Cell Survival and Tissue Dose Response Curves

Cell Survival Curves *In Vitro*

A. **The toxic (cell killing) effects of ionizing radiation exposure present additional risks to irradiated individuals in the form of:**

- Normal tissue complications that develop following radiation therapy
- Acute, whole-body radiation syndromes
- Embryonic and fetal effects (teratogenesis)
- Cataract formation

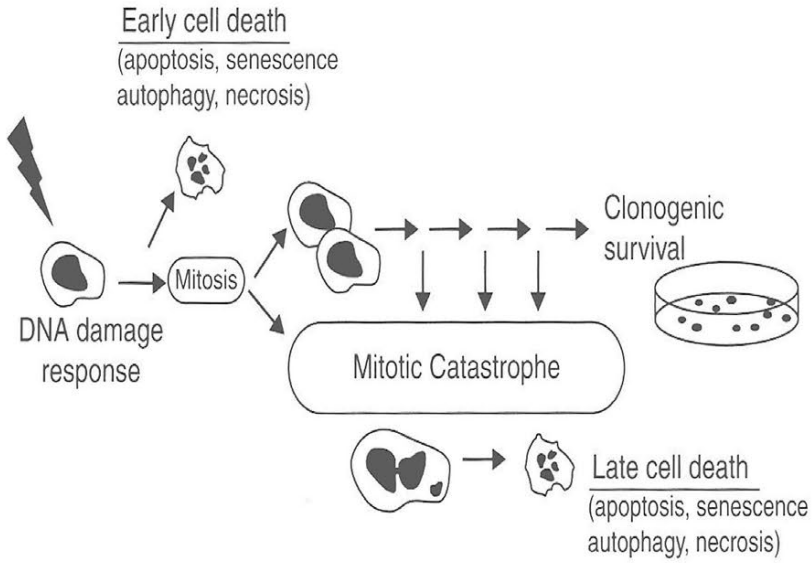
(...and, of course, let's not forget the eradication of tumors!)

In order to determine the likelihood that a certain effect will occur following irradiation, it is necessary to have some measure—either direct or indirect—of the radiosensitivity of the cells whose deaths precipitate the effect. *This is accomplished by generating either a cell survival curve, and/or a tissue dose response curve.*

B. **What is meant by cell death? (not necessarily what you might think!)**

1. **to the radiation biologist and oncologist, cell death means "loss of reproductive integrity" or "CLONOGENIC death", not necessarily** literal death

a) historically, radiation biologists recognized two types of cell death, one type was "prompt" (as in, within hours of irradiation) and the other was delayed (up to several days after irradiation), and that when the cells died in the literal sense, it always seemed to occur while they were in the process of going through mitosis



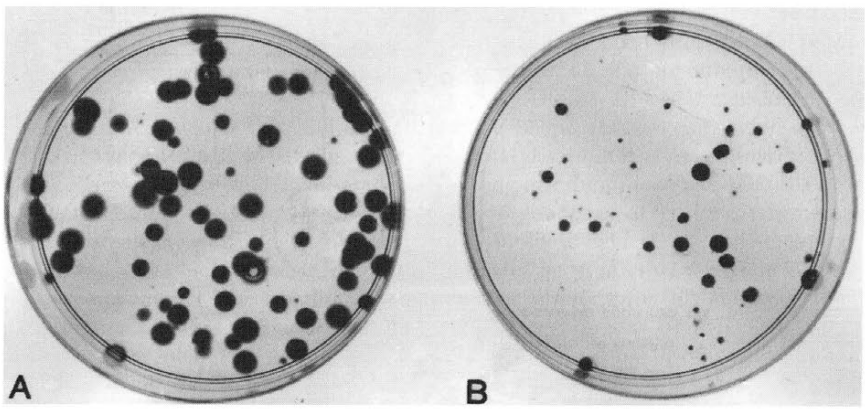
Schematic of cell death following irradiation. DNA damage induced by irradiation elicits activation of the DNA damage response, which leads to induction of cell-cycle checkpoints and DNA repair. In certain rare cells this response also induces apoptosis or other forms of cell death. However, in most cases cells die only after attempting mitosis. Remaining or improperly repaired DNA damage causes mitotic catastrophe, which subsequently leads to cell death. Mitotic catastrophe and cell death can take place after the first attempt at cell division, or after several rounds of proliferation.

Joiner and van der Kogel, *Basic Clinical Radiobiology, Fourth Edition*, 2009

b) we now know that cells classified as clonogenically dead can die by several different mechanisms, some of which result in literal death pretty promptly, others that take a while for literal death to occur, and others that still may never culminate in literal death; the bottom line is that any one of these modes of cell death qualifies as "clonogenic death" in that the affected cell **WILL NOT BE ABLE TO REPRODUCE INDEFINITELY**

C. How do you measure the survival of individual cells?

1) Answer: by determining whether, after irradiation, they can form "colonies" in a petri dish, with each colony arising from a single cell that has reproduced hundreds if not thousands of times (meaning that such cells are NOT "clonogenically dead"; the ones that are cannot form a macroscopic colony)



From Hall, Radiobiology for the Radiologist, 5th Edition

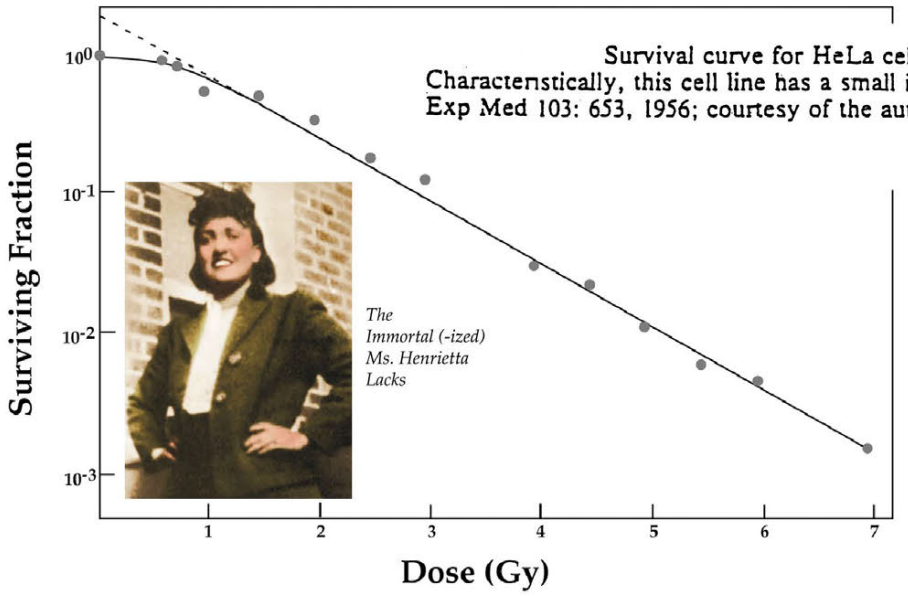
note the broader dispersion in colony sizes in the irradiated sample compared to control

Colonies obtained with Chinese hamster cells cultured in vitro. **A:** In this unirradiated control dish 100 cells were seeded and allowed to grow for 7 days before being stained. There are 70 colonies; therefore the plating efficiency is 70/100, or 70%. **B:** Two thousand cells were seeded and then exposed to 800 rad (8 Gy) of x-rays. There are 32 colonies on the dish.

2) by analyzing colony counts for different cells irradiated with different doses, a survival curve is generated

a. *the very first survival curve for mammalian cells was generated by Puck and Marcus in 1956*

1] *this technological achievement ushered in the so-called "modern era of radiobiology"*



Survival curve for HeLa cells in culture exposed to x-rays. Characteristically, this cell line has a small initial shoulder. (Puck TT, Marcus PI: J Exp Med 103: 653, 1956; courtesy of the author and Rockefeller University Press)

The Immortal (-ized) Ms. Henrietta Lacks

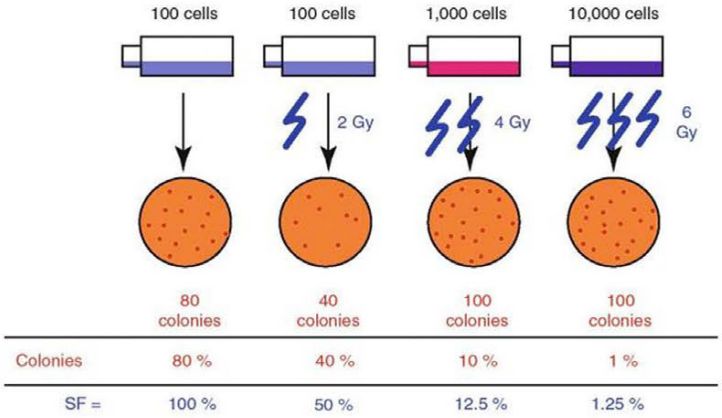
The cell type used for the first mammalian cell survival curve was designated "HeLa", derived from a human cervical carcinoma biopsy taken from an indigent Black woman named **He**nrietta **La**cks; the cells were then used for biomedical research without her consent or for any compensation to her or her heirs

HeLa cells were also the very first (mammalian) cell line to be established *in vitro*, and arguably, launched the entire field of cell biology

3) Where do the "points" on the survival curve come from?

The fraction of cells surviving a radiation dose, "D" = $\frac{\text{plating efficiency after dose "D"}}{\text{plating efficiency after no irradiation}}$

where "plating efficiency" = $\frac{\text{number of colonies obtained on a dish}}{\text{number of cells originally plated in dish}}$



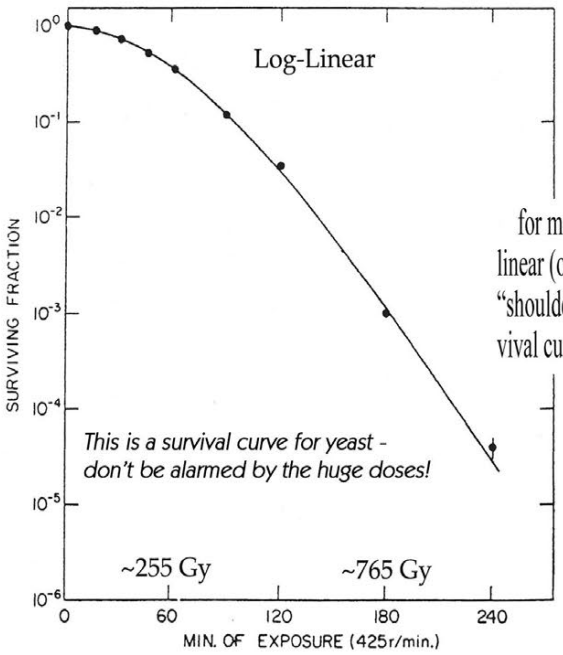
The clonogenic survival assay allows the serial determination of cell surviving fraction after a series of radiation doses.

The best "statistics" are obtained when the number of colonies that form per (small) dish is between ~40 and 100, so the original number of cells plated per dish prior to irradiation is adjusted accordingly. *(This presupposes that you have a general idea of what the surviving fraction is going to be after a given dose.)*

4) Survival Curve Terminology, Plotting Conventions and Mathematical Models

a. ***survival curves are always plotted as the surviving fraction of cells on a log scale (Y-axis), as a function of the radiation dose on a linear scale (X-axis)...in other words, a SEMI-LOGARITHMIC PLOT***

1. this plotting convention is preferred for a number of reasons, not the least of which is that mathematical modeling is easier this way



for most cell types – normal or malignant – the shape of the cell survival curve is roughly linear (on a log scale, "linear" means **exponential**) at high radiation doses, and a more curved, "shoulder" region at low doses...although there are a few cell types that show little or no survival curve shoulder (e.g., bone marrow stem cells, cells bearing certain DNA repair defects)

c. **three descriptive, mathematical terms are commonly used to describe the shapes of radiation survival curves: D_0, n and D_q**

A sometimes handy calculation: $\ln n = D_q/D_0$

D_0 is defined as:

"the increment of dose--in units of Gy--to reduce cell surviving fraction to 37% of its initial value on the exponential portion of the survival curve"

Translation: **D_0 is a the reciprocal of the slope of the straight portion of the curve, i.e., a large value for D_0 means a shallow slope and a small value for D_0 means a steep slope**

n the extrapolation number, is defined as:

"a unit-less number corresponding to the back extrapolate of the exponential portion of the survival curve to the point where it intersects the y-axis"

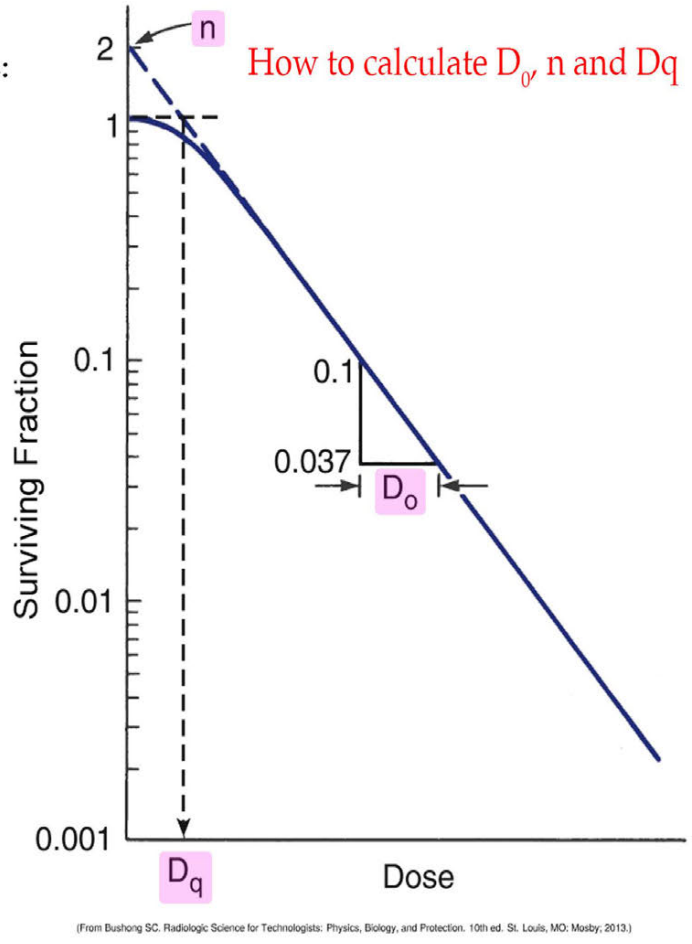
Translation: **n provides partial information about the "steepness" of the shoulder region of the cell survival curve, i.e., the larger the extrapolation number, the steeper the pitch of the shoulder (also note: a survival curve for which n = 1.0 has NO shoulder)**

D_q the quasi-threshold dose, is defined as:

"the increment of dose – in units of Gy – at which the back extrapolate of the exponential portion of the cell survival curve intersects an imaginary X-axis drawn at a surviving fraction of 1.0"

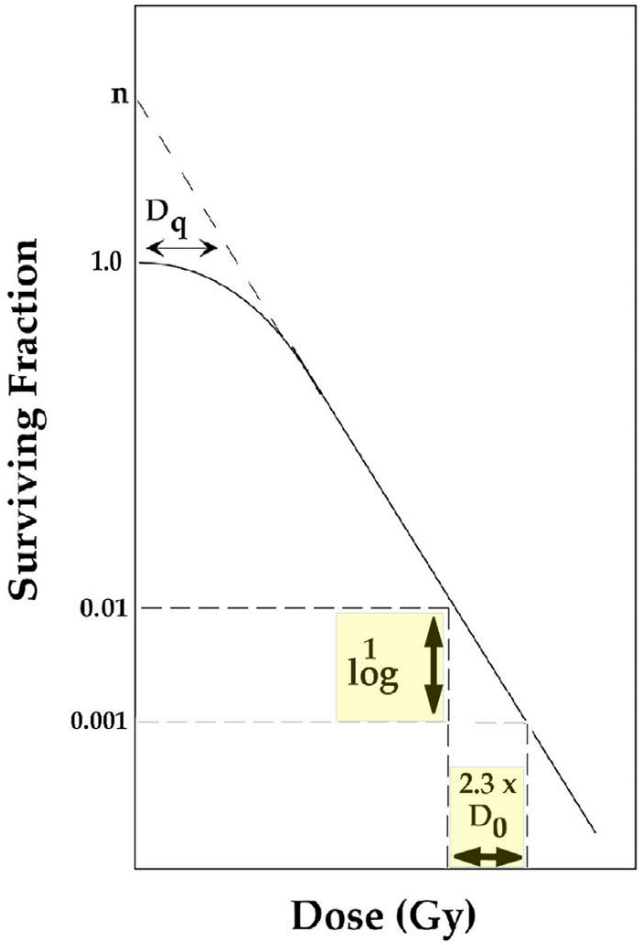
Translation: **Dq provides the rest of the information needed to fully describe the "width" of the shoulder region of the survival curve.**

The larger the value for Dq, the broader the survival curve shoulder.



(From Bushong SC. Radiologic Science for Technologists: Physics, Biology, and Protection. 10th ed. St. Louis, MO: Mosby; 2013.)

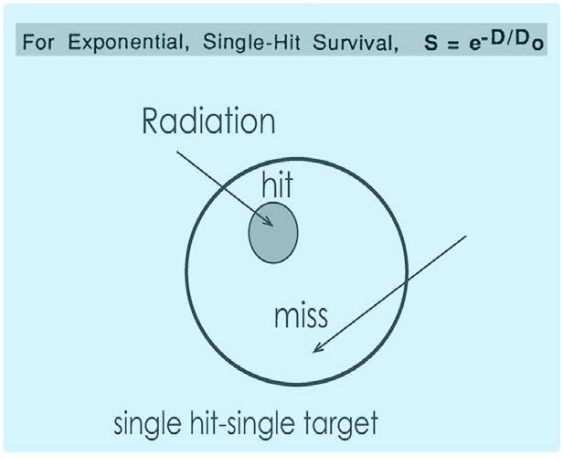
Pssssst! There's actually an easier way to calculate D_0 without having to deal (as much) with log scales or 37%'s...and this can come in handy for certain types of Boards questions.



5) in an attempt to understand why radiation survival curves were "shouldered, and then exponential", radiation physicists and biologists developed mathematical models that fit the sets of data points

a. first came Hit Theory (1920's), the idea that the dose response curve is due to the fact that absorption of energy is not a continuum but a quantized, random and discrete process of independent "hits"

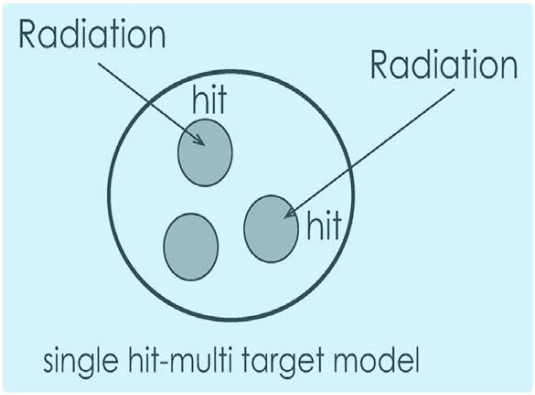
1] the distribution of hits could be determined statistically (using Poisson statistics), and an exponential survival curve would be obtained by postulating that the organism would be killed when a hit was received:



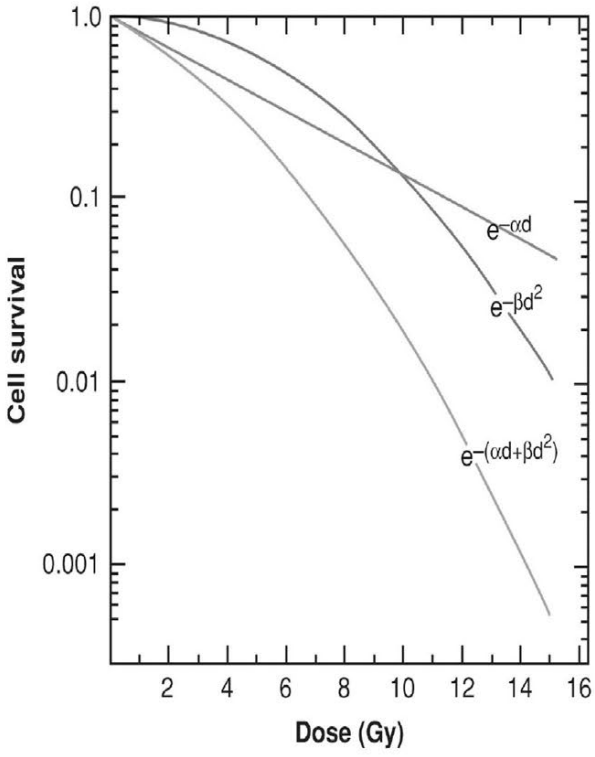
b. then came Target Theory (1940's), which suggested that the cell had a number of targets that each needed to be hit before death would occur; this presented a way of explaining the shoulder region of the curve, i.e., that there was a sublethal region at low doses while the required number of hits were accumulating before death would occur

For a Multitarget Equation, $S = 1-(1-e^{-D/D_o})^n$

where **n** = the number of targets, each of which requires a single hit, before cell death would occur (later called the extrapolation number)



c. **Linear-Quadratic, α, β, Dual Radiation Action, or Sublesion Model:** another approach to explaining the shape of the radiation survival curve, based originally on microdosimetric considerations, but also with some biological basis as well



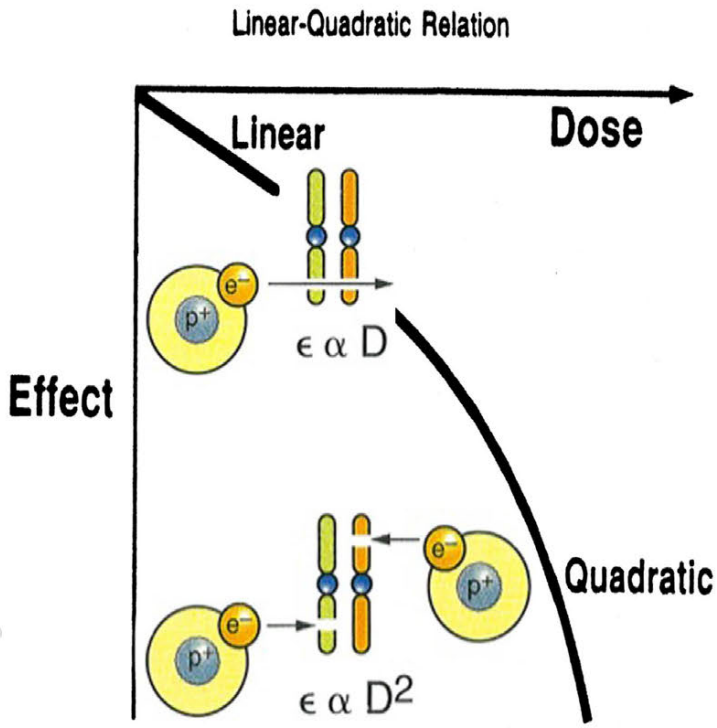
this model assumes that there are two components of radiation-induced cell killing, one which is proportional to dose, and another proportional to the square of the dose; the survival curve expression for this model is:

$$S = e^{-(\alpha D + \beta D^2)}$$

where α and β are proportionality constants related to the relative contributions of linear and quadratic cell killing to the overall cell survival curve

Boards!

the most useful descriptive parameter for this model is **the α/β ratio, the dose at which the linear and quadratic components of cell killing are equal**

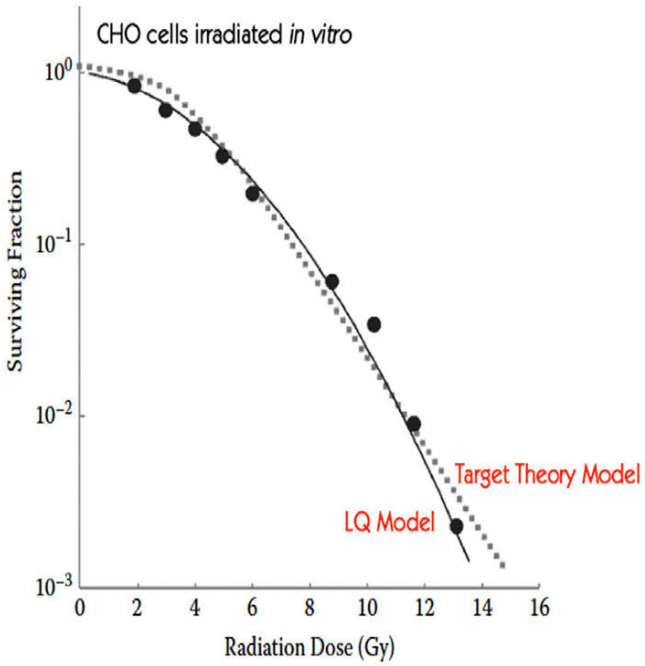


Suggested radiobiological underpinnings of the alpha-beta model:

Two-hit chromosome aberrations are assumed to be responsible for cell death after irradiation. These may be produced by the passage of a single particle track causing both "hits", or else by the passage of two different tracks, each causing one "hit".

The former process, which would predominate at lower radiation doses, would be a linear function of dose, and the latter, which predominates at higher doses, would be a quadratic function of dose.

Target Theory and Linear-Quadratic Models Compared:



Similarities and Differences:

• The models are similar in that they both do a reasonably good job fitting the data points!

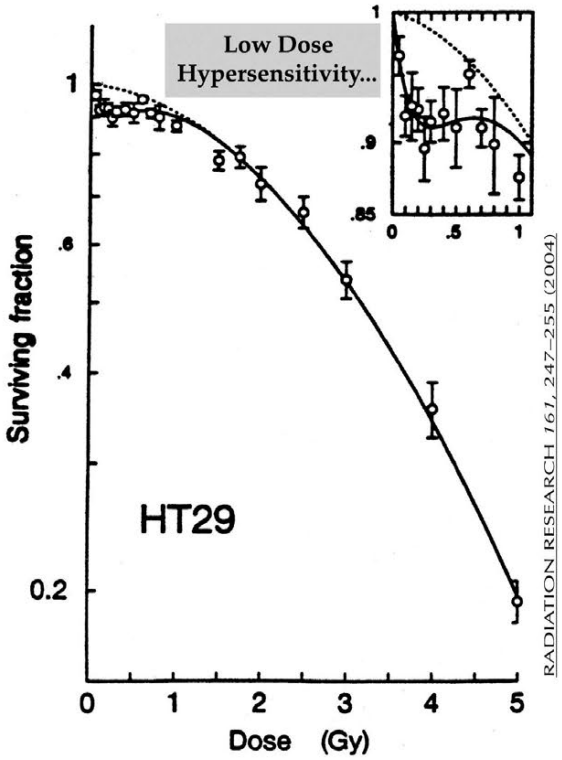
• The linear-quadratic model does a better job in the low-dose region of the survival curve.

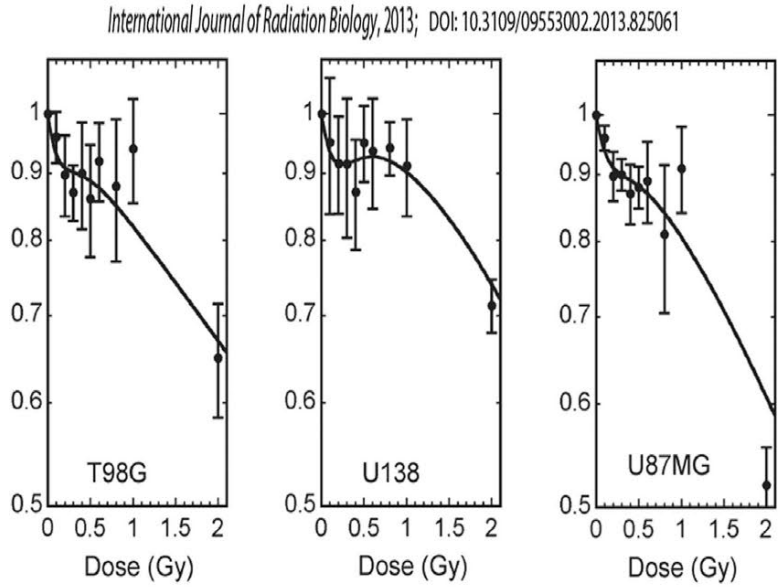
• The target theory model predicts that the low-dose region of the survival curve should be flat, i.e., with no initial slope, and the high dose region should be exponential.

• The linear quadratic model has a non-zero initial slope in the low dose region, and the high dose region is continuously bending.

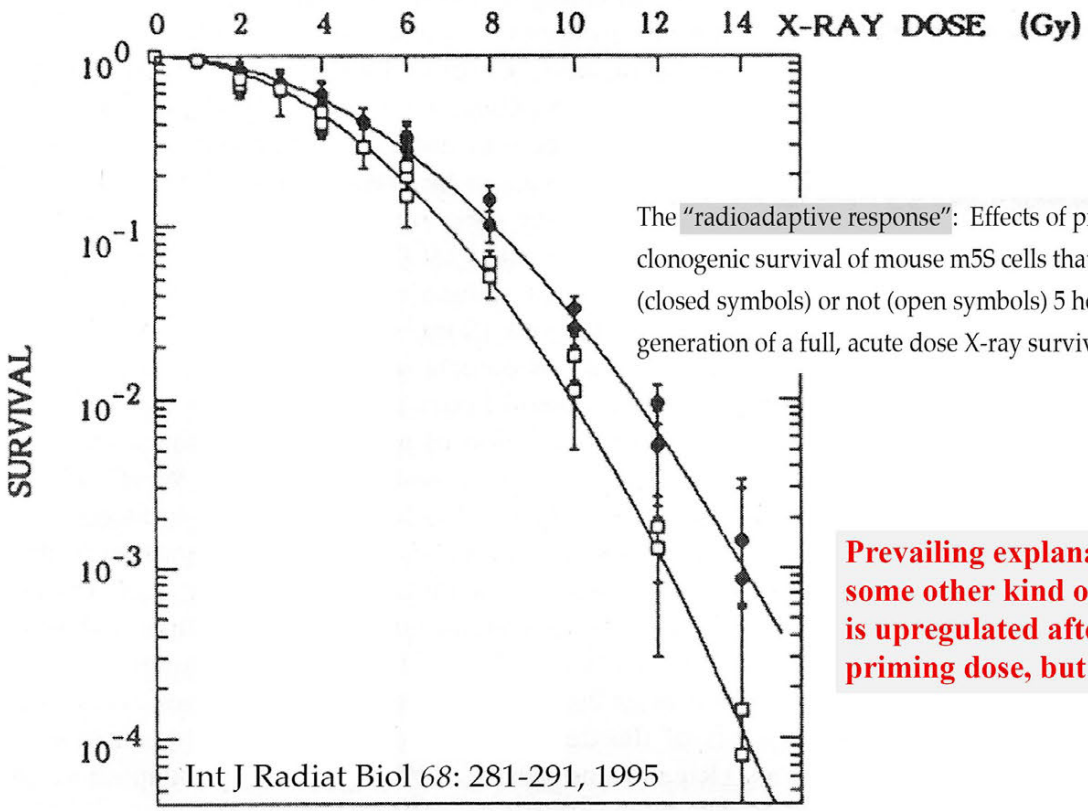
There are laboratory observations however that are difficult to reconcile with either the target theory or the linear-quadratic model: "Low Dose Hypersensitivity" and the "Adaptive Response"

Survival curves for four human cell lines demonstrating the phenomenon of low dose hypersensitivity.





Prevailing explanation: that NHEJ doesn't kick in until a cell type dependent, (low) dose threshold is reached



The "radioadaptive response": Effects of preirradiation on the clonogenic survival of mouse m5S cells that received 2 cGy (closed symbols) or not (open symbols) 5 hours prior to the generation of a full, acute dose X-ray survival curve.

Prevailing explanation: behaves as if some other kind of repair process is upregulated after a very low priming dose, but what, exactly?

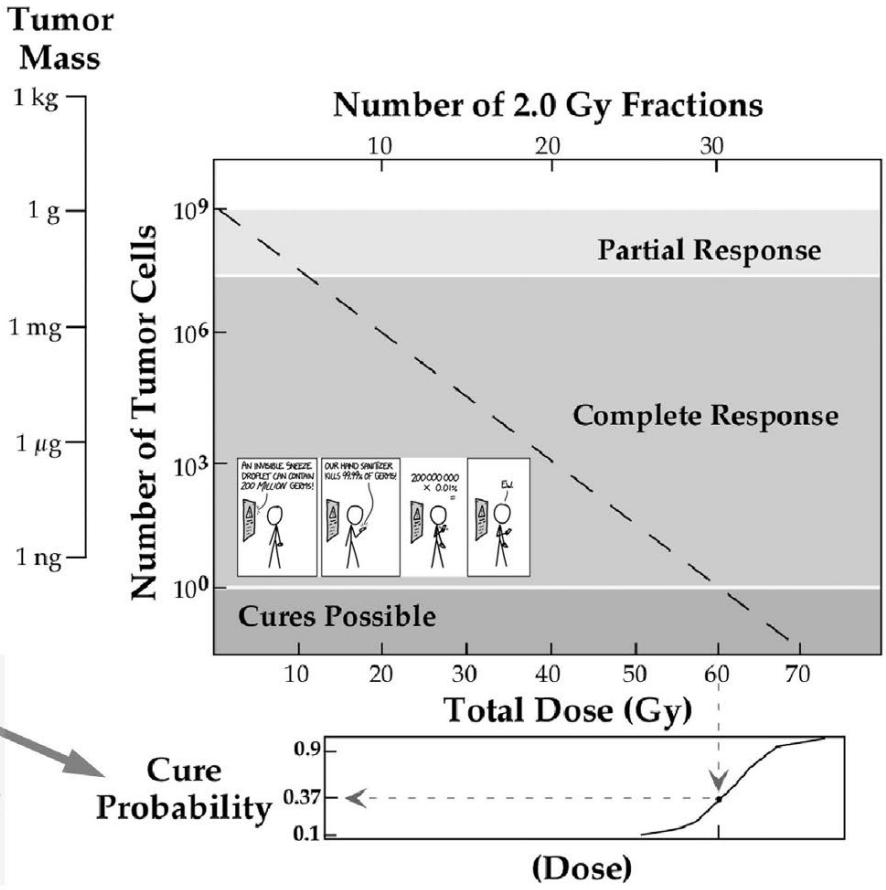
E. Putting Survival Curves into Perspective for Radiotherapy

1. regardless of the particular survival curve model, the bottom line is: *THE KILLING OF TUMOR CELLS IS THE BASIS FOR HOW RADIOTHERAPY WORKS*

2. the problem is, however, that tumors have so many, many cells in them that it takes large radiation doses to kill every one of them (which is the only way to *guarantee* that the tumor won't grow back)

a) How many cells? **It has been estimated that even the smallest tumor we can currently detect probably has a billion cells in it (10^9, or "9 logs")**

1] therefore, in order to get rid of such a tumor, the radiation dose would have to kill at least 10 logs worth of cells!



A tumor containing a single, remaining clongenic cell after radiotherapy will, on average, only be cured about a third of the time!

2] don't be fooled though – tumors can start to shrink, and even disappear altogether, WAY before the dose is high enough to be sure they're gone for good

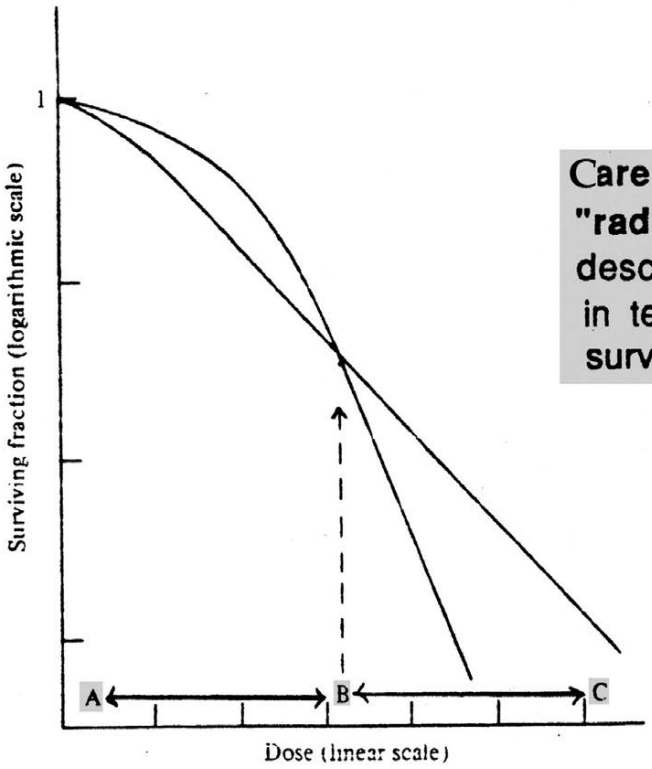
(a) for example, a **"partial response"** (tumor shrinks) occurs when the tumor has been depleted to only 30% (0.3) of its original cell numbers

(b) a **"complete response"** (tumor disappears) occurs when the tumor has been depleted to 1% (0.01) of its original cell numbers

3. on the other hand, the normal tissues in the radiation field that you *don't* want to cause too much damage to, can, at best, tolerate being depleted to 5% (0.05) of their initial cell numbers

F. And one final thing about cell survival curves...

...WATCH YOUR LANGUAGE!

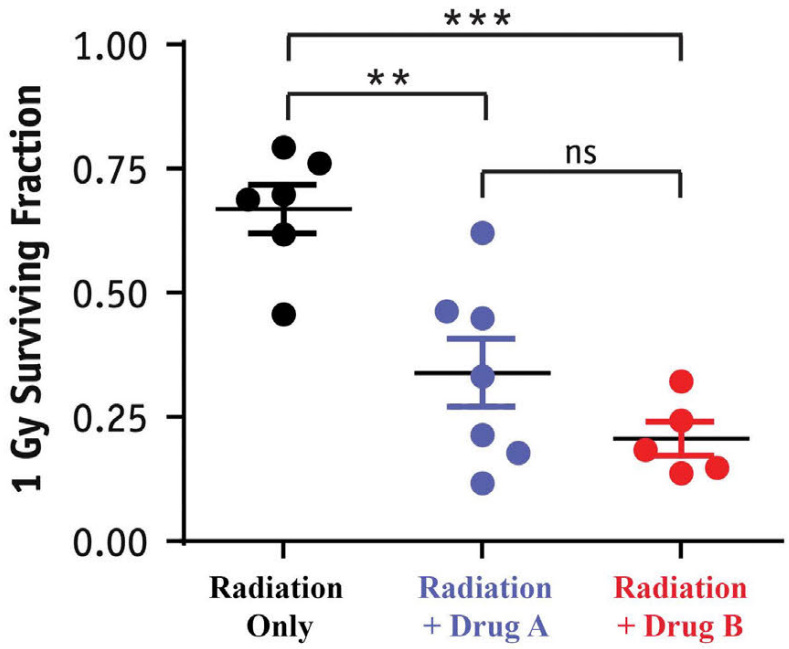


Care must be taken in the use of the words "radiosensitive" and "radioresistant" when describing survival curves; i.e., "sensitive" in terms of what?--final slope, initial slope, survival for a given dose?

To show that the description of one population as 'more radiosensitive' than another may be inadequate, without more specification.

Based on this figure, can you conclude that drugs A and B are "radiosensitizers"? Why or why not?

Do you notice anything(s) wrong with the figure?



Survival and Dose Response Curves *In Vivo*

A. Are cell survival curves generated from cells in petri dishes (*in vitro*) representative of what's going on in intact tissues and organs (*in vivo*)?

1) the answer to this question was particularly of interest to radiation oncologists, because there was NO WAY they were going to collect biopsy samples from every single patient, send them off to a lab to have survival curves generated, and then get a report back (which would take a minimum of 3 weeks) to help guide them with treatment planning

2) therefore, *it became critically important to develop ways of estimating cellular radiosensitivity, but without actually taking samples of the patient's tissues (including the tumor) – enter the so-called* ***In Vivo Assay or Tissue Dose Response Curve***

(a) there are two types of tissue assays: clonogenic and non-clonogenic

1] *a clonogenic tissue assay is similar to a cellular survival assay in that it involves the counting of colonies...the only difference being that the irradiated subject serves as its own petri dish (instead of a glass or plastic vessel)*

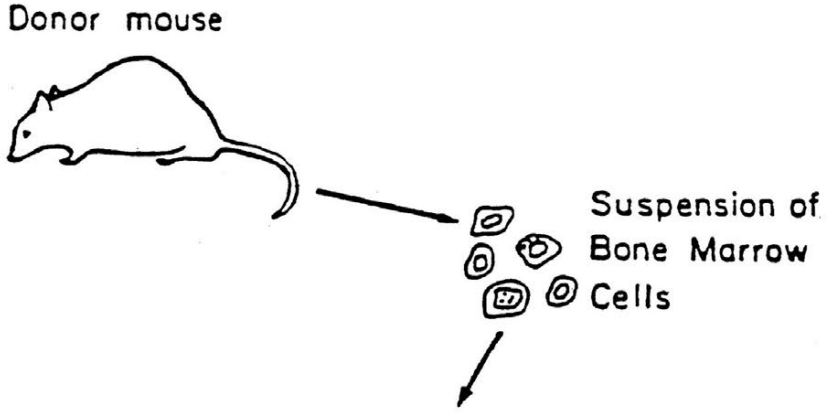
2} *a non-clonogenic tissue assay uses either structural or functional integrity of the tissue (i.e., is the tissue still intact and "working") as a surrogate endpoint for the survival of cells*

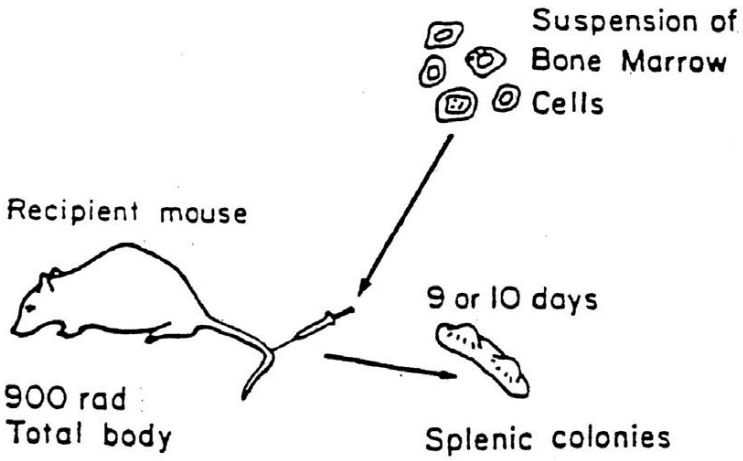
Dose Response Curves for Normal Tissues

- *Examples of Clonogenic Assays In Vivo*

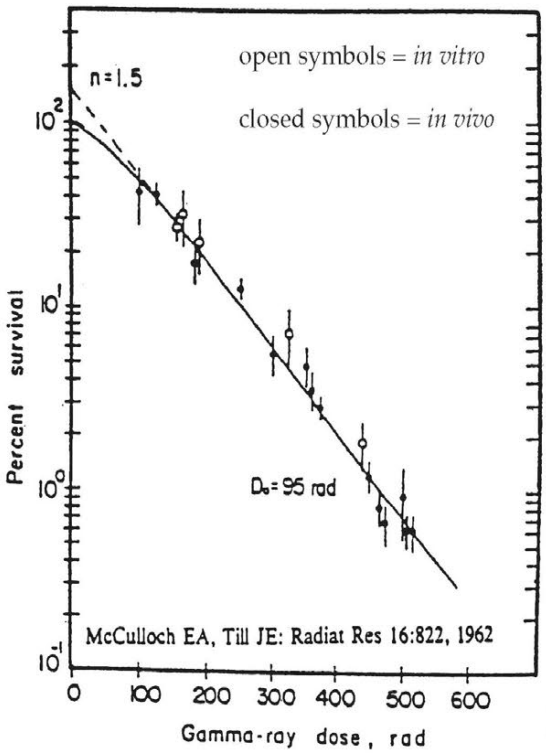
Spleen Colony Assay (Till and McCulloch, 1961) - could measure the survival curve for mouse bone marrow cells *in vivo*

1] the first step was to lethally irradiate (about 10 Gy single dose) a recipient mouse, and try to "rescue" it with injection of normal bone marrow from a syngeneic (genetically identical) donor mouse; the number of injected cells necessary to produce a visible nodule (of regrowing cells) in the previously sterilized spleen of the recipient was then determined and compared to how many colonies were obtained after the donated marrow was irradiated

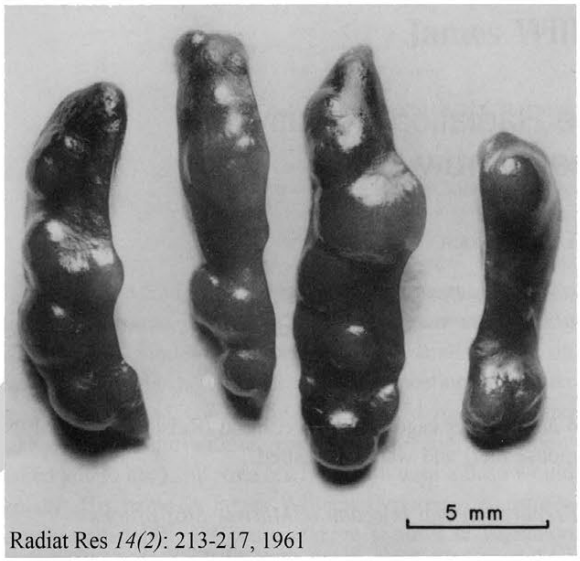




2] by irradiating the donor bone marrow, either while still in the donor mouse, or after its removal, the apparent number of cells necessary to produce a spleen colony increased as a function of dose



this method allowed survival curves to be generated in vivo



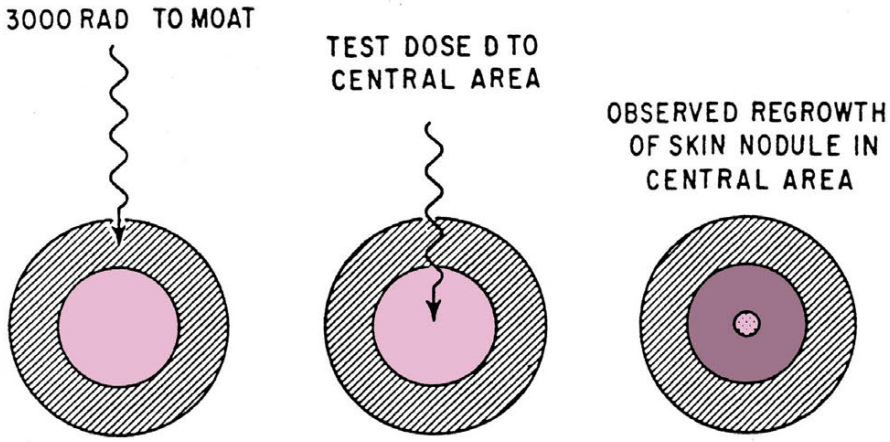
Spleens of irradiated mice 10 days after injection of 6×10^4 nucleated cells. The nodules on which the assay is based are readily seen.

Survival curve measured in the mouse (*in vivo*) was identical to the one for the same cells grown in a petri dish (*in vitro*).

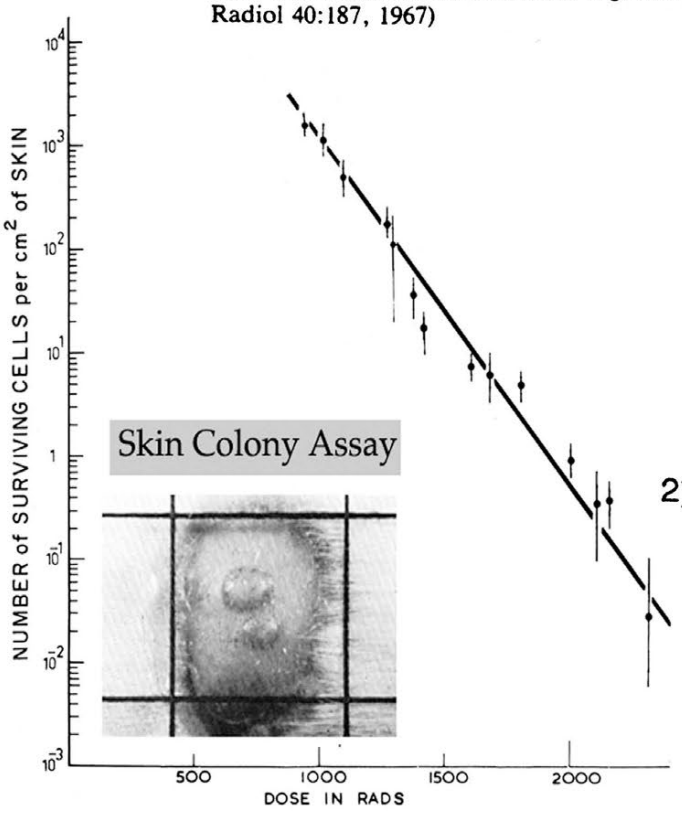
Gamma-ray survival curve for the colony-forming ability of mouse bone marrow cells. The cells are irradiated *in vivo* in the donor animal and grow into colonies in the spleens of supralethally irradiated recipient animals.

Skin Colony Assay (Withers, 1967) - a clever way of determining the survival curve for mouse skin cells; after different radiation doses, an isolated area of skin was examined over time for the appearance of "nodules", each of which roughly corresponded to a regrowing colony of cells (derived from a single survivor)

1] to prevent cells from outside the irradiated area from migrating to the damaged site and influencing the results, a large "moat" was produced by lethally irradiating an annulus of skin around the test area



Technique used to isolate an area of skin for experimental irradiation. A superficial (30-kV) x-ray machine is used to irradiate an annulus of skin to a massive dose of about 3000 rads. An isolated island of intact skin in the center of this "moat" is protected from the radiation by a metal sphere. The intact skin is then given a test dose and observed for nodules of regrowing skin. (Redrawn from Withers HR: Br J Radiol 40:187, 1967)



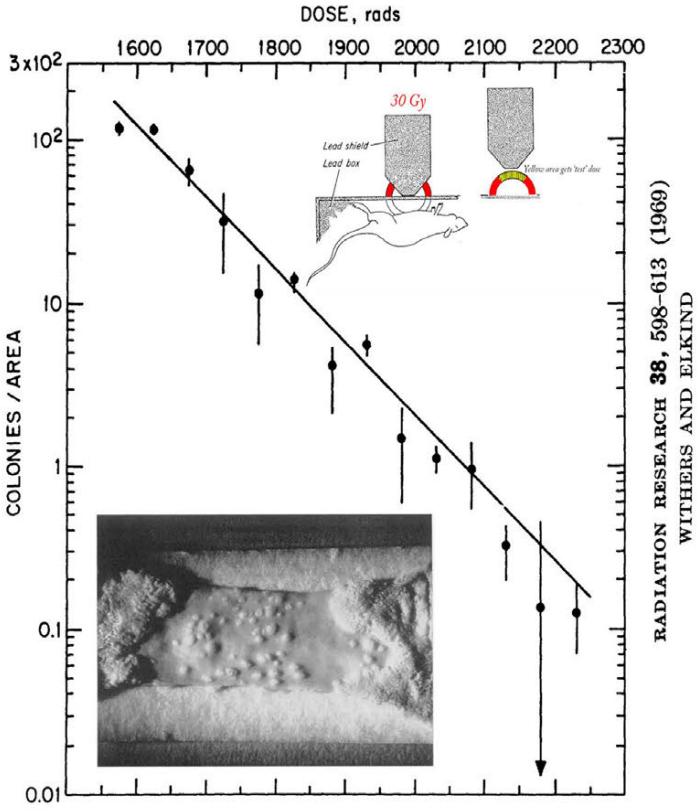
Dose-response curve for epithelial cells of mouse skin exposed to x rays. The 37% dose slope (D_0) is 135 rads. The ordinate is *not* the surviving fraction as in the survival curves for cells cultured *in vitro*, but is the number of surviving cells per square centimeter of skin. (From Withers HR: Brit J Radiol 40: 187, 1967.)

2] the resulting survival curve had a D_0 of about 1.35 Gy, also very much like the *in vitro* survival curves

Jejunal Crypt Assay (Withers and Elkind, 1968) - similar to the skin colony assay, except that what is scored in this case are colonies of regrowing crypt cells of the irradiated mouse jejunum

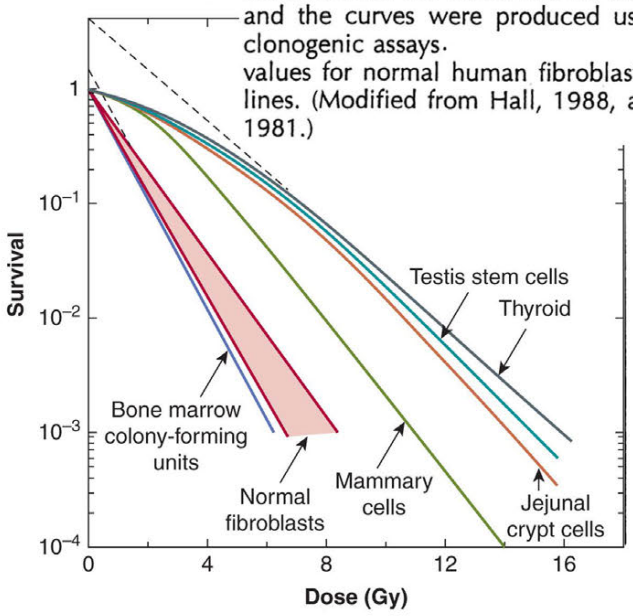
1] this assay is faster than the skin colony method, since cross sections of the jejunum can be examined microscopically after only a few days after irradiation

2] a typical D_0 for this type of experiment was about 1.3-1.5 Gy (for single radiation doses; likewise comparable to results for gut-derived cells irradiated *in vitro*)



Dose response curve for crypt stem cells in a 2 cm length of mouse jejunum surgically-exteriorized and exposed to single doses of X-rays. Like the skin colony assay, moats were created around the test volume to prevent migration of unirradiated cells from outside the field.

Select Other Normal Tissues Amenable to Clonogenic Assay In Vivo

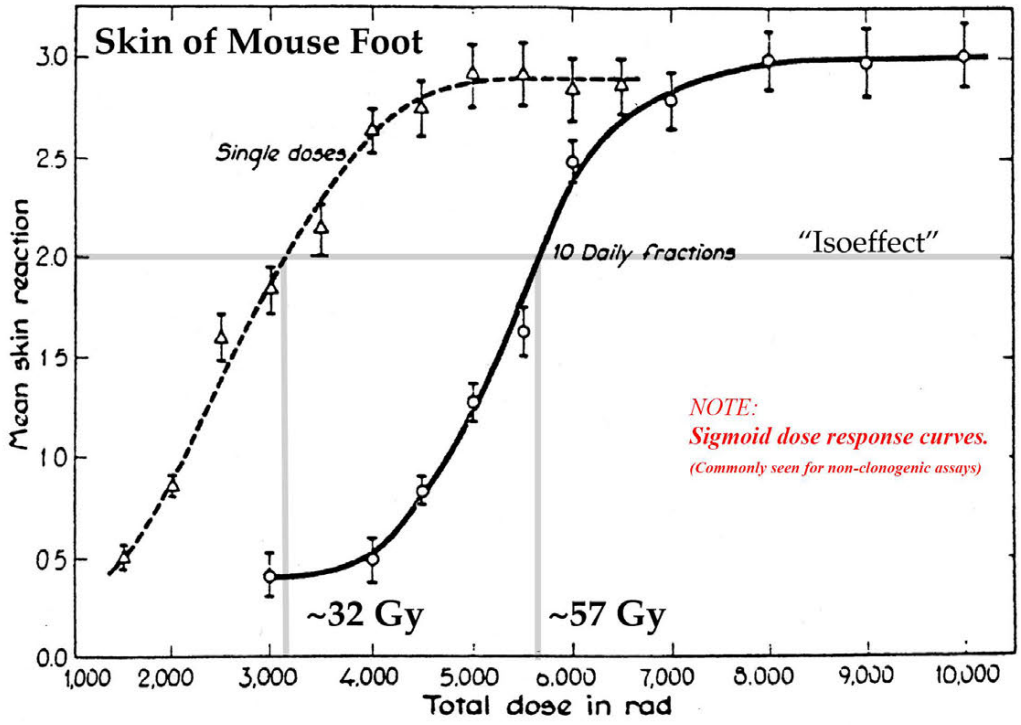


Survival curves for cells from some normal tissues. Most of the curves are for cells from rodent tissues, and the curves were produced using in vivo or in situ clonogenic assays. The range of values for normal human fibroblasts are for cultured cell lines. (Modified from Hall, 1988, and Fertil and Malaise, 1981.)

Examples of Non-Clonogenic Assays

(Pig and Rodent) Skin Reaction Assays - an assay based on an arbitrary scale of skin reactions following irradiation, ranging from a score of "1" (corresponding to erythema) up to approximately "3" (corresponding to necrosis)

1] usually, some **isoeffective** level of skin reaction is chosen, such as "attainment of a skin score of 2.0 by 25 days after irradiation" or "having an average skin score of 1.5 over a 30 day observation period", and then different treatment patterns are compared in terms of producing the chosen level of effect



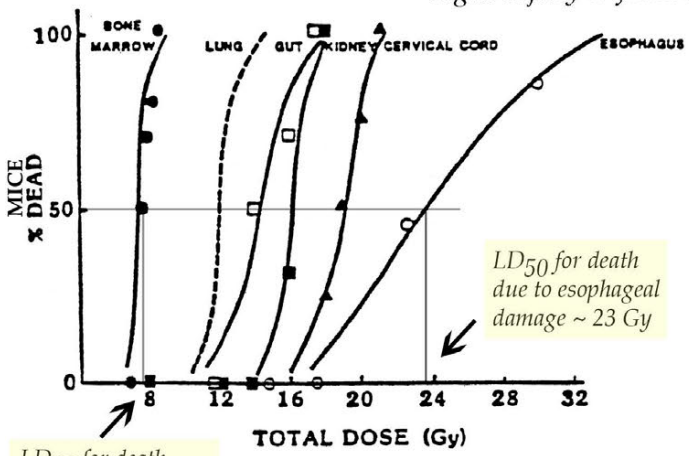
Dose-response curves for skin reactions in the mouse foot for single doses and for 10 daily fractions. The mean skin reaction for single doses was obtained by averaging the daily skin reaction scores for the eighth to 29th postirradiation days. The scores for the fractionated treatment were averaged over 13 to 34 days from the start of treatment.

(From Brown JM, Goffinet DR, Cleaver JE, Kallman RF: J Natl Cancer Inst 47:75–89, 1971)

Other Functional Assays for Normal Tissues

Lung:	Breathing Rate ← higher breathing rate implies more lung damage
Spinal Cord:	Myelopathy
Kidney:	^{51}Cr Clearance Urinary Frequency
Colon/Rectum:	Fecal Deformities *(don't ask)*

LD_{50} Assays - an assay applicable for a number of different target organs where the endpoint evaluated is *"what dose does it take to kill half of the irradiated subjects due to a specific organ injury or failure?"*



LD_{50} for death due to esophageal damage ~ 23 Gy

LD_{50} for death due to bone marrow destruction ~7.5 Gy

Number of animals dead is a function of dose for six different normal tissues. In general, the curves are very steep, although they are displaced on the dose axis, bone marrow being most "sensitive," and esophagus, most "resistant." (Redrawn from Travis EL: Relative radiosensitivity of the human being. *Adv Radiat Biol* 1987; 12:205–238.)

In this example, you can conclude that the bone marrow is more radiosensitive than the esophagus, based on the LD_{50} value

Dose Response Curves for Tumors

Examples of Clonogenic Assays

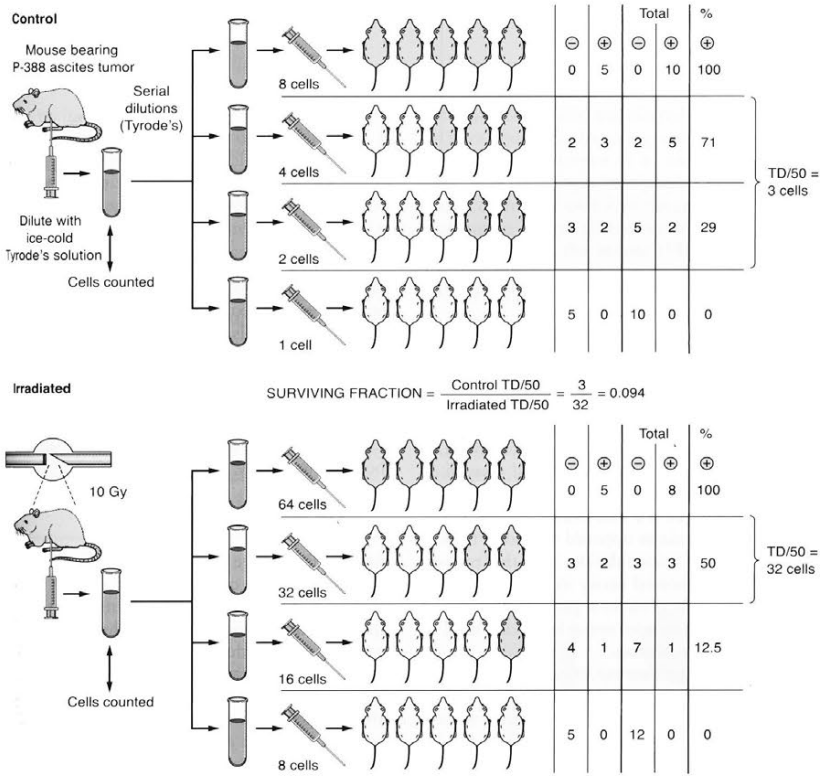
Endpoint or "Limiting" Dilution Assay
a donor/recipient assay similar in principle to the spleen colony technique, except that the endpoint is the ability of donor cells to produce a tumor in recipients

the assay is particularly amenable to tumors that can grow as ascites

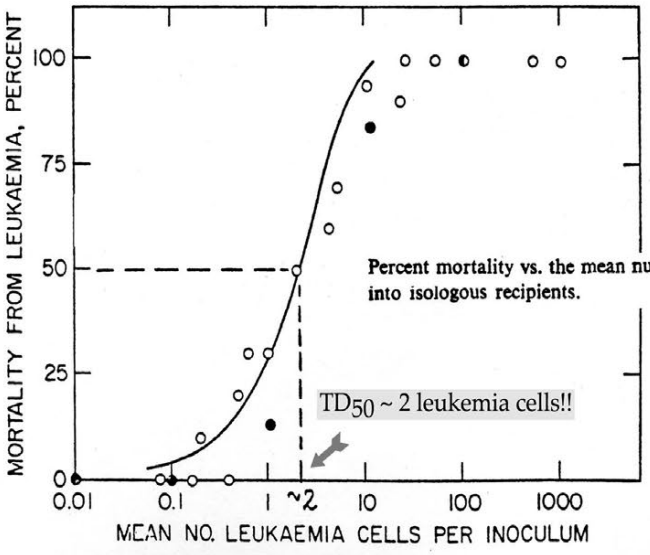
Murine Lymphocytic Leukemia Model (Hewitt and Wilson, 1959):

different numbers of leukemia cells from donors are first iniected into recipients and the **TD_{50}** that is, the *"50% take dose"* or **the number of cells it would take, on average, to produce tumors in half of the recipient animals,** determined

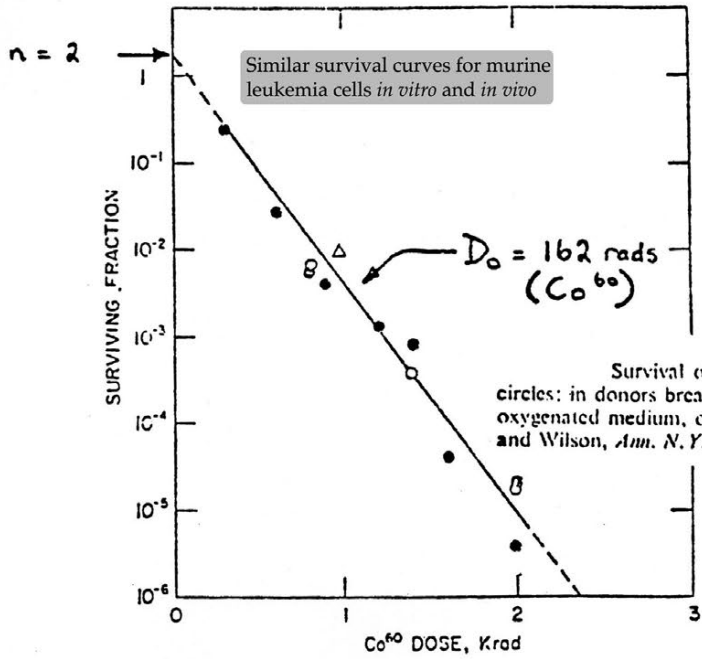
akin to the plating efficiency



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

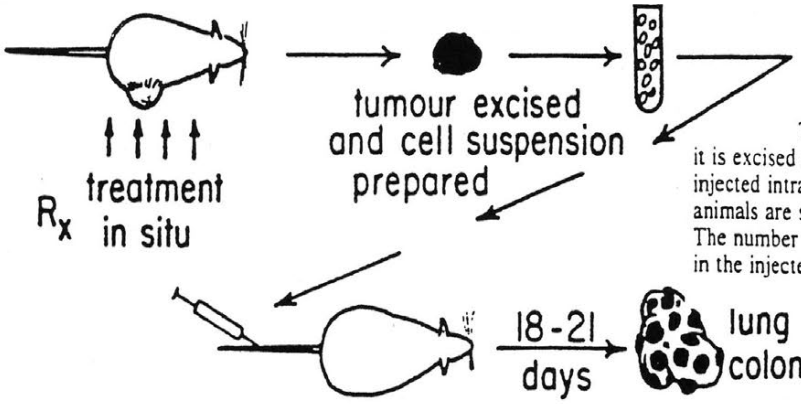


Percent mortality vs. the mean number of CBA leukemia cells inoculated into isologous recipients.



Survival of CBA leukemia cells irradiated in donors breathing air, closed circles; in donors breathing 5% CO_2 plus 95% O_2, open circles; and *in vitro* in a well-oxygenated medium, open triangle. Uncertainties not specified. (Courtesy of Hewitt and Wilson, *Ann. N.Y. Acad. Sci.* 1959)

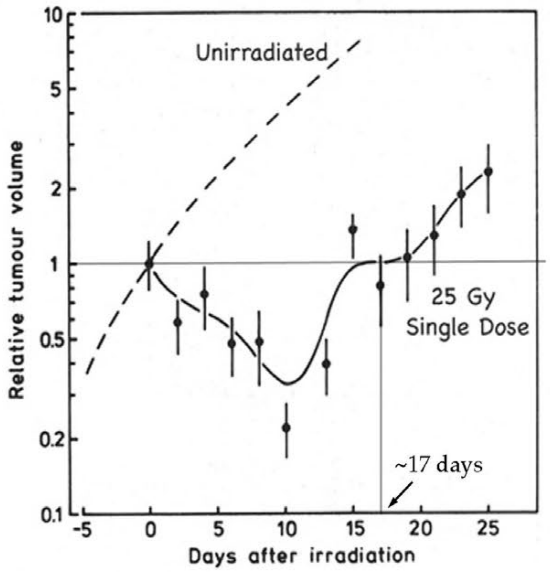
Lung Colony Assay (Hill and Bush, 1969) - based on the ability of viable tumor cells from a donor mouse to form colonies or nodules in the lungs of recipient animals following intravenous injection; this method works with several different mouse tumor types including the KHT sarcoma and Lewis lung carcinoma



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 is then 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)

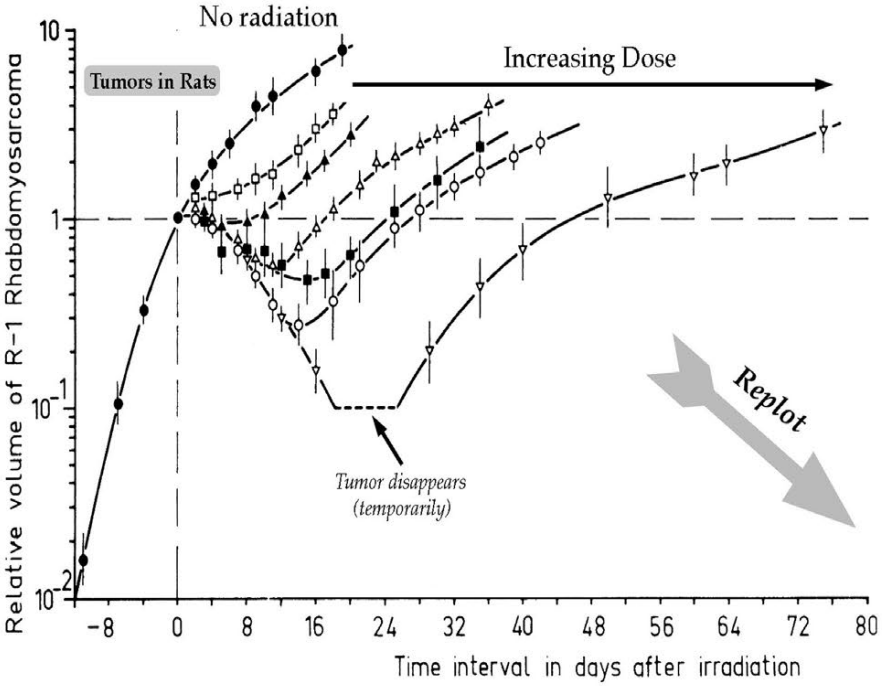
Examples of Non-Clonogenic Assays for Tumors

Tumor (Re-)Growth Delay Assay - uses changes in tumor size and/or growth rate during and after radiation therapy as an indirect indicator of the radiosensitivity of the tumor cells; for example, *a given tumor may slow its growth, stop its growth, regress in size (either slowly or quickly), or even disappear altogether during treatment or soon thereafter*

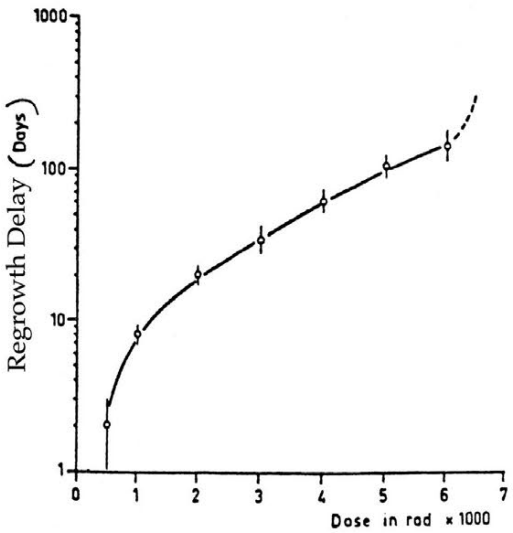


The longer the time it takes for a tumor to re-grow back to its pre-irradiation size, (its "regrowth delay"), the more radiosensitive the cells it contains...to a first approximation, anyway.

Growth characteristics of two, same-sized, mouse tumors, one irradiated with a large dose of X-rays (25 Gy) and the other unirradiated. The regrowth delay is around 17 days in this case.



Another example of tumor regrowth delay for several different radiation doses of increasing size. Growth delay increases as the dose increases, implying that there is greater tumor cell kiling the higher the radiation dose.



From: Hall, *Radiobiology for the Radiologist, 5th Edition*, 2000.

50% Tumor Control Dose (TCD_{50}) - another non-clonogenic assay that measures how much radiation it takes to "locally control" a tumor (that is, for the main tumor mass to disappear and not grow back within a specified period of time); however, the presence of distant metastases is **not** taken into account in this type of assay, so it is not quite the same thing as saying "tumor cure"

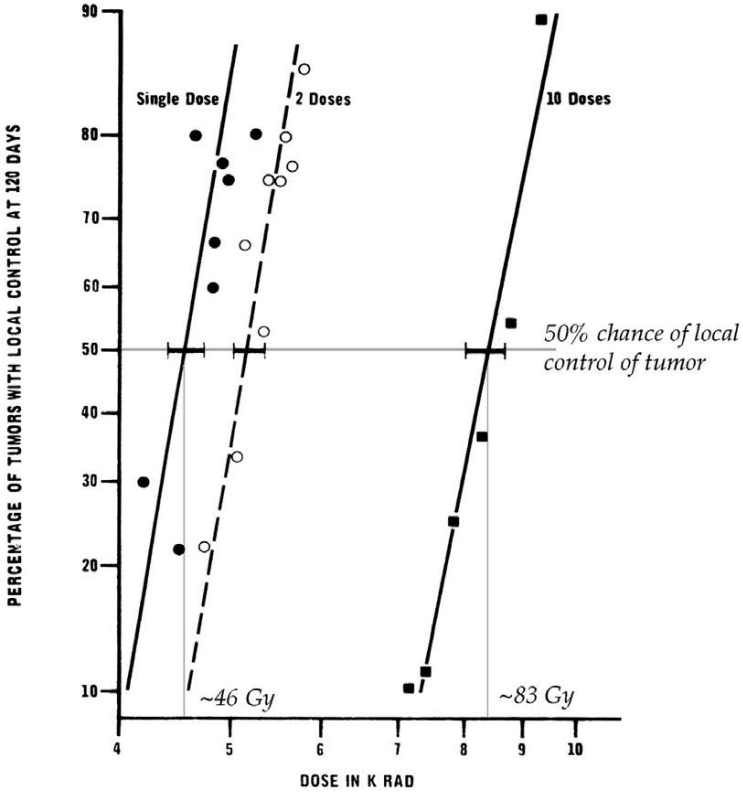
(From Suit H, Wette R: Radiation dose fractionation and tumor control probability. Radiat Res 29:267–281, 1966.)

Note in this example how the TCD_{50} increases with increasing fractionation of the total dose (total dose given as a single fraction, versus 2 fractions or 10 fractions), illustrating that protracting treatment over longer time intervals decreases the cell killing efficiency.

Also of interest is the fact that these dose response curves are very steep, and the data points are tightly distributed.

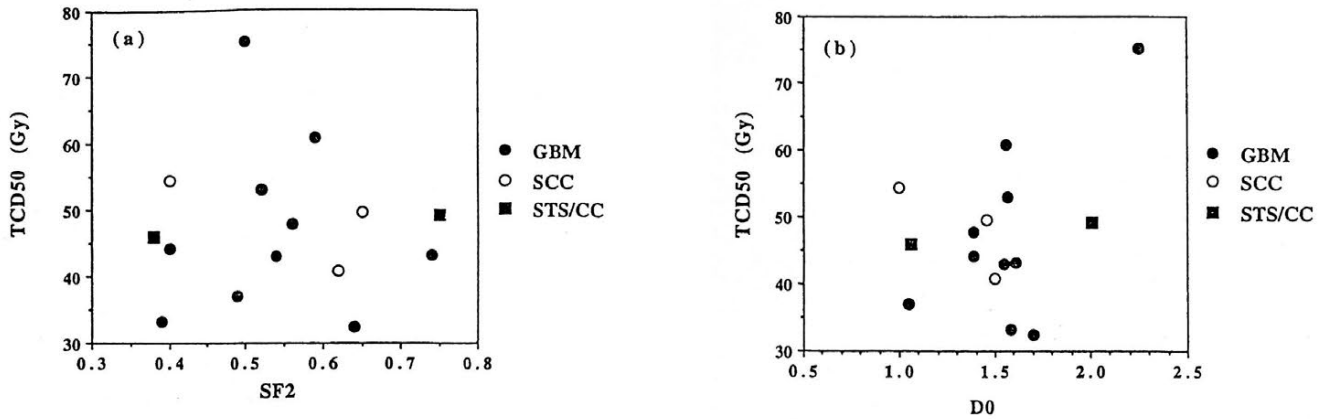
This is commonly seem in dose response relationships for very HOMOGENEOUS populations of cells within a tissue or tumor.

(This is true in this case because inbred strains of mice and serially transplantable mammary tumors such as these are practically clones of each other!)



Percentage of mouse mammary tumors locally controlled as a function of x-ray dose, for single exposures and for two different fractionation patterns.

Unfortunately, TCD_{50} curves tend to look more like scattergrams for spontaneous tumors (that are much more heterogeneous)



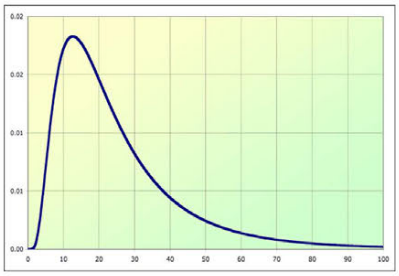
The correlation between the *in vivo* sensitivity described by the TCD_{50} and the *in vitro* sensitivity described by the SF_2 (a) or the D_0 (b) of 15 tumor lines: 10 glioblastoma multiforme (GBM), 3 squamous cell carcinoma (SCC) (SCC21 did not have a corresponding *in vitro* cell line), 1 soft tissue sarcoma (STS), and 1 cancer colon (CC) xenografts.

TCD_{50} is a measure of Tumor Control Probability (TCP) - the below equation can be used to determine *any* TCP, not just the TCD_{50}

the likelihood of controlling a tumor as a function of how many remaining clonogenic cells it contains follows a probablistic function (Poisson statistics to be exact); therefore, it is possible to estimate, to a first approximation, how many clonogenic cells remain in tumors that have achieved a TCD_{50}, TCD_{90}, TCD_{99}, etc., using:

$$TCP = e^{-X} = e^{-SFxN}$$

where X is the average number of surviving tumor clonogens, which in turn can be calculated from the product of SF (fraction of cells surviving) and N (initial number of tumor clonogens)



Poisson (aka "Log Normal") distribution

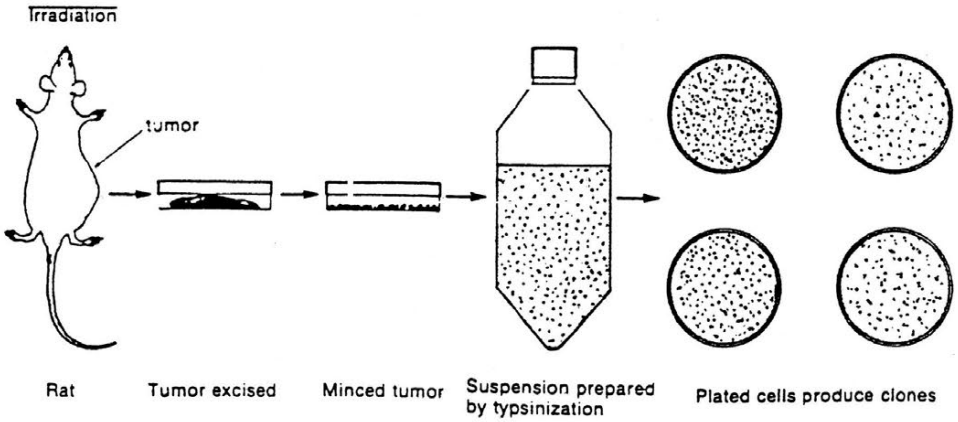
- At an average of **X surviving tumor cells per patient:**
  - (e^{-X}) patients are cured (no tumor cells).
  - $(1 - e^{-X})$ patients recur. (at least 1 tumor cell).
- Based on this equation,
  - @ 1 tumor cell per pt: **TCP = 0.37**
  - @ 0.5 tumor cells per pt: **TCP = 0.61**
  - @ 0.1 tumor cells per pt: **TCP = 0.90** *"TCD90"*
  - @ 0.05 tumor cells per pt: **TCP = 0.95**
  - @ 0.01 tumor cells per pt: **TCP = 0.99** *"TCD99"*

Useful Rule of Thumb: To achieve a particular TCP, aim for a tumor cell surviving fraction of (1-TCP)

@ 0.7 tumor cells per pt: **TCP = 0.5** *"TCD50"*

"Hybrid" Model Systems - assays that incorporate elements of both *in vitro* and *in vivo*

***In Vivo/In Vitro* Excision Assay** - takes advantage of the fact that several cell lines have been adapted for growth both in tissue culture and as solid tumors in mice (including EMT6, RIF-1, KHT, SCC VII, rat rhabdomyosarcoma etc.); the usual technique involves the irradiation of tumors *in vivo* followed by tumor excision, digestion with enzymes into a single cell suspension, and plating of cells into petri dishes for colony formation *in vitro*

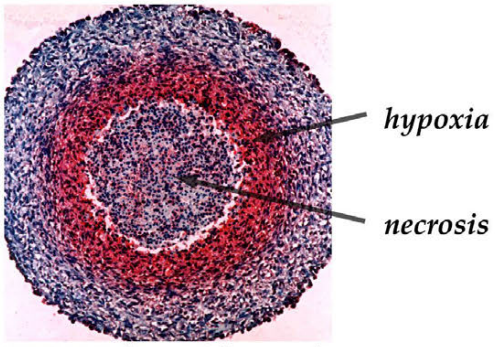


The principle of the *in vivo/in vitro* assay system using the rhabdomyosarcoma in the rat. The solid tumor in the animal can be removed and the tumor cells assayed for colony formation in petri dishes. This cell line can be transferred to and fro between the animal and the petri dish.

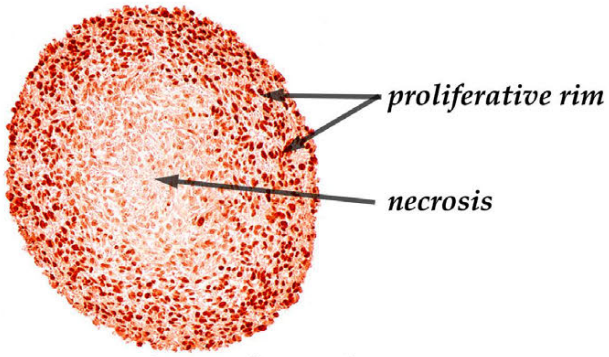
Multicellular Tumor Spheroids

- tissue culture cells, most notably rodent lines, that can grow as little balls when prevented from attaching to glass or plastic surfaces by being maintained in stirred suspension

a. spheroids can grow as large as 1 mm in diameter, and begin to take on various solid-tumor like characteristics, such as:



EMT6 Spheroid (mouse)



HT1080 Spheroid (human)

- ✱gradients of oxygen and other nutrient levels
- ✱central necrotic regions distant from medium supply
- ✱heterogeneous cell cycle kinetics at different points within the spheroid
- ✱cell-to-cell contact effects

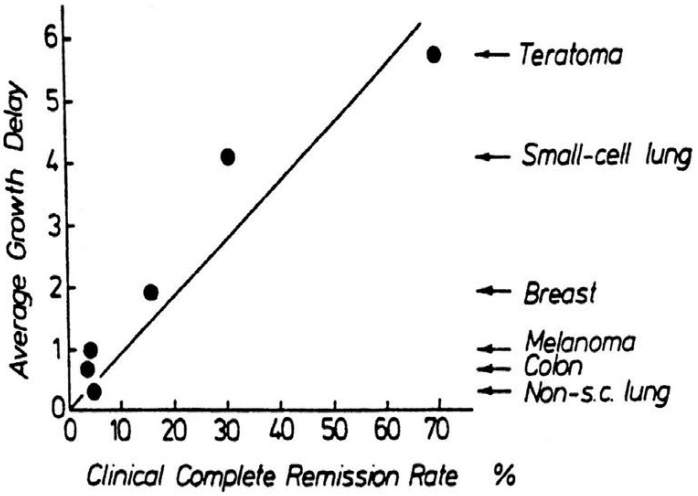
b. yet, at the same time, spheroids can be easily disaggregated and the responses of certain subpopulations evaluated after irradiation using the conventional clonogenic assay

Human Tumor Xenografts

- involves the implantation of chunks of human tumor tissue into immunodeficient mice (e.g., nude or SCID mice)

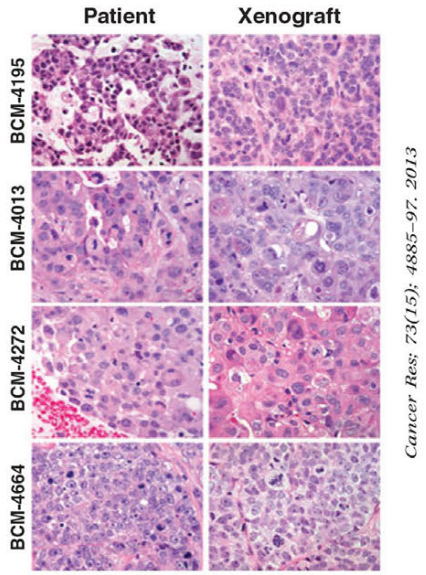
a. these xenografts retain many – but not all – of the characteristics of the human tumor they were derived from

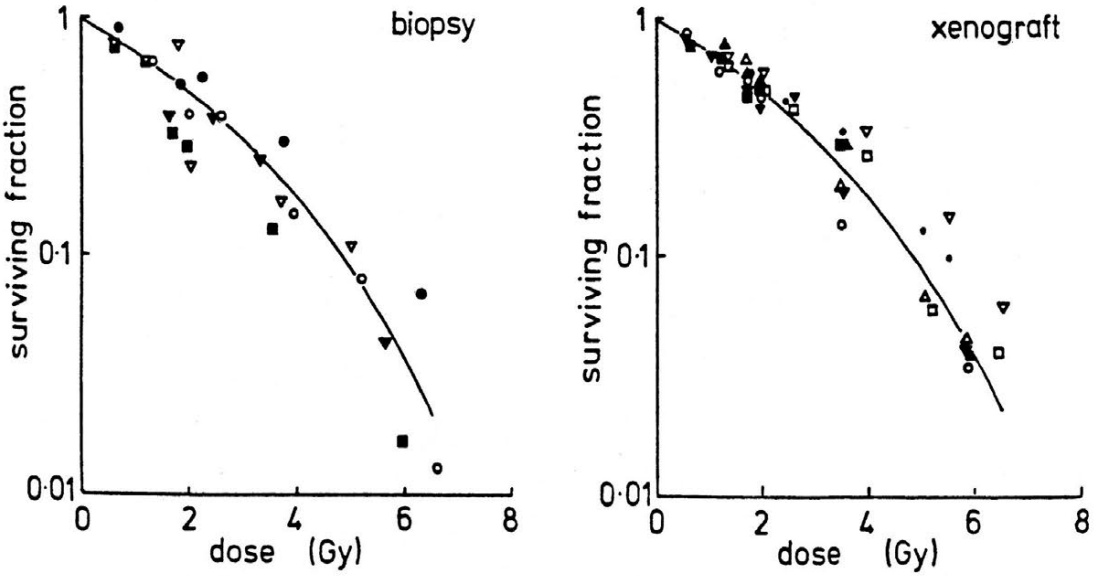
b. the endpoint commonly measured is growth delay, and a good correlation has been found between the drug sensitivity of a select group of xenografts and the clinical response of human tumors of the same histological type



Correlation between the response of human tumor xenografts to chemotherapy and the clinical complete remission rates. The ordinate indicates the average specific growth delay of 3–10 xenograft lines treated with those clinically used drugs that were most effective in the xenografts.

Diverse (molecularly and ethnically) human breast tumor xenografts grown in nude mice. In general, the appearance and behavior of the xenografts mimic what is observed in tumor biopsies directly from the patient.





Cell survival measurements in human melanomas following in vitro irradiation under well-oxygenated conditions. The left-hand panel indicates data on cell suspensions made directly from tumor biopsies. The right-hand panel shows data on cell suspensions prepared from xenografts. Symbols indicate different tumor specimens. The full line is a linear-quadratic equation fitted to the biopsy data, and it is repeated in the right-hand panel.

(1) on the other hand, many types of human cancer do not form xenografts that well, or else require months of adaptation to growth in the mouse, and a resulting loss of their human characteristics

What *can't* you do with human tumor xenografts (other than for comparative purposes)**?**

> anything related to the tumor's vasculature (e.g., pharmacokinetics, drug delivery, tumor hypoxia, angiogenesis, etc.), ***because the vasculature is murine***, even though the tumor is human

> anything related to ***immune response or immunotherapy***, because the host has little to no immune system; may also be true when it comes to studies of tumor metabolomics and/or the microbiome...

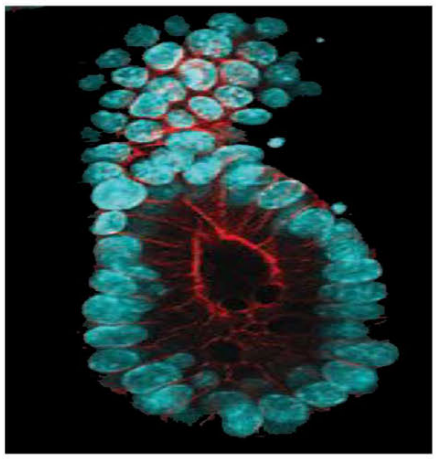
Emerging Science: Organoids as cancer models

A. Organoids are similar to spheroids in that they combine the 3D architecture of tissues or tumors with the experimental ease of using 2D cell lines...**except** that spheroids contain a single (tumor) cell type, ***whereas organoids incorporate additional elements, such as a 3D extracellular matrix, the presence of other cell types,*** etc.

1) another advantage of organoids is that ***cells from normal tissues can be maintained as organoids, whereas they can't grow as spheroids*** (i.e, normal cells need to be anchored to an appropriate extracellular matrix or else they die by apoptosis)

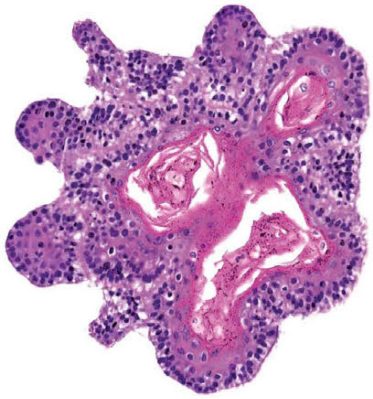
2) organoids were originally developed to study stem cell biology, i.e., how and under what conditions stem cells differentiate and self-organize into normal tissues

3) with respect to the use of organoids as models of cancer however, it is tumor cells, not normal tissue ones, that are most commonly cultured

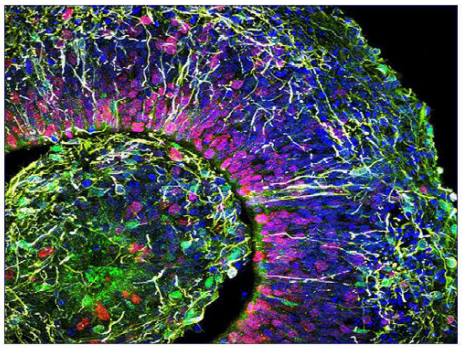


Annu. Rev. Pathol. Mech. Dis. 2016.11:199-220.

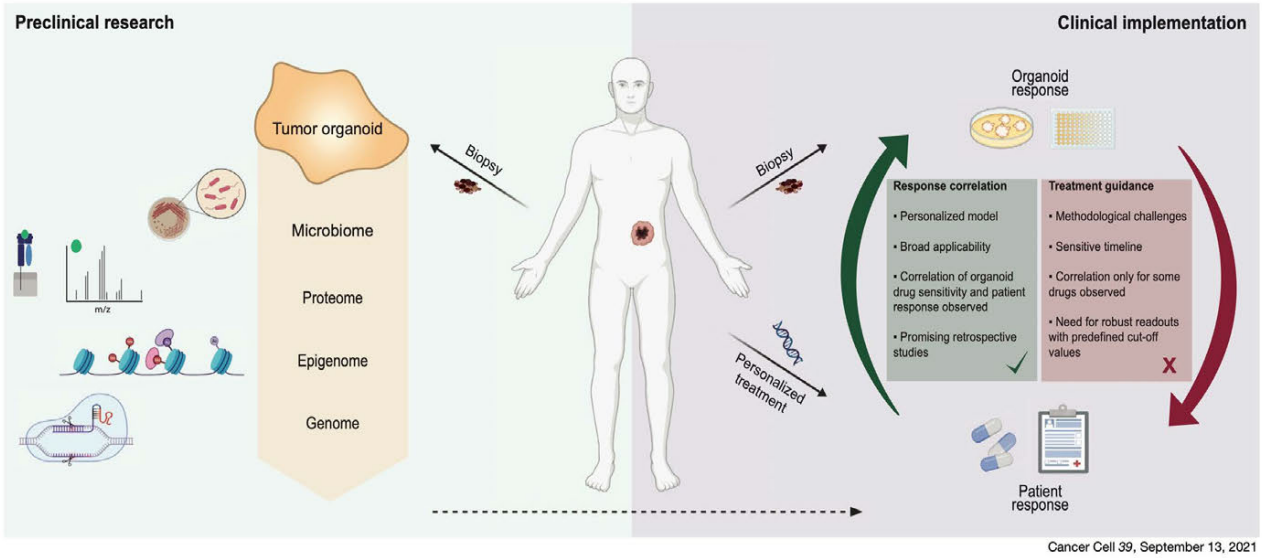
Human colorectal cancer organoid



Human head & neck SCC organoid



Mouse brain organoid



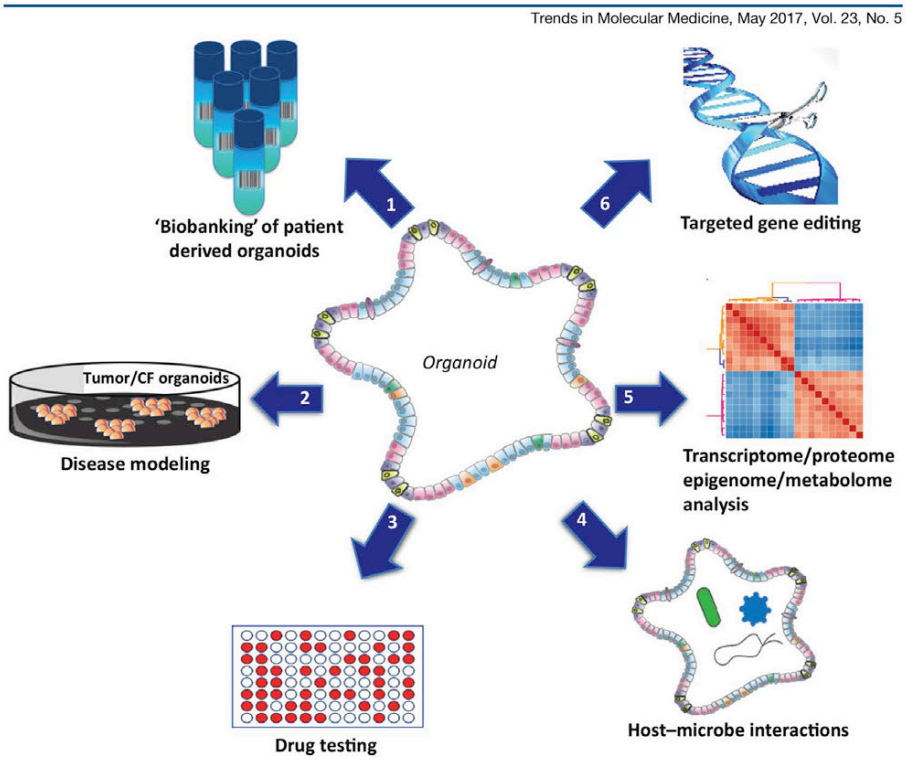
Cancer Cell 39, September 13, 2021

Tumor organoids in preclinical research and clinical implementation

As a versatile model system, organoids can be used to address fundamental research questions in various omic disciplines and deepen our knowledge of cancer biology. Due to their resemblance to the original tumor, organoids offer great opportunities in preclinical research that may shape the future of precision medicine. In the clinical setting, organoids can be used to analyze sensitivity to treatments on an individual level. While retrospective correlations to patient response data are encouraging, the predictive value of tumor organoids in prospective studies is so far accompanied by methodological challenges such as initial culture success. Another major challenge for potential treatment guidance is the development of robust readouts with predefined cutoff values for drug sensitivity to allow the translation of heterogeneous intra- and inter-patient drug responses.

Organoids have lots of potential for basic and translational cancer research, and for precision oncology in general.

The hope is that organoids will someday serve as "patient avatars" that help match treatments to the molecular specifics of individual tumors.



What *don't* organoids have that would make them an even more attractive model?

Answer:
innervation
blood vessels
(usually) an immune system