# The Four, Five (and maybe even Six) R's of Radiotherapy

A. What are the various R's of radiotherapy, and why are they called that?

**Repair**
**Repopulation/Regeneration**
**Reoxygenation**
**Redistribution/Reasssortment**

**More R's Added Later:**
**Radiosensitivity**
**Reactivation**


**The Four R's of Radiotherapy**

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1] **the original term "Four R's", was coined by Dr. Rod Withers in his seminal 1970's publication of the same name** (Withers, HR. Adv Radiat Biol 5:241-247, 1975), among the first concerted efforts to bring the then "new biology of radiotherapy" to the clinical community in an easily digestible form

a. about 15 years later, another R, "**Radiosensitivity**", was added to the list, and then, within the past 5 years, yet another R has been proposed, "**Reactivation**" (as in "radiation-induced reactivation of the host immune system")

2] *of the various R's, it is important to know which ones are at work in both normal tissues and tumors versus which ones are specific for either the normal tissue or tumor, but not both*

a. **the R's that apply to both normal tissues and tumors** – ***not always to the same extent*** – **include:**

**Radiosensitivity**
**Repair**
**Repopulation**
**Redistribution**
**Reactivation** (although the tumor is also involved!)

b. **the only R that is tumor-specific is Reoxygenation** (on the assumption that normal tissues aren't hypoxic)

3] *it is likewise important to know what influence each of the R's would have on the outcome of radiation therapy, i.e., will the effect result in increased or decreased responsiveness*

a. for example, **the inherent radiosensitivity and/or repair capacity of constituent cells may make a tumor either easier or harder to cure, or a normal tissue more or less prone to radiation-induced complications**

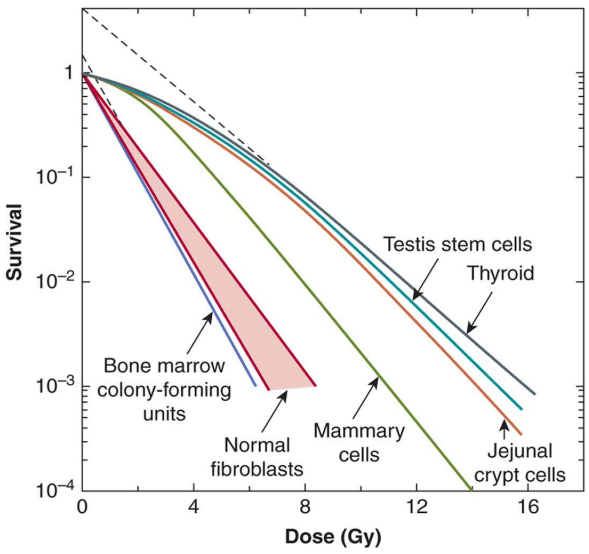
b. **if a tumor undergoes reoxygenation during the course of radiotherapy, it will have a net sensitizing effect; ditto for the case where sizeable cell cycle redistribution occurs during treatment**

c. **if tumor cells repopulate during treatment, this will have the net effect of making the tumor seem more "resistant"; however on the other hand, repopulation of normal tissue cells is desirable as a means of recovery from acute reactions**

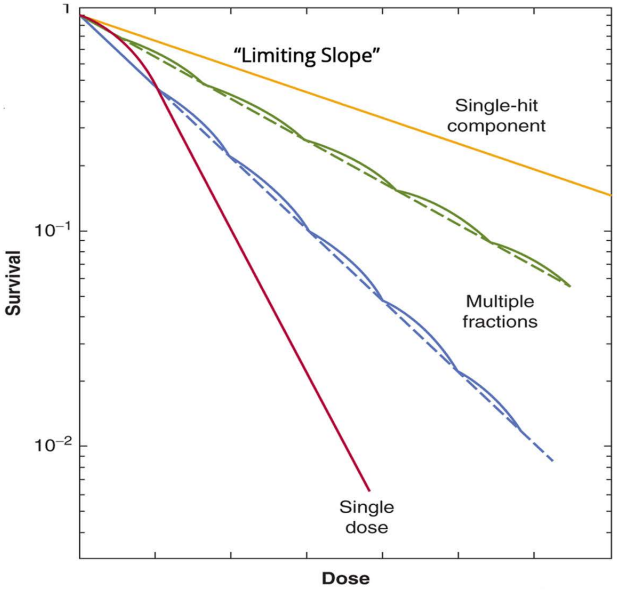
**RADIOSENSITIVITY** - the inherent radiosensitivity of the tumor cells you are trying to eradicate *and* the critical normal tissue cells you are trying to spare, ultimately can make or break the success of radiation therapy, everything else being equal

1] the single dose survival curve for cells has a finite initial slope, due to a nonrepairable, "single hit" component of radiation damage

a. therefore, there is a limit below which further reduction in the fraction size will no longer reduce the effective slope of the initial part of the survival curve, and at this limit, essentially all repairable damage is being repaired and what's left is, by definition, non-repairable




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.

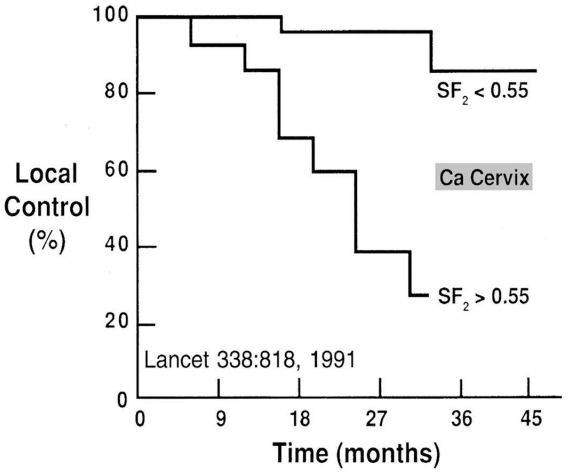



The influence of fractionating the radiation treatment on the shape of cell survival curves. When repair occurs between the fractions, the shoulder of the survival curve is repeated for every fraction.

b. however, the fraction size at which this limit is reached depends critically on the exact shape of the initial portion of the cell survival curve, and varies from cell type to cell type

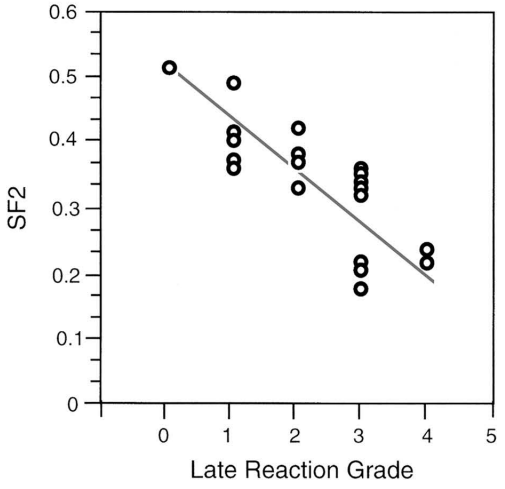
3] **a reasonably good way to "guesstimate" the inherent radiosensitivity of a cell type is to determine its $SF_2$ or surviving fraction at 2 Gy**; *this is not completely definitive however insofar as other factors (such as tumor hypoxia for example, or rapid repopulation) in addition to inherent sensitivity can also influence the overall outcome of radiotherapy*

## $SF_2$: Clinical Correlates



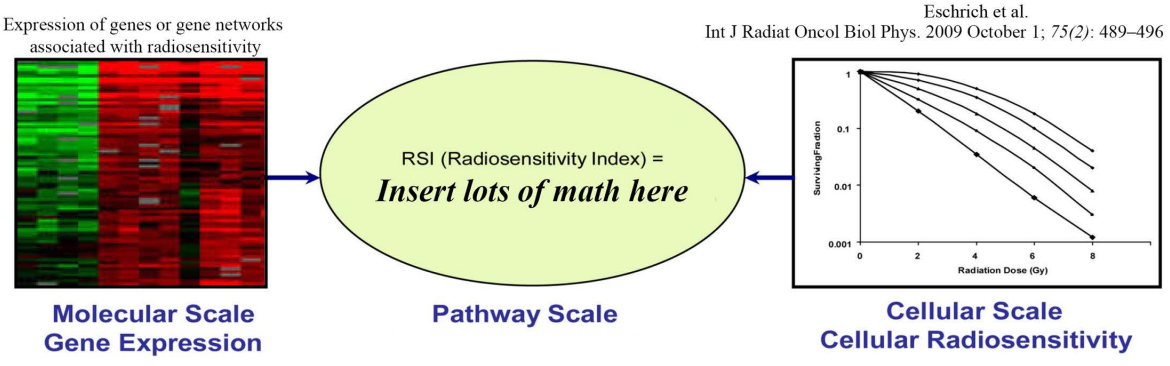

Local control as a function of time after treatment with radiotherapy alone for carcinoma of the cervix (Stages I. II, and III).

Patients were separated into two groups based on a pre-treatment measure of $SF_2$ from a tumor biopsy.




Data for 20 patients showing the correlation between late reaction to radiation therapy and the *in vitro* radiosensitivity of fibroblasts obtained from a biopsy. $SF_2$ is the fraction of cells surviving an acute dose of 2 Gy. The correlation is significant with a probability of 0.0001. (Redrawn from the data of Geard et al.: Int J Radiat Oncol Biol Phys 27:1173–1179, 1993.)

a) **a genomics- and systems biology-era take on the $SF_2$ idea has been termed the "radiosensitivity index" (RSI)**: expression patterns for a panel of 10 genes or gene "hubs" associated with radiation sensitivity and that vary proportionally with the $SF_2$ are reduced to single numerical values between 0 and 1




1] a further use for tumor-specific RSIs would be to modify α/β ratios accordingly, allowing the calculation of a **"genome adjusted radiation dose" (GARD)**

a. ***a higher GARD means that a given total dose has a greater effect on one individual's tumor than another individual's tumor with a lower GARD***

b. ***GARD has been validated in both preclinical and some clinical studies, and was shown to be a better predictor of time to first recurrence and overall survival than physical dose***

**REPAIR**–the basic importance of repair for radiotherapy is that, with a few caveats, **the total dose required to achieve a certain level of cell killing increases with increasing number of fractions or decreasing dose rate (for continuous irradiation), all of the other "R's" being equal**

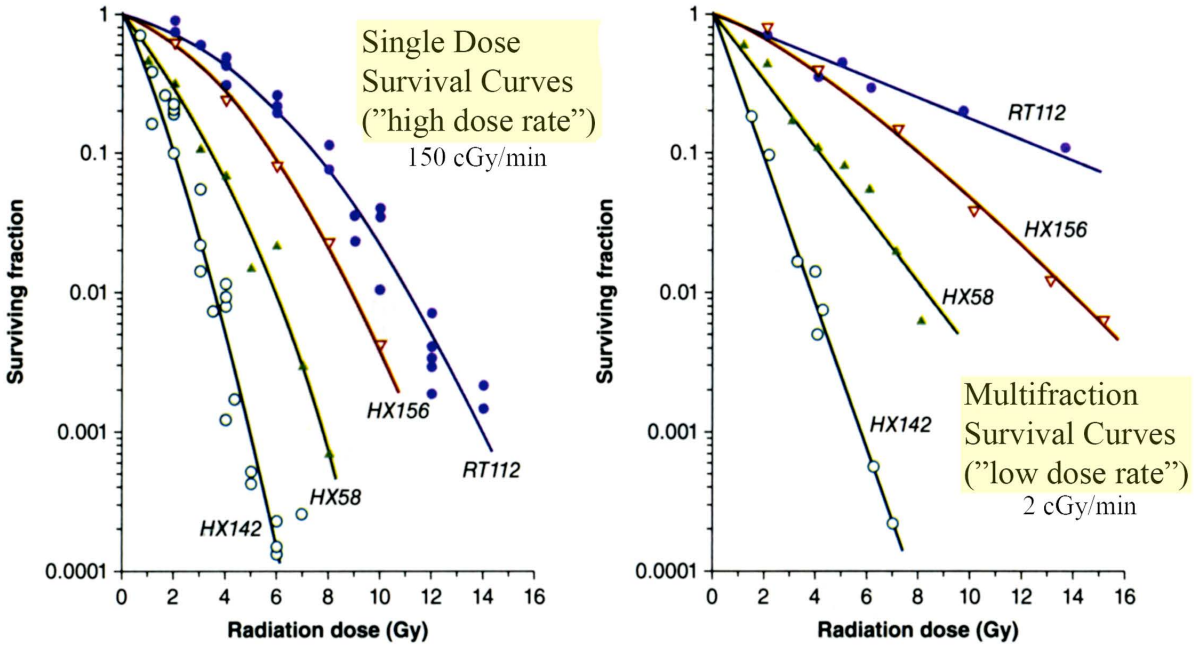
1] Which radiobiological processes are at play?

Chemical "Repair" - the very fast interactions between radiation-induced free radicals and cellular biomolecules that can "fix" or "restitute" radiation damage

DNA Repair - takes minutes to hours, and involves the cell recognizing that damage has occurred, and attempting to repair it enzymatically

Cellular "Repair" - sublethal and potentially lethal damage recovery; survival increases after irradiation that are a reflection of the repair of DNA damage and the shapes of the radiation survival curves for the cell types involved

Tissue "Repair" - how a tissue as whole responds to radiation damage to its constituent cells; involves both the cellular repair processes, the tissues's structural and functional organization, and its proliferation kinetics (see also below)




INT. J. RADIAT. BIOL., 1989, VOL. 56, NO. 6, 1045-1048

Multifraction dose survival curves compared with a single dose curve. The effective survival curves for multifraction regimens that produce an equal (proportionate) decrement in survival from each dose are linear, with shallower slopes than the single dose curve at the same dose. The slopes of the multifraction curves become less steep with decrease in fraction size until the dose per fraction is so low that multihit killing contributes negligibly and the slope is the limiting one determined by single-hit killing.

a) **fractionation sensitivity** - for a particular type of cell or tissue, how much sparing occurs with increasing fractionation?

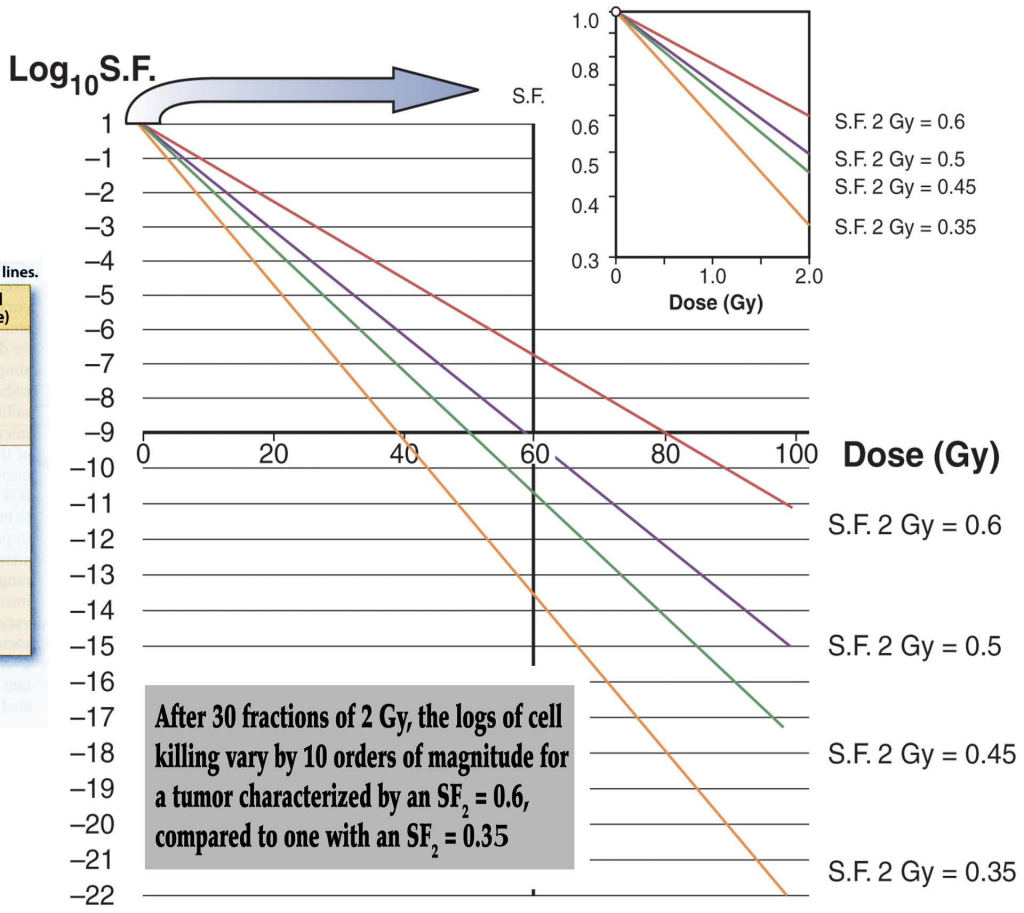
## *You can employ a survival curve model-free parameter like SF2...*

**Values of the surviving fraction at 2 Gy for human tumor cell lines.**

| Tumor Cell Type* | Number of Lines | Mean Survival at 2 Gy (Range) |
|---|---|---|
| 1. Lymphoma | 14 | 0.20 (0.08-0.37) |
| Neuroblastoma | | |
| Myeloma | | |
| Small-cell lung cancer | | |
| Medulloblastoma | | |
| 2. Breast cancer | 12 | 0.43 (0.14-0.75) |
| Squamous-cell cancer | | |
| Pancreatic cancer | | |
| Colorectal cancer | | |
| Non-small-cell lung cancer | | |
| 3. Melanoma | 25 | 0.52 (0.20-0.86) |
| Osteosarcoma | | |
| Glioblastoma | | |
| Hypernephroma | | |

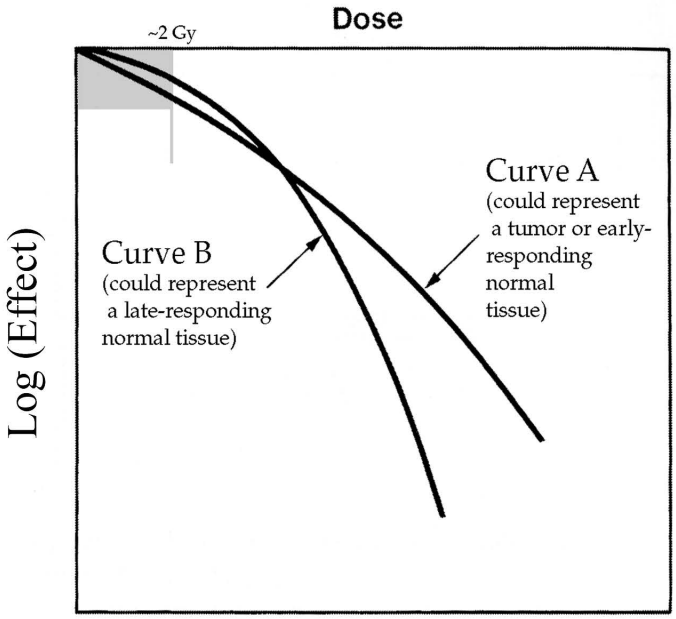
*Tumor types are grouped (1 to 3) approximately in decreasing order of their likelihood of local control by radiation treatment.

*The Basic Science of Oncology, 6th Edition (2021)*




## *...OR you can use the α/β ratio (although this locks you in to a particular survival model)*

Step #1: Assume that you have two tissues, "A" and "B", one characterized by dose response Curve A (a tumor you're trying to cure), and another by dose response curve "B" (a surrounding normal tissue that you'd really like to avoid producing a complication in)

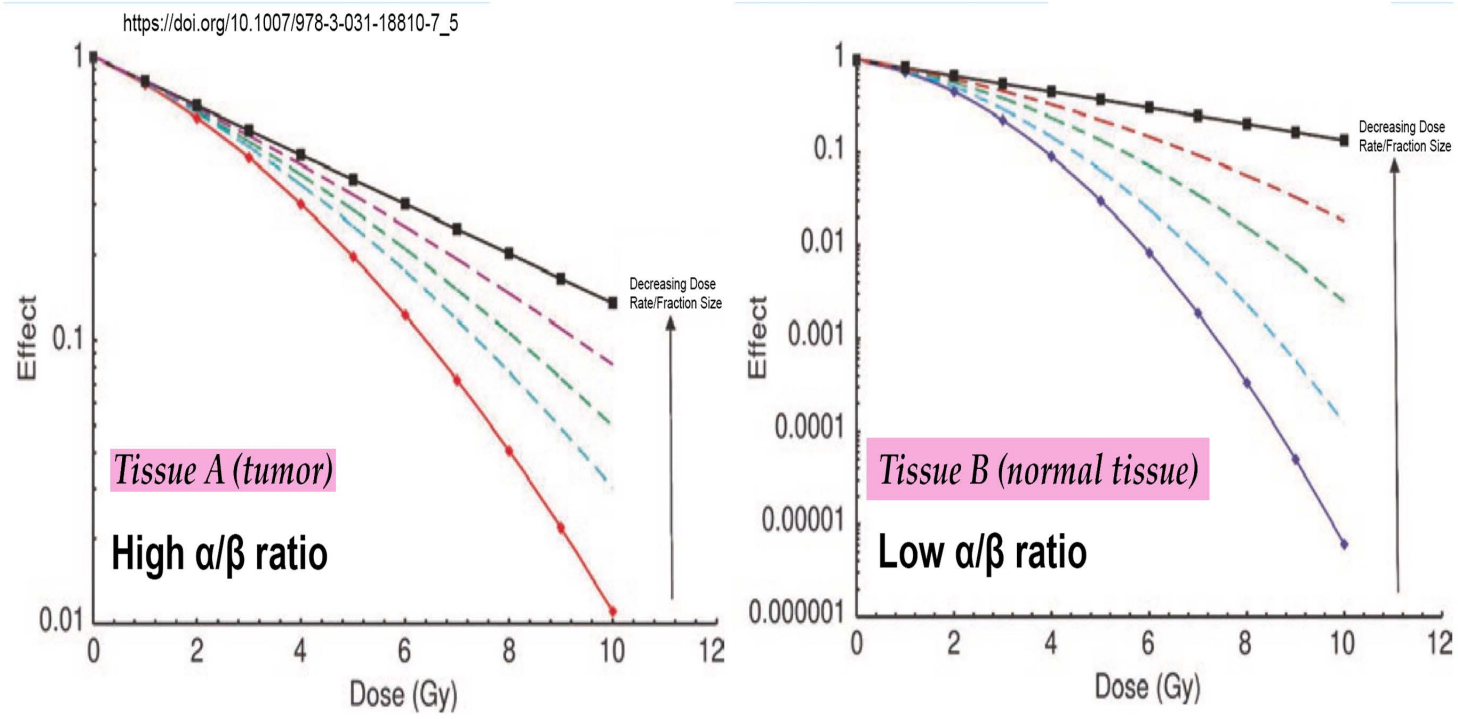



**Dose response Curve A has a gradually bending initial slope in the low-dose region of the curve, and dose response Curve B has a more steeply bending initial slope region.**

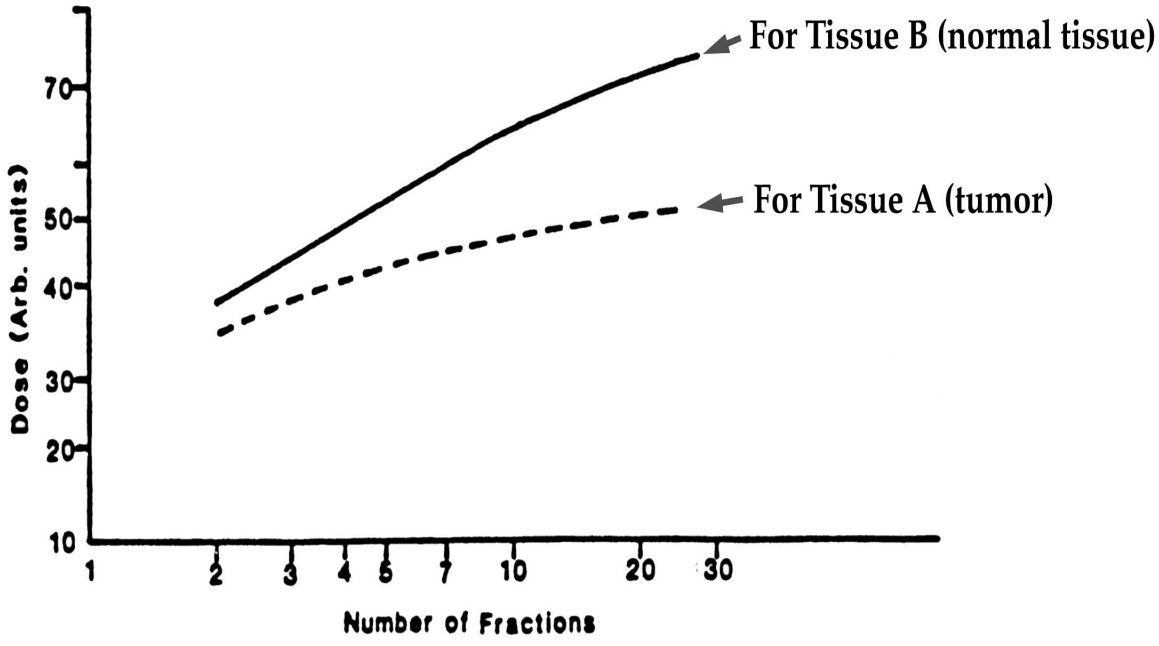
**In the language of the linear-quadratic model, this is the same thing as saying that Curve A has a large α-component and therefore a large α/β ratio, and Curve B has a small α-component and therefore a small α/β ratio.**

**(And in the language of $SF_2$, Curve A would be expected to have a lower surviving fraction at 2 Gy than Curve B.)**

Step #2: Now, subject each tissue to a range of doses per fraction (or dose rates) until the so-called limiting slope has been reached. Notice that this family of curves "fans out" more for Tissue B than Tissue A, owing to differences in the intial slope regions for each.




Step #3: Replot the multifraction data for both tissues in a more clinician-friendly (and clinically-relevant) way, that is, plot the total dose that yields isoeffect "E" as a function of the number of fractions (or, could also use dose per fraction or dose rate) required to reach that isoeffect



## So, what can we conclude?

- The "tolerance" or "curative" doses for tissues with large α/β ratios change less with changing fractionation than tissues with small α/β ratios.

- This is a reflection of subtle differences in the initial slopes (α-components) of the dose response curves for these tissues. These differences are magnified when lower dose rates or many, small doses per fraction are used.

- An isoeffect curve plots the total dose required to reach a particular endpoint (y-axis), as a function of one of the varied treatment parameters (x-axis), in this case, number of fractions...although dose per fraction, dose rate, or overall treatment time could also be used. Isoeffect curves were developed empirically by radiation oncologists MANY decades ago, in the absence of knowlege about the underlying biology at work.

- Based on this type of analysis, **it appears that tissues with low α/β ratios are also characterized by steeper isoeffect curves than those for tissues with high α/β ratios. In other words, the overall fractionation sensitivity, and potential for tissue sparing at lower doses per fraction or dose rates, is greater for tissues with low α/β ratios.**

**Note that the above exercise makes an important assumption: that all the (sublethal) damage capable of being repaired has been, i.e., that sufficient time has been allowed between fractions for full recovery. But what if it's not?**

1. If the time between fractions *isn't* long enough (i.e., less than 6 hours, typically), then there will be "incomplete repair", and the normal tissue tolerance or tumor control dose would be lower than expected

a) For tumors, this would be a good thing, however it could be catastrophic for normal tissues, because going to the same total dose would increase the complication frequency (possibly by a lot)

2. What information would help avoid this? ***Knowing the RATE of repair for the tissues at risk, usually expressed as the "half-time" of repair***

***Estimated repair half-times for early-responding normal tissues***

| *Tissue (ref.)* | $T_{1/2}$ *(hours)* |
|---|---|
| Jejunum (Thames *et al.* 1984) | 0·45(0·43,0·47) |
| (Huczkowski & Trott 1986; Dale *et al.* 1987) | 0·2–0·7 |
| Colon (Thames *et al.* 1984) | 0·79(0·76,0·83) |
| Skin (Henkleman *et al.* 1980) | 1·3(1·0,1·6) |
| Lip mucosa (Ang *et al.* 1985c, 1987a) | 0·8(0·6,1·3) |
| Hair follicles (Withers *et al.* 1987) | 1·5(1·4,1·6) |
| | (telogen) |
| | 0·63(0·58,0·7) |
| | (anagen) |

95% confidence intervals enclosed in parentheses.

Radiother Oncol 14: 303-305, 1989

Consequences of different repair half-times for the choice of clinical fraction intervals for 2 fractions a day.

| Interval between fractions (h) | Percentage of reparable lesions still unrepaired if repair half-time is: | | | | |
|---|---|---|---|---|---|
| | 0.5 h (%) | 1.0 h (%) | 1.5 h (%) | 2.0 h (%) | 2.5 h (%) |
| 1 | 25 | 50 | 63 | 71 | 76 |
| 2 | 6 | 25 | 40 | 50 | 57 |
| 3 | 2 | 13 | 25 | 35 | 44 |
| 4 | <1 | 6 | 16 | 25 | 33 |
| 5 | – | 3 | 10 | 18 | 25 |
| 6 | – | 2 | 6 | 13 | 19 |
| 8 | – | <1 | 2 | 6 | 11 |
| 10 | – | – | <1 | 3 | 6 |
| 12 | – | – | <1 | 2 | 4 |

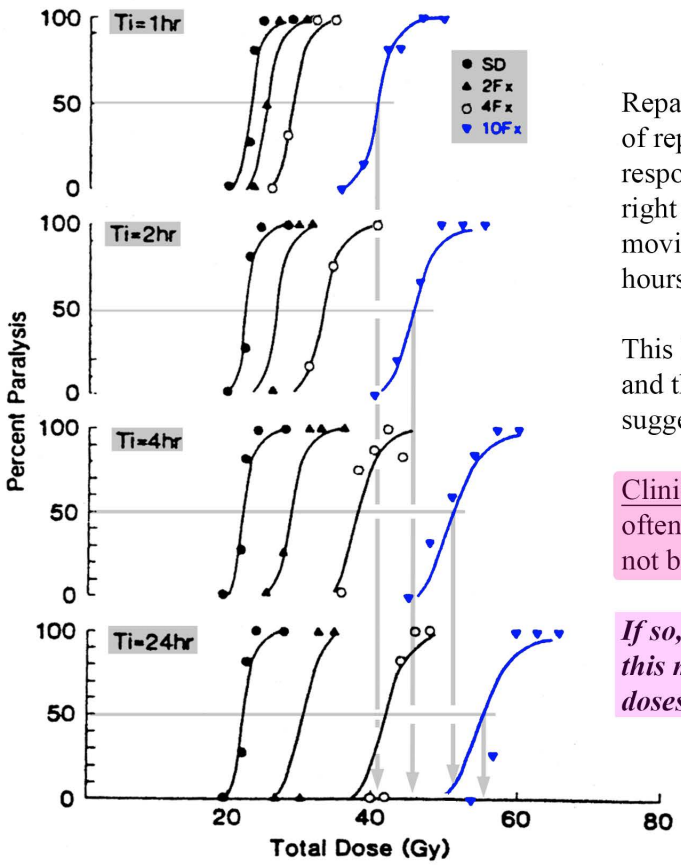
These calculations assume that all damage is repaired at the same rate, i.e. a simple mono-exponential function.

## $T_{1/2}$ for late-responding tissues

| Endpoint | $T_{1/2}$ (h) | 2.5%-tile (h) | 97.5%-tile (h) |
|---|---|---|---|
| Laryngeal oedema | 4.9 | 3.7 | 6.1 |
| Skin telangiectasia | 3.8 | 2.9 | 4.5 |
| Subcutaneous changes | 4.4 | 4.0 | 4.8 |

Bentzen et al. *R&O* 53: 219 (1999)

***The half-times of repair for these late-responding normal tissues are significantly longer than for their early-responding counterparts***




Repair kinetics in the spinal cord (from Ang *et al.*, 1987). The effect of repair is evidenced by increasing displacement of the dose response curves for paralysis (secondary to transverse myelitis) to the right with increasing fractionation interval. Note that the curve is still moving toward higher doses even for intervals between 4 and 24 hours.

This has been seen in experiments with both rodents and primates, and there's even some data from human fractionation studies suggesting that this is true.

Clinical implication: That a minimum of 6 hours between fractions – often used as a rule of thumb when using hyperfractionation – may not be long enough when it comes to spinal cord injury.

***If so, and assuming the interfraction interval was increased further, this means that the spinal cord might actually tolerate higher total doses than we think it can!***

**REPOPULATION/REGENERATION** - for some normal tissues and tumors, the ability to accelerate proliferation in response to the "injury" caused by radiation exposure is probably the second most important factor determining clinical outcome

1. all other factors being equal, repopulation in a tissue during the course of radiotherapy will have the net effect of making it seem more "radioresistant", i.e., higher normal tissue tolerance doses and higher tumor control doses

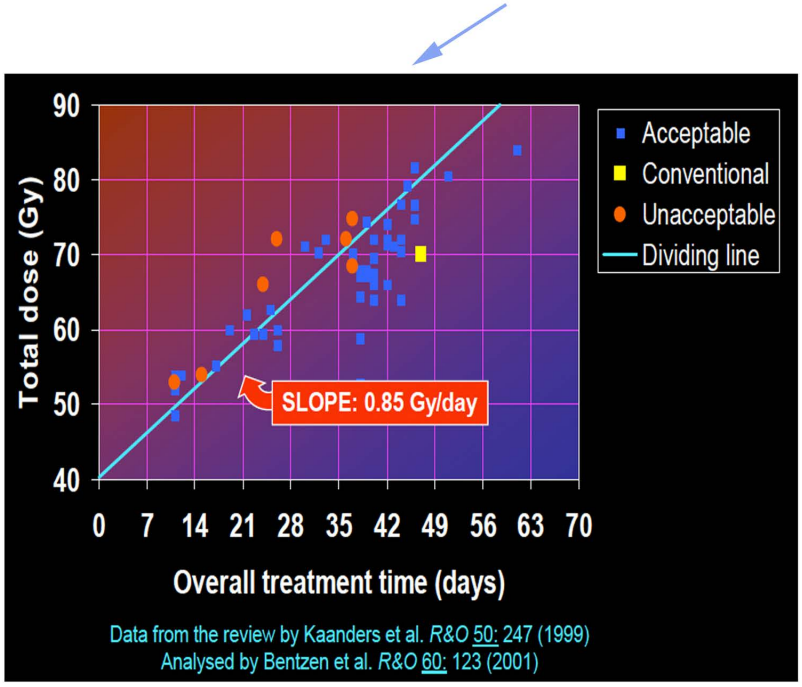
Which clinical parameters are influenced by repopulation?

- overall treatment time (also, whether to schedule a gap during treatment)
- whether to intensify or boost during the last 2 weeks of treatment
- whether to add a chemical modifier that targets rapidly-growing cells (e.g., BUdR, or many traditional chemotherapy agents)

**2] estimates are that up to 50% of the killing effect of each dose fraction may be "recovered" (in the case of early-responding normal tissues) or "wasted" (in the case of tumors) due to compensatory proliferation of surviving cells during an extended treatment**

Clinical Examples of Repopulation in Normal Tissues and Tumors during Radiotherapy

***Proliferative response in oral mucosa during radiotherapy*** - assuming the daily dose per fraction was 1.8-2.0 Gy, approximately half of this dose was "recovered" once compensatory proliferation began

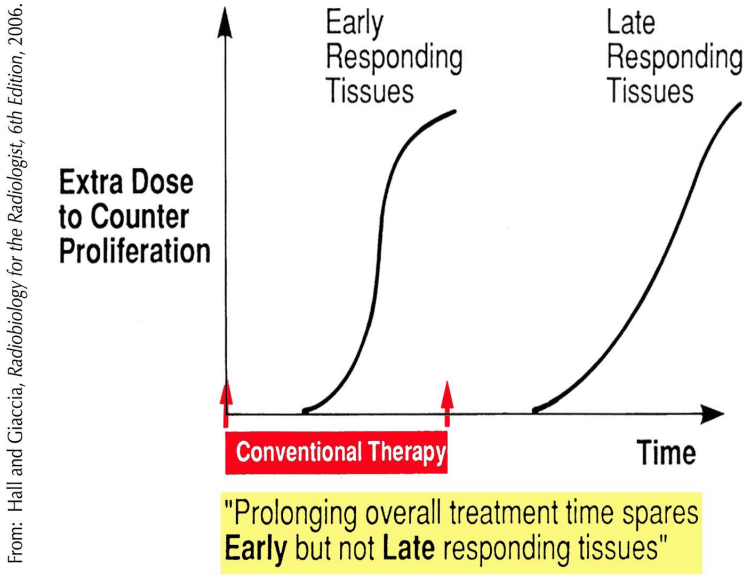



Values for $D_{prolif}$ from clinical studies

| Tissue | Endpoint | $D_{prolif}$ (Gy day$^{-1}$) | 95% CL (Gy day$^{-1}$) |
|---|---|---|---|
| *Early reactions* | | | |
| Skin | Erythema | 0.12 | [−0.12; 0.22] |
| Mucosa | Mucositis | 0.8 | [0.7; 1 1] |
| Lung | Pneumonitis | 0.54 | [0.13; 0.95] |
| *Tumours* | | | |
| Head and neck | | | |
| Larynx | | 0.74 | [0.30; 1.2] |
| Tonsils | | 0.73 | |
| Various | | 0.8 | [0.5; 1 1] |
| Various | | 0.64 | [0.42; 0.86] |
| Breast | | 0.60 | [0.10; 1 18] |
| Oesophagus | | 0.59 | [0.18; 0.99] |
| Non-small cell lung cancer | | 0.45 | N/A |
| Medulloblastoma | | 0.52 | [0.29; 0.75] |
| Prostate | | 0.24 | [0.03; 0.44] |

$D_{prolif}$ values estimated for other normal tissues and tumors

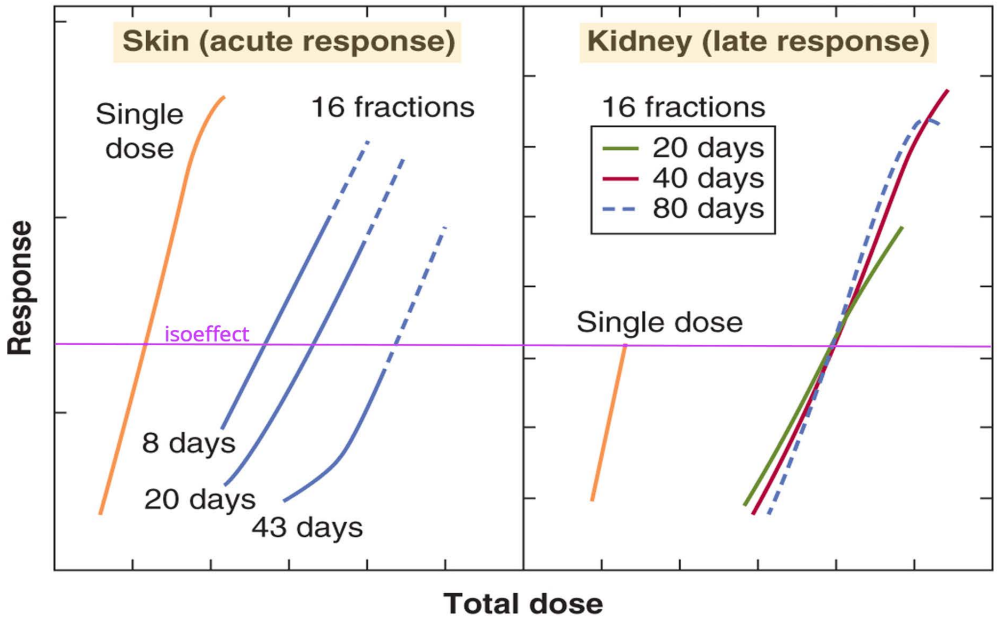
3] however, **this is NOT the case for late-responding normal tissues that have little or no proliferative potential or for especially slowly-growing tumors**




From: Hall and Giaccia, *Radiobiology for the Radiologist, 6th Edition*, 2006.

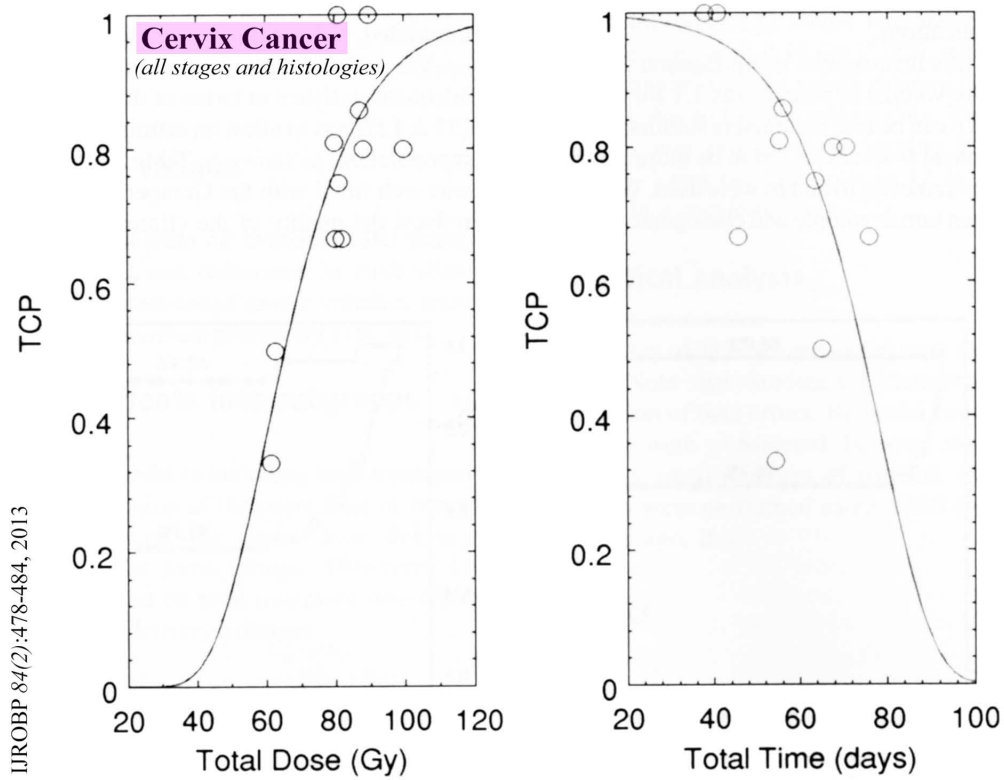
The extra dose required to counter proliferation in early-responding tissues begins to increase after a few weeks into a fractionated regimen, certainly during the time course of conventional therapy. By contrast, conventional protocols are never sufficiently long to include the proliferation of late-responding tissues.

a. ***one implication of this is that attempts to intensify treatment (such as, concomitant boost, or two fractions per day) late during the course of therapy so as to combat proliferation may overdose late responding normal tissues that don't benefit from repopulation during treatment***



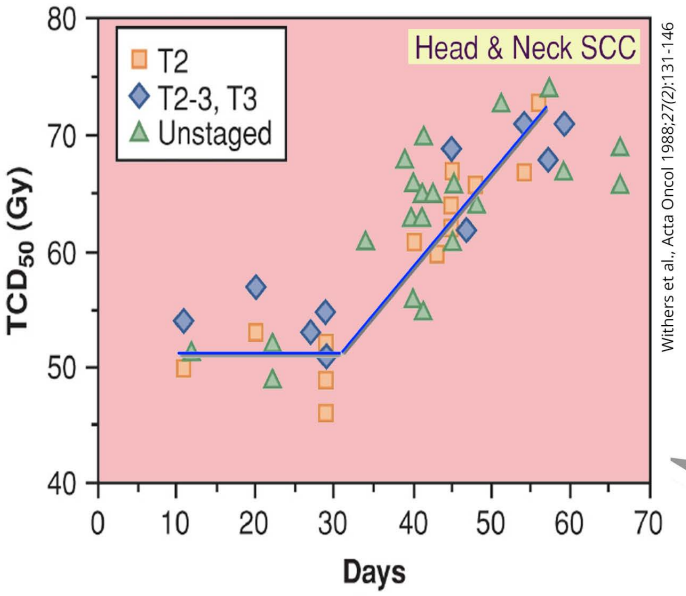

For acute skin reactions, extending the time over which a course of 16 fractions is given results in an increase in the total dose required for a given level of effect. In contrast, for late response of kidney, there is no change in the isoeffective dose for 16 fractions regardless of whether the treatment is given over 20, 40 or 80 days. (Modified from Denekamp, 1986.)

## *Proliferative Response of Tumors During Radiotherapy*




Tumor control probability for human cervix cancer as a function of total dose (left...a reflection of radiosensitivity) and overall treatment time (right...a reflection of repopulation).

***For a given total dose, local control of the tumor is lost the longer the overall treatment time.***




Estimated total doses of fractionated irradiation required to achieve 50 percent probability of tumor control for squamous cell carcinomas of the head and neck (various stages) plotted as a function of the overall treatment time.

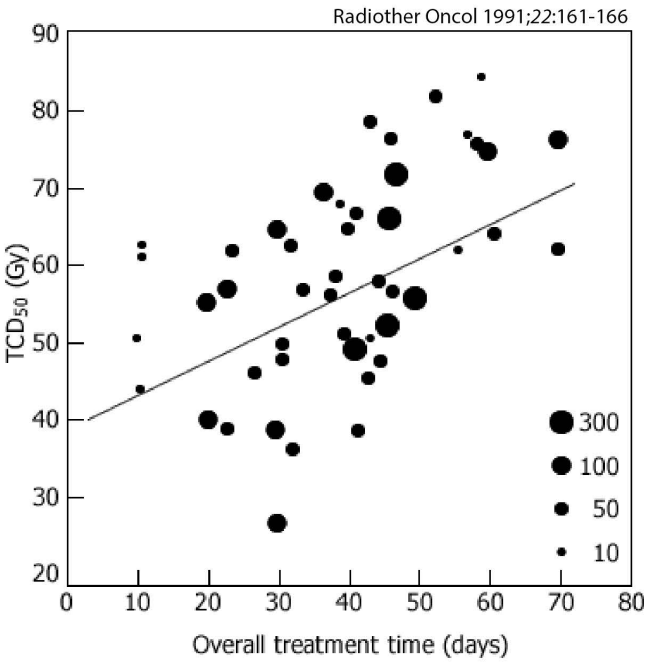
*(Data pooled from over 50 different studies over many years, involving nearly 500 patients.)*

***The infamous Withers "hockey stick" plot...beware, not everybody believes it!***

***Insofar as this graph is believable ...***

➠ **it suggests a linear time factor once proliferation begins**

➠ **it also suggests that the proliferative response is delayed 3-4 weeks after the start of radiation therapy**




A re-analysis of the data included in the Withers hockey stick plot by Bentzen and Thames (1991), where weighting factors have been assigned based on the number of patients in each treatment group.

***While it does not dispute the general finding that the tumor control dose for head and neck cancers increases with increasing overall treatment time (suggestive of compensatory repopulation), it does dispute the 4-week "lag time" before proliferation commences.***

Loss of percent local control with one week's extension of overall time in larynx radiotherapy (Taylor *et al.*)

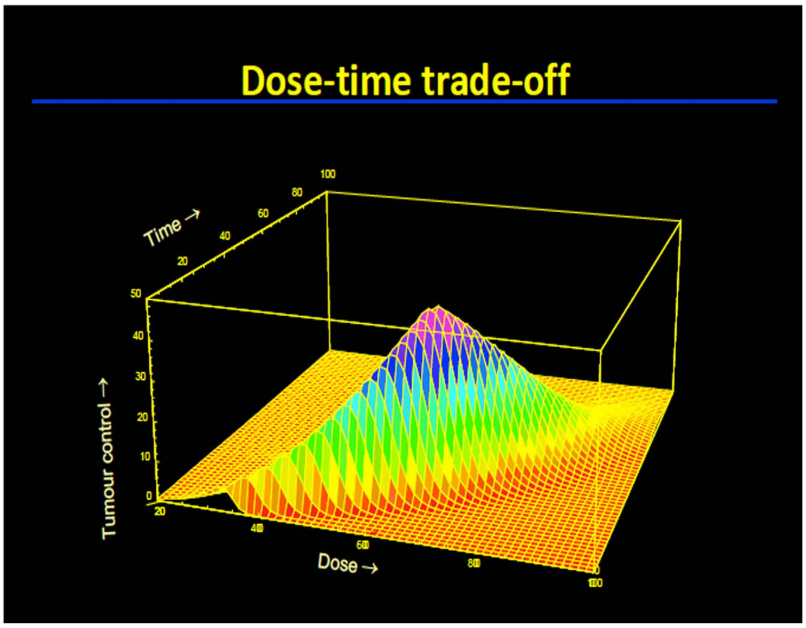
| Falling from (%) | Pharyngeal wall | Vocal cord | Supraglottic larynx | Pyriform sinus | Average (%) |
|---|---|---|---|---|---|
| 90 to | 82 | 68 | 72 | 82 | 76 |
| 60 to | 43 | 26 | 30 | 43 | 36 |

Conclusion: Average loss of 24% local control in one week at steepest part of regression curve.

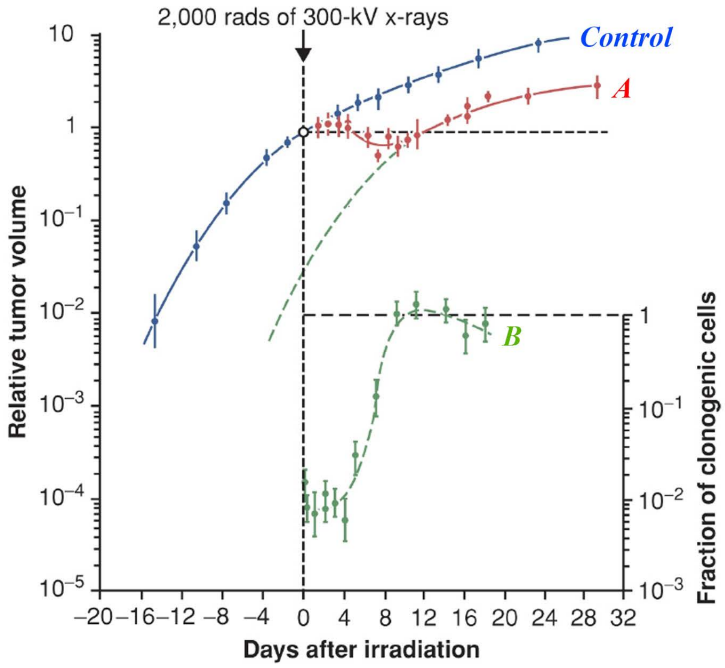
Increase in fitted (actuarial) local control at 3 years on shortening from 7½ to 6 weeks overall time (C.C. Wang)

| T-stage | b.i.d.-q.d. 7½ weeks | b.i.d.-b.i.d. 6 weeks | *p*-value for difference due to random chance in survival curves |
|---|---|---|---|
| $T_1T_2$ | 81% (13) | 97% (44) | 0.53 |
| $T_3T_4$ | 47% (39) | 77% (44) | 0.017 |
| $T_1$–$T_4$ | 56% (52) | 85% (88) | 0.0013 |
| $N_0$ | 46% | 93% | 0.00043 |

Figures in brackets show numbers of patients at risk.




***Trying to balance a tumor's radiosensitivity (i.e., total dose it takes to achieve local control) and its ability to repopulate (i.e., longer overall treatment time means more repopulation) is always a trade off!***




**A final note about tumor repopulation:**

Don't be fooled by a tumor that responds slowly to irradiation as a whole, when there might be a subset of clonogens repopulating rapidly!

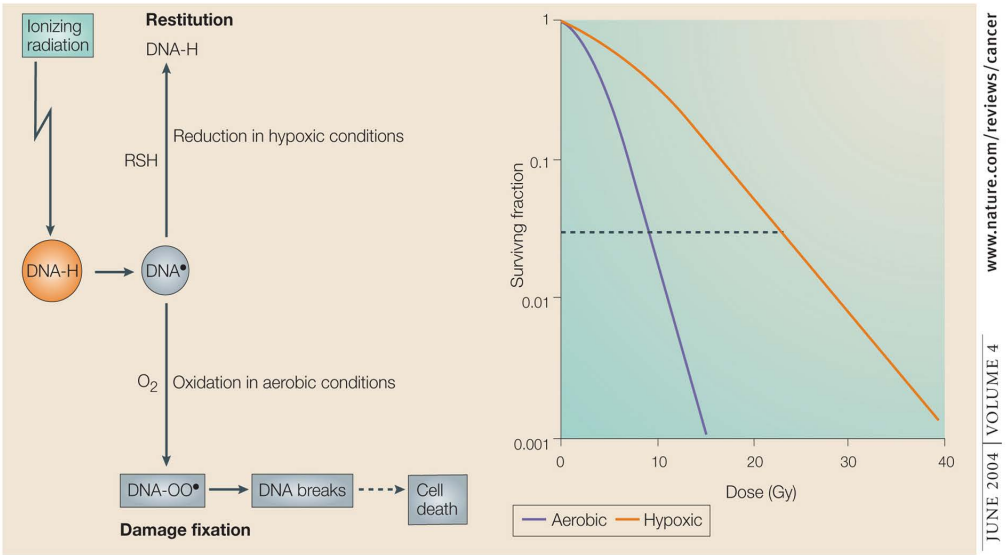
The best (if far from ideal) indicator of a tumor's potential for accelerated repopulation is its $T_{pot}$ (or $T_{eff}$, if it can be measured).

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)

**REOXYGENATION** – potentially, the most important "R" of all...for those tumors containing clonogenic, hypoxic cells that is

1] quickie review of the hypoxia problem in radiotherapy:

a. both for cells maintained *in vitro*, and for tumors *in vivo*, hypoxic cells are anywhere from 1.5 - 3.0 times more radiation resistant (in terms of $D_0$) than their aerobic counterparts, thanks to differences in free radical reactions that occur during, and within a few milliseconds of, low LET irradiation

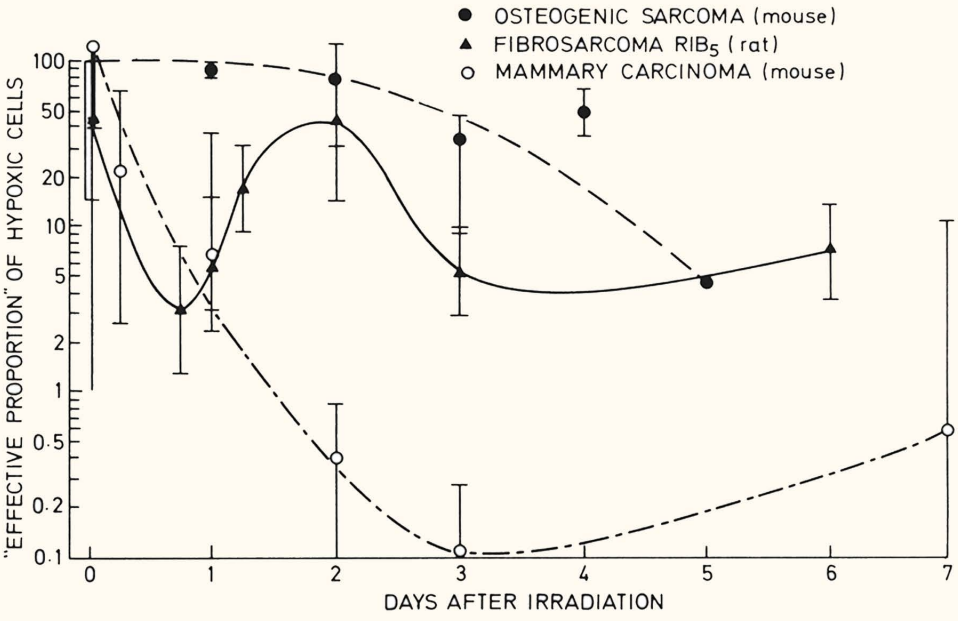


b. many rodent tumors are known to contain clonogenic, hypoxic cells that limit the tumor's curability, and this is likewise assumed to be the case for human tumors as well

c. however, *if for whatever reason, hypoxic cells in tumors are able to reoxygenate during the course of protracted radiation therapy lasting several weeks, then the "hypoxia problem" would not turn out to be a problem at all*

1) we know that reoxygenation does occur in rodent tumors, and that it can proceed rapidly (minutes to hours), more slowly (hours to days) or be somewhat cyclic in nature

a] the patterns and extent of reoxygenation can vary with tumor type, size, site, and how the tumor is treated




The temporal pattern of re-oxygenation of three experimental animal tumours after a high single dose of radiation (from Thomlinson 1970)

2) What about reoxygenation in human tumors?

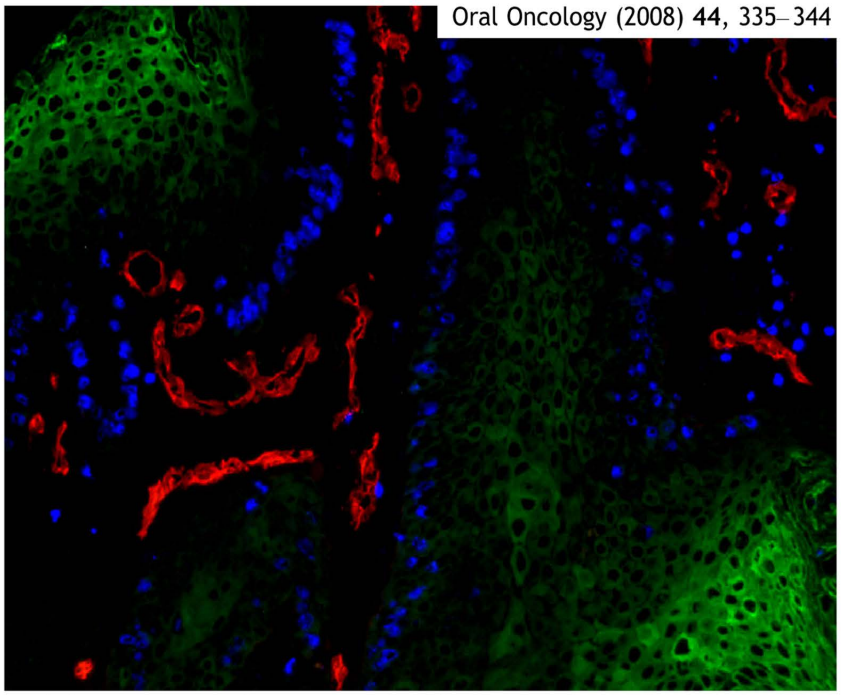
a] *we assume that the patterns of reoxygenation in rodent tumors are fairly representative of the human situation*; some (very limited) data from human tumors support this in general, although not necessarily sufficiently to make sweeping statements

b] despite this uncertainty however, we're pretty sure that many types of human tumors do reoxygenate – if not, the dose-limiting radioresistance of hypoxic cells would mean that we'd barely cure any cancers at all

2] Spotlight on Reoxygenation and Its Clinical Implications

a. the clinical, "operational" definition of **reoxygenation**: ***the re-establishment, between subsequent radiation doses, of approximately the same hypoxic fraction in a particular tumor as was present prior to the first radiation dose, leading to a net tumor radiosensitization over an extended course of radiotherapy***

1) **diffusion-limited or chronic hypoxia occurs secondary to the consumption of oxygen by cells close to tumor vasculature, and the resulting deprivation in the case of cells beyond oxygen's diffusion distance**




Fluorescence microscopy of a biopsy specimen from a human hypopharynx carcinoma showing the "geographic" relationship between blood vessels (red), proliferating cells (blue) and hypoxic cells (green).

a) ***for the chronically hypoxic cells***, there are two ways for them to become reoxygenated (assuming they haven't died in the interim):

1. ***well-aerated cells are killed by the radiotherapy, such that less oxygen is consumed and it can therefore diffuse further into the previously hypoxic regions***

2. ***many (but not all) tumors shrink once cells start to die in large numbers, which would have the effect of bringing the remaining hypoxic cells "closer in" to the tumor blood vessels such that they likewise receive more oxygen***

3. because it usually takes quite a while for dead cells to be cleared out of tumors or for it to start shrinking, ***it follows that chronically hypoxic cells would likely takes days or more to reoxygenate***

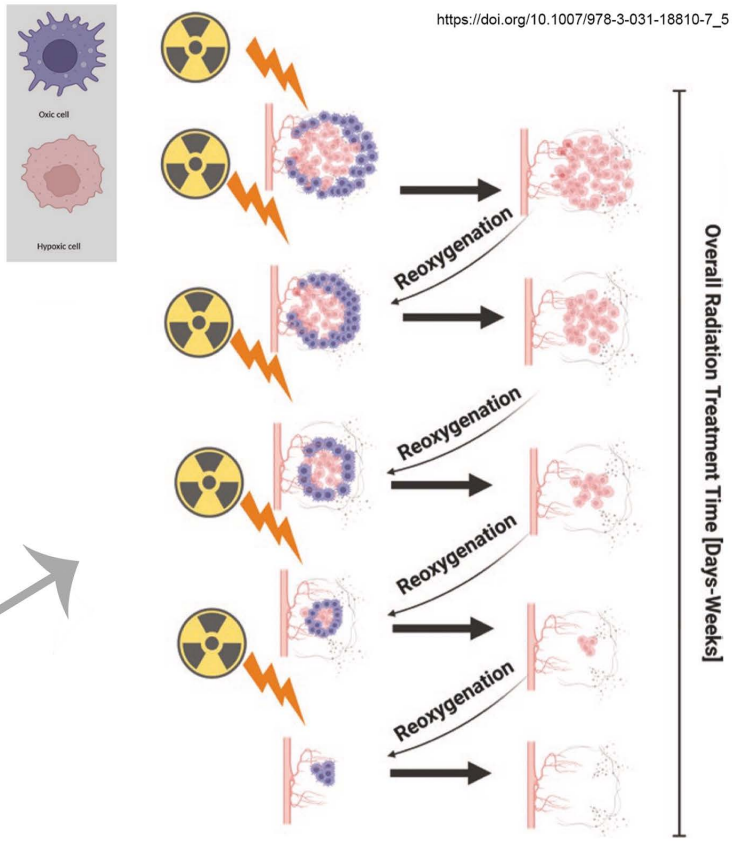
b) Does this slow type of reoxygenation occur in human tumors?

1. Answer: Likely. Evidence for such is the success of at least some clinical trials in which interventions targeting chronically hypoxic cells were employed (e.g., ARCON, hyperbaric oxygen, hypoxic cell radiosensitizers, etc.), along with various studies using hypoxia markers.

https://doi.org/10.1007/978-3-031-18810-7_5

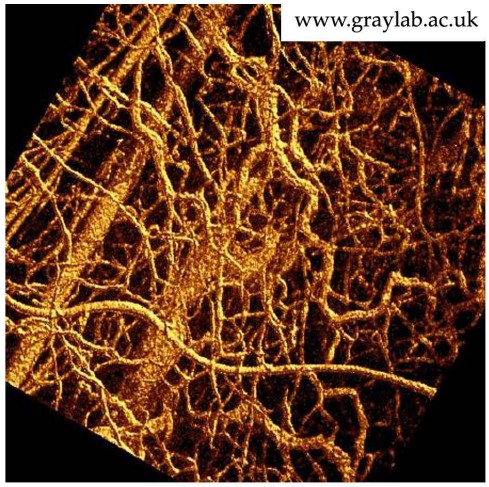
The process of reoxygenation.

Tumors contain a mixture of aerated and hypoxic cells. A dose of x-rays kills a greater proportion of aerated cells than hypoxic cells because aerated cells are more radiosensitive. Therefore, immediately after irradiation, most cells in the tumor are hypoxic. However, the preirradiation pattern tends to return because of reoxygenation. If the radiation is given in a series of fractions separated in time sufficient for reoxygenation to occur, the presence of hypoxic cells does not greatly influence the response of the tumor.

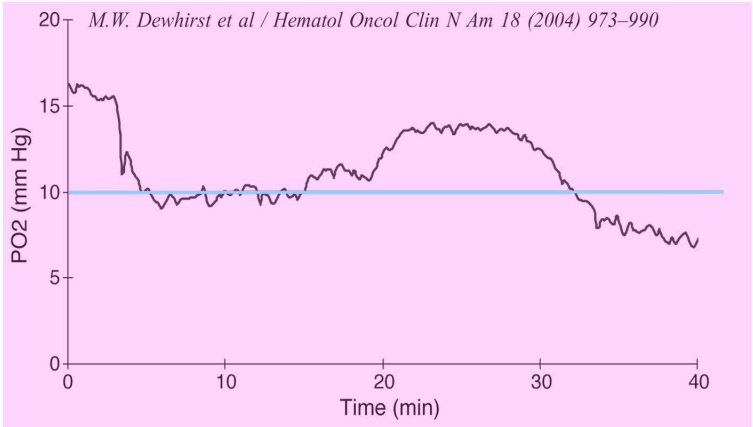



Oft referred to as the "Thomlinson and Gray Model" of reoxygenation...a pity then that it fails to explain the rapid types of reoxygenation observed in rodent tumors.

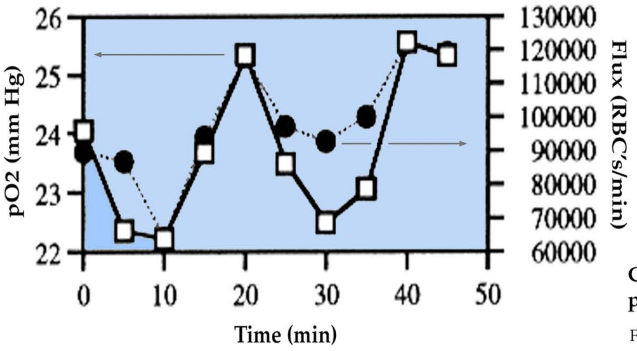
2) **perfusion-limited (or, for the more enlightened, "intermittent" or "fluctuant"), acute hypoxia occurs in tumors due to any number of aberrant physiological processes common to tumor vasculature**




abnormal microvasculature in human tumors demonstrated using confocal microscopy




Data from 9L rat glioma measured with oxygen microelectrode showing spontaneous fluctuations in oxygen concentration under air-breathing conditions.




**Cyclic changes in red blood cell flux (and therefore, oxygen partial pressure in the nearby tissue) in small mouse tumor arterioles.**

From: Kimura *et al*. Cancer Res *56*: 5522-5528, 1996

b) **for acutely hypoxic cells, reoxygenation would be expected to be fast(er) – minutes to hours – but also, very variable depending on what, exactly, is going on physiologically**

Mechanisms and time-scales of tumour reoxygenation

| Mechanism | Time |
|---|---|
| Recirculation through temporarily closed vessels | Minutes |
| Reduced respiration rate in damaged cells | Minutes to hours |
| Ischaemic death of cells without replacement | Hours |
| Mitotic death of irradiated cells | Hours |
| Cord shrinkage as dead cells are resorbed | Days |

Joiner and van der Kogel, *Basic Clinical Radiobiology, 4th Edition*, 2009

c) Does this fast type of reoxygenation occur in human tumors?

1. Answer: (Again) Presumably, to the extent that experimental studies of tumor physiology and clinical studies targeting acute hypoxia are/were successful (e.g., nicotinamide, vasculature-"normalizing" anti-angiogenics, vasculature-destroying agents, etc.)

c. Which clinical parameters are important vis-a-vis reoxygenation?

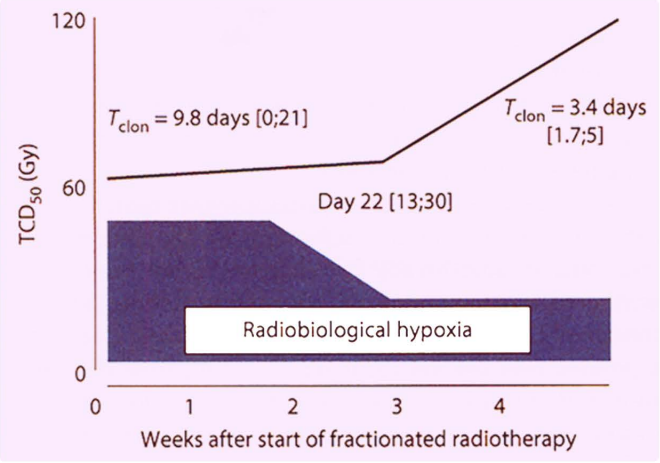
- Overall treatment time, i.e., is it long enought to allow complete reoxygenation? (Cases where it might NOT be long enough: brachytherapy, intraoperative radiotherapy involving a single large dose, stereotactic radiosurgery involving only one or a few large doses)

- Whether or not to add a hypoxic cell radiosensitizer (although it would *certainly* help if we knew whether the patient's tumor contained hypoxia in advance)

d. some final thoughts about reoxygenation...

1] even though reoxygenation is consider a good thing overall (i.e., that it overcomes the radioresistance of hypoxia during protracted-duration radiotherapy), it also has its dark sides

a) the reactive oxygen species generated when a microregion of a tumor is reoxygenated (especially on a cyclic basis) can initiate several "bad" processes:




Rate, kinetics and underlying mechanism of repopulation of clonogenic tumour cells in FaDu squamous cell carcinoma growing in nude mice. As the result of repopulation, the tumour control dose ($TCD_{50}$) increases with time.

- increased tumor cell proliferation
- preservation/protection of existing tumor vasculature (increased resistance of vascular endothelial cells to apoptosis )
- increasingly-aberrant vascular-"remodeling" and/or new angiogenesis
- increased genomic instability in nearby tumor cells

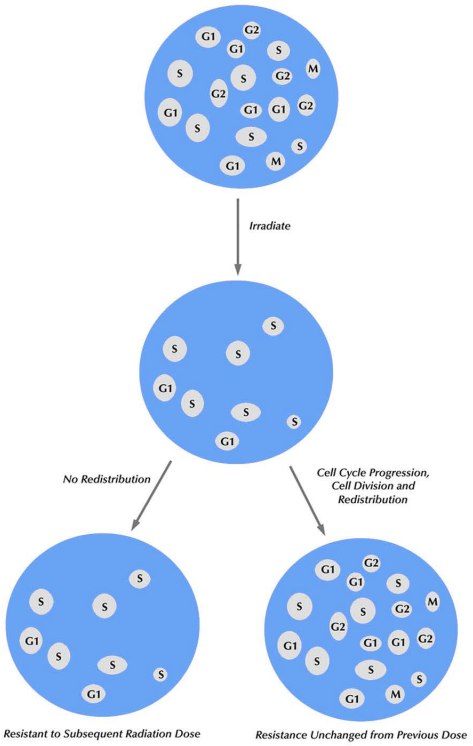
## REDISTRIBUTION/REASSORTMENT – the "R" that don't get no respect...but does it deserve any?

Definition: a consequence of the age response through the cell cycle and radiation-induced cell cycle blocks and delays, redistribution is the tendency of fractionated radiotherapy to first synchronize and then reassort, rapidly growing cells in tissues (normal or tumor) such that, by the time of the next dose fraction, the cells' age distribution effectively matches that at the time of the previous dose fraction

*Because this redistribution of surviving cells occurs, the tissue would therefore NOT become enriched with radioresistant S phase cells, and its radisensivity would be maintained for each successive dose fraction.*

1] there are two subtypes of cell cycle redistribtution, arbitrarily called (mostly by me) Type 1 and Type 2; both of them produce the net effect of sensitizing rapidly-proliferating cells to a subsequent dose fraction

"Type 1"




When a mixed ("asynchronous") batch of cells receives a dose of radiation, the most resistant ones (S phase and a few G1 phase) tend to be the ones that survive.

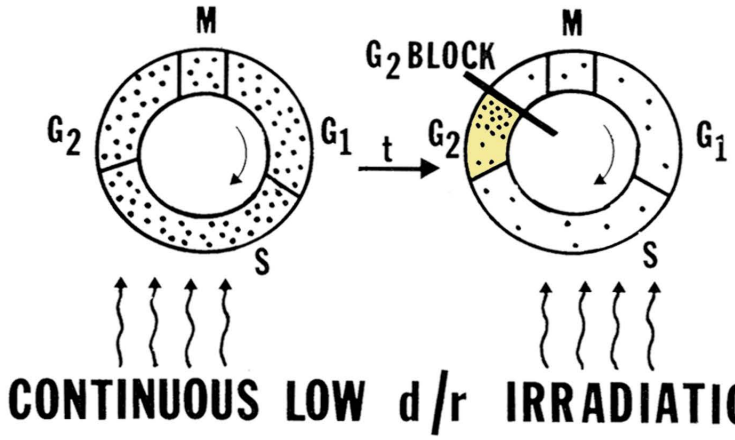
If the situation remains unchanged by the time of the next dose fraction, the tumor will be more radioresistant as a whole due to enrichment with resistant cells.

***However, if, during the time between the first and second dose, the surviving, resistant cells continue to move around the cell cycle and divide, they will re-establish the conditions of the original tumor prior to irradiation, and have the same overall radiosensitivity. This process is called "redistribution or reassortment".***

Redistribution has the net effect of making each subsequent dose fraction equally as effective as the prior one; if redistribution *didn't* occur, the tumor would become more and more resistant with each dose.

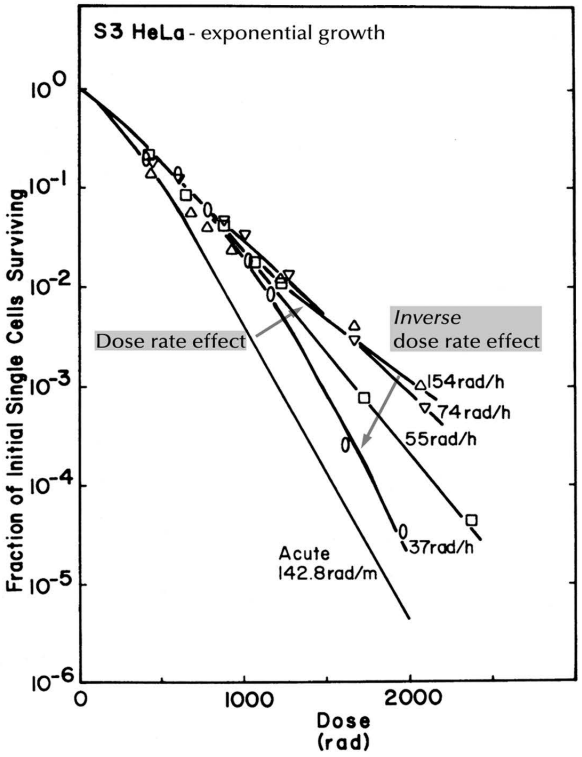
"Type 2"

A second type of redistribution requires continued cell cycle progression of surviving cells after irradiation (in a synchronous manner), but NO subsequent cell division, due to activation of the G2 checkpoint




Hall EJ: The biological basis of endocurietherapy: The Henschke Memorial Lecture 1984. Endocurie Hypertherm Oncol 1:141–151, 1985

a) **if this type of redistribution occurred, one would expect the tissue to become more and more sensitive as low dose rate irradiation progressed and more and more cells became blocked in G2 phase...this has been termed the "inverse dose rate effect", and mostly has been studied *in vitro***




The inverse dose-rate effect

A range of dose rates can be found for HeLa cells such that lowering the dose rate leads to more cell killing. At 1.54 Gy/h (154 rad/h), cells are "frozen" in the various phases of the cycle and do not progress. As the dose rate is dropped to 0.37 Gy/h (37 rad/h), cells progress to a block in $G_2$, a radiosensitive phase of the cycle. (From Mitchell JB, Bedford JS, Bailey SM: Dose-rate effects on the cell cycle and survival of S3 HeLa and V79 cells. Radiat Res 79: 520–536, 1979)

4] ***because the conditions minimally necessary for either type of redistribution to occur (e.g., a tumor with a high growth fraction and short potential doubling time) don't occur all that often in humans, it's hard to say how influential redistribution is - or isn't - in terms of clinical outcomes***

# SUMMARY GRAPHIC:
# HOW THE 4 R'S INFLUENCE RADIOTHERAPY EFFECTIVENESS