Normal Tissue and Tumor Kinetics

A. Models for the Proliferative Organization of Normal Tissues

1) normal tissues reactions to irradiation vary both in severity (related to dose, i.e., the radiosensitivity of component cells) and in the timing when the tissue damage is manifest (related to the **turnover kinetics** of the tissue)

a] *"turnover kinetics" refers to how, and at what rate, a tissue responds to damage, and the way in which it replaces lost cells (if at all)*

b] tissues that replace lost cells quickly exhibit early reactions, and those that replace lost cells more slowly, or barely at all, show late reactions

2) How long has it been known that the proliferative status of tissues governs radioresponsiveness?

Nearly 120 years!

a] **Bergonié and Tribondeau** were two French scientists working in the very early days of radiation therapy (1906), who formulated basic "laws" governing the behavior of tissues after irradiation; ***they predicted that tissues would be "radiosensitive"*** (not exactly the correct terminology..."radioresponsive" would have been a better choice in retrospect) ***if the cells comprising that tissue:***

1} **had a high mitotic rate** (i.e., rapidly dividing)

2} **had a long mitotic future** (i.e., that under normal circumstances, the cells would be capable of dividing indefinitely)

3} **were of the primitive, undifferentiated type** (like stem cells)

b] *these laws, although simplistic by today's standards, nevertheless can still be used as general rules of thumb for predicting the radioresponsiveness of tissues, and moreover, formed the basis of a tissue classification system developed decades later that is still in use (by some) today*

3) **The Rubin and Casarett Tissue Classification System** - developed during the 1960's and based on the original ideas of Bergonié and Tribondeau

a] in this model, tissues are classified as being one of four "types" based on their state of differentiation and proliferation kinetics

VIM or "Class I" cells = **"vegetative intermitotic" cells, i.e., undifferentiated stem cells**

DIM or "Class II" cells = **"differentiating intermitotic" cells, i.e., cells that are on the path to terminal differentiation, but aren't quite there yet** and are still capable of cell division (by today's terminology, these would be called "progenitor" or "transit-amplifying" cells)

RPM or "Class III" cells = **"reverting post-mitotic" cells,** i.e. terminally differentiated, functioning cells of a tissue that, under certain circumstances, can temporarily "revert" to a less-differentiated state, and regain the ability to divide, usually for the purposes of replenishing lost cells due to an injury

FPM or "Class IV" cells = "fixed post-mitotic" cells, i.e., cells that are terminally differentiated and incapable of division, even under pathological conditions

CHARACTERISTICS AND RADIOSENSITIVITY OF CELL POPULATIONS

CELL TYPE	CHARACTERISTICS	EXAMPLES	RADIOSENSITIVITY
VIM	Rapidly dividing; undifferentiated; do not differentiate between divisions	Type A spermatogonia Most primitive bone marrow cells Crypt cells of intestines Basal cells of epidermis	Most radiosensitive
DIM	Actively dividing; more differentiated than VIMs; differentiate between divisions	Intermediate spermatogonia Oocytes and spermatocytes Intermediate keratinocytes	Relatively radiosensitive
RPM	Do not normally divide but retain capability of division; differentiated	Hepatocytes Lymphocytes* Some salivary gland cells ? Kidney tubule cells ? ? Fibroblasts ?	Relatively radioresistant
FPM	Do not divide; differentiated	Neurons Myocytes, Osteocytes Erythrocytes	Most radioresistant

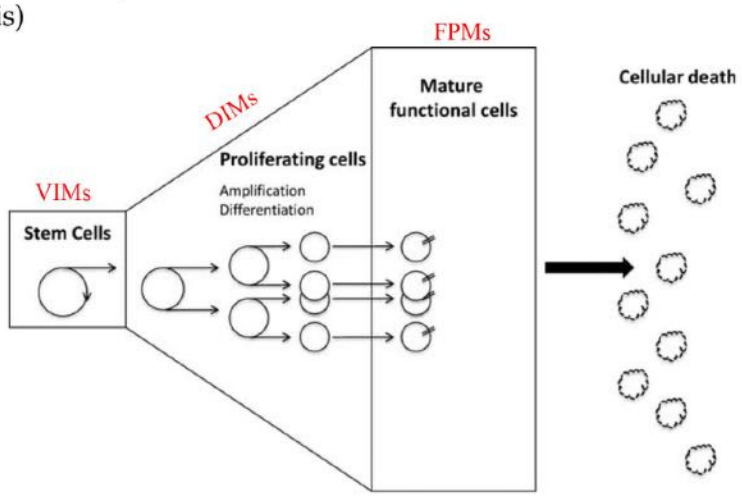
*Lymphocytes, although classified as relatively radioresistant by their characteristics, are very radiosensitive.

4) **The Michalowski Tissue Classification System** - a simplification of the VIM-DIM-RPM-FPM system

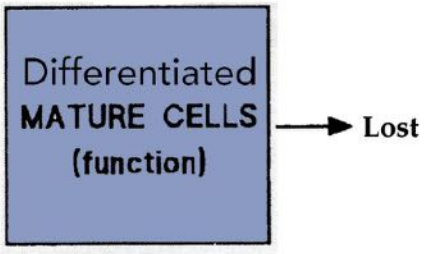
a] this system classifies tissues according to whether they *clearly* contain stem cells, transit cells and mature, functioning cells, or whether they appear to only (or mostly) contain mature, functioning cells (technical name: parenchymal cells)

b] ***"Type H" or Hierarchical Tissues*** - contain, stem, transit and mature cells, each of which is discretely located in its own "compartment" within the tissue, and for which the cells only move from primitive to more mature, but not backwards (examples: bone marrow, intestinal epithelium, epidermis of the skin, ovary and testis)

1. compared to the Rubin and Casarett system, Michalowski's Type H tissues are composed of VIM's, DIM's and FPM's

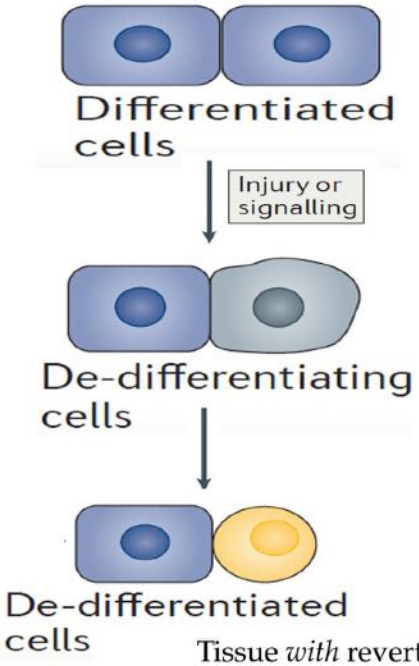


c] ***"Type F" or Flexible Tissues*** - show no strict hierarchy or histologically-identifiable compartments, but only appear to be composed of differentiated, functioning cells (examples: brain, muscle, bone, most mature blood elements), or else, in a few cases (examples: liver, lymphocytes, maybe kidney), mature cells that can revert to stem cells and back in response to injury



Tissue with *no* reverting behavior

(FPMs)



(RPMs)

Tissue *with* reverting behavior in response to injury or other stimulus

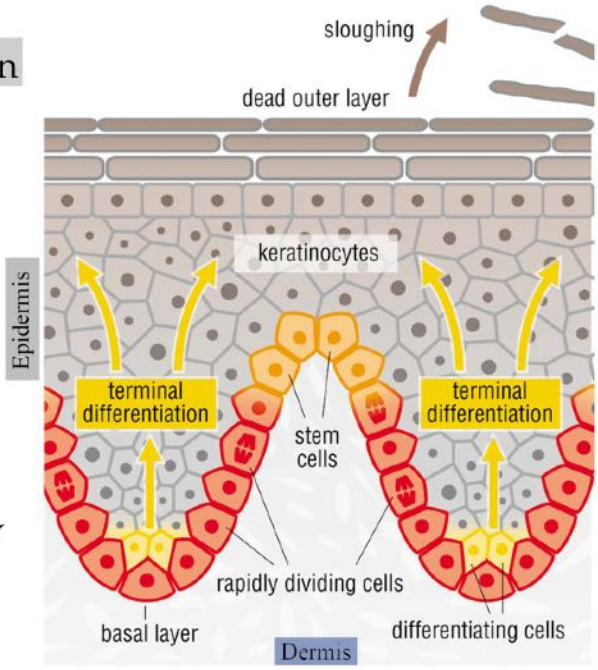
1. compared to the Rubin and Casarett system, Michalowski's Type F tissues are composed of RPM's and FPM's

B. Examples of Hierarchical Tissues

1) for these types of tissues, it is first necessary to identify the critical **"target cell"**, that is, the cell whose death is largely responsible for the subsequent radiation-induced tissue injury (a simplification of the real world, of course)

while the severity of the tissue injury is a function of the dose, in most cases (unless the dose is truly huge), the onset time for the appearance of the tissue injury is independent of dose and instead, depends on the natural rate of the flow of cells from stem cell to transit cell to mature cells

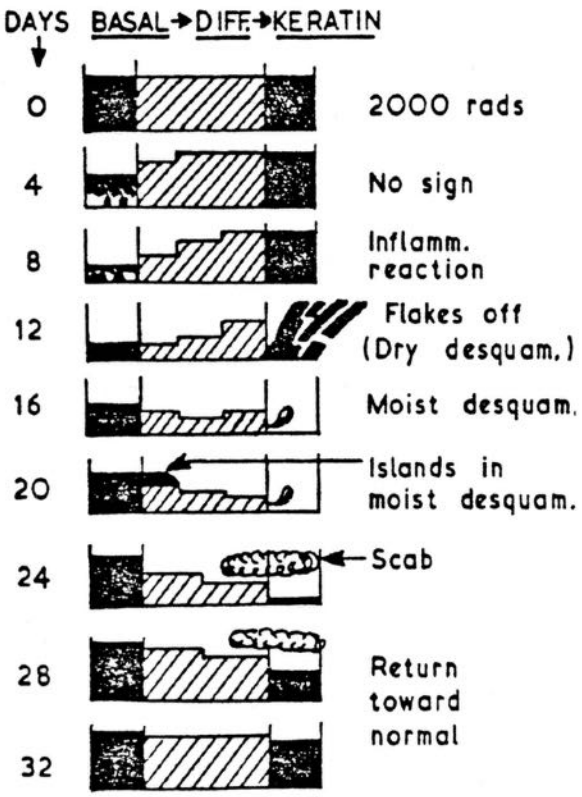
Skin Before Irradiation



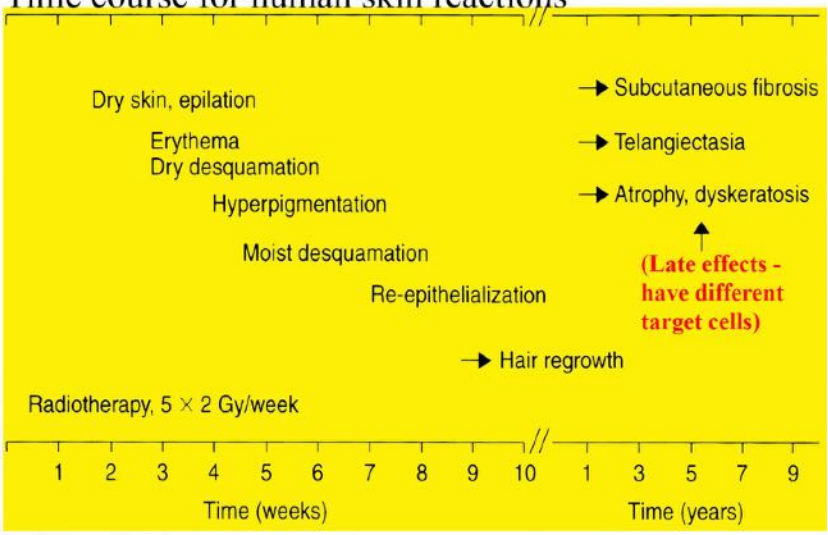
Based on the Laws of Bergonie and Tribondeau, for a hierarchical tissue, it is usually the tissue's stem cells that are the most radiosensitive...in this case, the basal cells of the epidermis

Target Cell: the basal stem cells of the epidermis

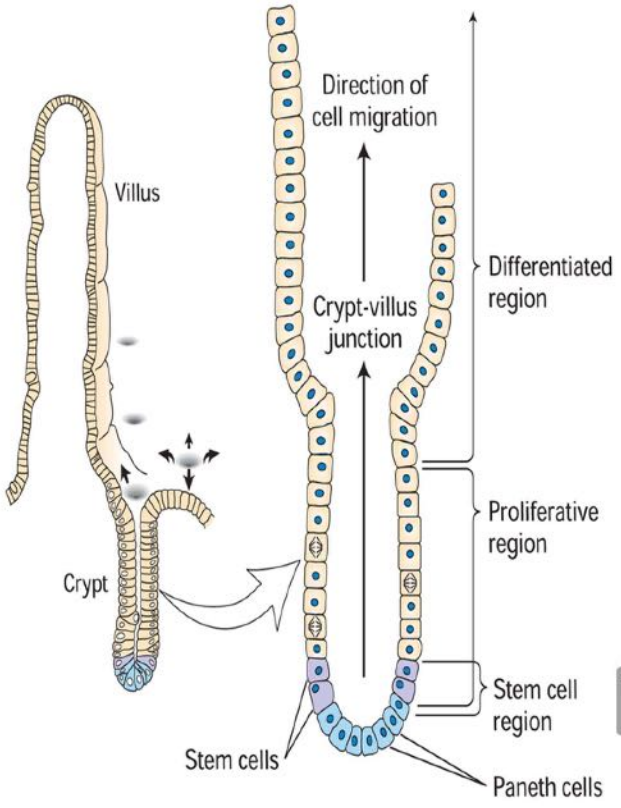
Skin After Irradiation (Mouse)



Time course for human skin reactions



Intestinal Villi Before Irradiation



Schematic drawing of the lining of the small intestine, which contains numerous villi formed from a column of cells. These epithelial cells are born near the base of pits (crypts) located between the villi. Located at the very bottom of crypts are Paneth cells, a type of support cell; just above these are four to six stem cells, which divide about once a day, forming precursor cells that also actively divide. As the differentiated cells enter the epithelium of a villus, they stop dividing and begin taking up nutrients from the gut.

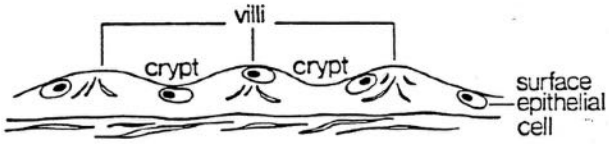
Target Cell: the "crypt" cells at the bottom of the villus

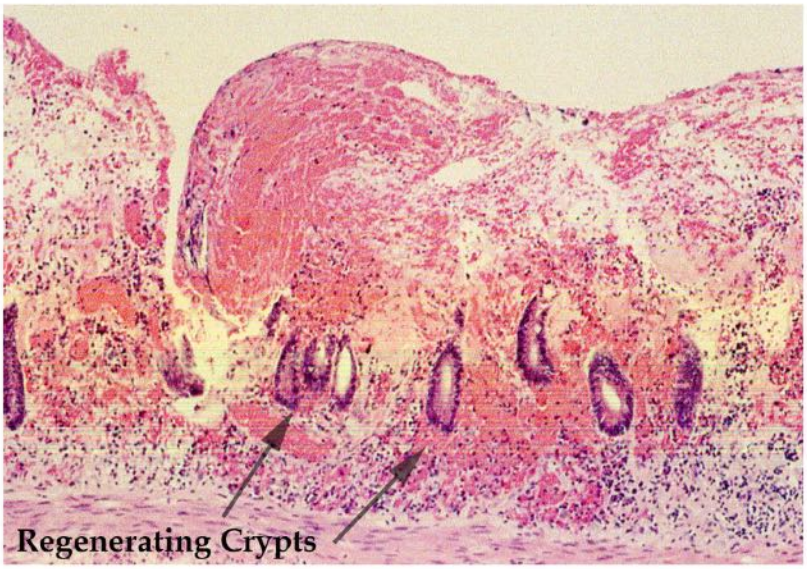
C. S. Potten, 1998, *Philos. Trans. R. Soc. London, Ser. B* **353**:821.

Intestinal Villi after Irradiation

As the epithelial cells that line the surface of the villi are lost, the villi eventually collapse...even though any surviving crypt cells may already be regenerating by this time

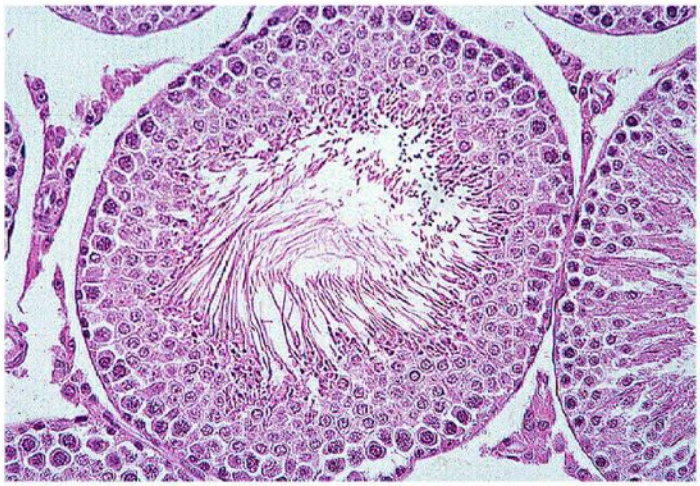
(No surviving crypt cells = no recovery possible)



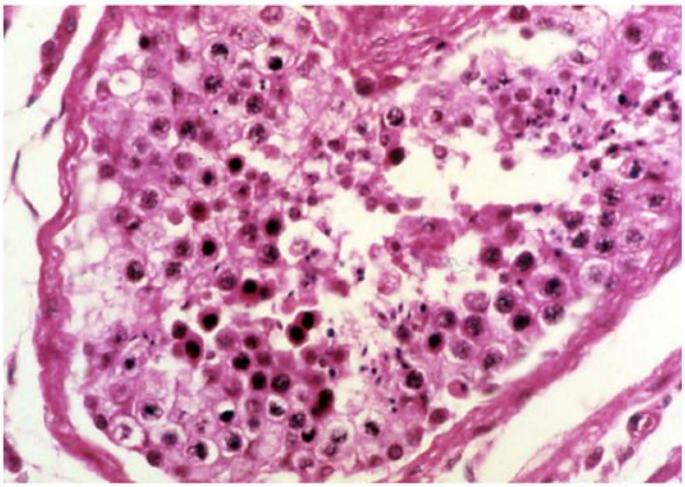


Seminiferous Tubules of the Testis

Before



After



Target Cell = spermatogonium, the undifferentiated precursor of more mature forms of the sperm lineage (i.e., spermatogonium - spermatocyte - spermatid - spermatozoa)

Note: ALL cells of the sperm (and egg) lineage are extremely radiosensitive, so it barely matters that the spermatogonium are technically the *most* sensitive

2) summary for hierarchical tissues

Gut Lining = transit time from a crypt stem cell to a mature cell sloughed off the upper surface of the villus is around 7-10 days; therefore, the radiation injury starts to manifest itself in approximately half that time

Epidermis of the Skin = transit time from a basal stem cell to a mature keratinized cell being sloughed off the skin surface is around 30-40 days; therefore, radiation-induced skin reactions start to become troublesome starting at about the 2-3 week point after (or during) irradiation [one note: oral mucosa, which is obviously skin-like, turns over a little faster than surface skin, such that mucositis starts to be apparent in less than 2 weeks)

Bone Marrow = transit time from a primitive bone marrow precursor to the loss from wear-and-tear of a mature, circulating blood cell normally takes from 60-90 days (depends on the exact blood cell type); so, the nadir in peripheral blood counts from the loss of the particular stem cell would be manifest in 4-6 weeks

Sperm Production in the Testes = normal transit time from a Type A spermatogonium to a mature spermatozoa takes around 60-90 days; however, because cell production is staggered, and many cells are lost naturally during the differentiation process, the nadir in sperm count actually takes a little longer than might otherwise be expected, i.e., about 60-70 days

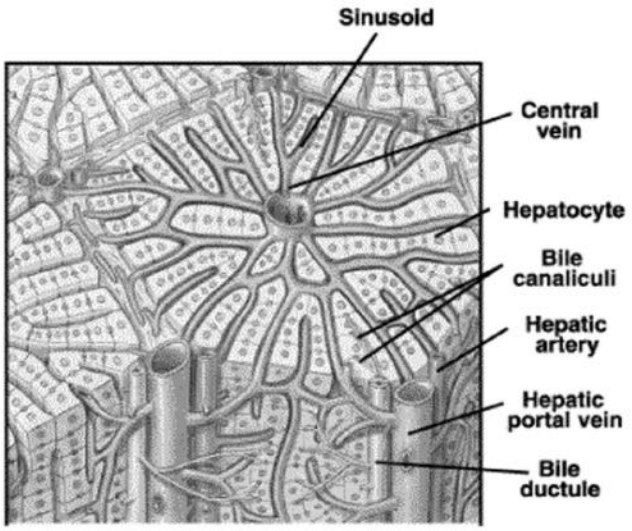
C. Examples of Flexible Tissues

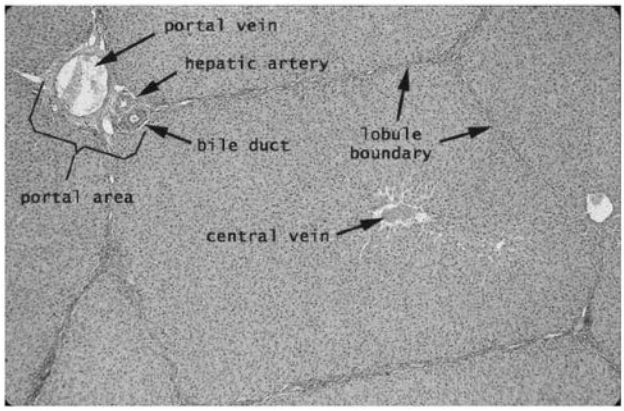
1) *for flexible tissues that proliferate very slowly if at all, the identification of specific target cells is more difficult, and often, may involve interactions between different types of cells that comprise the tissue*

a] *because of this, it is also harder to predict when the overt tissue injury will manifest itself, and often, it does depend on both the dose, the volume of the tissue irradiated, and "extenuating circumstances"*, i.e., if a separate injury unrelated to the radiation exposure helps precipitate the radiation reaction (think: osteoradionecrosis of the mandible years after radiotherapy brought on by a dental procedure)

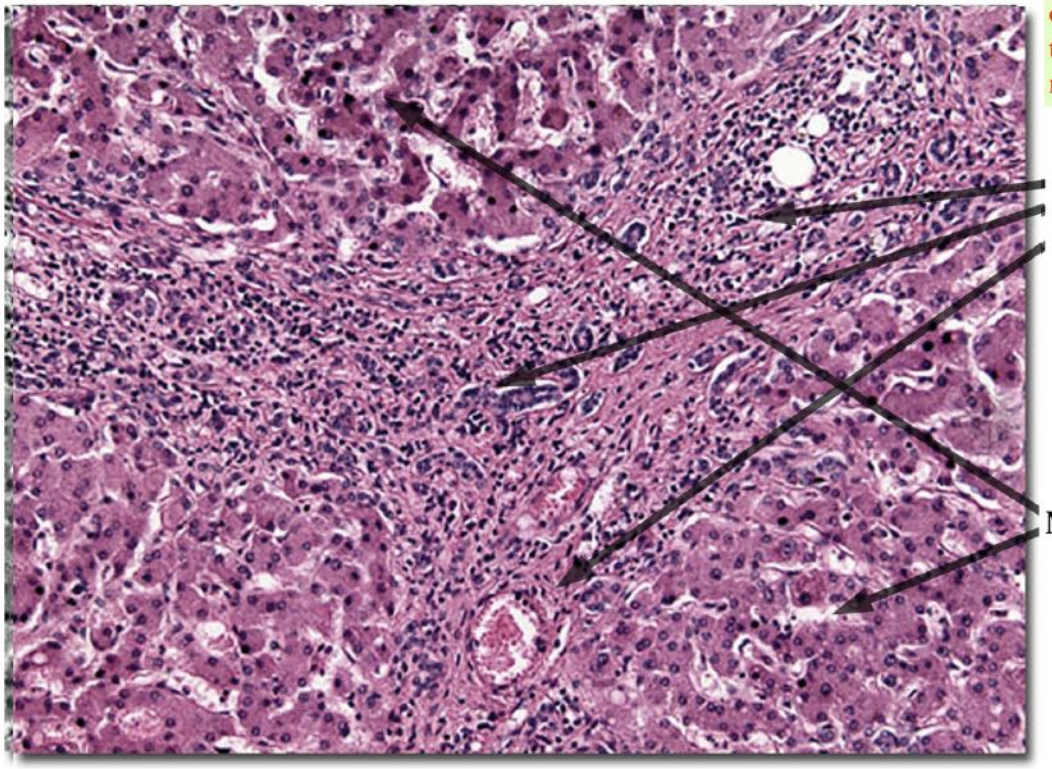
Liver Prior to Irradiation

Target Cell: the hepatocytes (mature liver cells) or the blood vessel cells or ????



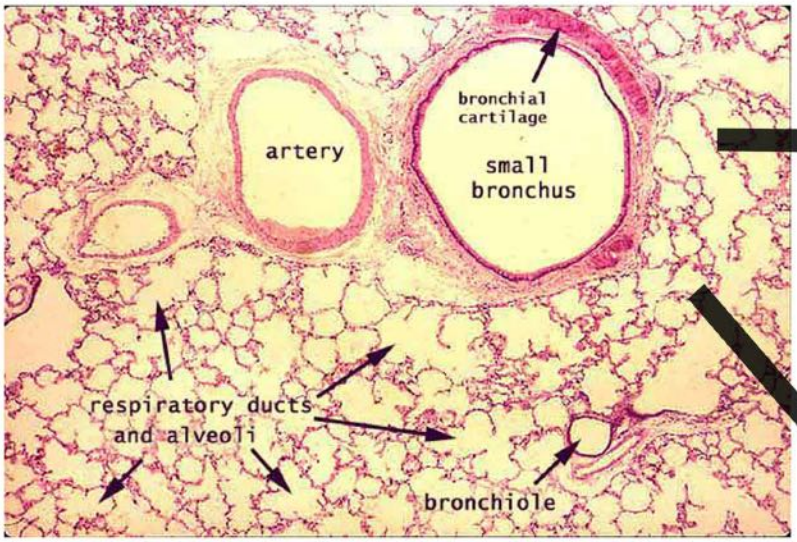


Liver after Irradiation

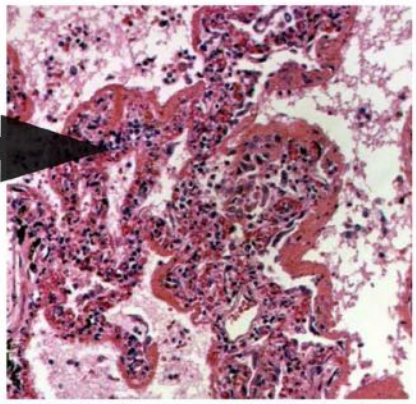


In flexible tissues, it is common for fibrosis to fill in the gaps left by the loss of mature, non-proliferating cells

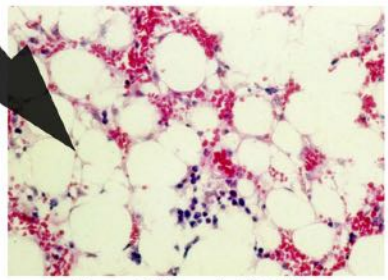
Lung Prior to Irradiation



Lung after Irradiation

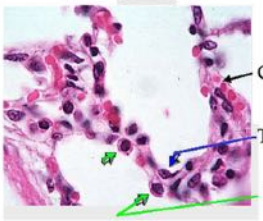


Radiation Pneumonitis (early)



Lung Fibrosis (late)

Alveoli

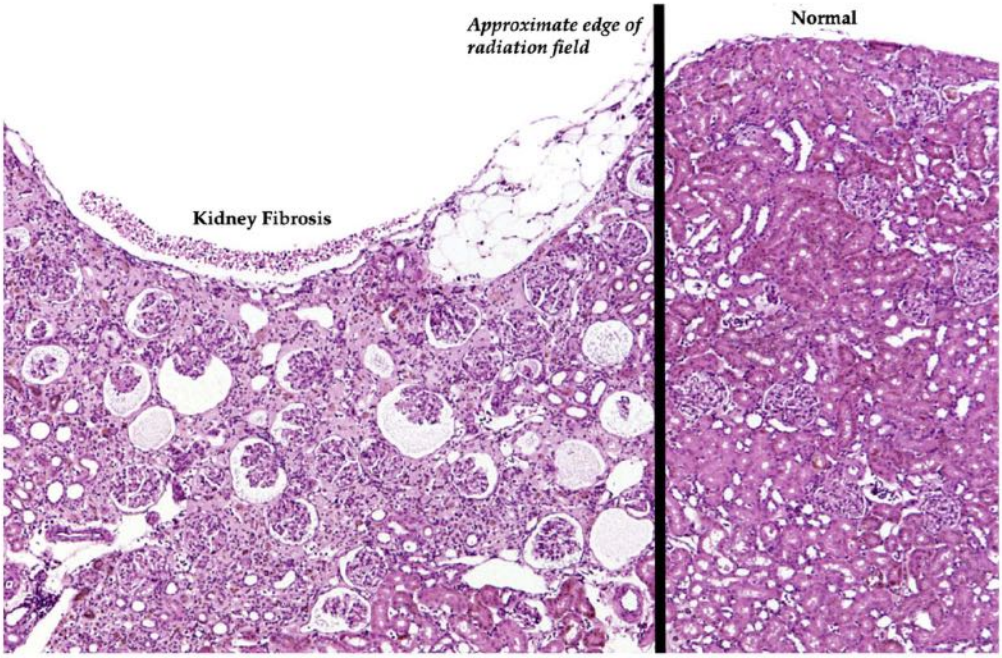


Like skin, the lung can show two different types of radiation responses, one early and one late. The exact target cells remain somewhat unclear though.

Target Cell = Type 1 or 2 pneumocytes or lung capillaries or ?

Cross section through an irradiated kidney showing a distinct boundary between dense fibrosis and atrophied or lost glomeruli and tubules in the irradiated area, next to normal kidney parenchyma

Target Cell = epithelial cells lining renal tubules (probably)



Thickening of glomeruli, thining of the linings of the renal tubules, and massive bleeding and fibrosis are seen in a heavily irradiated mouse kidney

D. **Growth Characteristics of (Already Well-Established) Tumors** – *because of all the abnormalities characteristic of tumor cells, it is much harder to understand the growth patterns of tumors as a whole, especially compared to normal tissues that are much more predictable in their behavior*

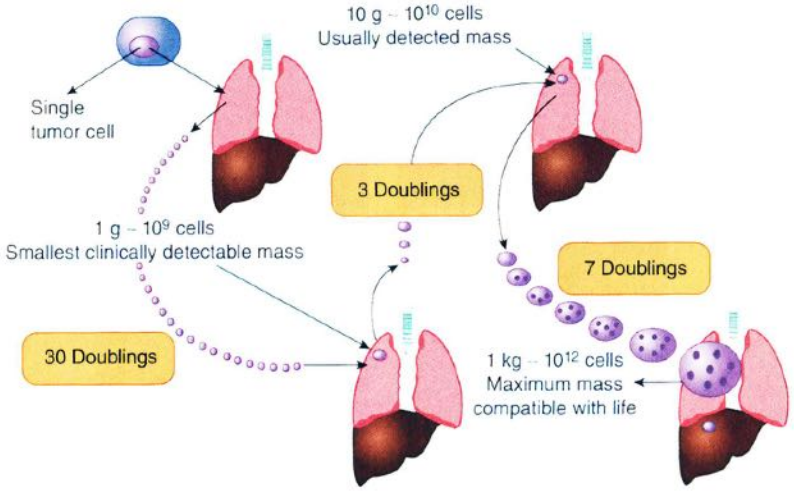
a) one thing that is clear however, is that **in tumors, cell production exceeds cell loss (although not necessarily by all that much)**

1. even though tumors continue to grow because of continuous gain of new cells, this doesn't necessarily mean that the tumor is growing rapidly or even that every cell in the tumor is capable of growing

b) because of this uncertainty, **we tend to describe tumor growth patterns according to size or volume changes in the tumor over time, such as "volume doubling time"**

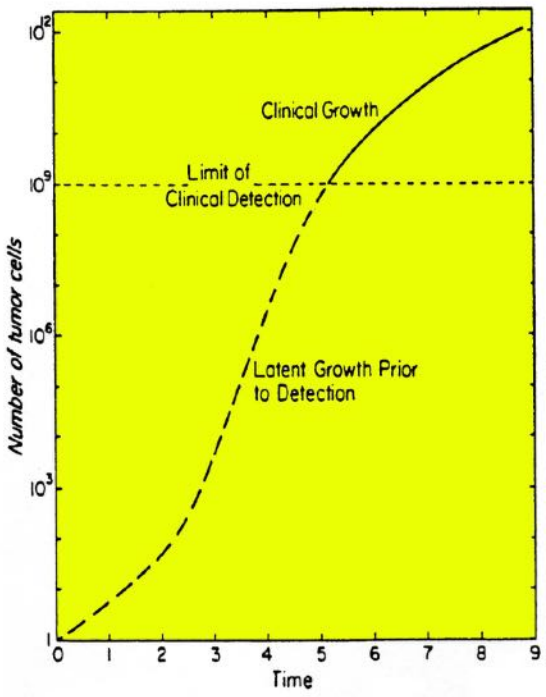
1. however, this can be somewhat deceiving in terms of what's going on with individual tumor cells, compared to the tumor as a whole

2. also, things get even more confusing when you realized that **most of the growth of tumors from a single cell to a clinically measurable tumor mass occurs prior to clinical detection** (*the clinically observable range of tumor growth is from about 10^9 cells to about 10^{12} cells, or about 30-40 cell divisions, and this is thought to represent only about 25% of the tumor's growth history*)!



A human solid tumor must undergo about 30 to 33 doublings in volume from a single cell before it achieves a detectable size of 1–10 g. Metastases may have been established prior to detection of the primary tumor. Only a few further doublings of volume lead to a tumor whose size is incompatible with life.

The Basic Science of Oncology, Sixth Edition (2021)

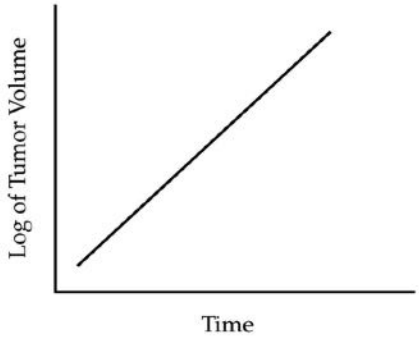


Hypothetical growth curve for a human tumor, showing the long latent period prior to detection. Tumors may show an early lag phase, and progressive slowing of growth at large size.

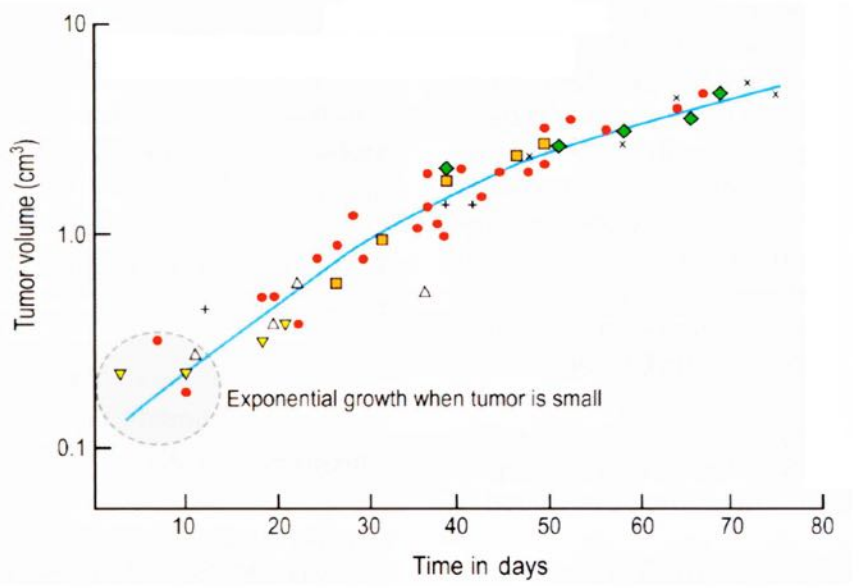
a} the only growth characteristics of the tumor that we can measure are in the tumor's "old age", and when it is a serious threat to the life of the host

c) keeping this in mind, what we do know is that tumors tend to increase in volume over time according to different growth patterns:

Exponential Growth: the log of the tumor volume increases linearly with time (more common in hematological malignancies, fast-growing tumors in rodents and in theory anyway, pre-detection human tumors and metastases)



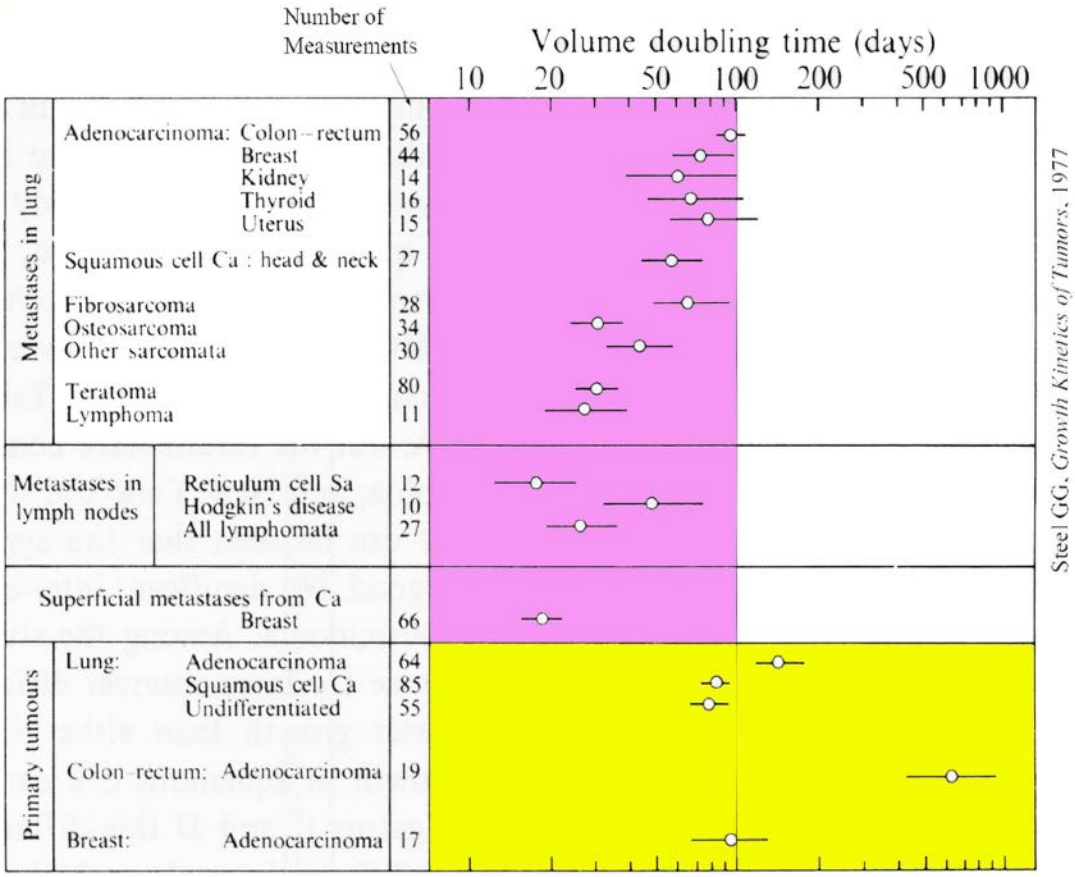
Gompertzian Growth: most common for human solid tumors – growth starts out roughly exponential, but progressively slows as the tumor gets bigger



d) another important point: ***although experimental (i.e., non-spontaneous, serially-transplantable) rodent tumors have been used historically to learn about the growth characteristics and cell cycle kinetic parameters of human tumors, the truth is that rodent tumors grow MUCH FASTER than nearly all human tumors and as such, may not be particularly representative of what is really going on in humans; most mouse tumors have volume doubling times in the 3-5 day range, sometimes even shorter***

Humans: Not Like Rodents

Volume doubling times for even the fastest growing human solid tumors are WAY longer than for mouse tumors!



For most solid tumors (carcinomas), the volume doubling time is ~3 months *(Best answer for Boards: 100 days).*

Metastases from these tumors typically grow faster than the primary tumor.

Sarcomas typically double slower than carcinomas; hematological cancers can grow much faster.

ALL of these numbers can be highly variable both within and between tumors.

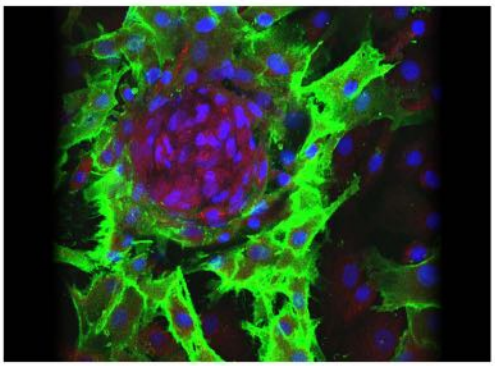
2. are tumors hierarchical, flexible, both or neither?

a] assuming we believe that **cancer stem cells** exist, *then tumors are probably closer to hierarchical than flexible tissues*, although these stem cells don't necessarily occupy a distinct anatomic compartment

b] however, they *do* seem to occupy a distinct physiological/microenvironmental compartment, termed the **stem cell niche**

A stem cell niche (blue = nuclei; red = stem cell specific proteins) embedded in a mass of cancer-associated fibroblasts (green).

Cancer stem cells are much easier to identify these days now that we know that they tend to express certain cell surface proteins that most of the rest of the tumor cells don't.



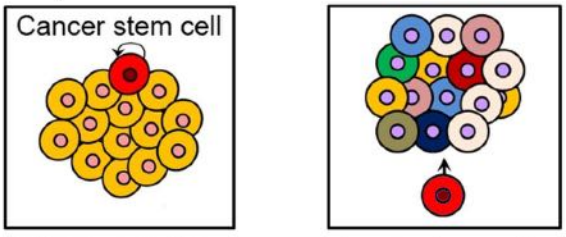
Useful stuff to know about cancer stem cells...

1, Where do they originate?

a. there are multiple theories as to where cancer stem cells come from, however the two most common are the **hierarchical model** and the **stochastic model** (sometimes termed the ***"plasticity"*** model); some kind of hybrid model is probably closest to reality though

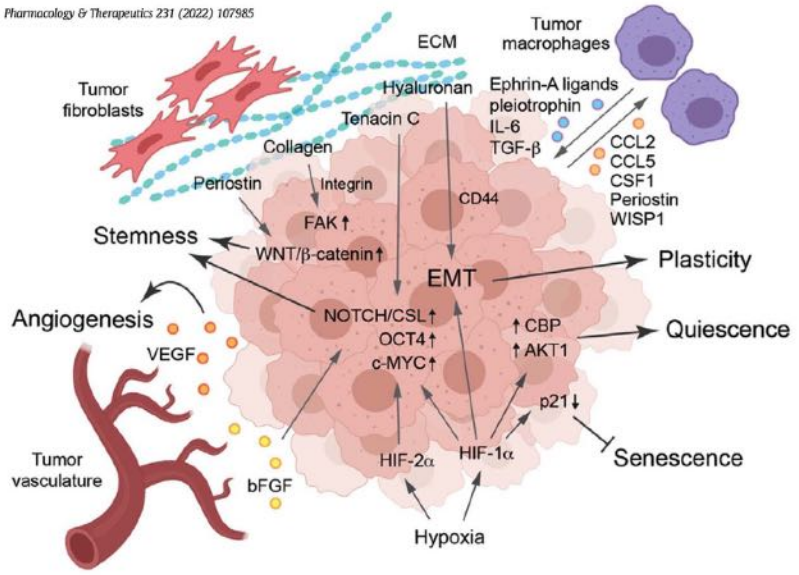
1) *the hierachical model imagines cancer stem cells to be similar to normal stem cells*, such as those found in hierarchical tissues like skin, gut or bone marrow - these are cells that are immortailized, and capable of both self-renewal and the generation of progeny (termed "transit amplifying" or "progenitor" cells) that gradually differentiate – at least to some extent – with each additional cell division

a] however unlike normal stem cells, tumor stem cells are genomically unstable, so all the progeny can end up being quite different from each other, which would help explain why tumors are so heterogeneous



2) *the stochastic model suggests that almost all the progenitor cells retain the ability to convert to cancer stem cells, sometimes at random, and at other times, in response to cues from the tumor microenvironment*, i.e., they remain "plastic"

a] What kinds of microenvironmental cues?

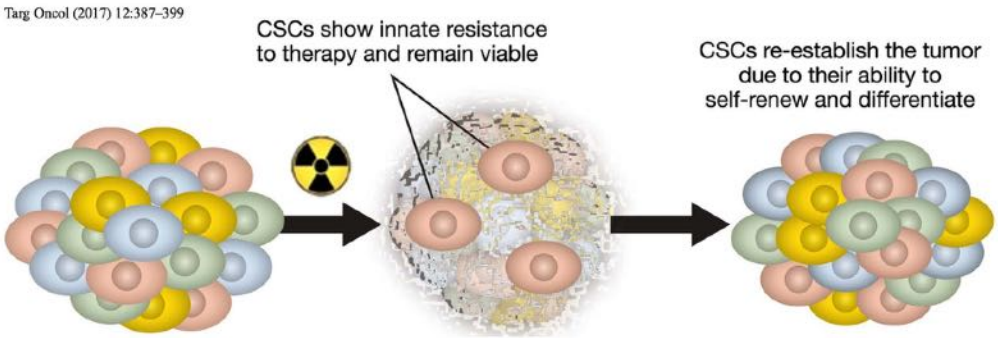


Tumour microenvironmental influences on liver cancer stem cells. The tumour-associated stroma has an important role in regulating cancer stemness in hepatocellular carcinoma. Regulation of cancer stem cells (CSCs) by their niche operates through cell–cell interactions, secreted factors, cell–matrix interactions and the biophysical properties of the niche. The most prominent players in liver CSC–stroma interactions include biophysical properties, hypoxia, nutrient (particularly glucose) deprivation and extracellular matrix (ECM) remodelling.

- Death of lots of their siblings
- Hypoxia and/or low pH
- Expression of specific cytokines
- Chronic inflammation
- Too many reactive oxygen species
- Traveling through the bloodstream to a new metastatic site

2. Why do we care about cancer stem cells?

a) because they are likely responsible for treatment failure, i.e., their progeny are killed, but they aren't

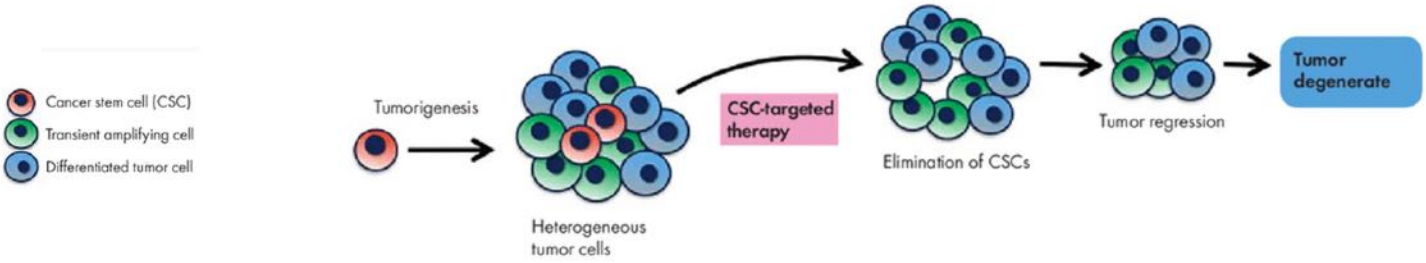


1] this suggests that **they must be at least somewhat resistant to radiation and/or chemotherapies - how?**

- conditions in the stem cell niche are such that they're mostly in a quiescent state (unless mobilized)
- in some cases, the niches are hypoxic, as well as less accessible to drugs
- in some cases, cancer stem cells contain higher concentrations of radical scavenging agents
- in some cases, they contain more multi-drug resistance transporters
- in some cases, they are better at initiating the DDR and repairing DNA than their progeny

2] this also suggests that **our current therapies must be targeting the progenitors, and not the cancer stem cells**

b) so **what we need are cancer stem cell-directed therapies**...any suggestions?



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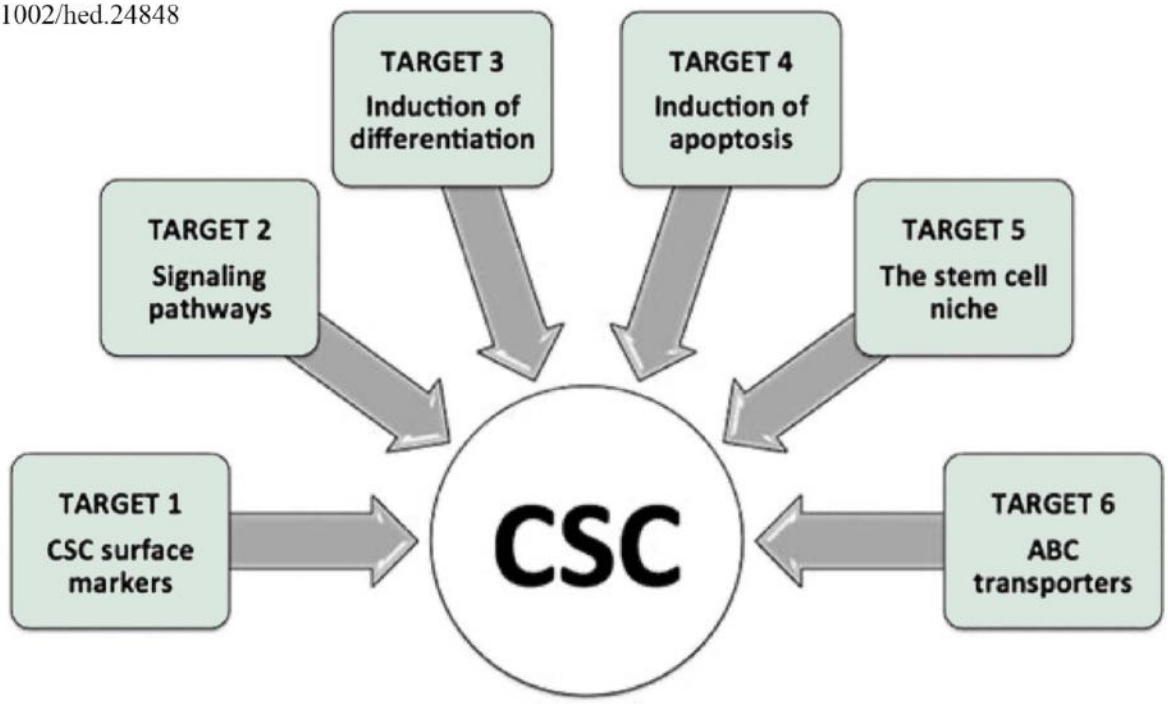


Targeting of cancer stem cells (CSCs) by novel therapies provides future directions that may potentially sensitize these cells to radiation, prevent recurrence of the cancer, and improve the survival rates.

E. Cell Cycle Kinetics of Tumors

1) when tumors are irradiated, they can...

- Continue to grow
- Continue to grow, but more slowly
- Stop growing (but not shrink), either permanently or temporarily
- Shrink to varying extents, either permanently or temporarily
- Shrink to below the limits of clinical detection, but grow back later
- Shrink to below the limits of clinical detection, and never grow back

...but what determines which of these actually occurs in a given situation?

a. Answer: it's impossible to predict, especially if the only thing you have to go on is gross measurements of tumor volume over time under pre-treatment conditions (and never mind that long-term observation of tumor growth patterns in the absence of treatment would be considered malpractice!)

2) because of this, it is necessary to know more about the behavior of tumor *cells*, rather than of the tumor as a whole...although this necessarily would require an invasive procedure (one or more biopsies) to determine

3) so, what is it about the cell cycle kinetics of tumor cells that would be useful to know?

a. ***T_C - the cell cycle time of the constituent tumor cells that are actively going through their cycles***

based on a cohort of 41 human tumor types (with each type sampled within and between multiple tumors), cell cycle times *in vivo* ranged from about **15 to 150 hours**

these values for Tc tend to be MUCH longer than for the same types of cells cultured *in vitro*

b. the ***cell cycle phase durations***, T_{G1}, T_S, T_{G2} and T_M, that together comprise T_C

1] *the duration of S phase (T_S) could be important as it might provide a rough estimate of radiation resistance of one tumor compared to another (because longer S phase durations = more S phase cells = more resistant)*

based on the same human tumor cohort, the **duration of S-phase *in vivo* varied between about 10 and 24 hours**

c. the (S phase) ***labeling index or LI - estimates how many cells are in the S phase of the cell cycle either instantaneously like a snapshot (pulse labeling index), or else that pass through S phase over an extended period of time (continuous labeling index)***

pulse labeling indices in human tumors varied from <1% to as high as ~35%

1. *a tumor with a high pulse labeling contains more S phase cells, meaning that the tumor as a whole has more cycling cells in it than one with a lower labeling index*

d. the ***growth fraction (GF) - the fraction/percentage of cells in a tumor that are actively going through the cell cycle (and that are assumed to be the clonogenic ones)***

Contrary to popular belief, on average, no more than about half of the cells in any given tumor are actively proliferating...

...this is no small part of the reason why the volume doubling time of an average solid tumor is months, whereas the cycle time of its individual cells is on the order of days

The Growth Fraction (GF) for Some Tumors in Experimental Animals

TUMOR	AUTHOR	GF(%)
Primary mammary carcinoma in the mouse (C_3H)	Mendelsohn	35–77
Transplantable sarcoma in the rat (RIB_5)	Denekamp	55
Transplantable sarcoma in the rat (SSO)	Denekamp	47
Transplantable sarcoma in the rat (SSB_1)	Denekamp	39
Mammary carcinoma in the mouse (C_3H)	Denekamp	30
Chemically induced carcinoma in the hamster cheek pouch	Brown	29

2. so, if the growth fraction of a solid tumor averages around 50%, what are the *other* 50% of tumor cells doing?

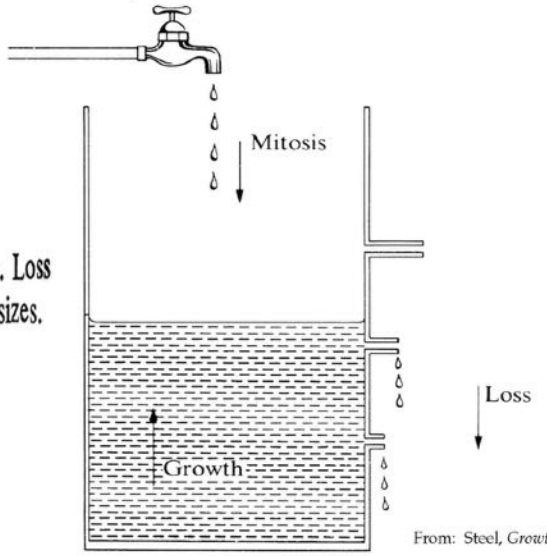
(a) at bare minimum, they have very long cell cycle times (if they're cycling at all)

1) possible reasons for not being in the cell cycle:

- quiescence secondary to nutrient or oxygen deprivation
- cellular senescence or differentiation
- cells clonogenically dead, yet still physically present

e. ***the cell loss factor, ϕ, - the fraction of cells physically lost from the tumor – for whatever reason – and that therefore never contribute to its volume increase over time***

1. the cell loss factor is a derived parameter based on knowledge of some of the other cell kinetic parameters for the tumor in question; as such, it cannot be directly measured



The interrelationship of cell production by mitosis, cell loss, and growth. Loss may occur by various mechanisms that come into play at different tumour sizes.

From: Steel, *Growth Kinetics of Tumors*, 1977

Calculation of CLFs for human tumours based on labelling with radiolabelled thymidine (*) or thymidine analogues (**) and volume doubling times, in separate series

Site	LI (%)	T_{pot} (days)	VDT (days)	CLF (%)
Undifferentiated bronchus Ca*,a	19.0	2.5	90	97
Sarcoma*,a	2.0	23.3	39	40
Childhood tumours*,a	13.0	3.6	20	82
Lymphoma*,a	3.0	15.6	22	29
Head and neck**,b	9.6	4.1	45	91
Colorectal**,b	13.1	3.9	90	96
Melanoma**,b	4.2	8.5	52	84
Breast**,b,c	3.7	9.4	82	89
Prostate**b,d	1.4	28.0	1100	97

[a] calculations assume $T_S = 14$ h, $\lambda = 0.8$.

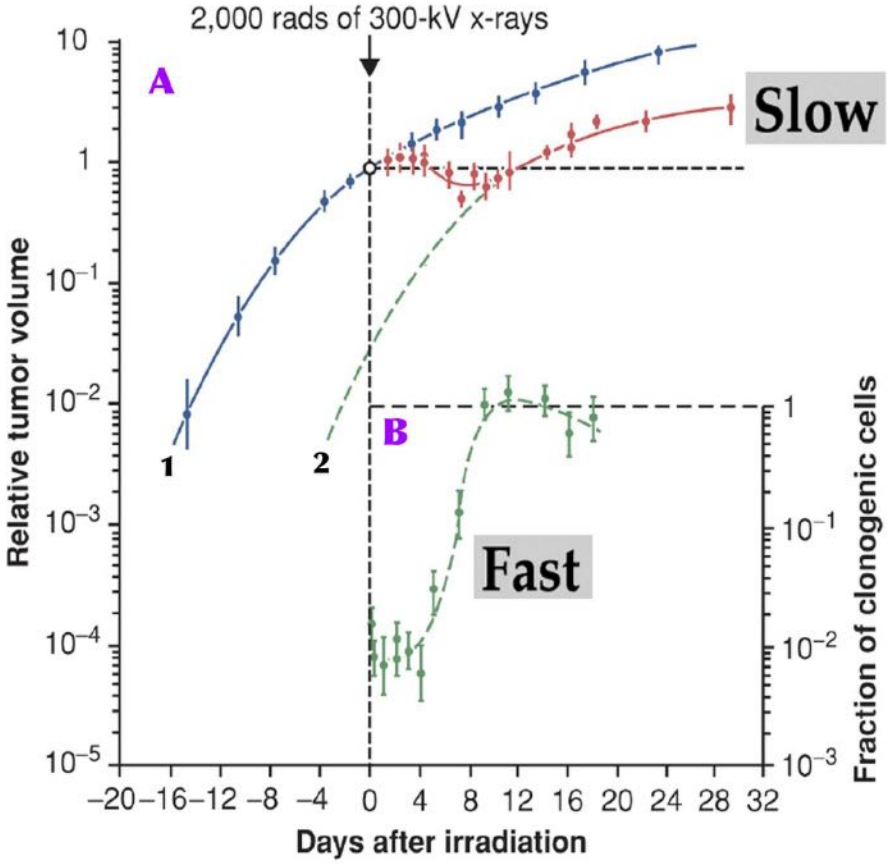
[b] LI, T_S, and T_{pot}; calculations assume $\lambda = 0.8$

[c] VDT values for pulmonary metastases

Cell loss factors for human tumors are VERY high, especially for carcinomas, averaging around 0.9 (90%).

Cell loss is the single largest contributor to the discrepancy between cell cycle times of proliferating tumor cells and the tumor's volume doubling time.

Another ramification of this is that the behavior of the tumor as a whole (such as, fairly slow shrinkage during the course of radiotherapy) can completely mask the presence of a small subset of cells growing rapidly (such as, tumor clonogens undergoing accelerated repopulation).



Classic example (translation: the *only* example) illustrating the rapid growth of clonogenic tumor cells after irradiation being masked by slow growth of the tumor mass overall.

Accelerated repopulation. Growth curves of a rat rhabdomyosarcoma showing the shrinkage, growth delay, and subsequent recurrence following treatment with a single dose of 20 Gy (2,000 rad) of x-rays. **A:** Curve 1: Growth curve of unirradiated control tumors. Curve 2: Growth curve of tumors irradiated at time t = 0, showing tumor shrinkage and recurrence. **B:** Variation of the fraction of clonogenic cells as a function of time after irradiation, obtained by removing cells from the tumor and assaying for colony formation in vitro. (From Hermens AF, Barendsen GW: Eur J Cancer 5:173–189, 1969)

2. where have all the "lost" cells gone?

- exfoliation from the tumor surface
- loss of cells via the blood stream or lymphatics*
- tumor cell death (most important):
  mitotic catastrophe, apoptosis, autophagy
  necrosis secondary to nutrient or oxygen deprivation
  immunological attack

**only a tiny fraction of which can go on to seed metastases (likely no more than 1-2 per 10,000 cells)*

e. ***the potential doubling time (affectionately known as T_{pot}) - the time it would take a tumor to double its cell number in the absence of cell loss***

1. T_{pot} is also a derived parameter (like the cell loss factor), and needs to be calculated based on values for other kinetic parameters

$$T_{pot} = \lambda \times T_S / LI$$

2. **T_{pot} had been a clinical (and radiobiological) favorite for many years, because it already has built into it a "correction" for the sometimes-misleading effects of cell loss; in that way, it gives probably the best indication of the rate at which what are thought to be the most critical tumor cells – the clonogenic ones that ultimately need to be killed – are doubling**

3. T_{pot} does, admittedly, have a clinical downside though, and that is that it is a PRETREATMENT kinetic measurement, and therefore, not necessarily representative of what is going on kinetically once radiotherapy is underway

a) because it is difficult to obtain and analyze biopsies during treatment, **a surrogate for Tpot has been proposed called the effective clonogen doubling time or T_{eff} (more recently termed "T_p")**

for example, several studies have shown that T_{eff} approaches T_{pot} toward the end of fractionated radiotherapy, suggesting that much of the accelerated repopulation of tumor clonogens ramps up toward the end of treatment, rather than from the outset

Cell kinetic parameters of human tumours derived from in vivo *labelling with Br-dUrd or I-dUrd and measured by flow cytometry*

Site	No. of patients	LI (%)	T_s (h)	T_{pot} (d)
Head and neck	712	9.6 (6.8–20.0)	11.9 (8.8–16.1)	4.5 (1.8–5.9)
CNS	193	2.6 (2.1–3.0)	10.1 (4.5–16.7)	34.3 (5.4–63.2)
Upper intestinal	183	10.5 (4.9–19.0)	13.5 (9.8–17.2)	5.8 (4.3–9.8)
Breast	159	3.7 (3.2–4.2)	10.4 (8.7–12.0)	10.4 (8.2–12.5)
Ovarian	55	6.7	14.7	12.5
Cervix	159	9.8	12.8	4.8 (4.0–5.5)
Melanoma	24	4.2	10.7	7.2
Haematological	106	13.3 (6.1–27.7)	14.6 (12.1–16.2)	9.6 (2.3–18.1)
Bladder	19	2.5	6.2	17.1
Renal cell ca	2	4.3	9.5	11.3
Prostate	5	1.4	11.7	28.0

Data derived from Haustermans *et al.* (1997); Rew and Wilson (2000).
Ranges represent variations in median or mean values between studies; ranges for individual tumours are considerably larger.

Median value for $T_{pot} \approx 5$ days.

Anything shorter than this (and especially, anything under 4 days) is considered "fast-growing", and such a tumor might be a candidate for accelerated fractionation.

F. Prognostic or Predictive Value of Tumor Kinetic Parameters?

1) Short Answer: not really, especially in terms of clinical endpoints such as survival

a. why not?

• kinetic parameters are virtually ALL pretreatment values and as such, may not be representative of the dynamics of what is happening during and after radiotherapy

2) Long Answer: for particular tumor types and under particular treatment conditions, one or more cell cycle kinetic parameters can, at best, be used to reasonably stratify patients into different treatment groups, or to make very rough estimates of the likelihood of treatment success

Selected Examples:

Tumor Type	Survival Advantage for Patients with†	
	Diploid Tumors	Tumors with Low S-phase Fraction
Breast	+	+ +
Ovary	+ +	+
Head/neck		+
Lung	+	+
Colon/rectum	+	+
Stomach	+ +	+ +
Melanoma	+	+
Brain	−	
Lymphomas	±	+ +
Myeloma	+ +	
Leukemia		
Acute myelogenous	+	
Acute lymphoblastic	−	

Both DNA ploidy (diploid = better prognosis, aneuploid = worse prognosis) and S phase fraction (low labeling index = better prognosis, high labeing index = worse prognosis) are sometimes associated with a survival advantage for certain tumors, less so for other types, and not at all for others.

A multi-institutional European study of the predictive value of cell cycle kinetic parameters on local control in head and neck cancer. LI, but not T_s or T_{pot}, showed a significant association with local control.

From: Begg *et al.* Radiother Oncol *50*: 13-23, 1999

Appendix Materials

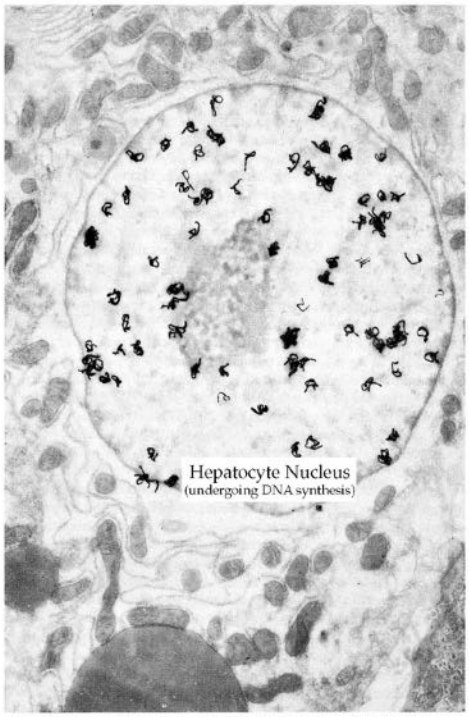
1. (Old school) techniques of cell cycle kinetics

2. Cell cycle kinetics cheat sheet

G. Techniques of Cell Cycle Kinetics

1) identification of S phase cells owing to their uptake of labeled DNA precursors

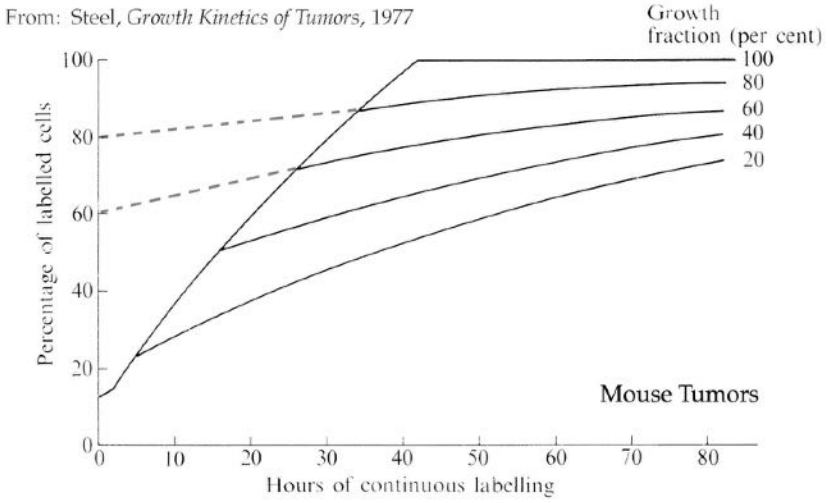
a. **tritiated thymidine (^{3}H-TdR) labeling** - needed for the determination of the cell cycle time, T_C, and individual cell cycle phase durations (see: percent labeled mitoses technique below); the labeling index, LI, and the growth fraction, GF



Electron micrograph of an autoradiograph of rat liver, labelled with ^{3}H-thymidine 20 h after partial hepatectomy.

From: Steel, *Growth Kinetics of Tumors*, 1977

The technique of autoradiography makes it possible to identify radio-labeled S phase cells based on their ability to expose a photographic emulsion.

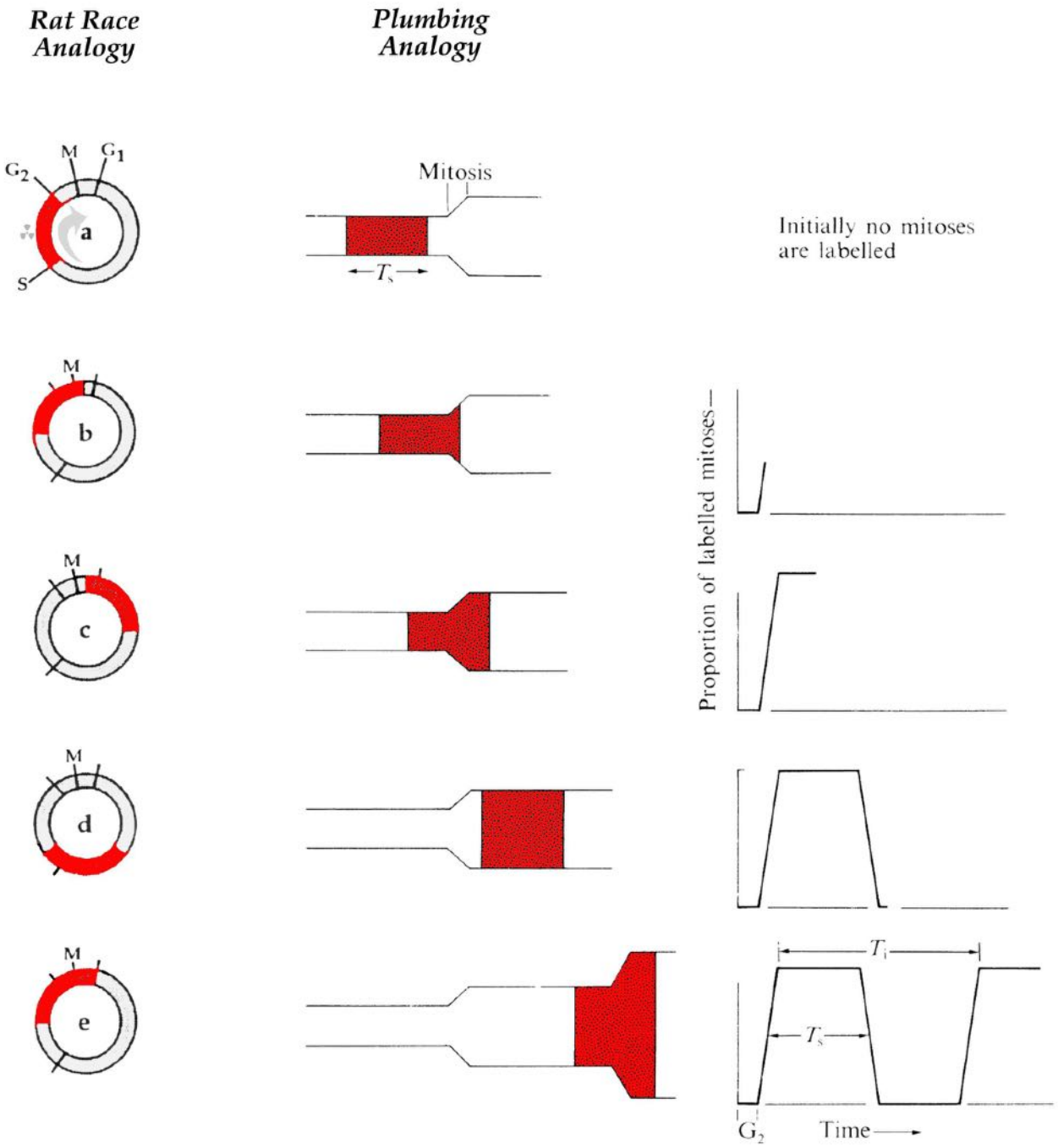


Theoretical continuous thymidine labelling curves

A tumor's growth fraction can be estimated from a continuous labeling experiment, provided the duration of labeling more than exceeds the estimated cell cycle time of the slowest growing tumor cell in the population.

1] **Percent Labeled Mitoses Technique** - the most accurate, and sadly, the most arduous, method of determining cell cycle phase durations (and the overall cell cycle time) for cells derived from a tumor *in vivo*

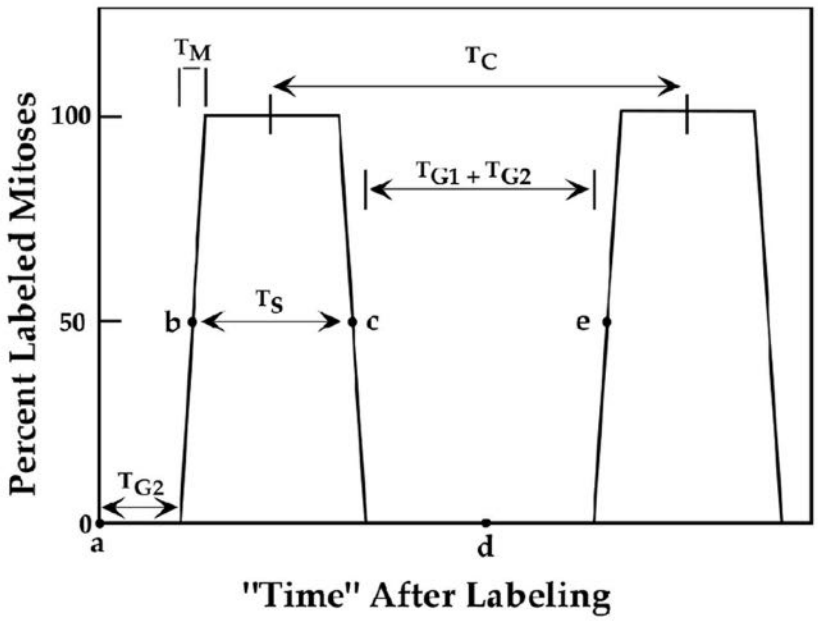
(a) the technique involves following a cohort of pulse-labeled S phase cells over time as they progress around the cell cycle, by detecting them autoradiographically as they enter mitosis



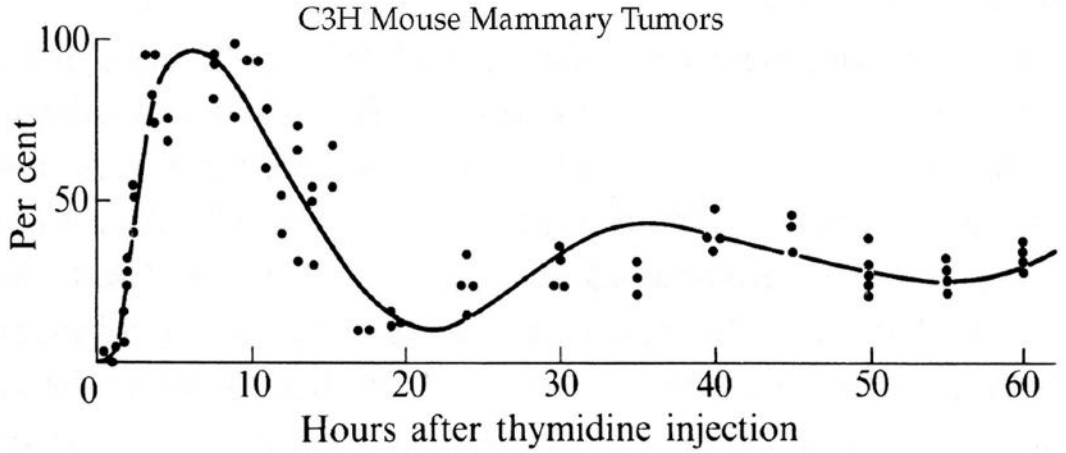
After a single administration of ^{3}H-thymidine the labelled cohort (the stippled area) moves through mitosis. As it does so, the proportion of labelled mitoses describes a peak whose width is equal to the duration of the DNA-synthetic period. Subsequently, the propotion of labelled mitoses peaks again; the peak-to-peak interval is equal to the inter-mitotic time (T_i).

From: Steel, *Growth Kinetics of Tumors*, 1977

Ideal World



Real World



From: Steel, *Growth Kinetics of Tumors*, 1977

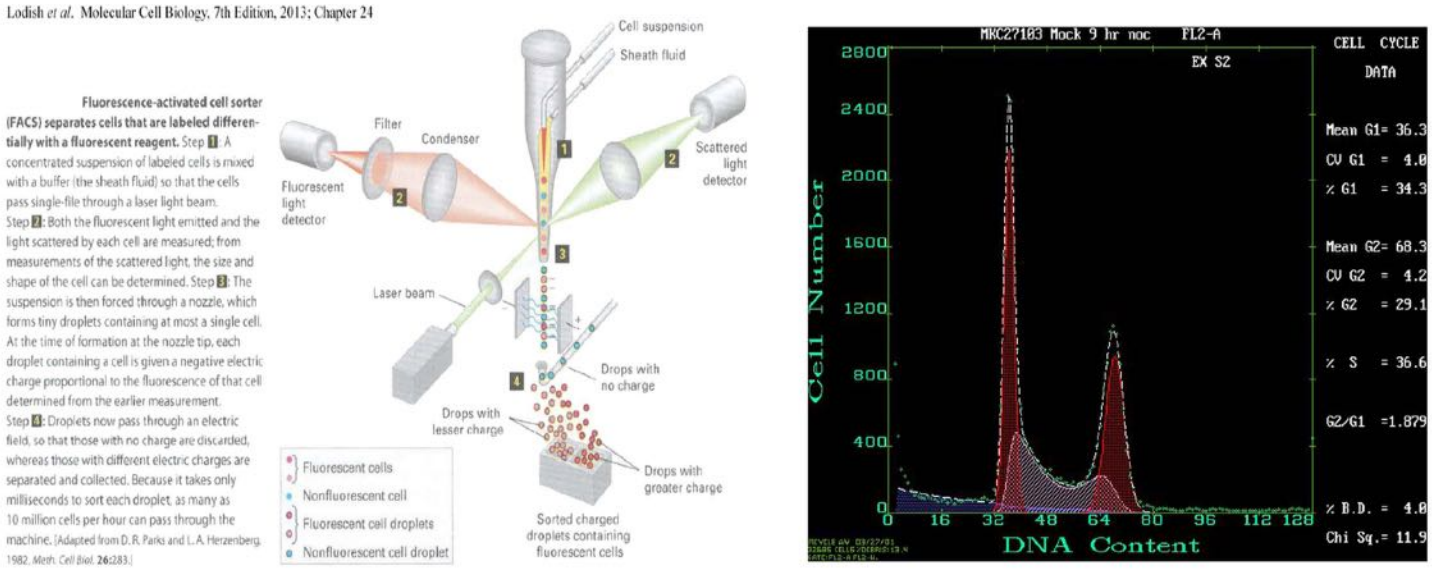
(b) although ideally, determining the cell cycle phase durations from a PLM curve would be easy, the situation is more complicated in the real world, where discerning peaks and troughs of the PLM curve can be difficult; in addition, subsequent waves of labeled mitoses are frequently "damped"

1] because of this, in practice, a mathematical expression is used to fit PLM data, and best-fit values for the various cell cycle parameters are then calculated

2) Flow Cytometry

a. flow cytometry is an analytical method of sorting and measuring types of cells by fluorescent labeling of one or more biomolecules on or in the cell; sometimes called "FACS" analysis (**f**luoresecence-**a**ctivated **c**ell **s**orting)

b. **DNA distribution analysis** - a fluorescent stain added to a cellular DNA extract binds to it in a quantitative manner, such that the amount of fluorescence is directly proportional to the amount of DNA; this type of flow cytometry can identify the four cell cycle phases (and G_0, indistinquishable from G_1) and their durations, ploidy measurements (diploid versus aneuploid), and to some extent, apoptosis (apoptotic cells have less than a diploid, variable DNA content)



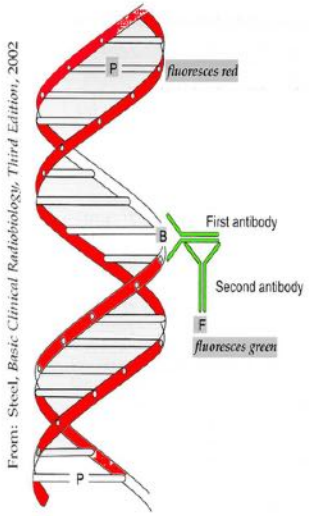
The principles of DNA distribution analysis of flow cytometry. Suspensions of fluorescent-stained single cells flow one at a time through a light beam with its wavelength adjusted to excite the fluorescent dye. The fluorescence stimulated in each cell is recorded as a measure of that cell's DNA content. Thousands of cells can be measured each second and the results accumulated to form a DNA distribution like that shown for asynchronously growing Chinese hamster ovary cells. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. Int J Radiat Biol 49:237–255, 1986.)

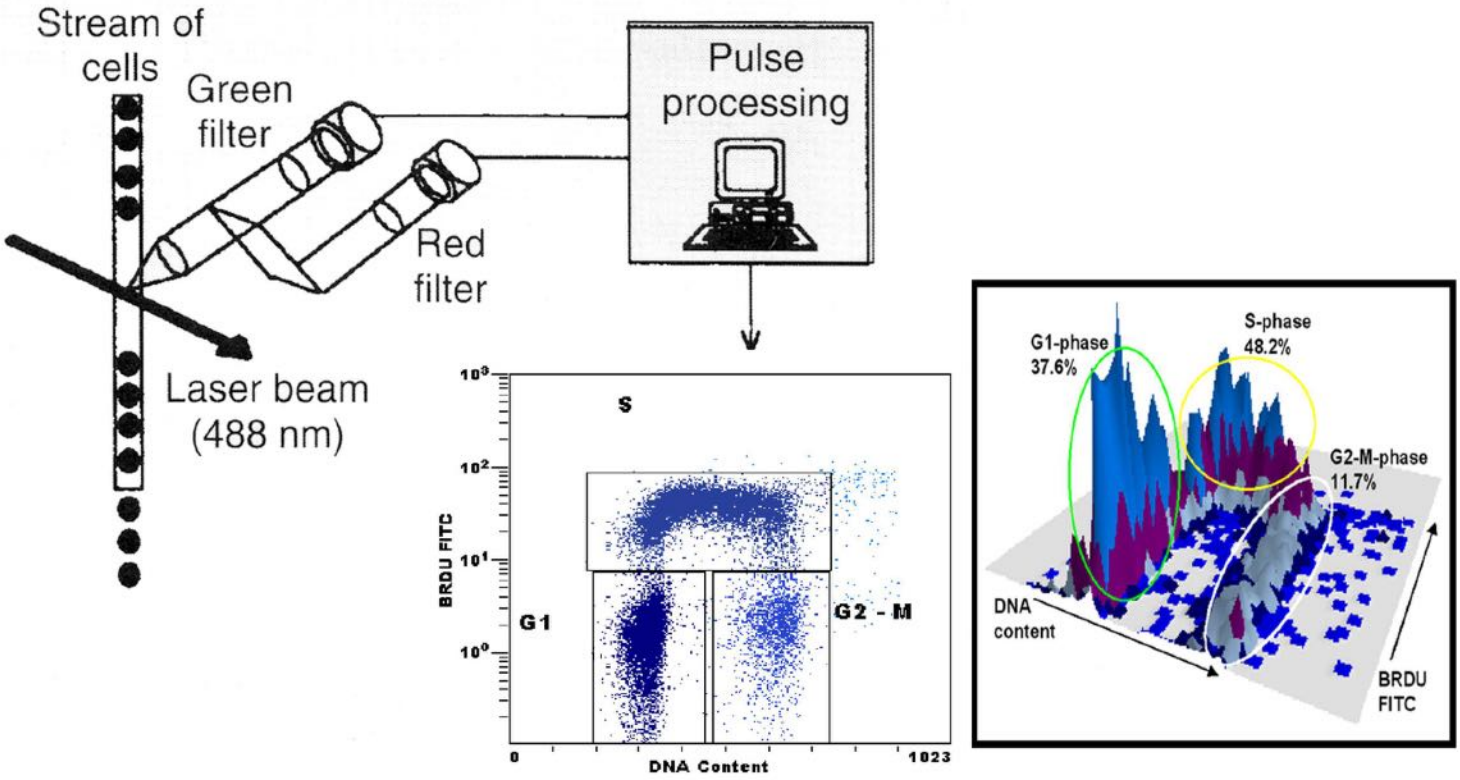
c. **the relative movement method** - *a double-labeling flow cytometry technique that allows the determination of the labeling index (LI), the duration of S phase (T_s) and the potential doubling time (T_{pot}) from a single tumor biopsy collected several hours after the injection of the DNA nucleotide analog bromodeoxyuridine (BUdR, or more correctly, but less easy to pronounce, BrdUdR), that is incorporated by S phase cells*

The DNA from the biopsy specimen is extracted and mixed with the dye propidium iodide (PI), which colors the DNA fluorescent red.

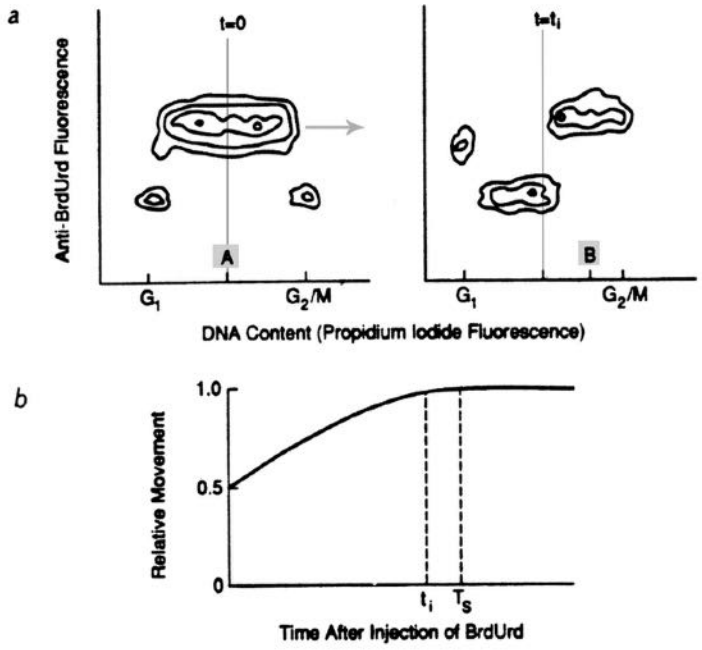
The DNA is then mixed with a monoclonal antibody to the BrdUrd incorporated into the S phase DNA. Then, a second, fluorescent antibody to the first antibody is added, making the S phase DNA glow fluorescent green (FITC, fluorescein isothiocyanate).

The sample is then run through the flow cytometer to record both the red and green signals.





The flow cytometric analysis of cellular bromodeoxyuridine (BrdUrd) and DNA content for cells stained with fluorescein (linked to BrdUrd) and propidium iodide (linked to DNA). The cells are processed one at a time through a blue (488-nm) laser beam that excites cellular BrdUrd content, and red fluorescence is recorded as a measure of cellular DNA content.



Use of BrdUrd and flow cytometry to estimate the duration of DNA synthesis, T_S. *a*, Two parameter distribution of cells with or without incorporated BrdUrd (vertical axis) versus DNA content (horizontal axis) immediately after injection of BrdUrd, and at time t_i later. Note that cells that label with BrdUrd initially have intermediate DNA content (i.e., are in S phase), and that this cohort of cells then moves through the cell cycle, divides and reappears in G_1 phase. *b*, Relative movement of BrdUrd-labeled cells.

The cohort of BrdUrd-labeled S phase cells moves out of S phase, and into G_2, M and the next G_1 in the time between the injection of the BrdUrd and the biopsy.

The "relative movement" of the cells during this time interval allows a determination of T_{pot} (after a couple of mathematical assumptions).

$$T_{pot} = \lambda \times T_S / LI$$

...with λ being a constant between the values of 0.7 and 1.0 (can usually assume 1.0 for Boards question purposes). LI is the fraction of the total number of cells that fluoresce green.

CELL CYCLE KINETICS CHEAT SHEET

GLOSSARY OF TERMS

Symbol	*Meaning*
T_C	Average cell cycle of the dividing cells of a population
T_M	Duration of mitosis (M)
T_{G_1}	Duration of G1 period
T_{G_2}	Duration of G2 period
T_S	Duration of DNA synthetic period (S)
GF	Growth fraction; proportion of viable cells in active cell division
T_d	Observed volume-doubling time of a tumor or tissue
T_{pot}	Potential doubling time of a tumor: theoretical doubling time calculated from the cell cycle and growth fraction, assuming no cell loss
Φ	Cell loss factor; rate of loss of cells is a fraction of the rate of new cell production $\Phi = 1 - T_{pot}/T_d$
MI	Mitotic index; proportion of cells in mitosis at any time
LI	Labeling index; proportion of cells in the population that take up tritiated thymidine
PLM	Percent-labeled-mitoses

Relationships Between Kinetic Parameters

Relationships		Values for C_3H Tumour	
t_C =	CELL CYCLE TIME	11.7	hours
↑ only equal if GF is unity	GROWTH FRACTION	0.54	
↓ t_{pot}	POTENTIAL DOUBLING TIME	21.5	hours
↑ only equal if no Cell Loss	CELL LOSS	0.35	
↓ t_D =	DOUBLING TIME	32.9	hours

Influence of Kinetic Parameters on Tumor Growth and on Regression After Irradiation (Representative Combinations Only)

ϕ	GF	T_C	Tumor Behavior
Low	Low	Long	Slow growth; slow regression
High	Low	Short	Slow growth; rapid regression
High	High	Short	Rapid growth; rapid regression
Low	High	Long	Rapid growth; slow regression

from Withers and Fletcher in Fletcher, 1980