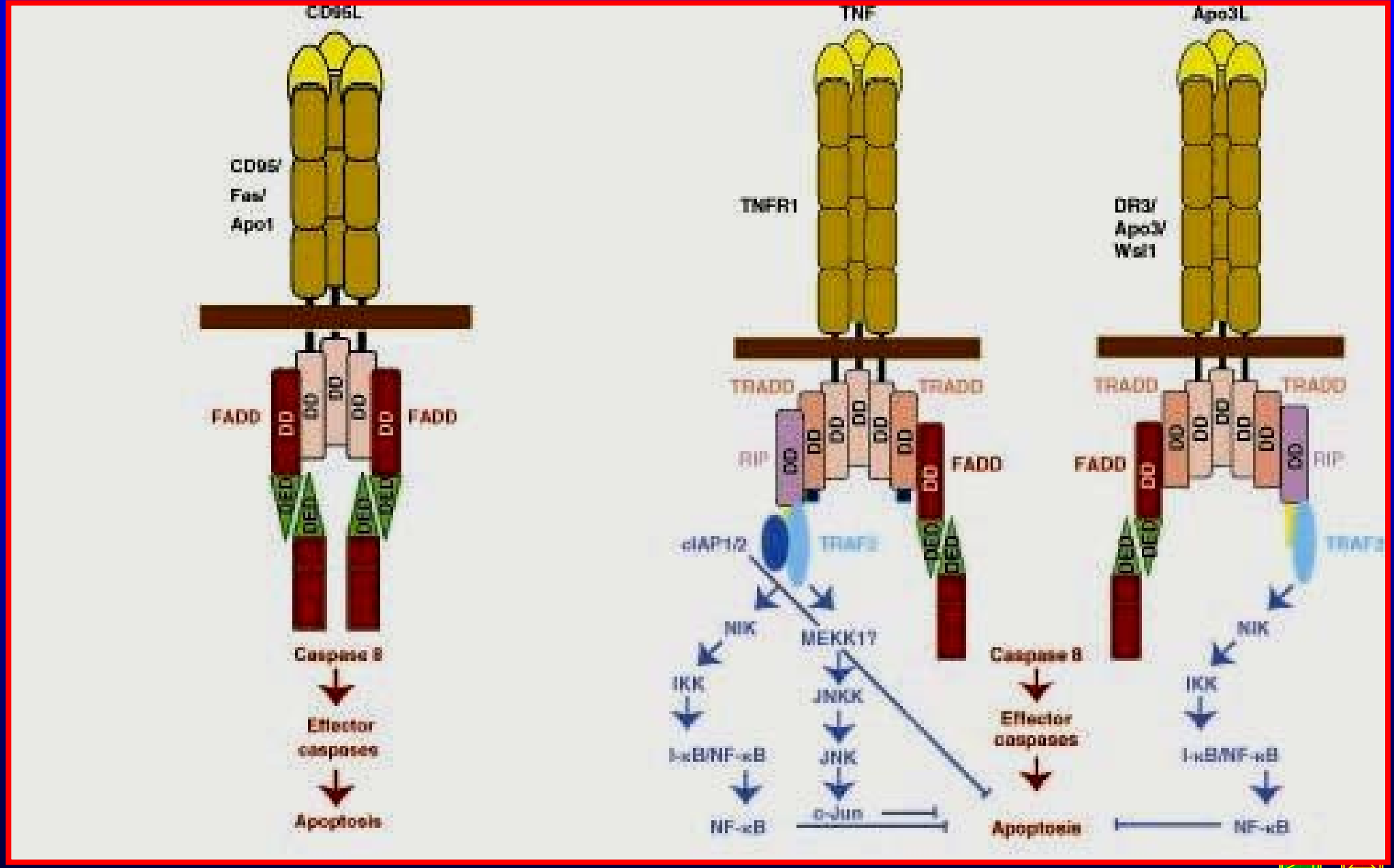
Assay of Apoptosis: TUNEL



Assay of Apoptosis: ELISA



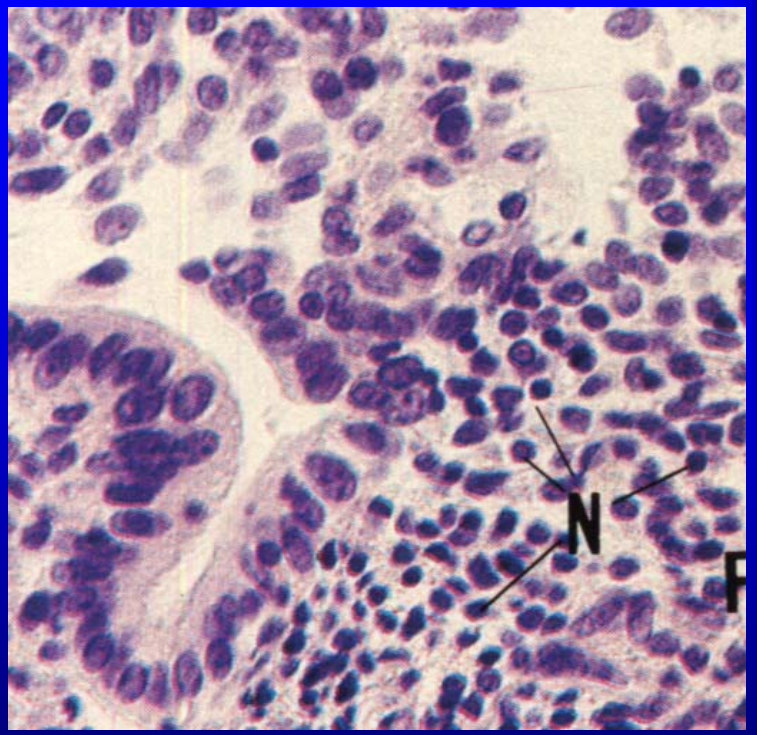
Apoptosis (Programmed Cell Death)



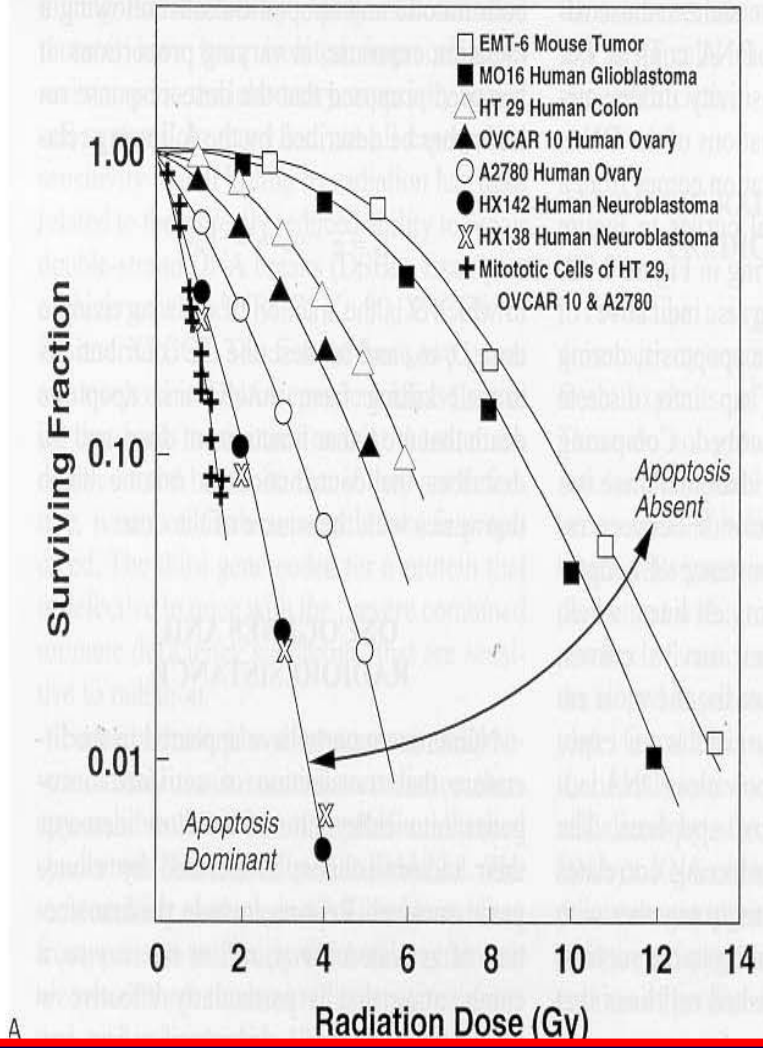
Features of Necrosis

Necrosis is a non-physiological process associated with

- Loss of plasma membrane integrity and deregulated ion homeostasis.
- Swelling and bursting of cells as water enters
- Groups of cells, rather than single cells, are affected
- DNA forms a random “smear” on agarose gel. There is no pattern to its fragmentation.
- Necrosis generates an inflammation



Application of Apoptosis in RT



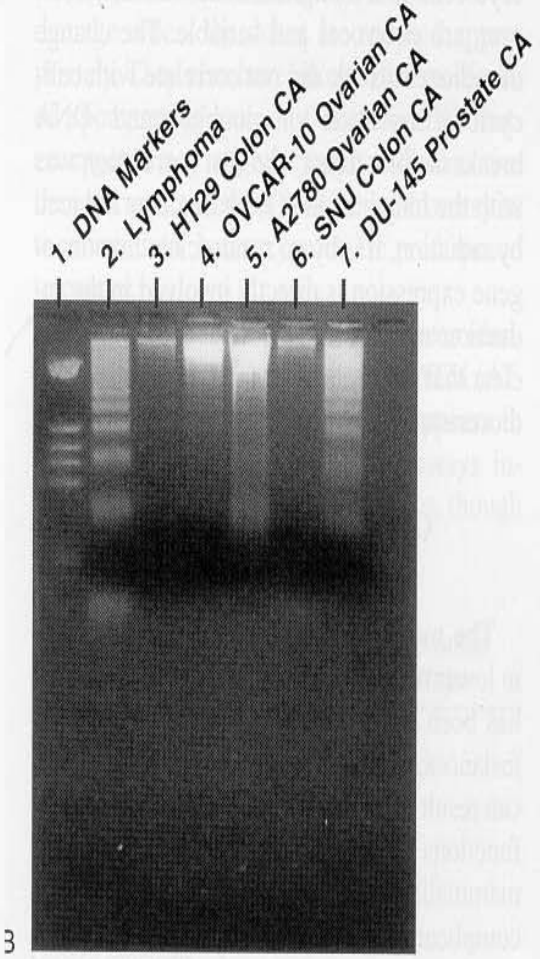
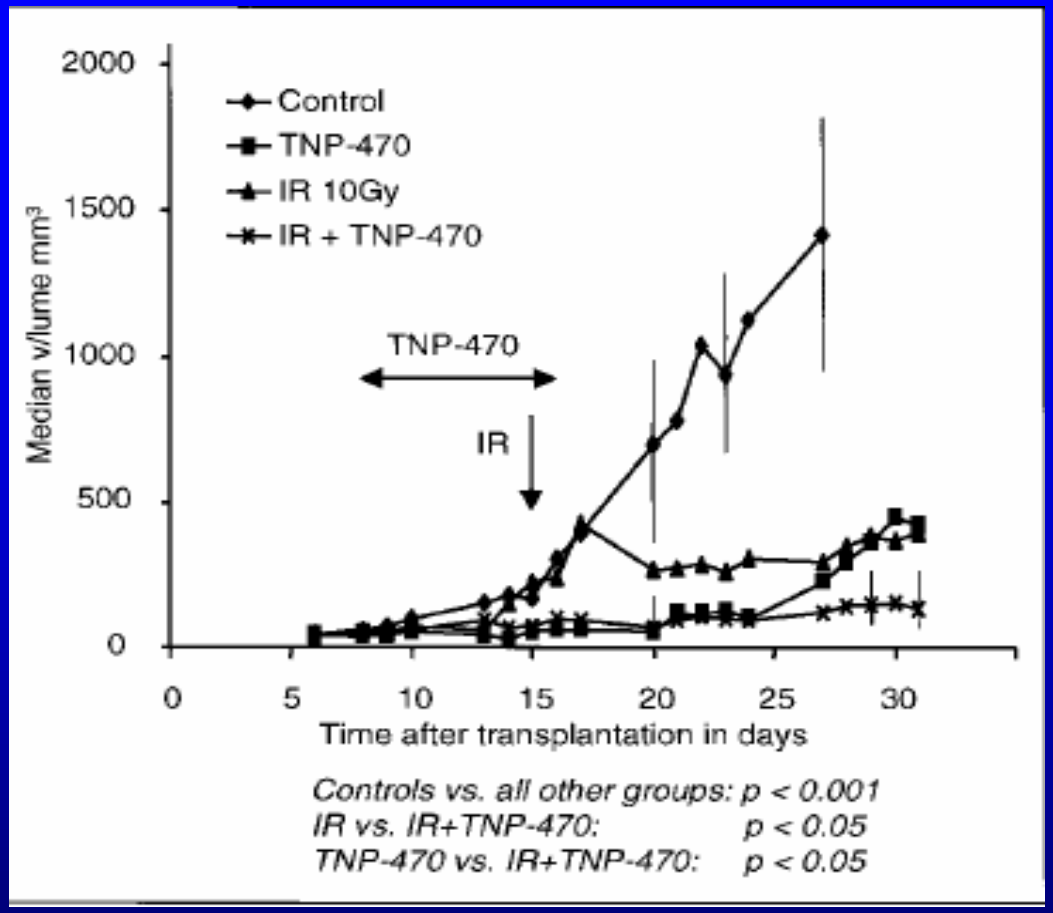


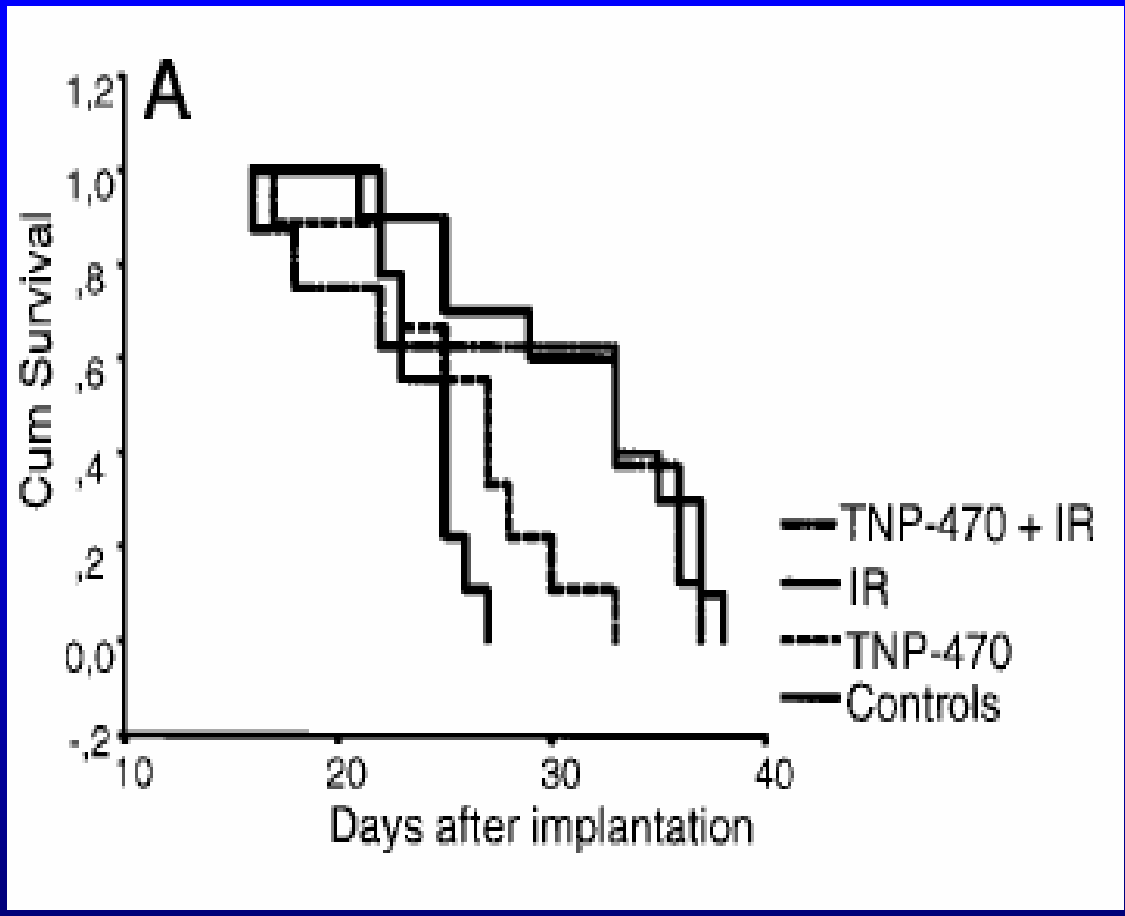
Figure 3.9. A: Compilation of survival curves for asynchronous cultures of a number of cell lines of human and rodent origin. Note the wide range of radiosensitivity (most notably the size of the shoulder) between mouse EMT6 cells, the most resistant, and two neuroblastoma cell lines of human origin (the most sensitive). The cell-survival curve for mitotic cells is very steep, and there is little difference in radiosensitivity for cell lines that are very different in asynchronous culture. (Data compiled by Dr. J. D. Chapman, Fox Chase Cancer Center, Philadelphia.) **B:** DNA purified from various cell lines (survival curves shown in Fig. 3.9A) 18 hours after irradiation with 10 Gy and electrophoresed for 90 minutes at 6 V/cm. Note the broad variation in the amount of "laddering"—which is characteristic of an apoptotic death. In this form of death, double-strand breaks occur in the liner regions between nucleosomes, to produce DNA fragments that are multiples of about 185 base pairs. Note that cell lines that show prominent laddering are radiosensitive. (Gel prepared by Drs. S. Biade and J.D. Chapman, Fox Chase Cancer Center, Philadelphia.)

$$S = e^{-(a_M + a_A)D - \beta_M D^2}$$

Influence of tumor model on therapeutic efficacy



S.C.



I.C.

Clin. Cancer Res., *6:* 971–978, 2000
Cancer Res., *59:* 5209–5218, 1999

Various immunodeficient mouse strains

Strain	Mechanism	Immune defect
Nude	Defect in the gene coding for the transcription factor Foxn1 (Chr 11). Thymic agenesis and lack of hair	T-cell deficiency
XID	Defect in B-cell signaling due to lack of Bruton's tyrosine kinase (Btk, chr X). Similar to Bruton's agammaglobulinemia in humans	Reduced antibody secretion
Beige	Deficiency of lysosomal trafficking regulator gene (Chr 13). Similar to Chediak – Higashi syndrome in humans	Impairment of phagocytosis and cytotoxicity. Reduced NK-cell function
NOD	Interaction between at least 3 genes important for antigen presentation and T-cell function (Chr 3, 9, and 17)	Autoimmune diabetes
SCID	DNA-repair defect, VDJ recombination defect due to deficiency of the catalytic subunit of DNA-dependent protein kinase (Chr 16)	T- and B-cell deficiency
XID/Nude	Combined effect of XID and nude mutations	T- and B-cell deficiency
XID/Nude/beige	Combined effect of XID, nude, and beige mutations	T-, B-, and NK-cell deficiency
SCID/beige	Combined effect of SCID and beige mutations	T- and B-cell deficiency and reduced NK-cell function
NOD/SCID	VDJ recombination defect added to various NOD anomalies	T- and B-cell deficiency, relative NK deficiency, no diabetes
$\beta 2M^{null}$ (KO)	No expression of MHC class I due to lack of β2-microglobulin (β2M, chr 2)	Lack of CD8 positive Tαβ cells, decreased T cytotoxicity
Pfp^{null} (KO)	No production of perforin (Pfp) in lytic granules (deletion of Pfp gene on chr 10)	No cytolysis made by T or NK cells
γ_c^{null} (KO)	Lack of receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (deletion of gene coding for the common γ-chain of the above mentioned receptors, chr X. Similar to X-linked severe combined immunodeficiency in humans)	Severe defect of T and NK function
$Rag1^{null}$ (KO)	Inability to form VDJ recombination, lack of T- and B-cell receptor, due to deletion of Rag-1 gene (chr 2)	No mature T and B cells
NOD/SCID/$\beta 2M^{null}$	VDJ recombination defect, no MHC class I. Various NOD anomalies	T- and B-cell deficiency, relative NK deficiency, no diabetes
NOD/$Rag1^{null}$	Lack of VDJ recombination combined with NOD defects	No T and B cells, deficiency of NK cells
NOD/SCID/γ_c^{null}	Combined effect of SCID and γ_c^{null} on NOD background	Severe defect of T-, B- and NK-cell function
NOD/$Rag1^{null}Pfp^{null}$	No VDJ recombination, nor perforin production + NOD defects	No T and B cells, NK cells non-functional

NK, natural killer; NOD, non-obese diabetic; SCID, severe combined immunodeficient; XID, X-linked immunodeficiency.

Humanized animals

- Mouse strains transgenic for human genes, for example, major histocompatibility complex (MHC) genes or T-cell receptor (TCR) genes.
- Immunodeficient mice reconstituted with human cells.