Chapter 21 – Model Tumor Systems

11/25/2024

Outline

- **Apoptosis in Tumors** Important implications on how tumor shrinks after RT
- Transplantable Solid Tumor Systems in Experimental Animals
  - Tumor Growth Measurements
  - Tumor Cure (TCD50) Assay
  - Dilution Assay
  - Lung Colony Assay
  - *In Vivo/In Vitro* Assay
  - Newer Models – Autochthonous and Transgenic Tumor Systems
- Human Tumor Models
  - Xenografts of Human Tumors
  - Patient-Derived Xenografts Models
  - Spheroids
  - Organoid Models

Mechanism of Tumor Death

- It is generally thought that irradiated cells die in attempting mitosis
- However, this is not the only form of cell death

Apoptosis occurs in normal tissues and **tumors**, *spontaneously*, *after radiation*, or can be triggered by *changes in tumor microenvionment*

Apoptosis in Tumors (*Spontaneously*)

Tumors grow much more slowly than would be predicted from the cell-cycle time of individual cells and the fraction of cells actively dividing (subject of Chapter 22)

One of the reason for this “cell loss” is random cell death resulting from apoptosis

Apoptosis in tumors have been studied with transplanted mouse tumors, as well as human tumors growing as xenografts in nude mice

Apoptosis in Tumors (*After Radiation*)

Radiation Therapy

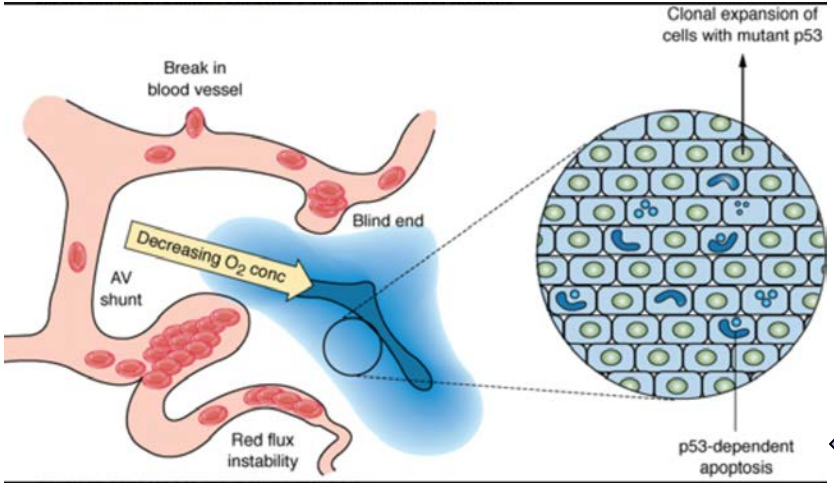
Different tumors vary substantially in their susceptibility of radiation-induced apoptosis

Apoptosis is the most important in **lymphomas**, essentially absent in **sarcomas**, and intermediate and variable in **carcinomas**

Following radiation, 50-60% of lymphoma cells may show signs of dying an apoptotic death

If a tumor responds rapidly to a relatively low dose of radiation, it generally means that apoptosis is involved, because the process peaks at **3 to 5 hours** after irradiation

Apoptosis in Tumors (*Microenvironment*)



⇦ Environment selects for cells with diminished apoptotic potential (e.g., *p53* mutation)

⇦ In the hypoxic regions, p53 induces apoptosis

Cells with diminished apoptotic sensitivity are probably more reflective of human solid tumors that are treated clinically.
Thsse cells can still die by a mitotic cell death.

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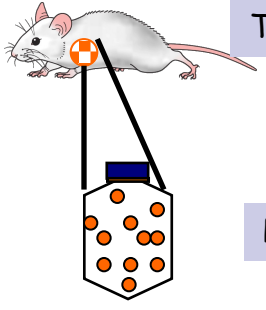
Model Tumor System

A wide range of experimental tumors of various histologic types have been developed for radiobiologic studies

These ***in vivo*** model tumor systems are necessary to confirm or refute *in vitro* results

A large number of virtually identical tumors will need to be produced, usually via inbred strains of rates or mice

Transplantable Solid Tumor Systems

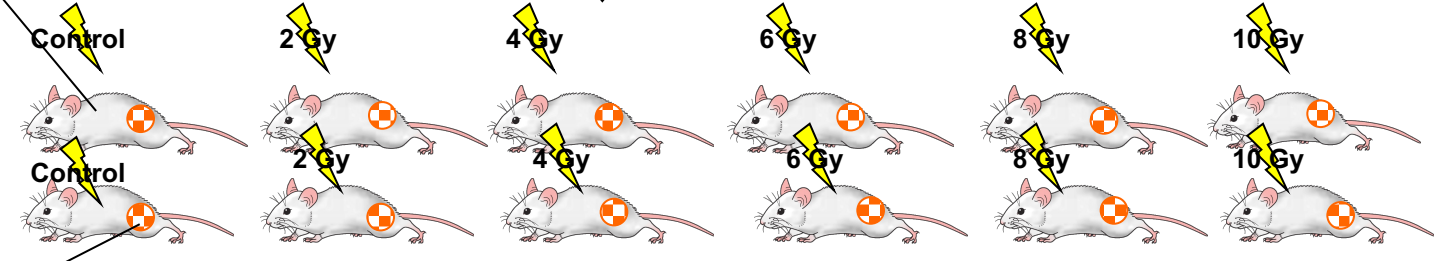


Tumor in donor animal

Prepared into single-cell suspension

Inoculated into recipient animals

Isogenic inbred rodents



Uniform in size & histologic type

Highly quantitative studies are possible

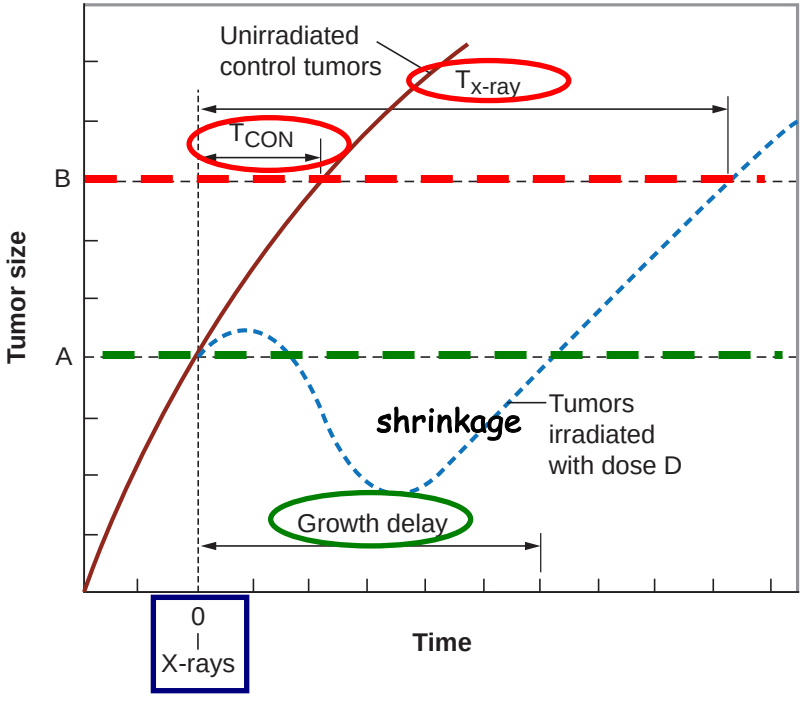
Commonly Used Assays

Commonly Used Assays

1. Tumor Growth Measurements (Growth Delay)
2. Tumor Cure (TCD50) Assay
3. Dilution Assay
4. Lung Colony Assay
5. *In Vivo/In Vitro* Assay
6. *Autochthonous and Transgenic Tumor Systems*

These assays take into account both intrinsic cell sensitivity to ionizing radiation as well as influence of the microenvironement.

1. Tumor Growth Measurements



Tumor size vs. Time after Treatment

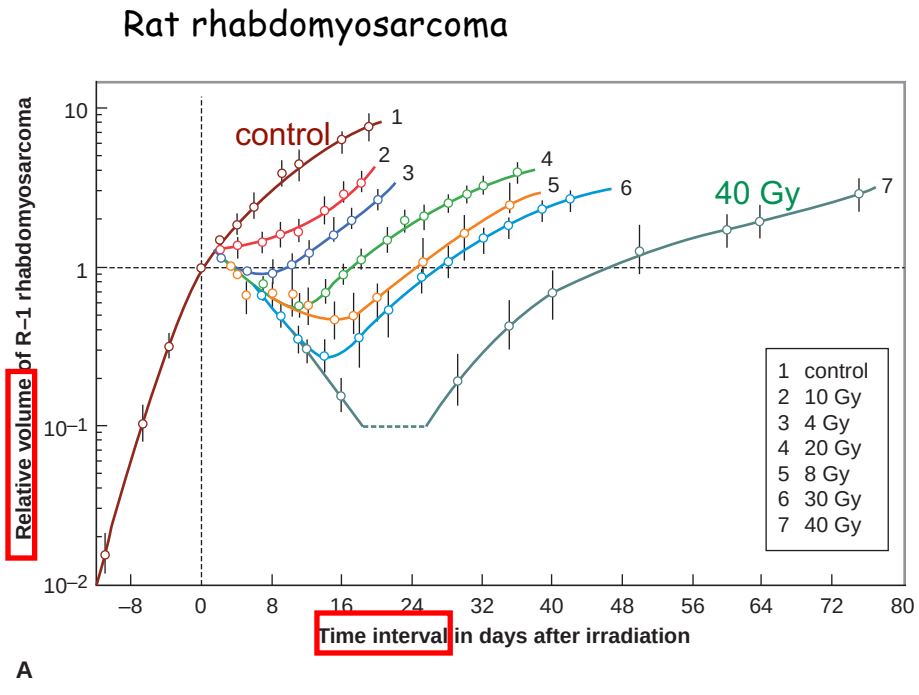
After irradiation, the tumor is measured daily to determine the mean diameter or volume

Unirradiated tumors will grow continuously

Irradiated tumors will show some shrinkage or delayed growth, then regrow

Score **growth delay** (i.e., regrow to initial size) or **time to grow to a specified size**

1. Tumor Growth Measurement

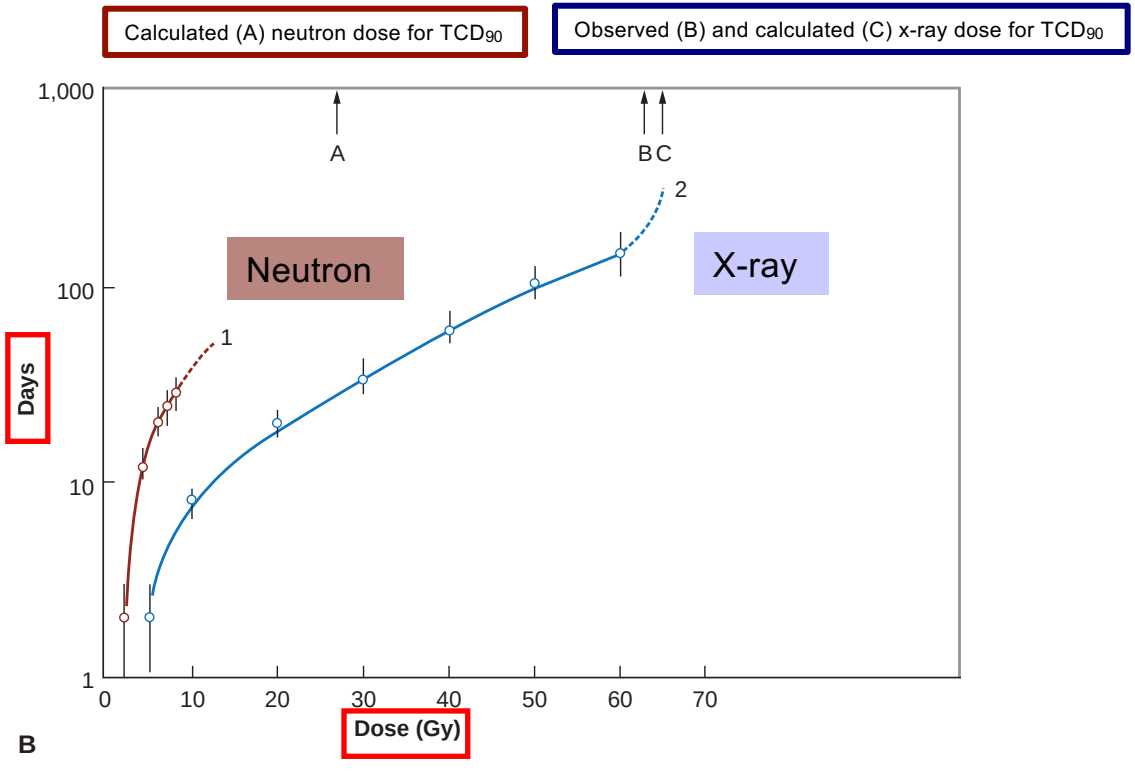


Can be used to study the effect of radiation dose, addition of a radiation sensitizer, different types of radiation, etc

Curve 3 and 5 are with fast neutrons

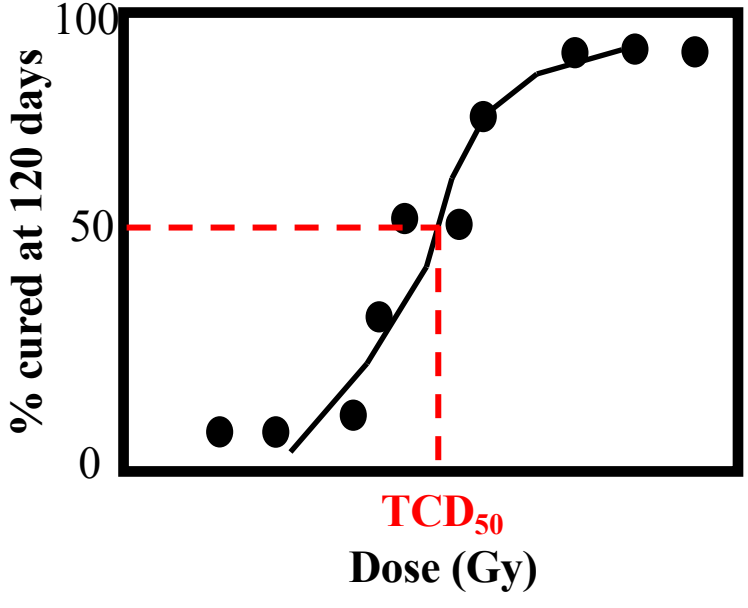
1. Tumor Growth Measurement

Growth delay plotted as a function of radiation dose



A growth delay of infinite # of days represents ???

2. Tumor Cure (TCD_{50}) Assay



Irradiate tumors of uniform sizes with various doses

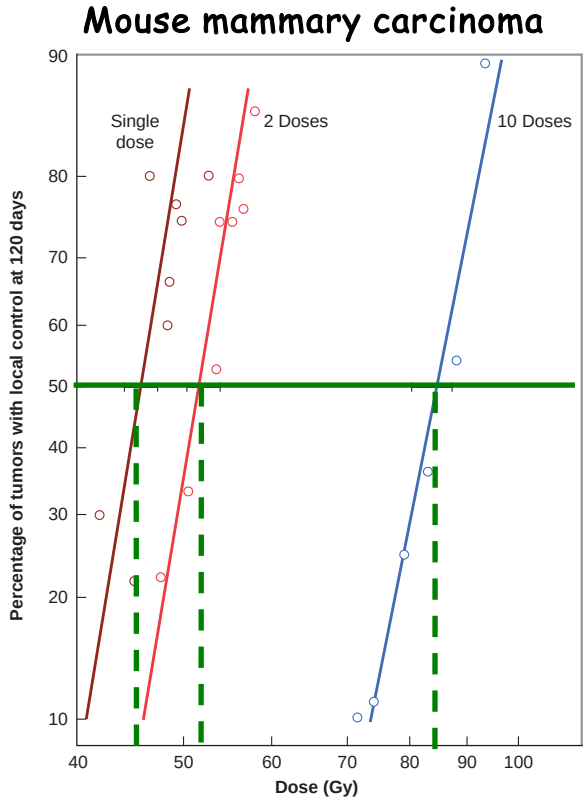
Observe for **local control** and **recurrence**

Plot % control vs. dose

TCD_{50} = dose to control 50% of tumors

This is a more relevant assay for radiotherapy than growth delay, but requires keeping greater number of animals for longer periods of time, so is more costly

2. Tumor Cure (TCD_{50}) Assay



Note that TCD_{50} ↑ in a multifractionated regimen, indicating SLD repair

Tumors were derived from a spontaneous mammary carcinoma in a C_3H mouse

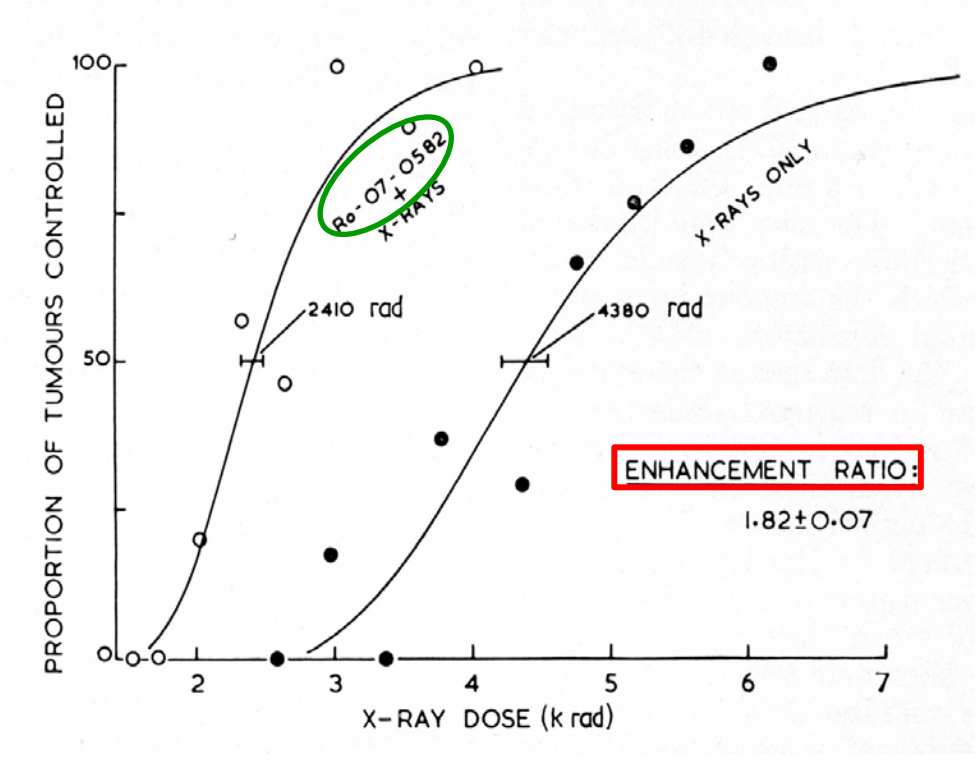
4 x 10^4 cells were transplanted into the outer portion of the mouse ear

The tumors were treated when they reach a diameter of 2 mm

Tumors could be made uniformly hypoxic by placing a brass circular clamp across the base of the ear and maintained for a minute before irradiation

2. Tumor Cure (TCD_{50}) Assay

Effect of a Radiosensitizer on TCD_{50}

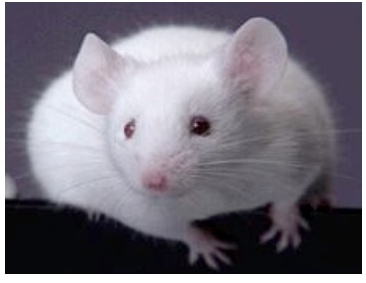


$$\text{Enhancement Ratio} = \frac{TCD_{50} \text{ w/o drug}}{TCD_{50} \text{ w drug}}$$

3. Dilution Assay Technique

Single cell suspension

Donor



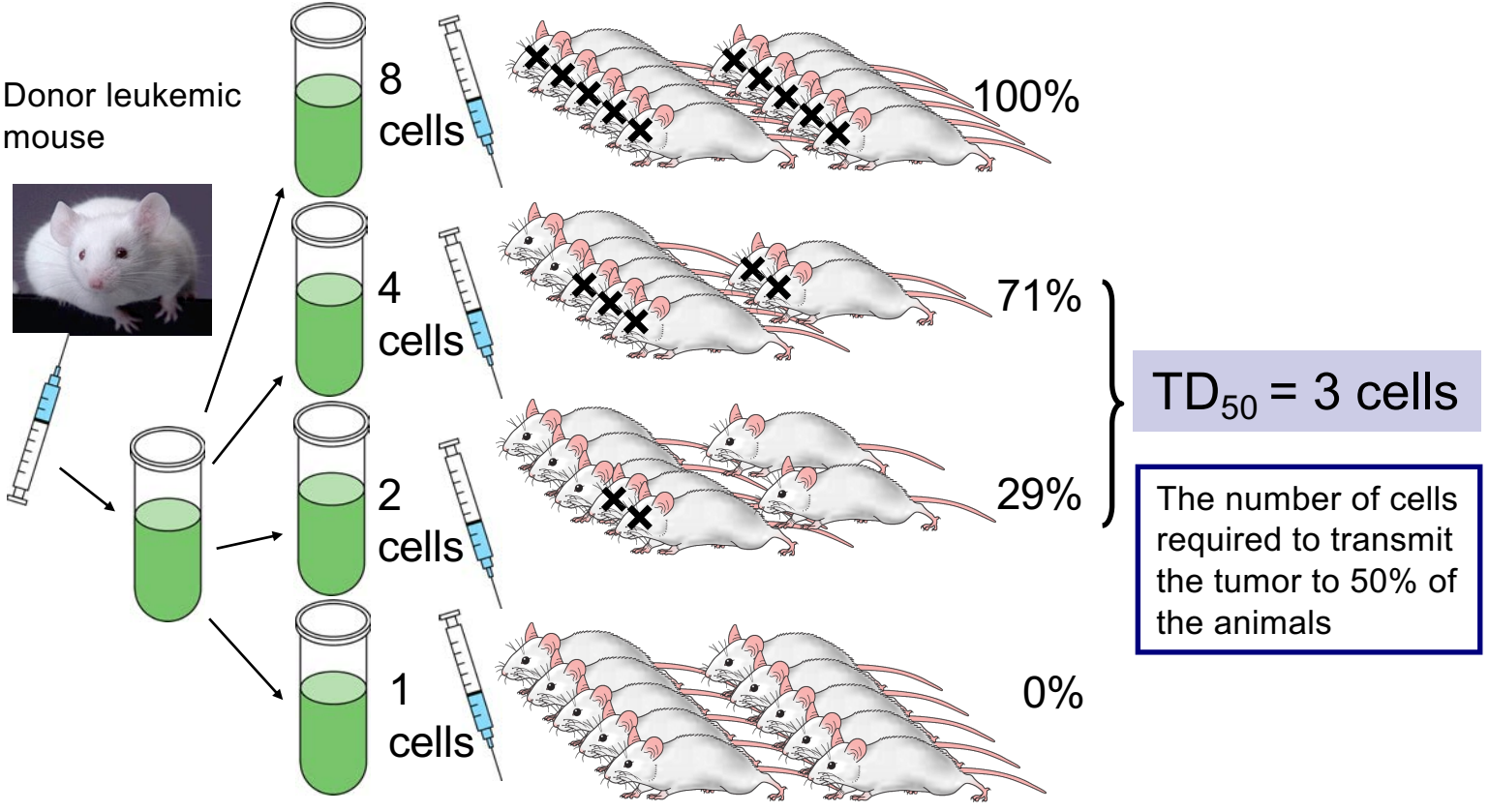
Recipient

Mouse with lymphocytic leukemia

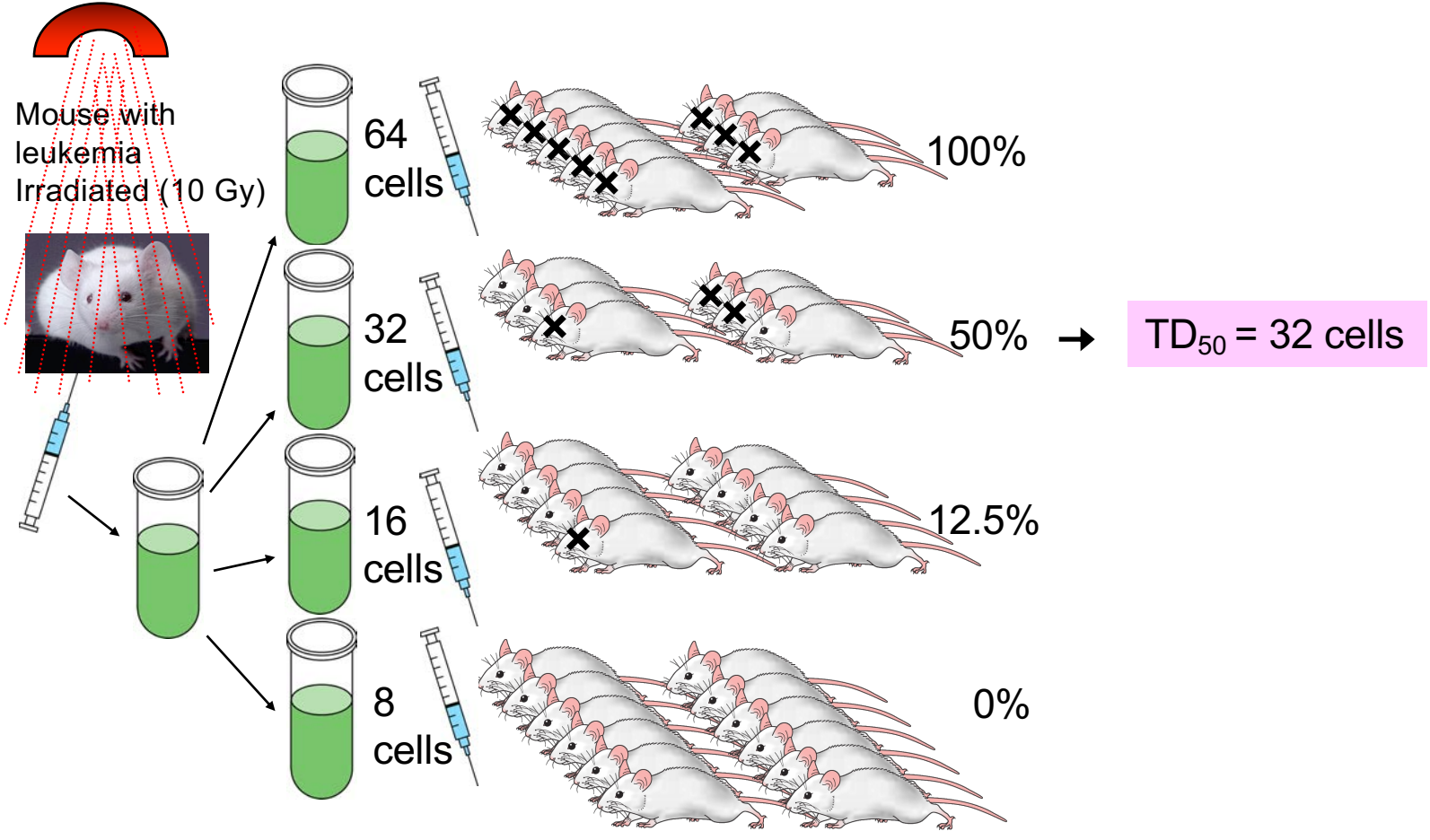
Single cell suspension prepared from the infiltrated liver

Known # of leukemic cells injected into the peritoneal cavities of recipient, which subsequently develop leukemia

3. Dilution Assay Technique

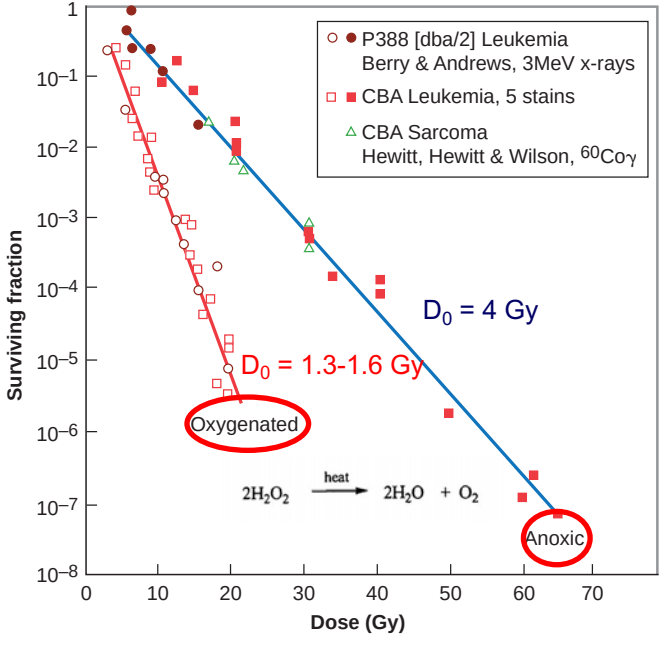


3. Dilution Assay Technique



3. Dilution Assay Technique

$$\text{Surviving Fraction (10 Gy)} = \frac{TD_{50}\ \text{(unirradiated)}}{TD_{50}\ \text{(10 Gy)}} = 3/32 = 0.094$$

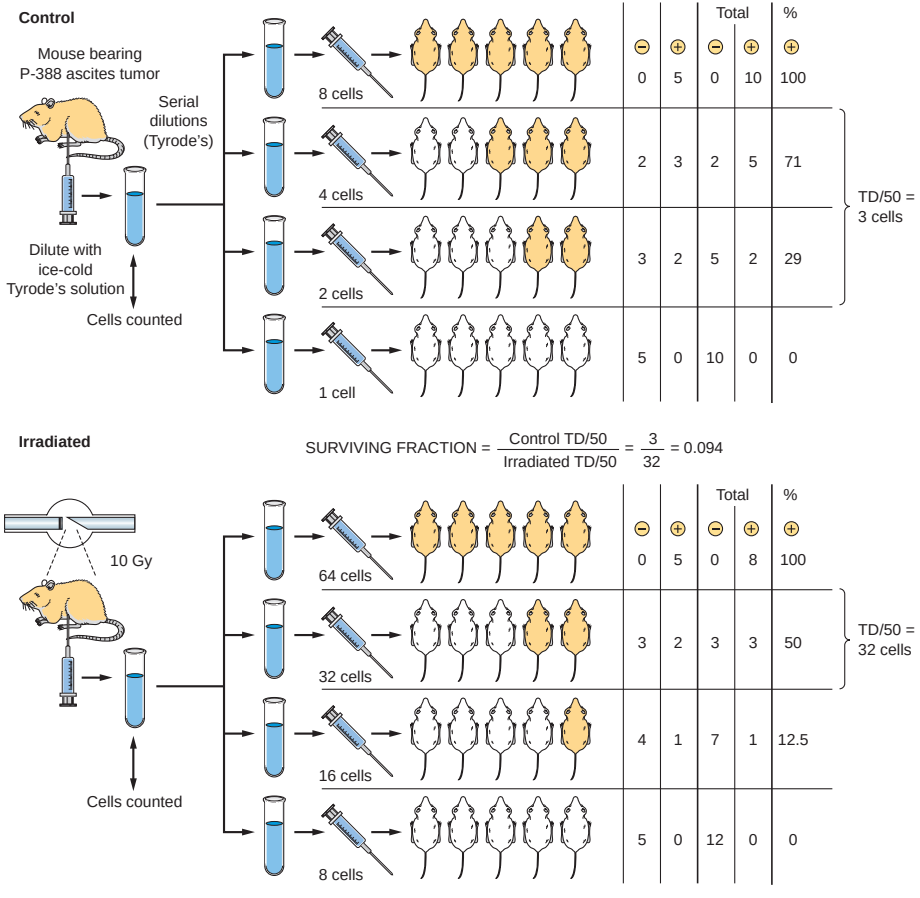


If the process is repeated for a number of doses of radiation, the *in vivo* survival curve can be constructed

We may also study the effect of hypoxia because cells grown in the peritoneal cavity are deficient in oxygen

Aerated condition could be achieved by injecting H_2O_2 into the peritoneal cavity of the mouse before irradiation

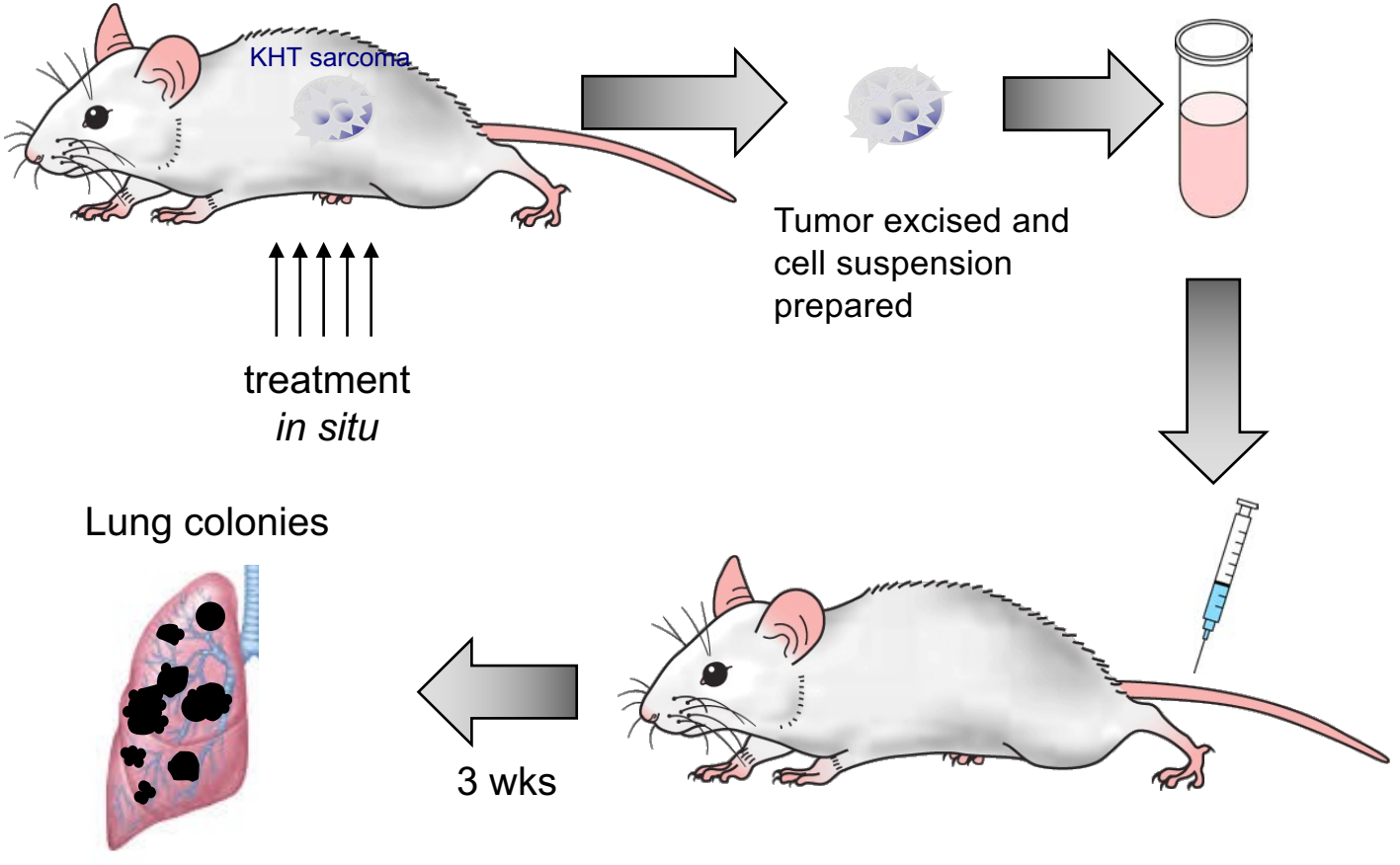
3. Dilution Assay Technique



4. Lung Colony Assay

- Same idea as the dilution assay, except uses solid tumor cells rather than leukemia cells
- The tumor is irradiated in a donor animal, removed and single cell suspension prepared, cells injected into recipient animal
- About 3 weeks later, lung colonies are counted

4. Lung Colony Assay

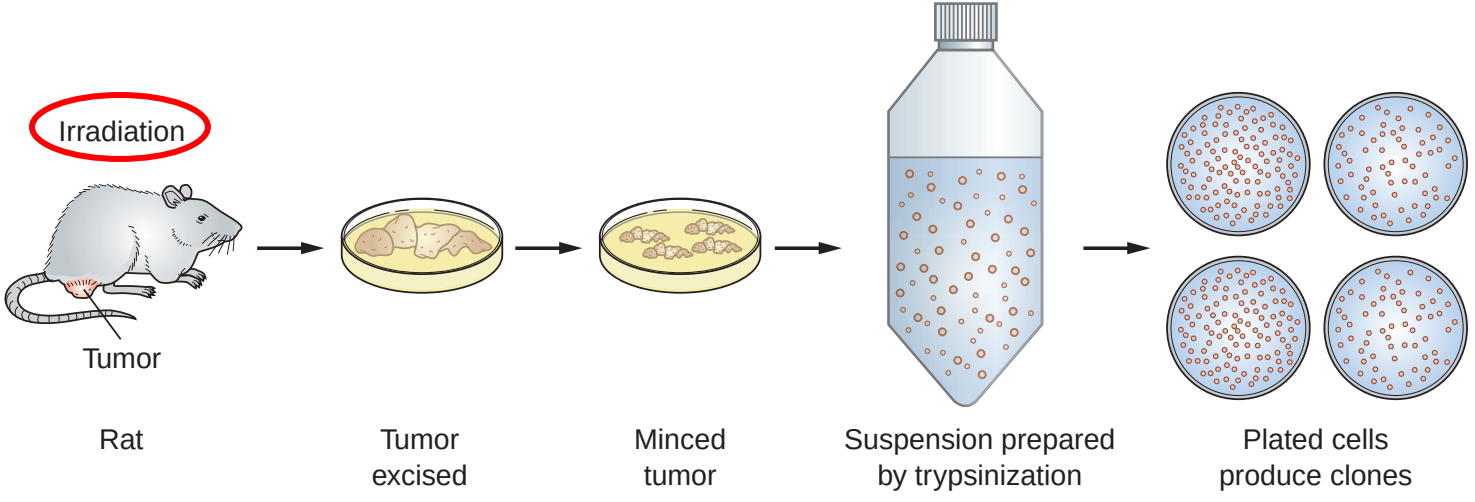


5. In Vivo/In Vitro Assay

- Some tumor cell lines have been adapted to grow both *in vivo* and *in vitro*
- Irradiate tumors in animals → remove tumors → prepare single-cell suspension → plate cells in suitable medium for colony formation

There is not necessarily qualitative or quantitative agreement between results of this assay and results obtained when tumors are left *in situ*

5. *In vivo/In vitro* Assay



Advantage – rapid, efficient, and less expensive
Disadvantage – the tumor that can be switched from petri dish to animal in alternative passages may bear little resemblance to a spontaneous tumor

Summary of Animal Tumor Models

Advantage

Tumor is treated *in situ* (rather than as single layer in petri dish) – the *in vivo* milieu is simulated, i.e., cell-cell contact, stroma, vasculature, central necrosis, heterogeneity

Assays can be highly quantitative

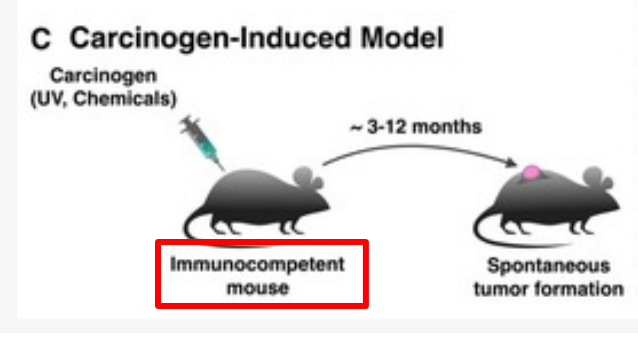
Disadvantage

Artificial – transplantable tumors tend to be fast growing, undifferentiated and highly antigenic, and are grown as encapsulated tumors in muscle or beneath the skin, not their sites of origin

Results must NOT be overinterpreted

6.1 Autochthonous Tumor Models

Spontaneous Tumors



Example: C_3H mice develop spontaneous mammary tumors as they can transmit the mouse mammary tumor virus in their milk

Advantages

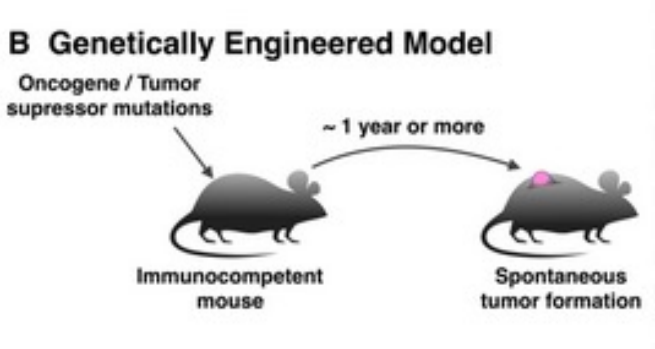
- They are the primary tumors that develop reproducibility in a certain organ
- Influenced by the host stroma and immune systems
- Able to metastasize through vasculature or lymphatic system

Disadvantages

- Variation in the number of primary tumors
- Variations in the time for tumor development

NOT suitable to study radiation response (A single experiment could take a over a year to perform

6.2 Transgenic Tumor Model



Spontaneous tumors develop in animals possessing specific mutations in a given oncogene or tumor suppressor genes

Advantages

- Effect of a single or few genetic alterations on the response of tumors to radiation could be examined in an immune-competent mouse in a reproducible manner

Disadvantages

- Timing of tumor development
- Variation in the number of primary tumors
- Normal tissues can't be easily spared with radiation

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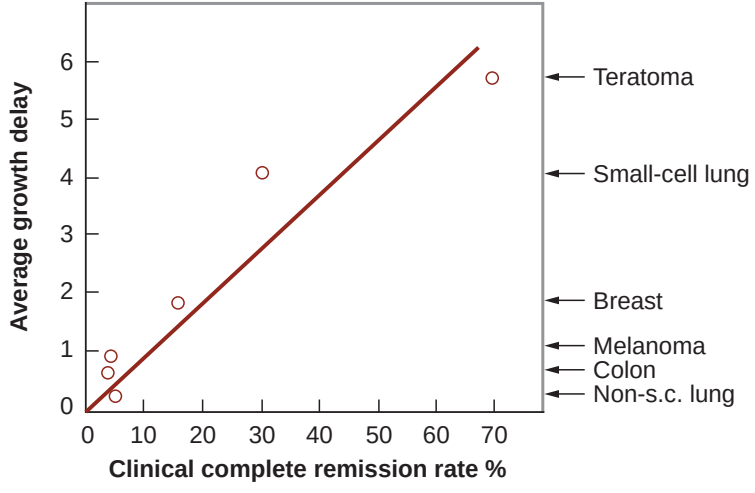
- **Human Tumor Models**
  - Xenografts of Human Tumors
  - Patient-Derived Xenografts Models
  - Spheroids
  - Organoid Models

1. Xenografts

- A **xenograft** is a transplant from one species to another
- In the cancer field, it means a human tumor growing in a laboratory animal
- Lab animals must be immune suppressed to accept human tumor graft
  - Athymic mice – lack thymus and are often nude
  - SCID mice lack B and T cells
  - Radiation or drugs can suppress the immune system

Advantages of Xenografts

- Cells retain the human karyotype and maintain some response characteristics of the tumor from which they were derived
- For many tumors, growth delay and clinical remission rate correlate

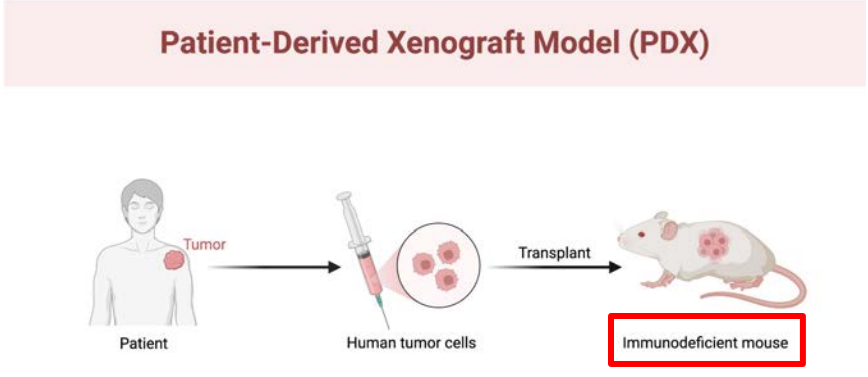


Growth delay assayed following treatment with a single chemo agent

Disadvantages of Xenografts

- Possible rejection by host – observing tumor control as an endpoint can be misleading
- Human tumor cells undergo kinetic changes and cell selection if transplanted into mice
- Stromal tissue is of rodent origin – makes studies where vascularity is important questionable
- Host is different – absence of an immune response is artificial
- Costly

2. Patient-Derived Xenografts (PDX)



Tumor tissue is surgically removed from a patient and implanted into immune-compromised mice

The mice can be “humanized” by introducing human $CD34^+$ hematopoietic stem cells.

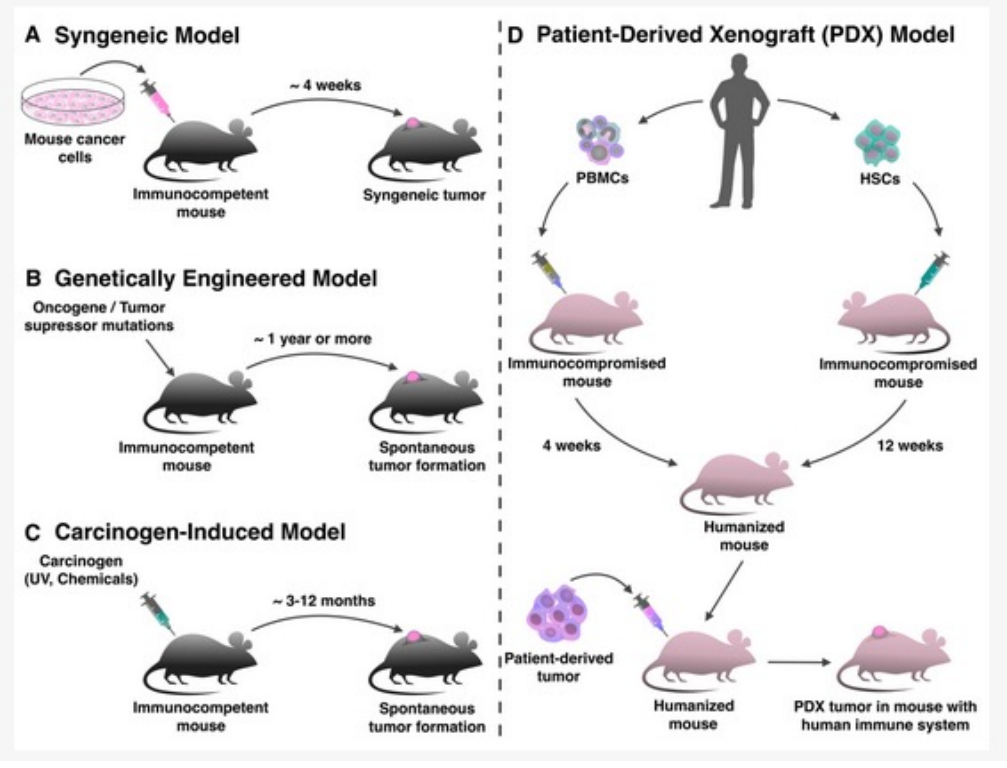
Advantages

- PDXs are not cultured *in vitro* and therefore are not exposed to the selective growth conditions of cell culture
- Tumor specimens, at least initially, possess stroma and cancer stem cell components of the human tumor

Disadvantages

- Long lag periods from implantation to growth
- Variability in tumor take rates
- Host is severely compromised (hence can’t be used to test immune modifying agents with radiotherapy)

Various Mouse Tumor Models



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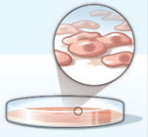
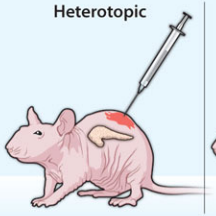
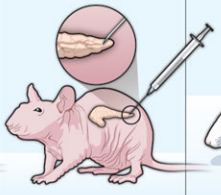
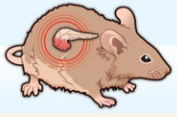
Xenograft Models Pros and Cons

	In vitro	Heterotopic	Orthotopic	PDX	Autochthonous (preinvasive)	Autochthonous (invasive/metastatic)
Platform						
Description	Established cell lines	Human cell lines in immune-deficient host (note: murine cells can be studied in syngeneic immune-competent host)		Patient-derived tumor pieces in immune-deficient host	GEMM of spontaneous PDA at preinvasive stage	GEMM of spontaneous PDA at invasive and metastatic stages
Processes modeled	Cell-autonomous proliferation/ survival of tumor epithelial cell	Growth +/- metastasis of invasive disease			Initiation and progression of preinvasive disease	Progression of invasive and metastatic disease with associated clinical sequelae
Strengths	Human tumor cells; ease; scalable	Human tumor cells; relative ease	Human tumor cells; growth in orthologous organ	Human tumor cells with some preserved human stromal elements	Spontaneous co-evolution of tumor epithelium and microenvironment in native organ; recapitulation of clinical and physiological spectrum of disease; amenable to rigorous correlative studies	
Limitations	2-dimensional growth on artificial substrate; in vitro clonal selection of tumor cells	Heterologous, cross-species growth of established clonally selected cell lines; absence of intact immunity	Cross-species growth of established, clonally selected cell lines; absence of intact immunity	Heterologous, cross-species growth; absence of intact immunity; absence of appropriate tumor architecture and mechanics	Murine cancer; multifocal disease initiation; labor- and resource-intensive	

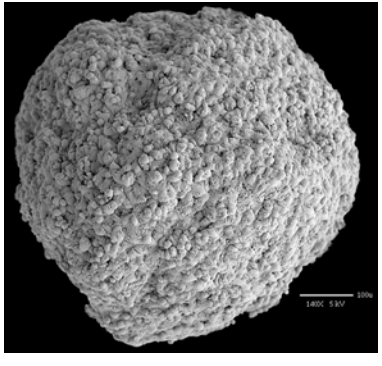
3. Spheroids

Mammalian cells in culture may be grown either as a monolayer or in suspension

Monolayer culture

“Spinner culture”

Most cells growing in suspensions remain as single cells
Some cell grow as **spheroids** – at each successive division, the progeny cells stick together, and grow into a large spherical clump

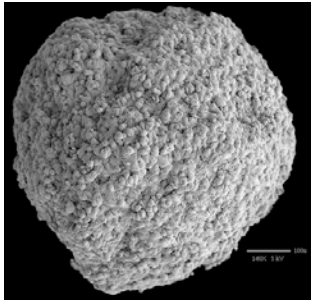


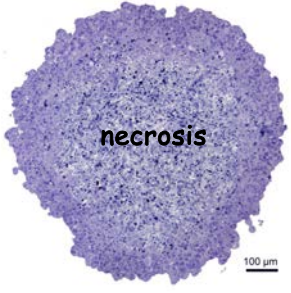
Advantages of Spheroids

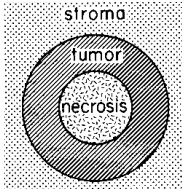
- Simpler, more reproducible, less expensive and easier to manipulate than animal tumors
- Spheroids are irradiated intact and then separated into single cells before plating out into petri dishes for survival assay

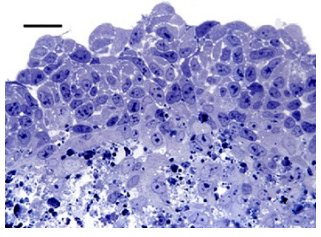
Advantages of Spheroids

800-μm spheroid contains 8 x 10^4 cells









Mature spheroids consist of 3 populations with varying radiosensitivity

- Asynchronous, aerobic cycling cells
- G_1-like, non-cycling, aerated cells
- G_1-like, non-cycling, hypoxic cells
- (Necrotic cells)

outside

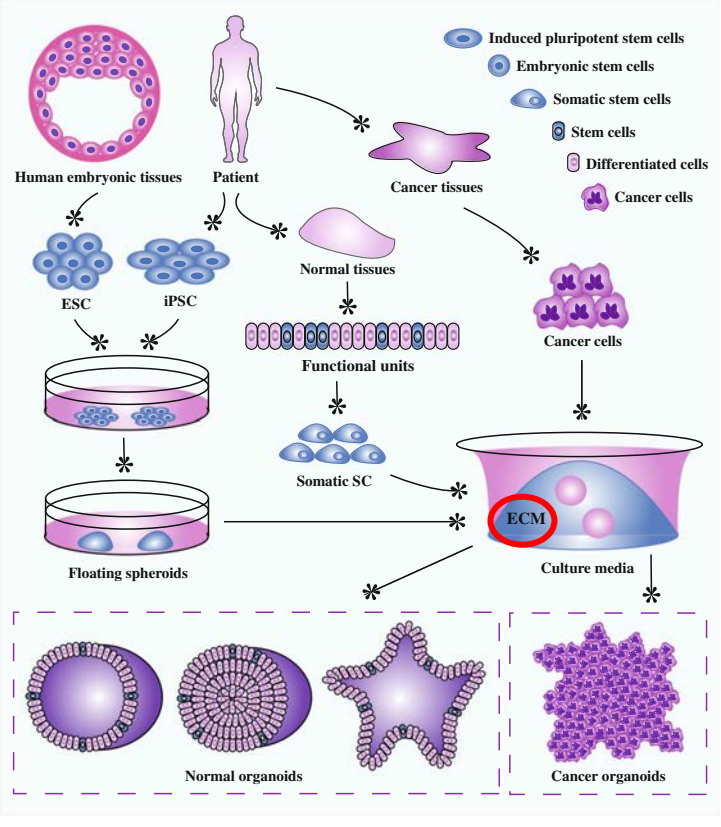
inside

Spheroids maintain the complexities of cell-to-cell contact and nutritional stress from diffusion limitations that are characteristic of a growing tumor

Spheroids of Human Tumor Cells

- Many types of human tumor cells can be cultured as spheroids
- Morphologically, they maintain many characteristics of the original tumor specimen and of the cells if grown as xenograft
- Radiobiologically, spheroids preserve characteristic radiosensitivity, because the dose-response curves for spheroids are virtually identical to those for cells growing as xenograft

4. Organoid Models of Human Tumors



- **Organoids** are 3D constructs developed from embryonic stem cells, induced pluripotent stem cells, somatic stem cells and **cancer cells** in specific 3D culture system
- They resemble the parent organ *in vivo* in terms of structure and function
- Compared to conventional 2D culture of cell lines, the most outstanding feature is the addition of **extracellular matrix substitutes** in 3D cultures
- Tumor-derived organoids represent a new and exciting approach to testing radiation-induced modifiers

Final Note

- No model system is ideal
- They represent a controlled environment in which to test specific responses
- Each has advantages and disadvantages that must be considered in the design of studies and the interpretation of results

Review Questions

Question 1

Which of the following assays would NOT be useful for the purpose of quantifying the response of a tumor to irradiation?

A. Lung colony assay
B. Number of tumors per animal
C. Time to reach a certain size
D. Growth delay
E. Colony forming ability of cells explanted from the tumor

Question 1

Which of the following assays would NOT be useful for the purpose of quantifying the response of a tumor to irradiation?

A. Lung colony assay
B. Number of tumors per animal (reflect metastatic spread)
C. Time to reach a certain size
D. Growth delay
E. Colony forming ability of cells explanted from the tumor

Question 2

The **TCD_{50}** assay

A. Measures radiation-induced tumor growth delay

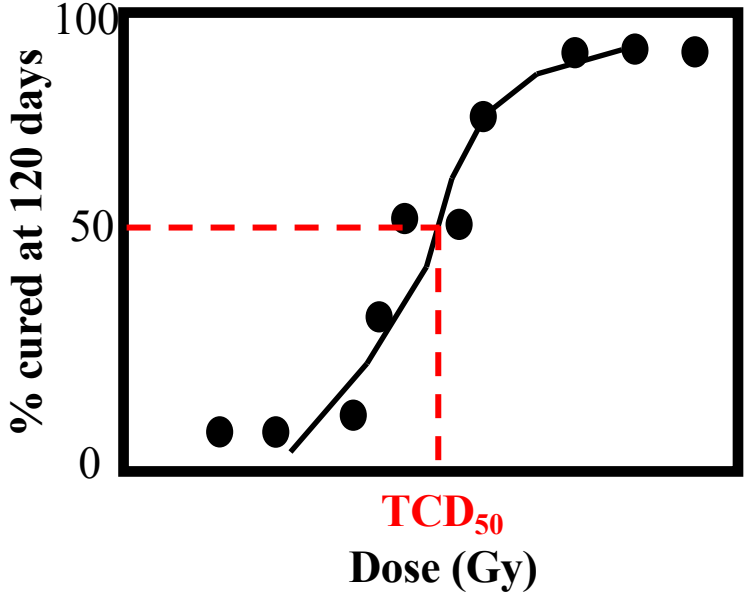
B. Can be conducted using mouse models but not human tumor xenografts

C. Gives a measure of the number of cells required to produce a tumor in a mouse

D. Yields results independent of the immune competence of the host animal

E. Measures tumor cure, making it a relevant endpoint for extrapolation to the clinic

Tumor Cure (TCD_{50}) Assay



Irradiate tumors of uniform sizes with various doses

Observe for **local control** and **recurrence**

Plot % control vs. dose

TCD_{50} = dose to control 50% of tumors

This is a more relevant assay for radiotherapy than growth delay, but requires keeping greater number of animals for longer periods of time, so is more costly