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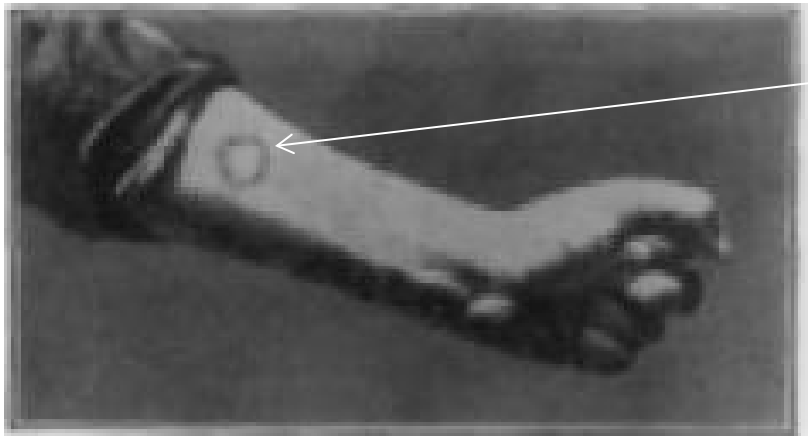
Non-cancer Tissue Effects from Radiation Exposure

Radiation Epidemiology & Dosimetry Course

National Cancer Institute www.dceg.cancer.gov/RadEpiCourse

The normal tissue effects of radiation became obvious very soon after its discovery. In 1900, Friedrich Walkoff wrote of a radioactive source *"An exposure of the arm has produced an inflammation of the skin which has now lasted already for two weeks, and exhibits the same aspect as that obtained after a long exposure to X-rays."* On the physicist who gave him the source he wrote *"Giesel's breath was so radioactive it would still discharge an electroscope 18 hrs after he left his laboratory"*

In 1905, Marie Curie wrote *"Pierre Curie voluntarily exposed his arm to the action of radium during several hours. This resulted in a lesion resembling a burn that developed progressively and required several months to heal. Henri Becquerel had by accident a similar burn as a result of carrying in his vest pocket a glass tube containing radium salt. He came to tell us of this evil effect of radium, exclaiming in a manner at once delighted and annoyed: "I love it, but I owe it a grudge.""*



Thought to be Pierre Currie's skin burn!

Marie Curie also experimented *"After some days, the red color increased without spreading. On the 20th day, scabs were formed and then an open wound needing covering up. On the 42nd day, the epidermis started to recover around the wound, reaching the center, and 52 days after the action of the radiations, an area of 1 cm^2 of a grey coloration remained, indicating a deeper necrosis.*

"carrying …. a little tube enclosed in a thin metallic box….during less than 30 minutes produced 15 days later a red spot which was transformed into a blister similar to that due to a superficial burn that takes 15 days to be cured. These facts show that the duration of the lesion's evolution varies according to the intensity of the radiation and the duration of the excitation action".

These studies were the first to indicate that radiation wounds displayed a time-dose relationship, take time to appear, and that lesions "evolved" with time.

Acute Radiation Effects - Acute Radiation Syndrome (ARS)

- High dose exposure
- Due to cell killing and functional tissue impairment

Late/Delayed Effects that are Independent of Acute Radiation Damage

- Most obvious in targeted radiation therapy

Late/Delayed Effects of Acute Radiation Exposure (DEARE)

- As in survivors of ARS

Multiple Organ Disease Syndrome (RI-MODS)

- As in patients exposed to high doses in Tokai-mura criticality accident, Chernobyl, etc.

Low Dose Late Effects

- A-bomb survivors, occupational workers, medical exposures….

What are the mechanisms that underlie these manifestations of radiation damage?

Acute Radiation Syndrome (ARS) after Whole Body Exposure

Prodromal Effects – within hours - not due to cell loss - time-dose dependent.

- Lowest dose it is seen is about 50cGy

After 6-8 Gy

- Vomiting: < 1 hr; Diarrhea: 1-3 hrs; Headache: 4-24 hrs; Temperature: <3hrs; Cognitive impairment > 6hrs (brain death with days after >20Gy)

Acute Deterministic Effects – Cell loss and functional tissue impairment

- Bone marrow failure (60 days) – 3-5Gy,
- Infection and sepsis 3Gy (1-2 weeks),
- Gut failure > 6Gy (5-10 days),
- hair loss: 2-5Gy; 2-3 weeks
- Skin erythema: 2-5Gy, desquamation and late damage: 20-40Gy

Dose Tolerance Varies with the Tissue

Low Dose Late Effects

RERF:

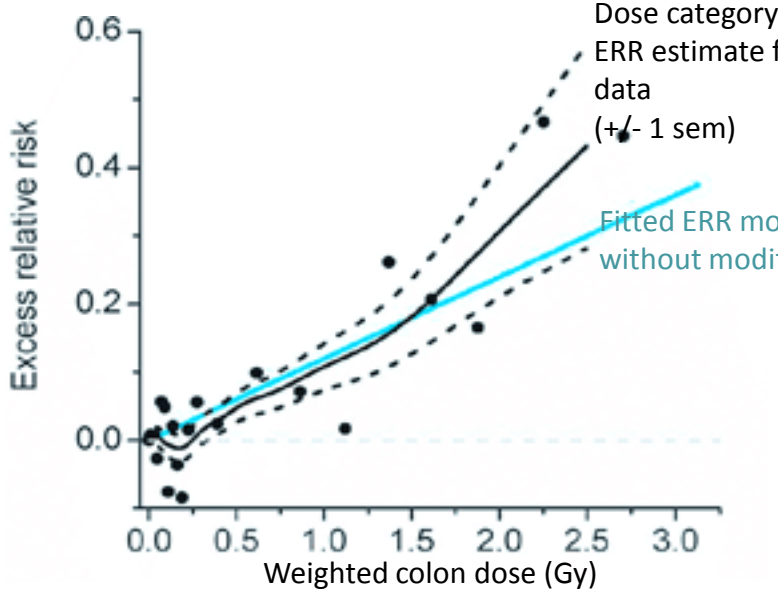
A-bomb survivors Life Span Study (LSS) mortality data (1950 – 1997; N = 49,114) with ≥0.005Gy colon dose.

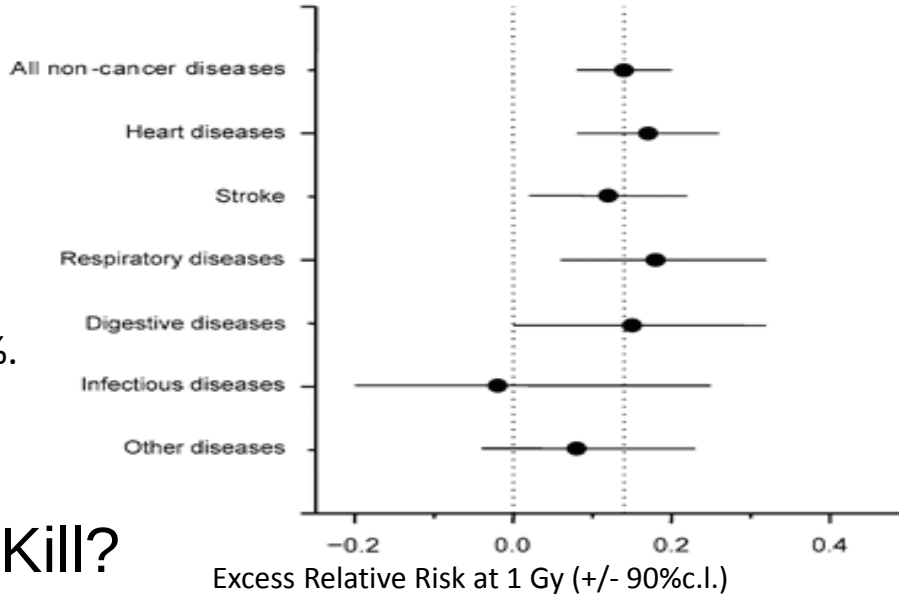
- Proportion of total life lost

60% solid cancer
10% leukemia
30% non-cancer
(Douple et al. 2011)

- 18,049 non-cancer deaths, excluding diseases of the blood. Non-cancer blood diseases studied separately as they may represent hematologic malignant/premalignant conditions.

60% circulatory diseases
15% liver diseases
10% respiratory diseases
Mean dose of 0.2Gy increases death rate by 3%.

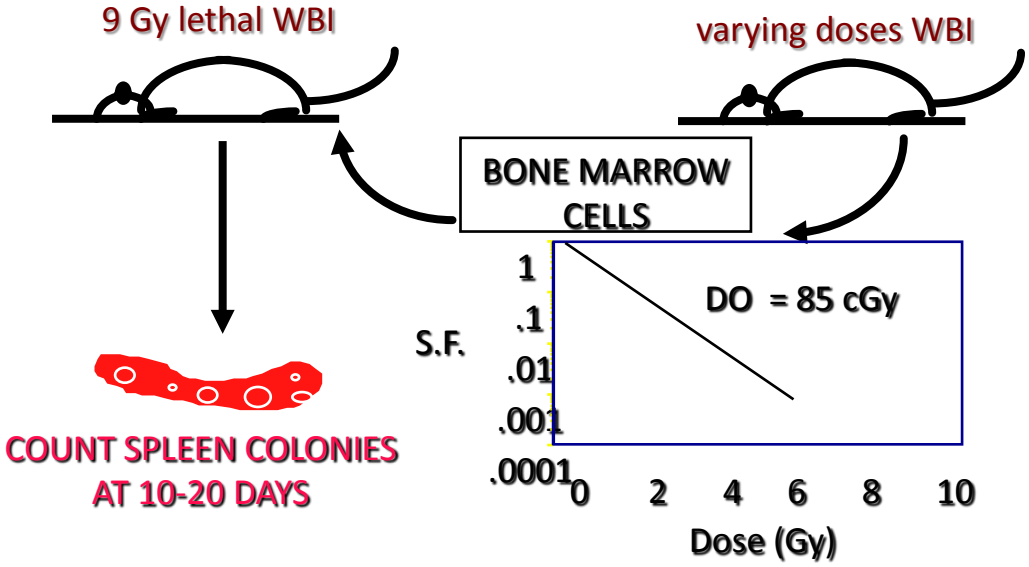




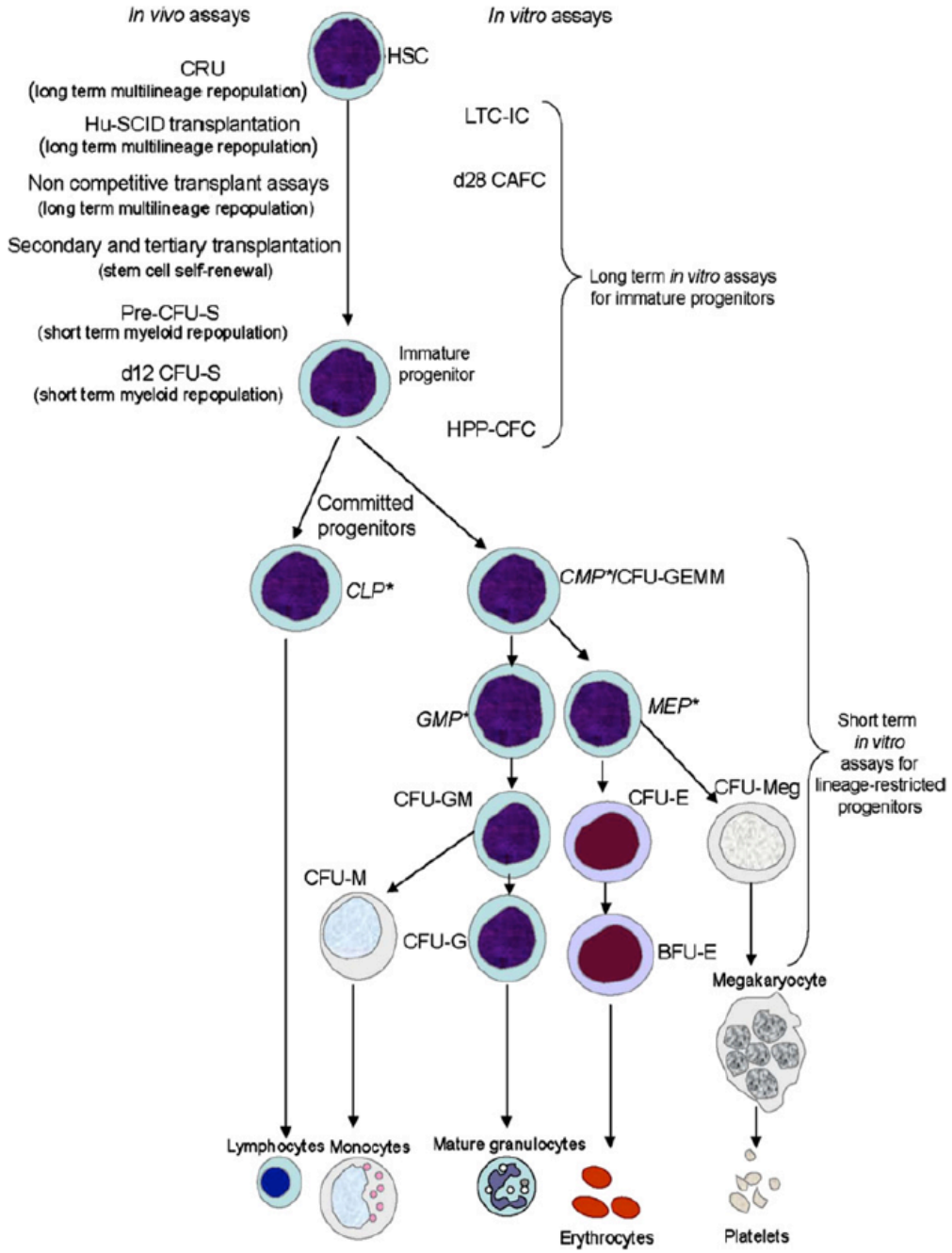
Are these Effects due to Cell Kill?

What underlies deterministic high dose effects? Evidence from "stem" cells

- Rescue of lethally irradiated mice by bone marrow cells
- Ford et al., Nature 177, 452, 1956, showed that donor cells were responsible using cytogenetics
- Till and McCulloch, Radiation Research 13 (1): 115, 1960, quantified this by counting colonies that developed in the spleen after transfer
- In humans, death within 2-8 weeks after > 4Gy W.B.I. is due to "bone marrow insufficiency"



Drs. James Till and Ernest McCulloch



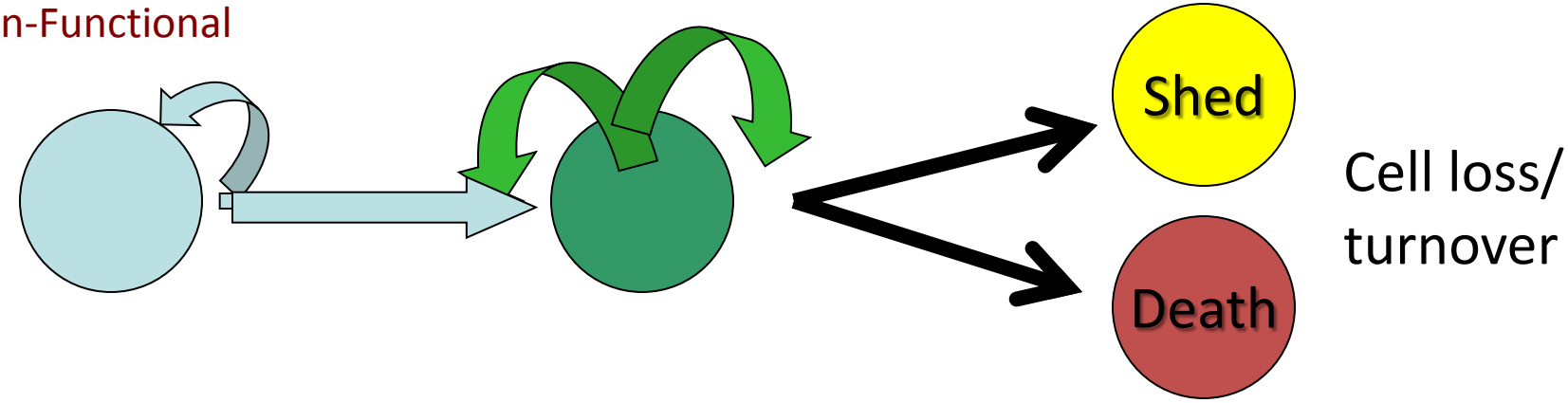
The type of colony that develops in the spleen varies with time after injection and reflect different lineages of progenitor/stem cells and their "primitiveness." Each has its characteristic dose-response relationship. "Rescue" requires function to be maintained and relies on rapid cell production.

Structural Organization of Tissues

Multipotent Stem cells
Small number
Quiescent - niche
Non-Functional

Pluripotent Progenitor cells
Highly proliferative – growth fraction
Generally non-Functional

Differentiated cells
Non proliferative
Functional

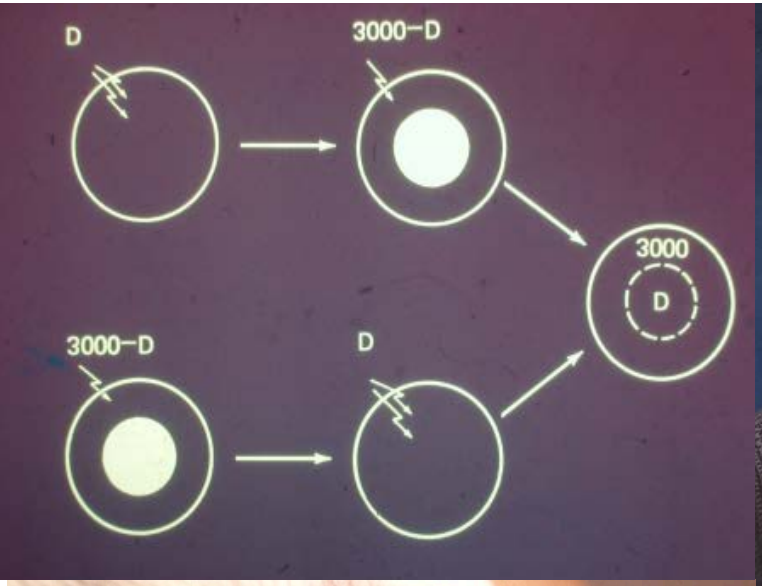


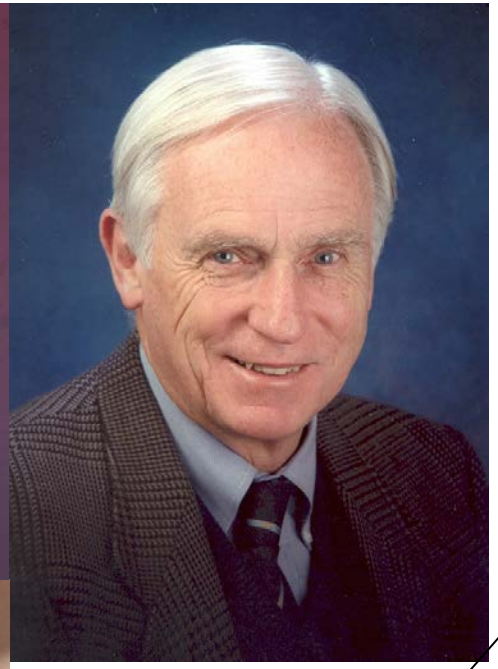
Under steady state conditions, a constant cell number is maintained by the balance between cell loss and cell proliferation. Cell loss i.e. $\Phi = 1.0$.
In regeneration, tumors and in embryos, $\Phi < 1.0$
After irradiation, the cell loss factor initially increases due to cell cycle arrest and death of progenitor cells which leads to decreased function and symptoms.

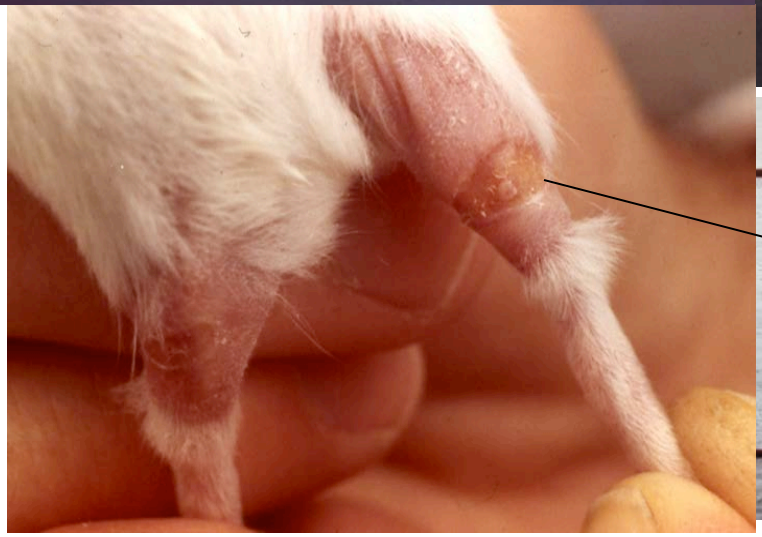
In turn, cell loss leads to regeneration and a decrease in the cell loss factor.
If regeneration fails to occur in time, lethality can occur.

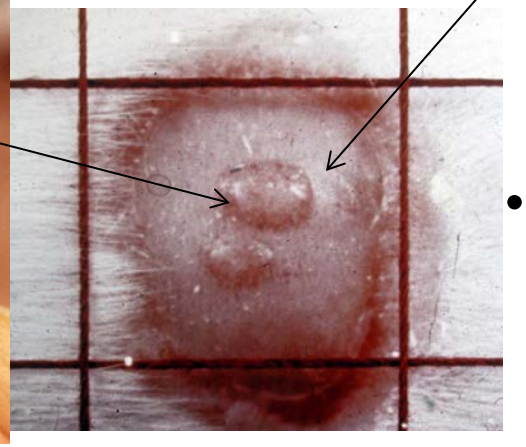
The time to loss of function is the LATENCY i.e. time to appearance of symptoms

In Vivo Skin CFU Assay







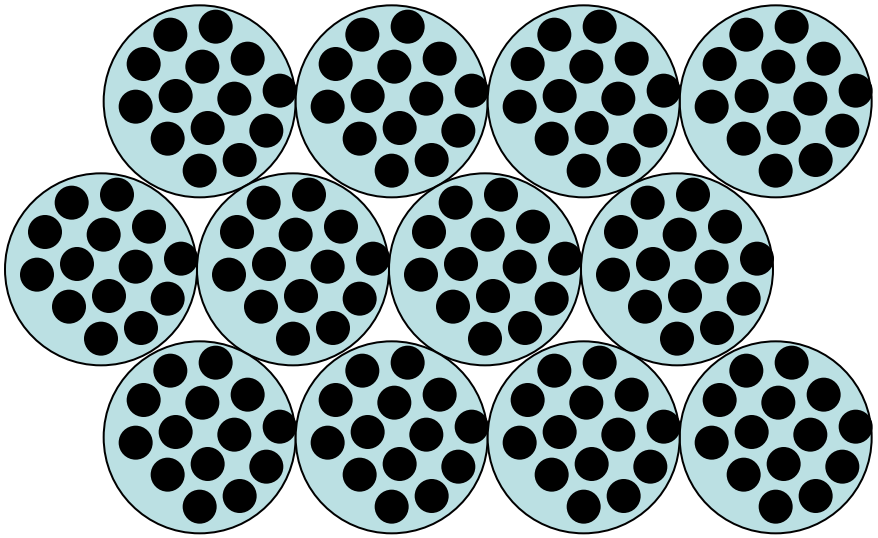


- Withers used shielding to create a central area of skin with a high dose “moat”. The center was then irradiated with varying doses. Discrete clones regenerated within a certain dose range.
- Two clonally-derived islands of rapidly regrowing epithelium in a 1cm diam radiation-induced skin ulcer.
- Moist desquamation results if there is <1 surviving clonogen/cm2, which equals SF of 10^{-6}.

Functional Subunits (FSU)

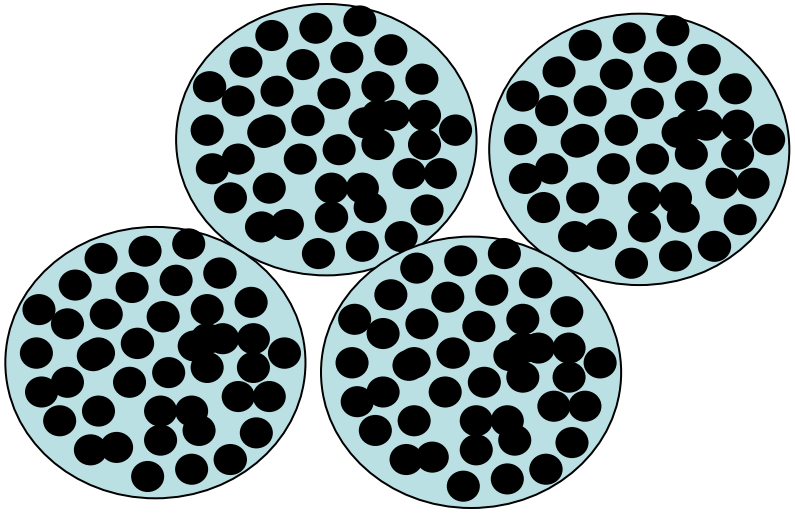
Tissue A

168 clonogens in 12 FSUs



Tissue B

168 clonogens in 4 FSUs

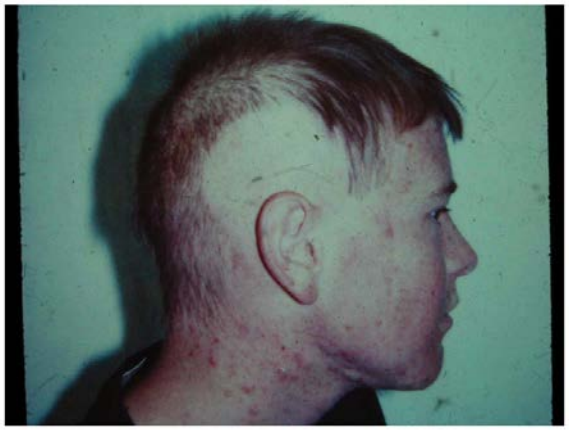


Which is more radiation resistant and by how much?

One reason why tolerance dose varies from tissue to tissue

Examples:

- Epilation occurs at a lower dose than desquamation because hair has fewer clonogenic cells per FSU. Epilation is reversible after 3Gy but irreversible after 6-7Gy. It occurs after 3 weeks.
- Hair depigmentation occurs at a lower dose than skin depigmentation because follicles contain fewer melanocyte clonogenic cells.

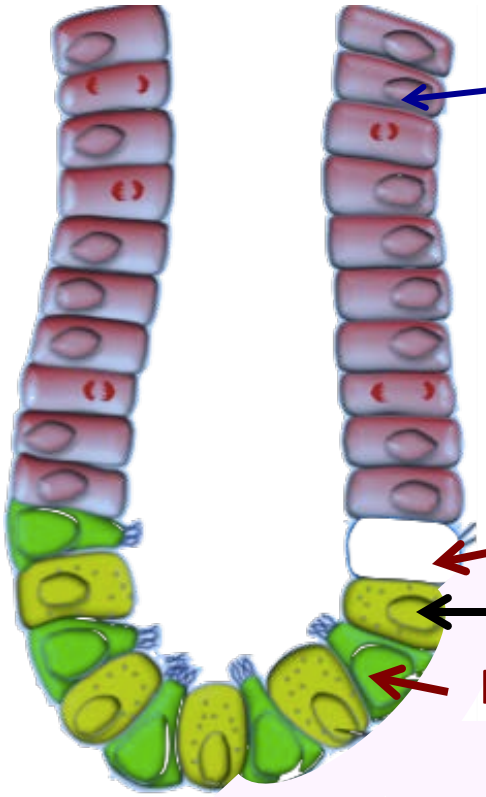


- Radiation can impair oocyte function, leading to infertility. The radiation tolerance dose decreases with age due to falling oocyte numbers.

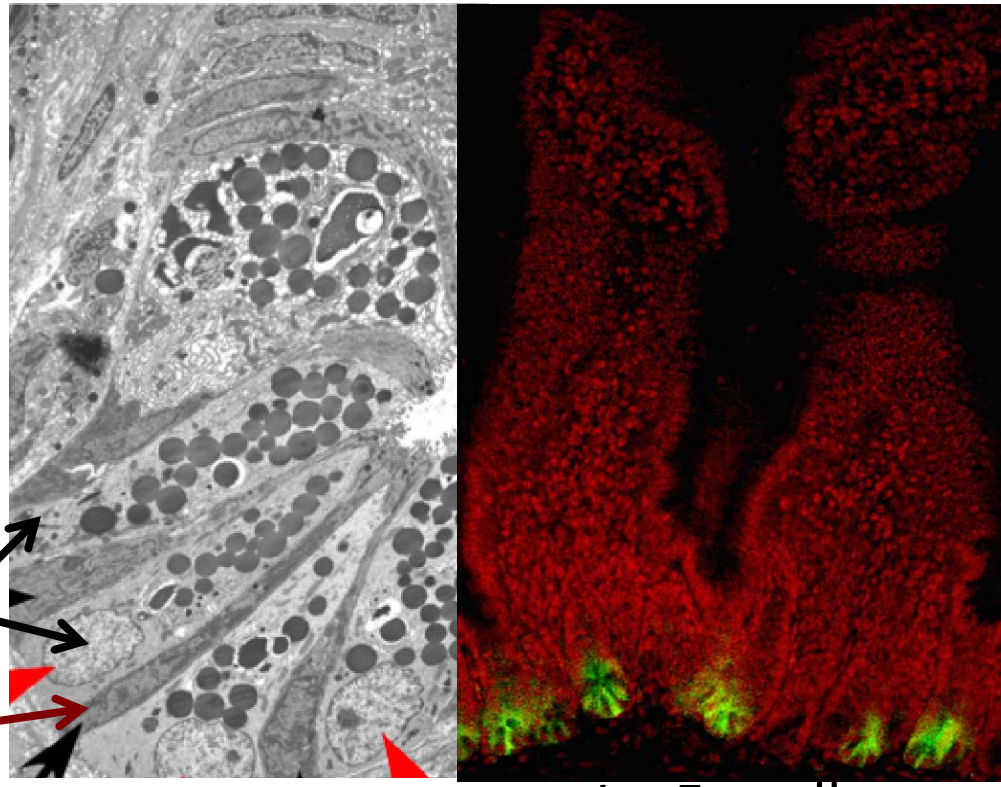
Tracing Events in the Gut after Irradiation

In the crypt, radiosensitive Lgr5+ and more resistant Lgr5- stem cells coexist with Paneth cells.

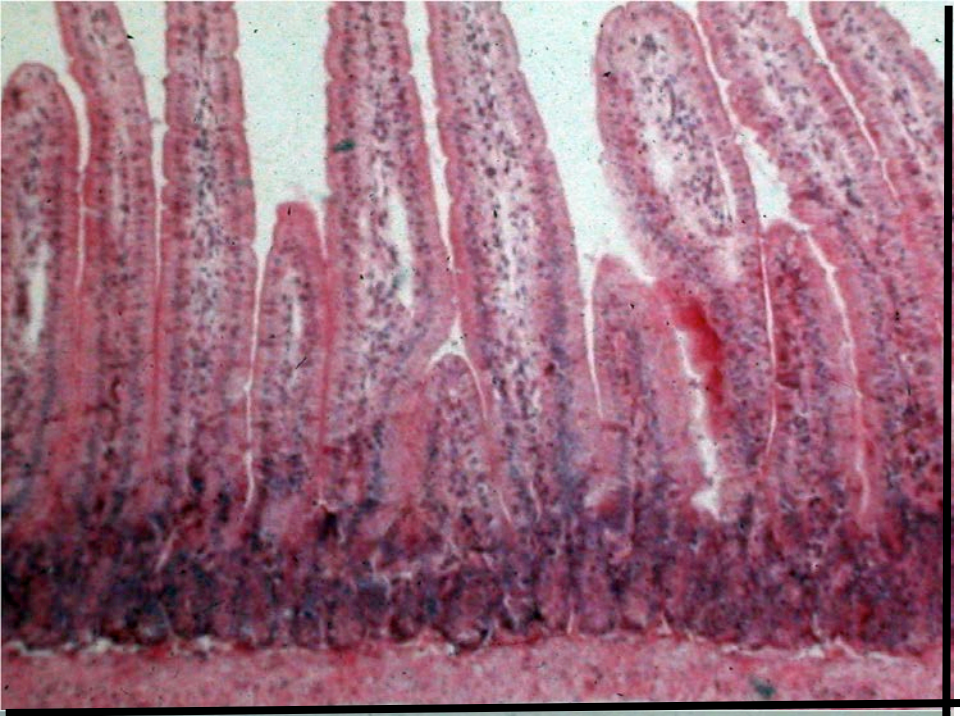
One theory is that after irradiation radiosensitive Lgr5+ CBCs are replaced by reprogramming of Lgr5- cells from position 4.



Cell Stem Cell 11, 452, 2012
Barker, et al.



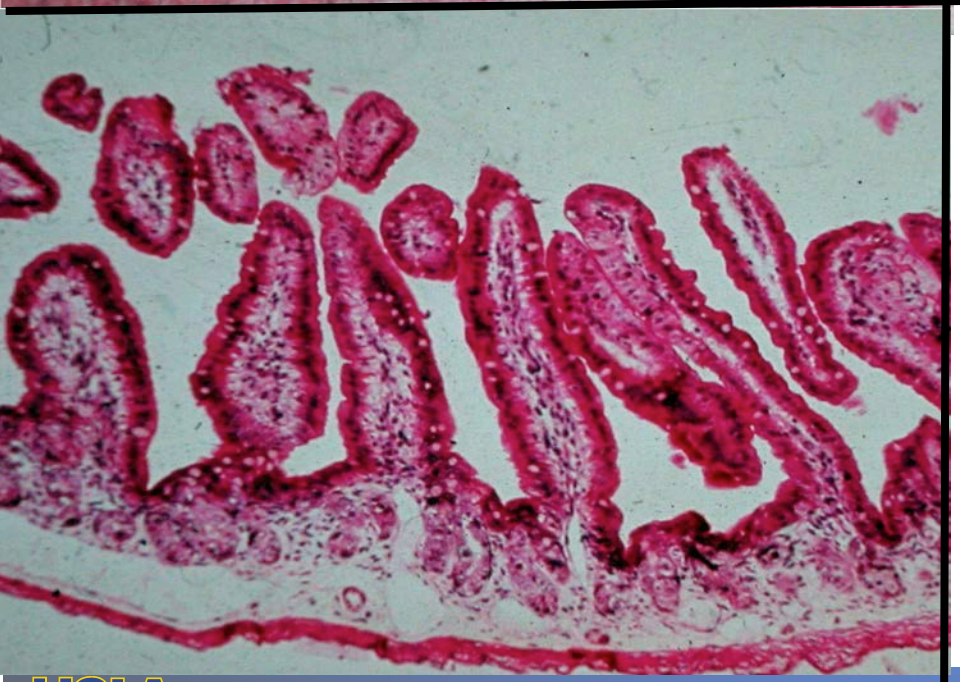
Lgr5+ cells (green)



Transmission light microscopy of mouse jejunum

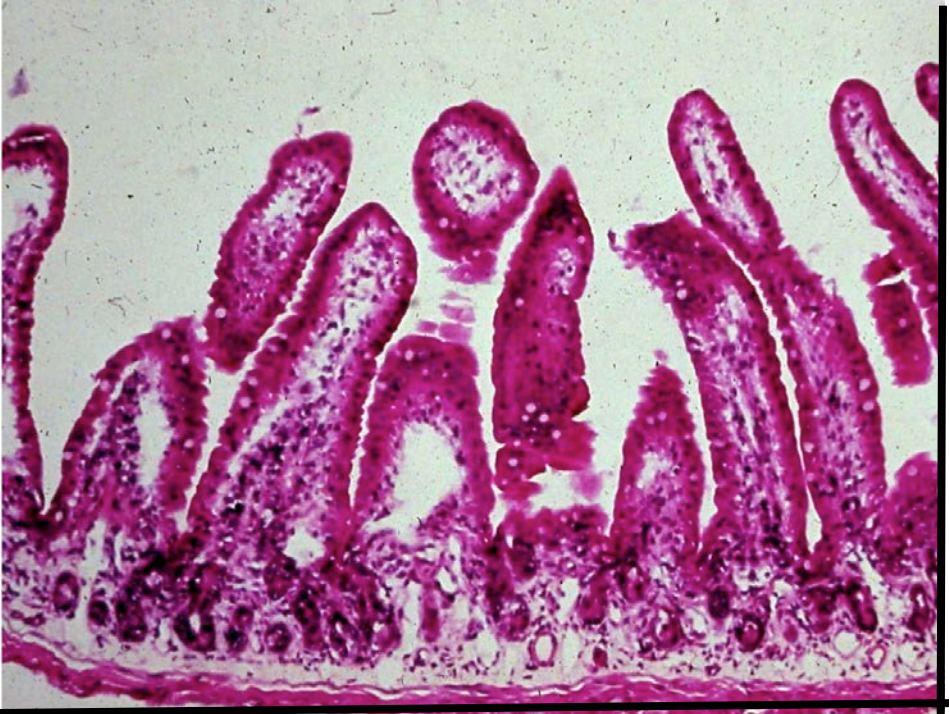
Normal:

- All mitotic activity is in crypts (dark stain)
- villous cells are post-mitotic, differentiated cells pink)



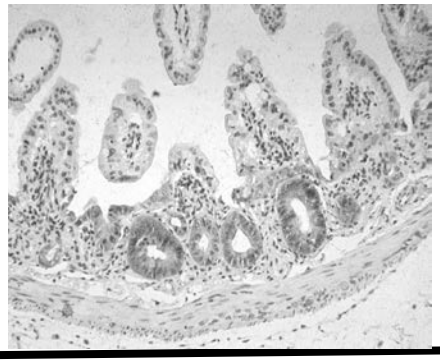
1dy after a sublethal dose of 10Gy:

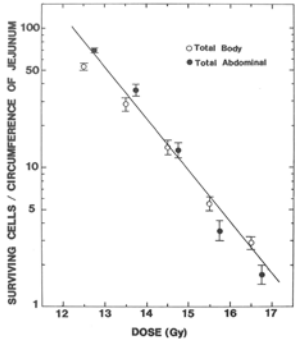
- Rapidly proliferating Lgr5+ crypt cells are depleted through apoptotic and mitotic cell death.
- Villous cells survive until lost through normal turnover kinetics (Φ)
- On average, about 5 of the original 130 clonogenes/crypt survive 10Gy.
- Lethality occurs when <1 survive/

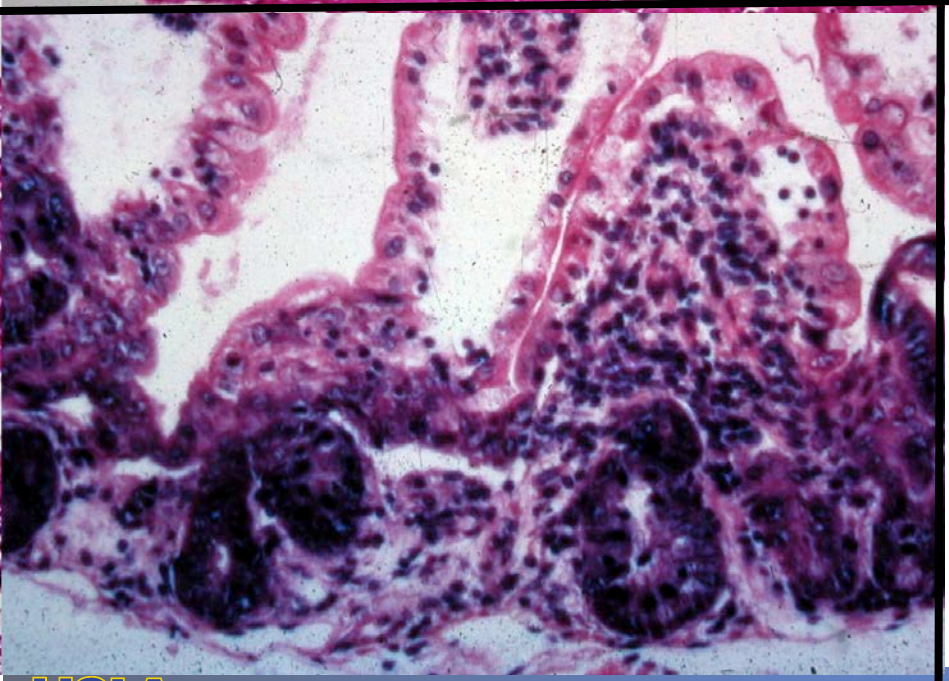


2dys after a sublethal dose of 10Gy:

- Villi getting shorter
- Crypts beginning to regenerate.
- Regenerating clones after 2.5dys are counted in a stem cell survival assay

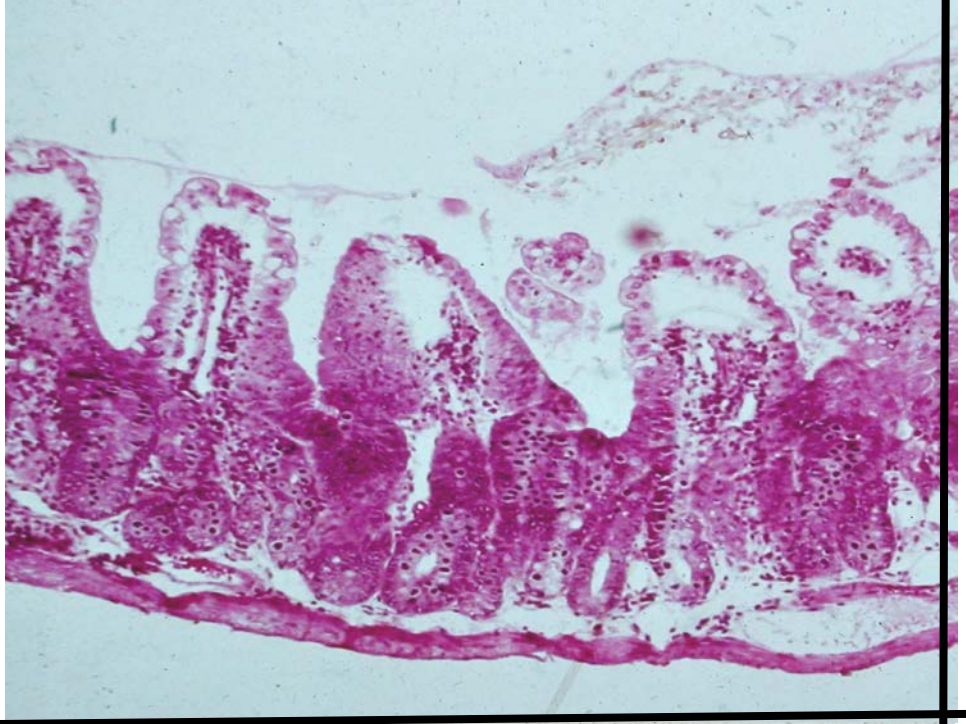






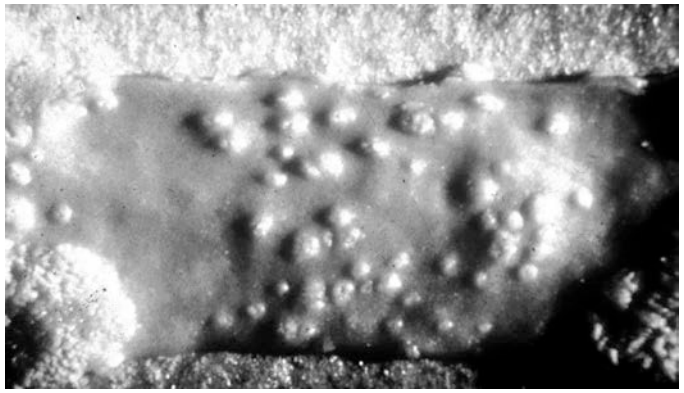
3dys after a sublethal dose of 10Gy:

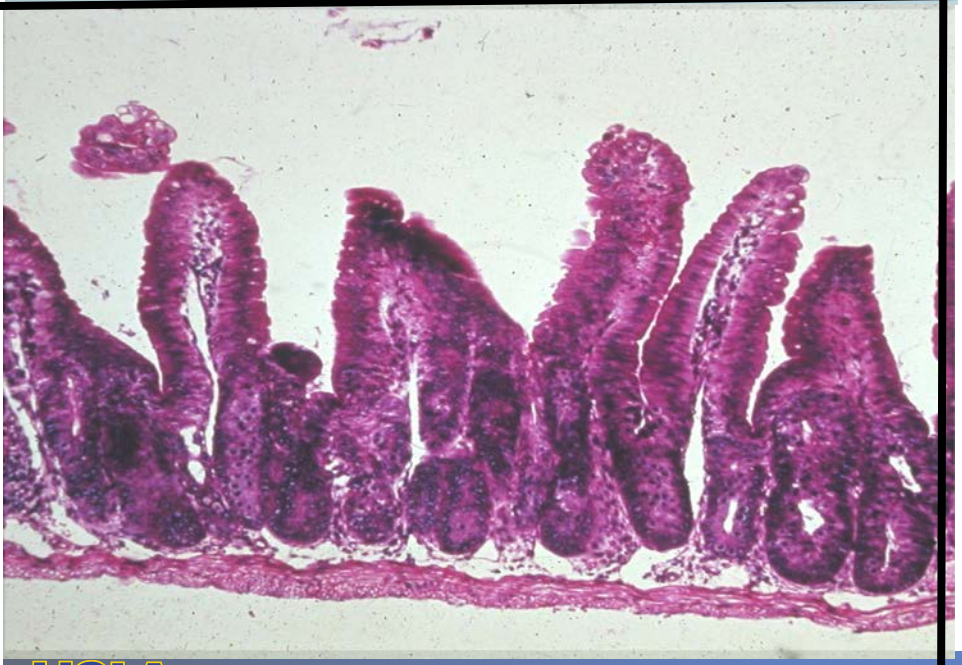
- Villi short and not replenished from crypts
- Clonogens in crypts exponentially increasing at same rate as cell cycle time of 8 hours, implying no cell loss i.e. $\Phi = 0$



4dys after a sublethal dose of 10Gy:

- Early reconstruction of villi
- Crypt regeneration “over-shoots” - macroscopic clones develop on gut surface





5days after a sublethal dose of 10Gy:

- Further recovery and reconstruction of mucosal structure to near normal

Latency

The time to expression of complete tissue failure (latency) depends on turnover time. This allows normal tissue complications to be divided into

Acute Responding causing Acute Radiation Syndrome (ARS) < 2.5 months

- Gut
- Skin
- Bone Marrow
- Mucosa

- Rapid turnover
- Failure due to loss of functional cells and lack of replacement due to a radiation-induced deficit in stem/progenitor cells
- Associated with acute inflammatory responses
- Regeneration leads to rapid and often complete recovery

Late Responding >2.5 months

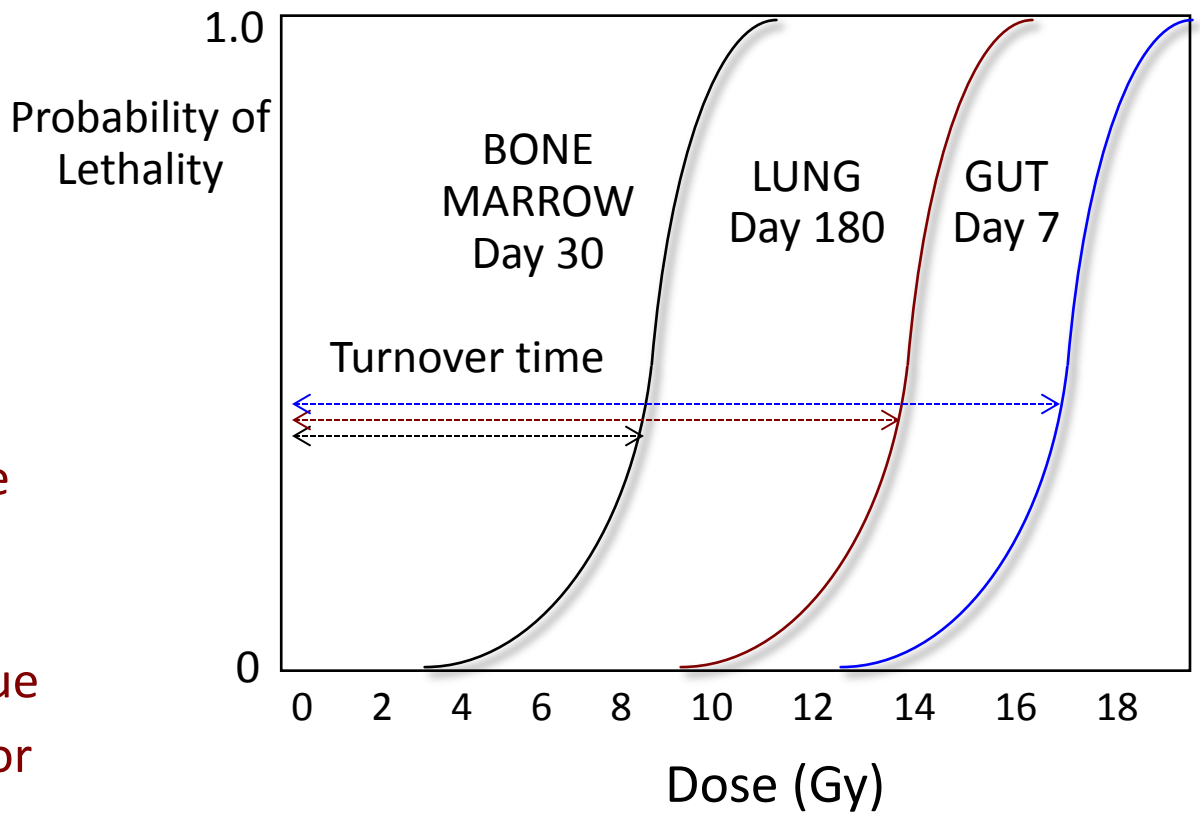
- Heart
- Lung
- Kidney
- Bladder
- CNS

- Slow tissue turnover rate
- Functional cells may be iforced to proliferate on demand after a long lag time.
- Can be forced to proliferate, eg surgery, resulting in an “avalanche” effect
- Late effects can be considered as dysregulated wound healing with molecular and cellular responses occurring during the latent period.
- Associated with chronic inflammatory responses

Acute and late complications can occur independently

Latency ≠ Dose Tolerance

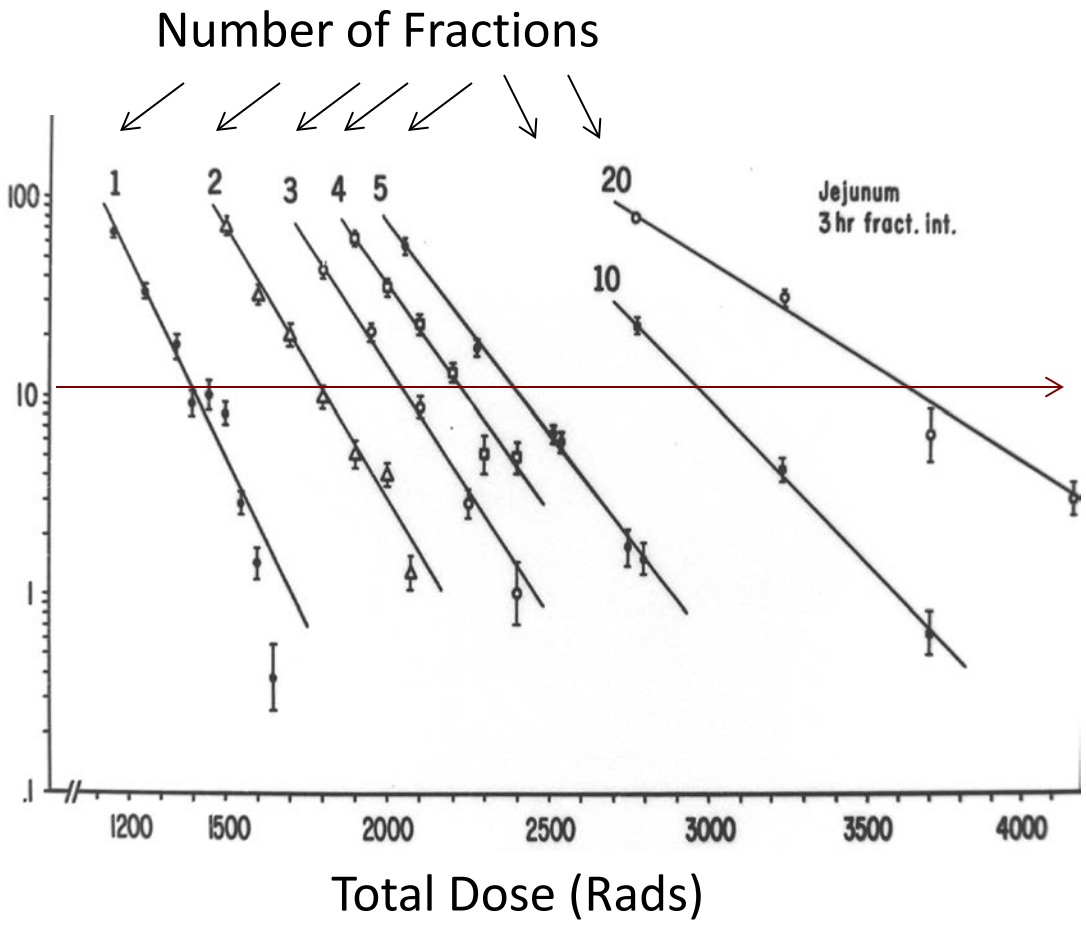
- For a specified endpoint, different tissues have different tolerances to irradiation and fail after different times (latency).
- There is some genetic component to radiosensitivity and to the nature of pathogenesis.
- Latency is determined by turnover kinetics in a tissue
- Latency is NOT an indicator of radiosensitivity.
- Late damage may occur after radiation doses that are insufficient to cause acute damage.



Threshold-sigmoid curves for overt damage, such as lethality, are very steep

In these experiments, mice were given varying doses of whole body, abdominal, or thoracic irradiation, and the dose and time to lethality assessed

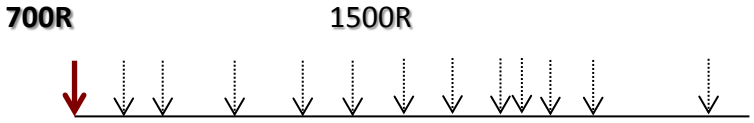
Factors Affecting Tolerance: Repair Between Fractions Measured by Gut Crypt Stem Cell Microassay

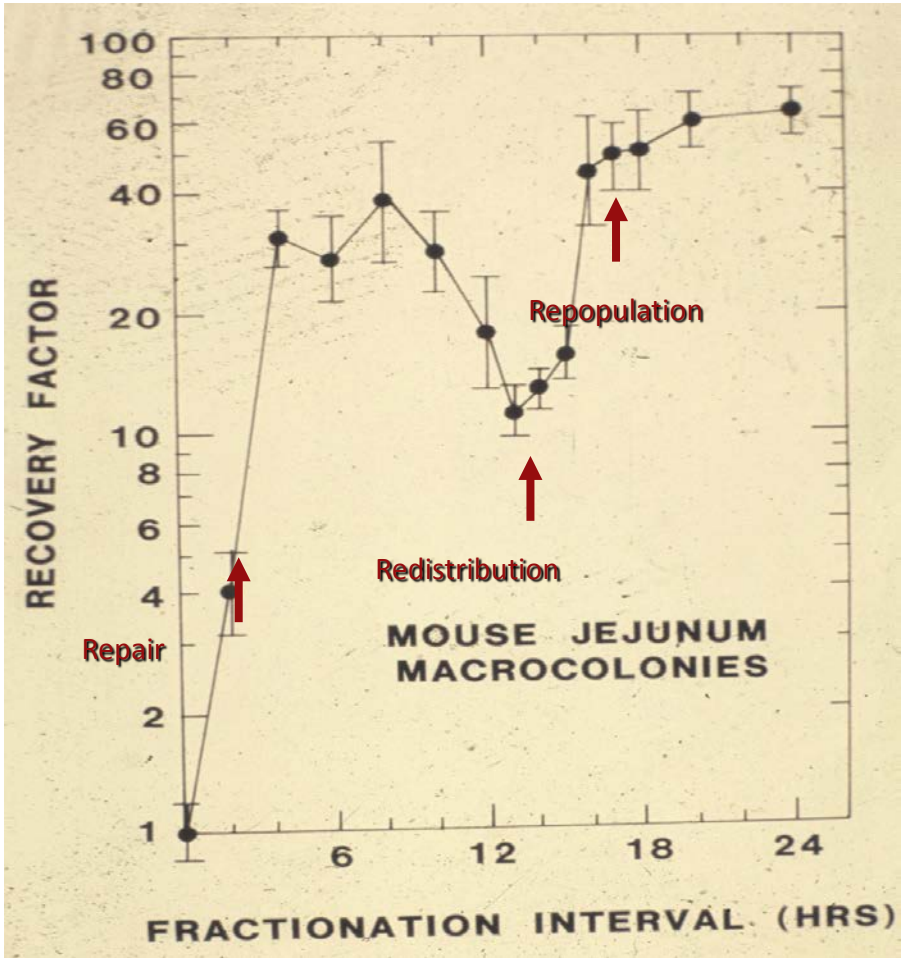


Sparing by dose fractionation -
Or by decreasing dose rate

Repair, Redistribution, Regeneration Measured using Jejunal Crypt Microassay

The success of fractionated RT was enshrined in the 4 Rs in the 1970s (Withers - Adv Radiat Biol 5:241, 1975) - repair, redistribution, repopulation, reoxygenation. This exploits differences between tumor and normal tissue in their time/dose responses. Late responding tissues are preferentially damaged by high single radiation doses



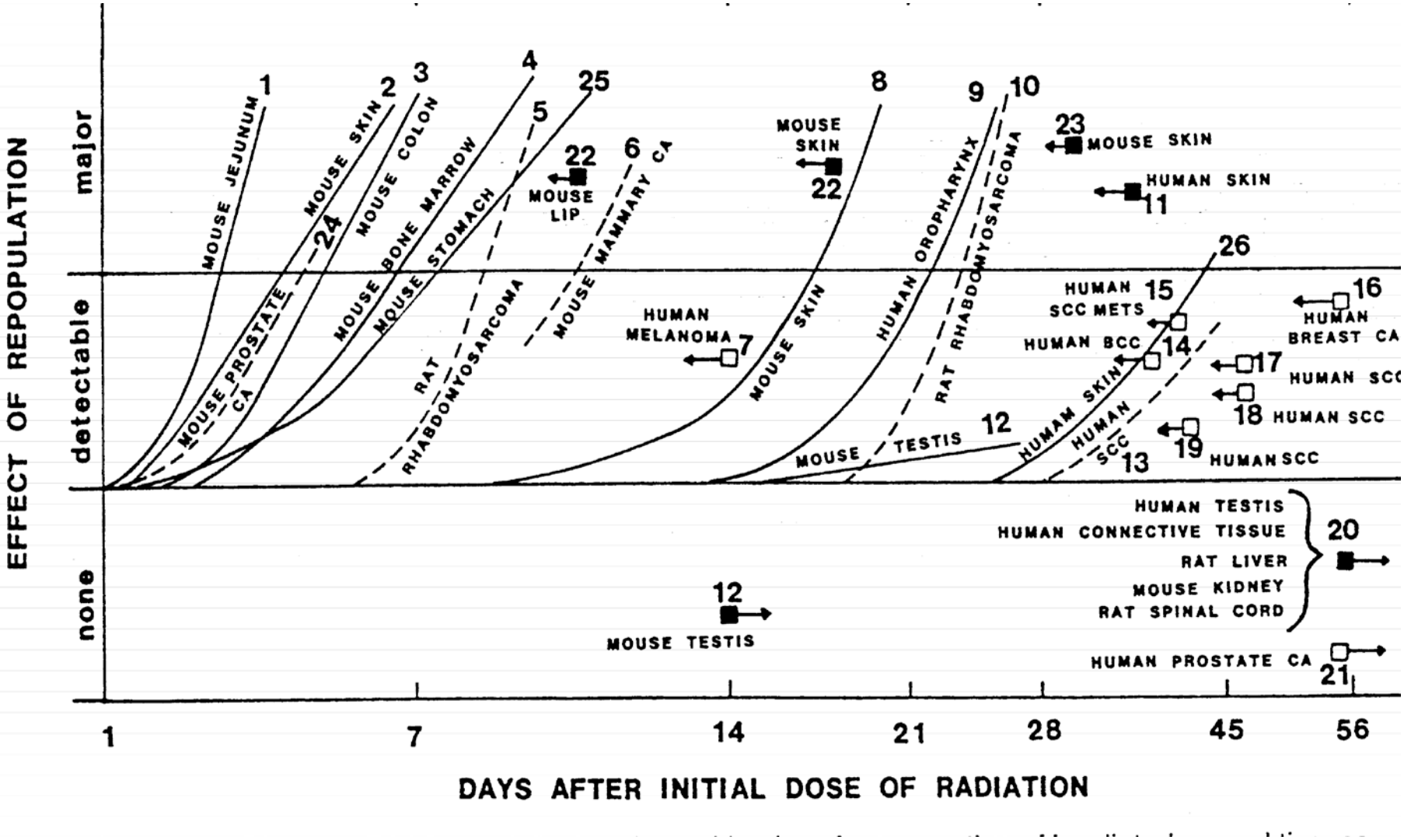


Tissues Vary in their Ability to Regenerate and this Affects Tolerance

Testis

- In testis ϕ remains 1.0, it does not regenerate
- Latency = 60 days - time for 1 spermatogenic stem cell to give 1000 sperm. This is why sperm counts remain normal for weeks after irradiation and then fall precipitously.
- Tolerance is about 3 - 5 Gy in 2 Gy fractions can cause permanent sterility. 0.1 - 0.15 Gy can cause temporary sterility.
- Regeneration: Unlike the gut, where recovery can be complete, sperm counts may not recover for years - there is little regeneration!

Lag Time to Regeneration Varies Greatly

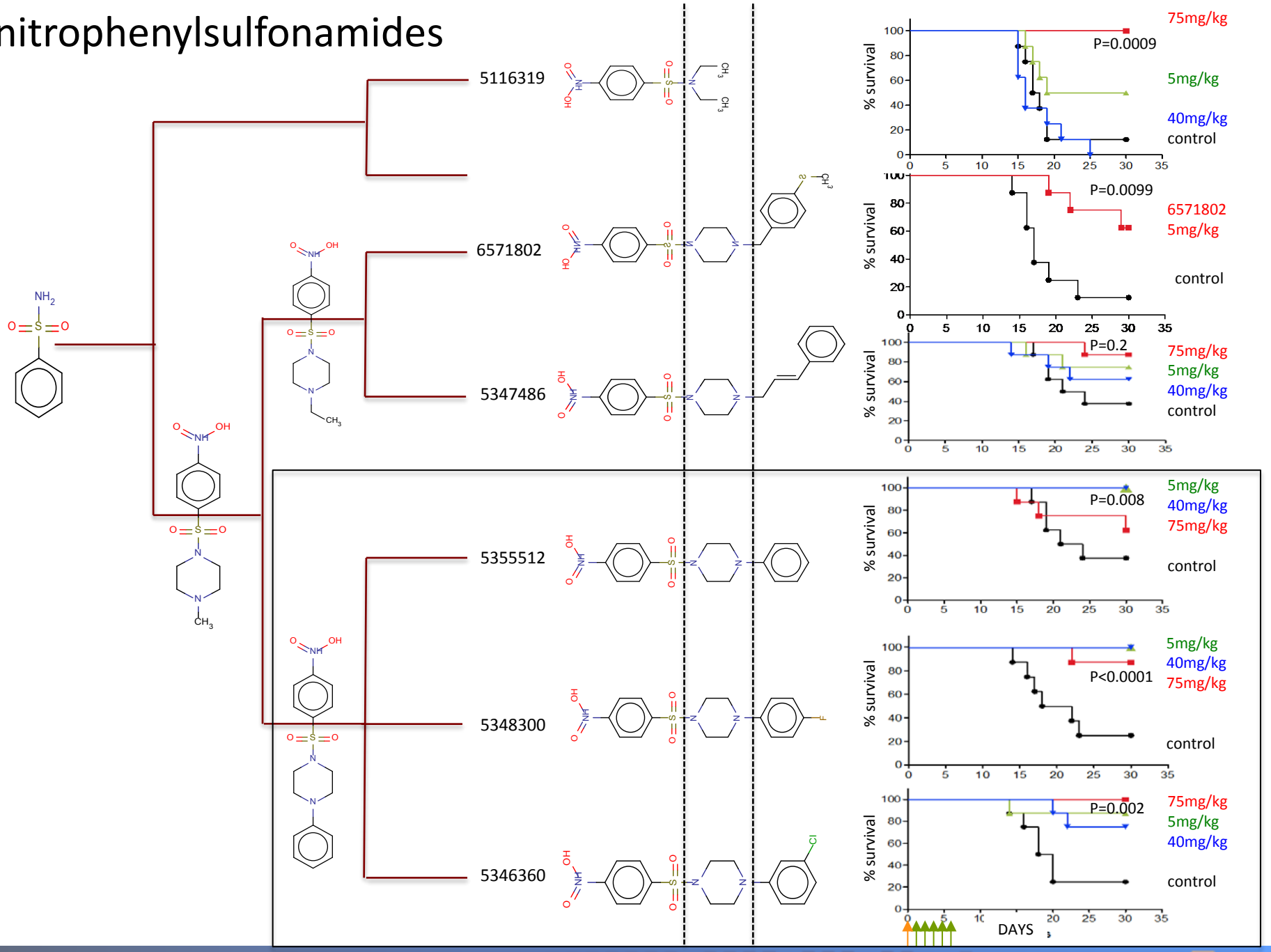


Kinetics of tissue regeneration. Withers and McBride, Perez, p82.

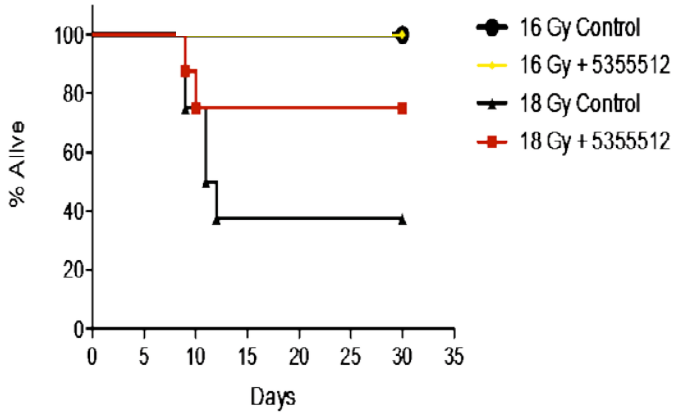
Can We Mitigate Lethal Radiation Damage?

Countermeasures given at least 24 hrs after potentially lethal whole body exposure.

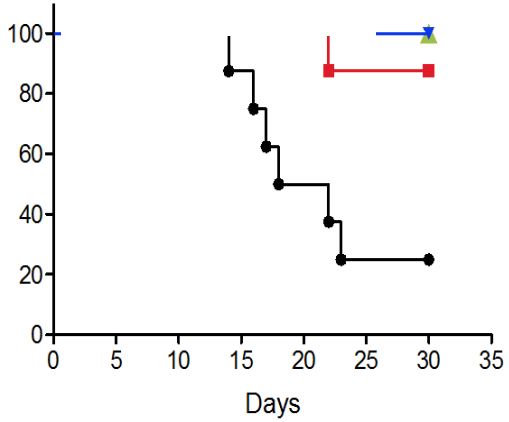
4-nitrophenylsulfonamides



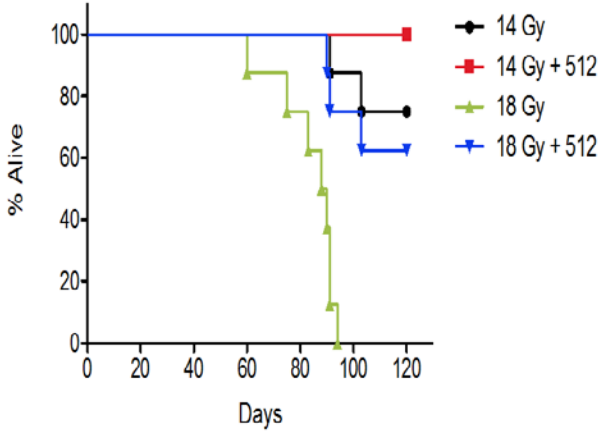
GI ARS in C57Bl/6 mice



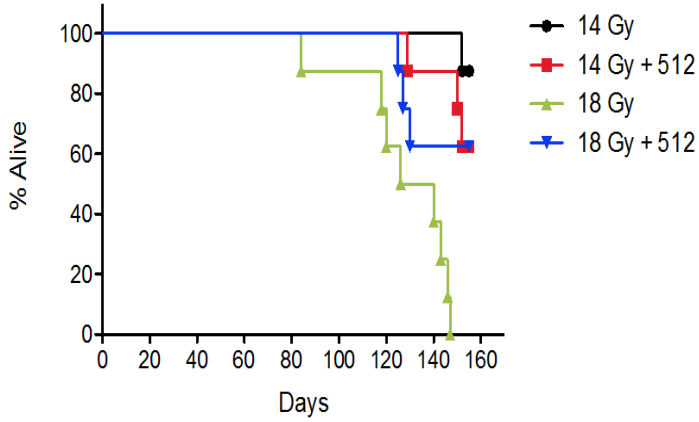
Hematopoietic ARS in C3H mice



Pneumonitis in C3H mice



Fibrosis in C57Bl/6 mice



One agent may mitigate against several radiation syndromes

Late Effects

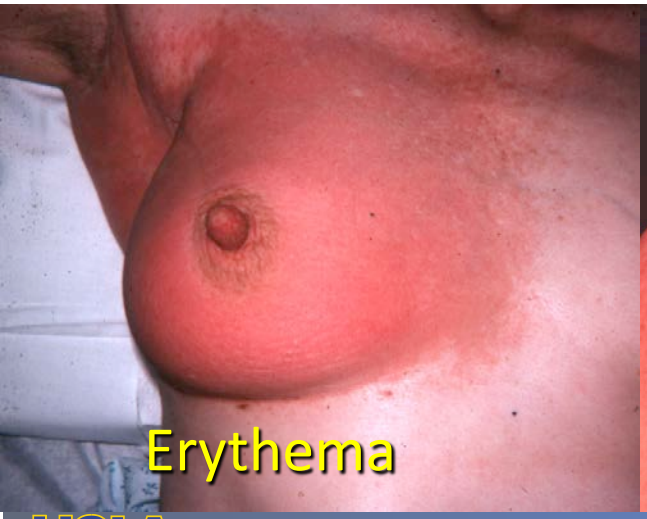
Late effects are complex:

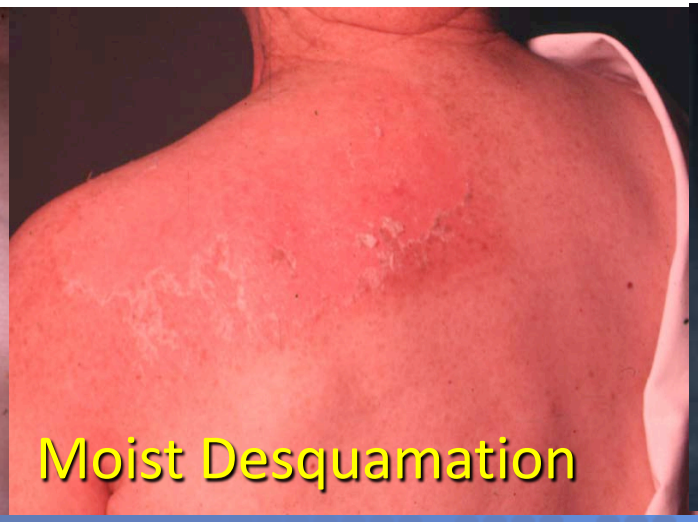
- *Late/Delayed Effects that are Independent of Acute Radiation Damage*
- *Late/Delayed Effects that Reflect Acute Radiation Exposure (DEARE)*
- *Late Multiple Organ Disease Syndrome (RI-MODS)*
- *Late Low Dose Non-Cancer Effects*

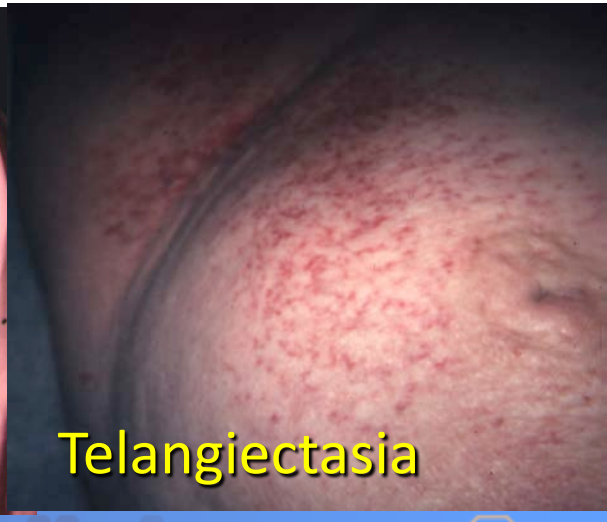
Acute and Late Effects Occur in One Tissue

For example - Skin

- Acute erythema can occur 1-2 days after 2-6 Gy
- Erythema and epilation can occur 2-3 weeks after 5-10 Gy
- Desquamation can occur 2-3 weeks after 15-20 Gy
- Re-epithelialization takes 6-8 weeks
- Late effects - atrophy, fibrosis, necrosis, telangiectasia.
- Some late effects may be consequential to acute damage, but certainly not all





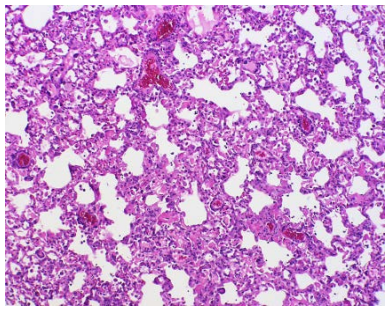


Lung

- The lung develops radiation-induced pneumonitis and/or fibrosis, the former occurs at 3-4 months after irradiation, the latter at >5 months.
- C3H mice develop pneumonitis, C57Bl/6 mice fibrosis - there is a genetic element.
- In humans, viral reactivation or infection can affect outcome.
- Inflammation and immune cell infiltration are more obvious hallmarks of radiation exposure.

Control

15 Gy XRT

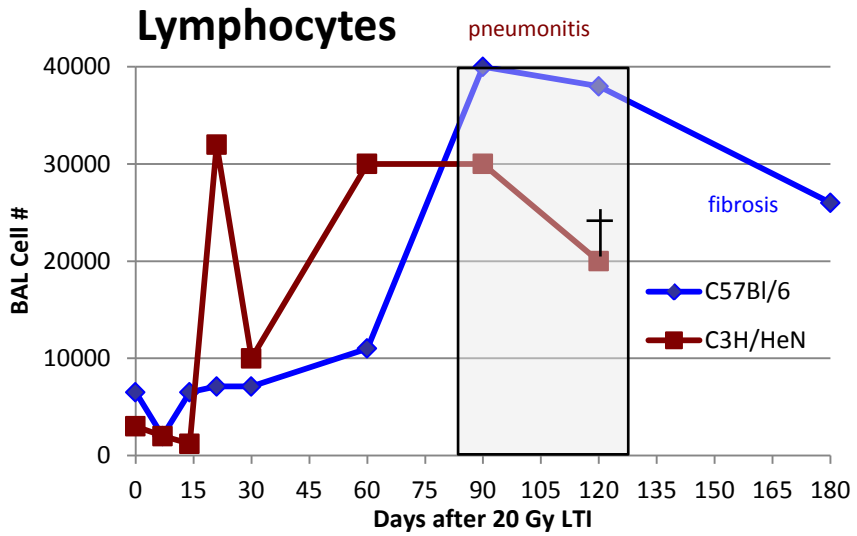


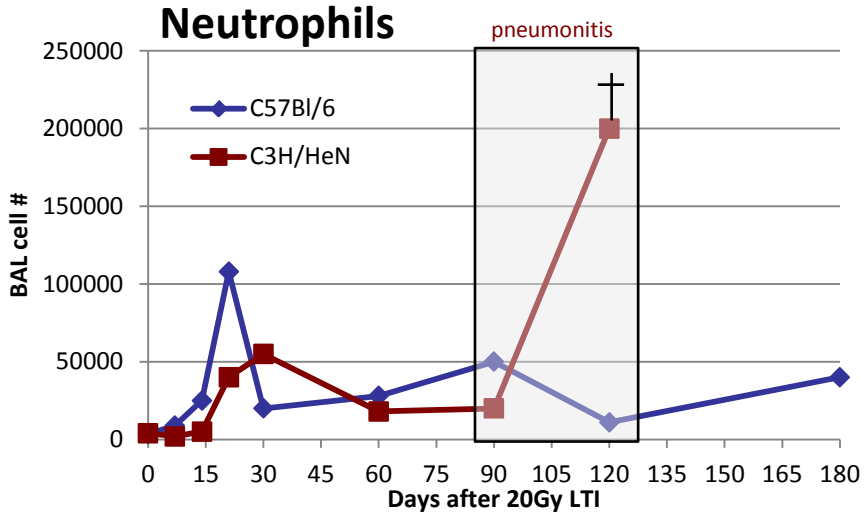
lagen

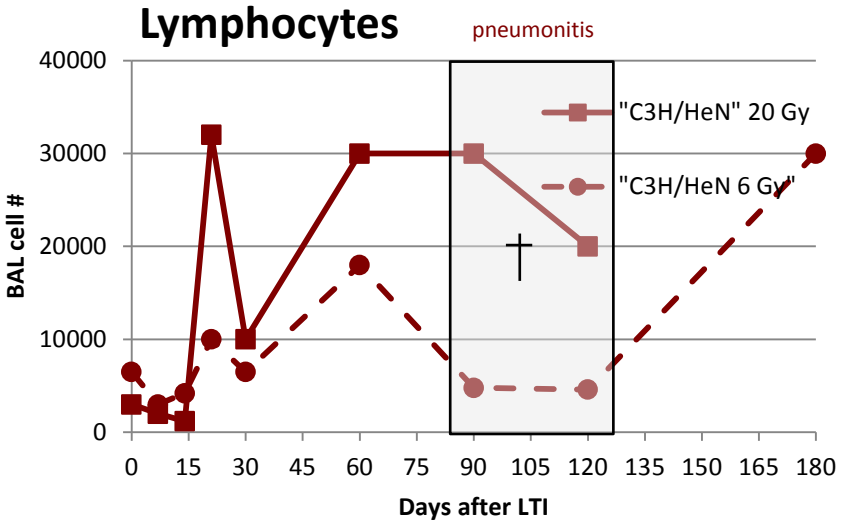
(Masson's Trichrome)

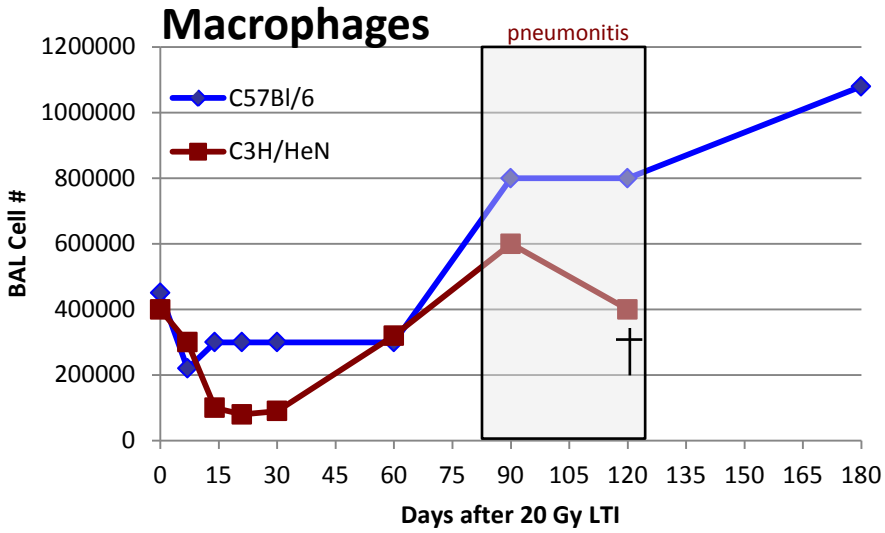
Macrophage

Radiation-induced inflammatory cells in bronchoalveolar lavage





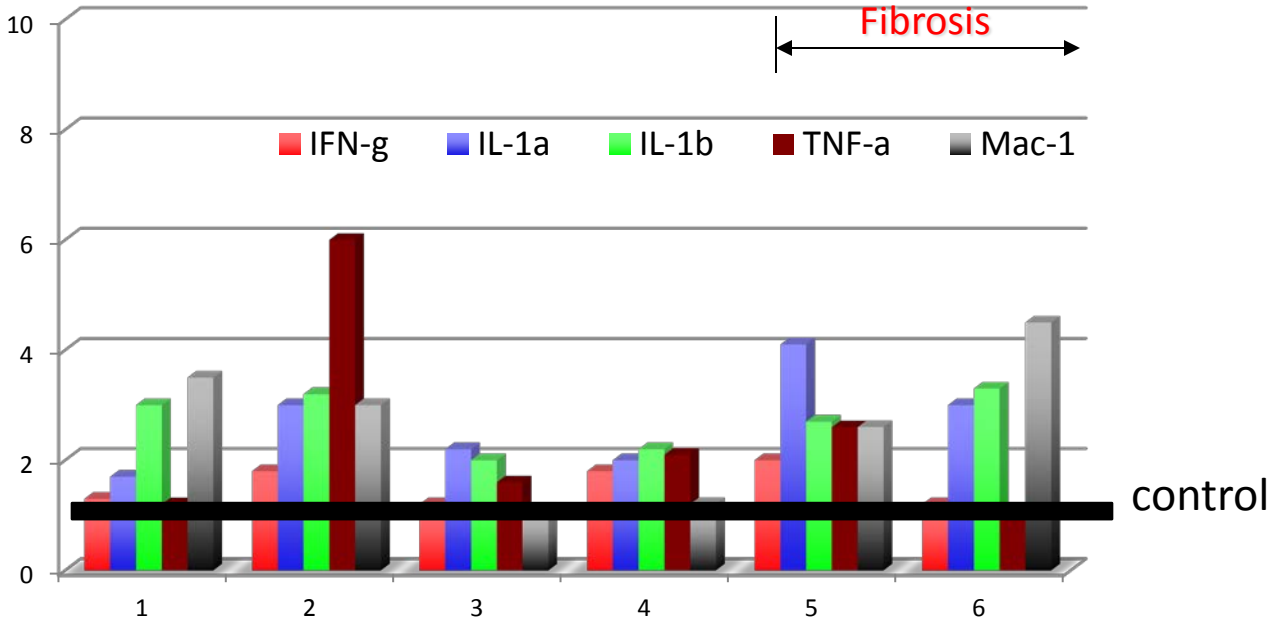




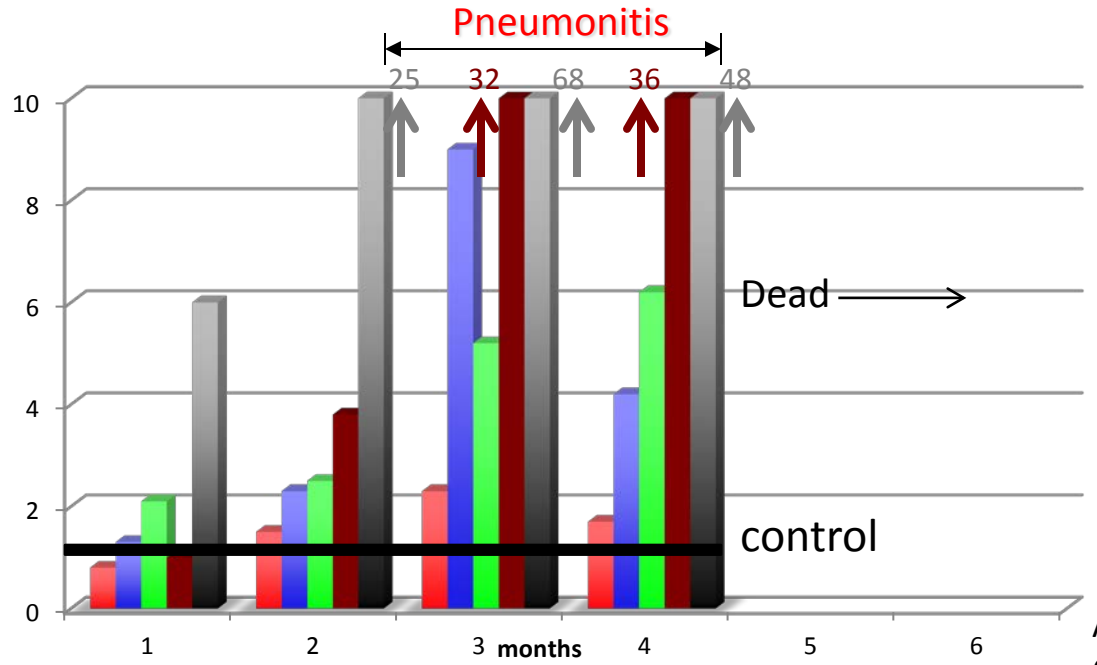
After: C-S CHIANG et al. Int. J. Radiation Oncology Biol. Phys., Vol. 62, No. 3, pp. 862–871, 2005

Whole lung irradiation:
C57Bl/6 fibrosis-sensitive mice
(Ratio mRNA to control)

20Gy LTI
RNP assay



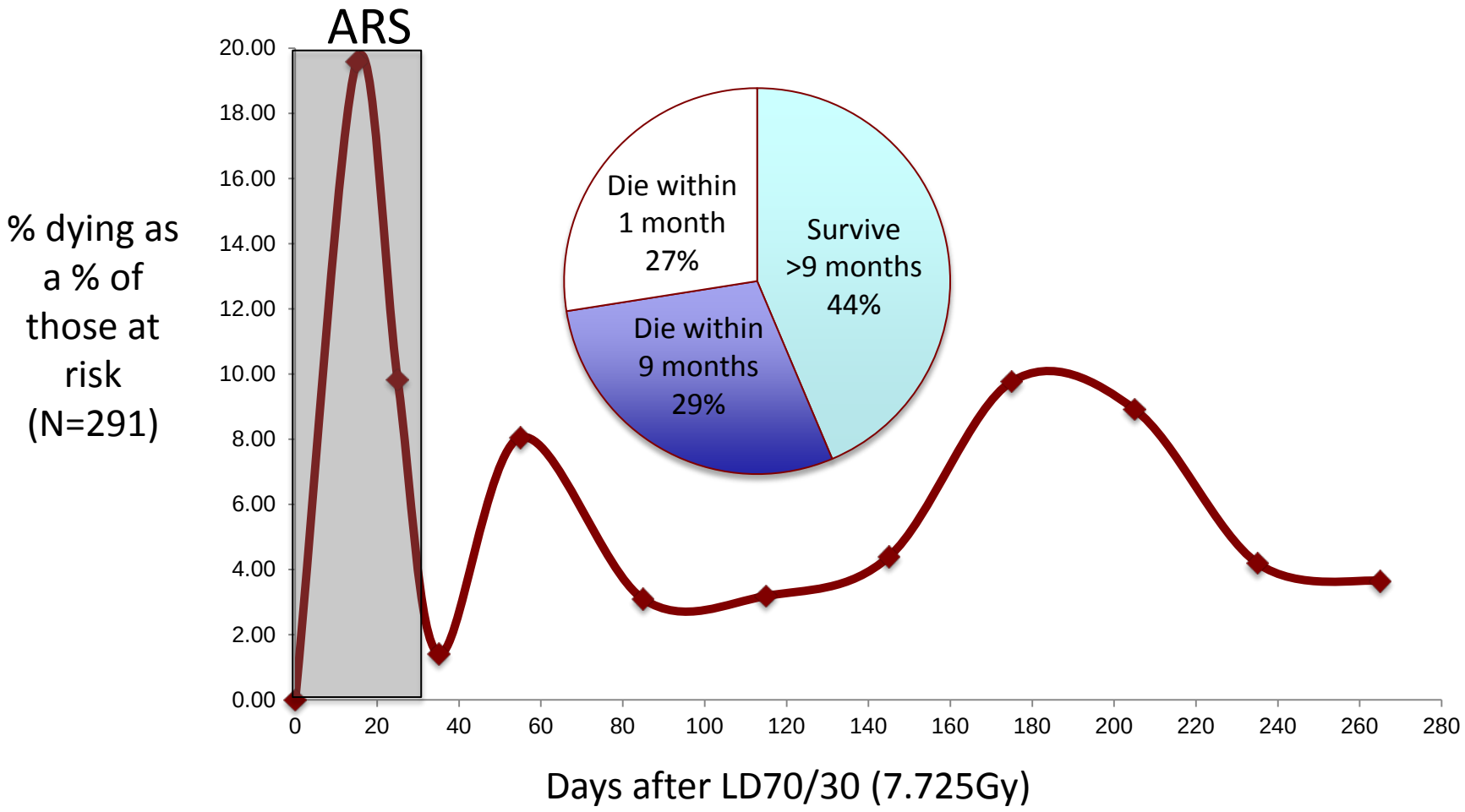
Whole lung irradiation:
C3H/HeN fibrosis-resistant mice
(Ratio mRNA to control)



Adapted from Chiang and Hong

- Irradiation induces an acute inflammatory response even in “late” responding tissues with cytokines expressed within hours of exposure
- Over subsequent weeks and months, a “perpetual (cyclical) cascade” develops - chronic inflammation.
- Cytokines release precipitate local and systemic damage and are a presumed cause of MODS.
- They may affect regeneration or may directly contribute to the pathogenesis of complications and may be responsible for some side effects of irradiation, such as fatigue, edema, somnulence, and nausea.
- Radiation is generally pro-inflammatory.
- Possible triggers for the chronic inflammatory response - dysregulated tissue kinetics, immune imbalance, or wound healing processes.

Survivors of Acute Radiation Exposure



After ARS, approximately 5.5% of survivors died each month.
Deaths occurred in 2 distinct waves and was sudden in most cases!

Histopathology

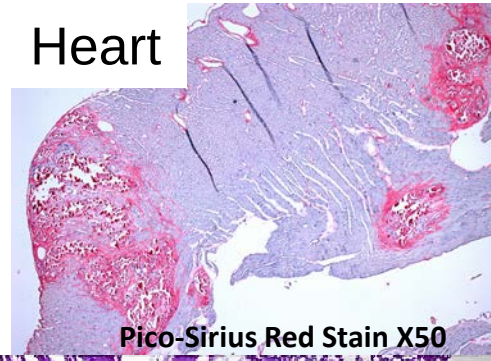
At 3 months after LD70/30 doses,

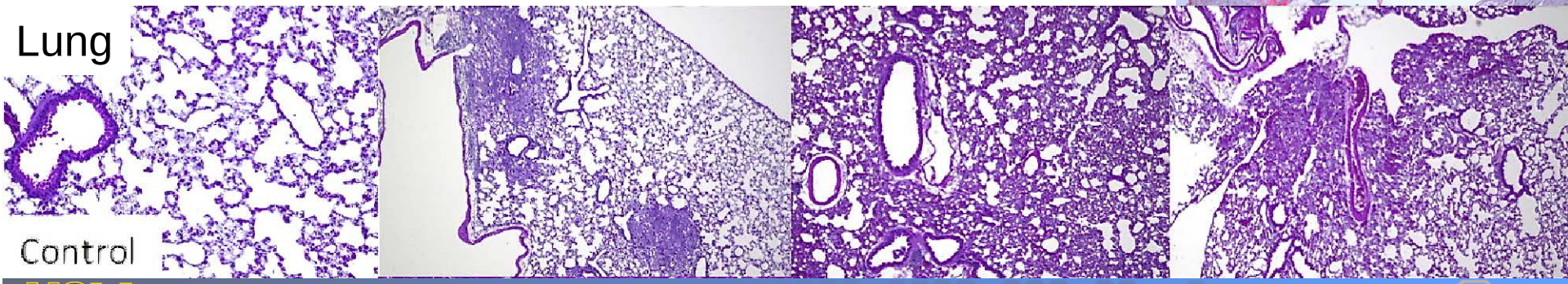
- Almost all mice had signs of cardiac fibrosis with mineralization of interventricular septum and ventricular free wall, and occasional necrosis.
- 50% had splenic lymphoid hyperplasia.
- Many had sporadic kidney, lung, liver inflammatory lesions

At 6 months after LD70/30 doses

- 40% had splenic lymphoid hyperplasia
- 20% had cardiac issues
- 20% had interstitial multifocal pneumonitis with some bronchial involvement or perivascular lymphoid hyperplasia
- 12% had liver nodular hyperplasia, fibrosis
- 11% had renal lymphoid hyperplasia

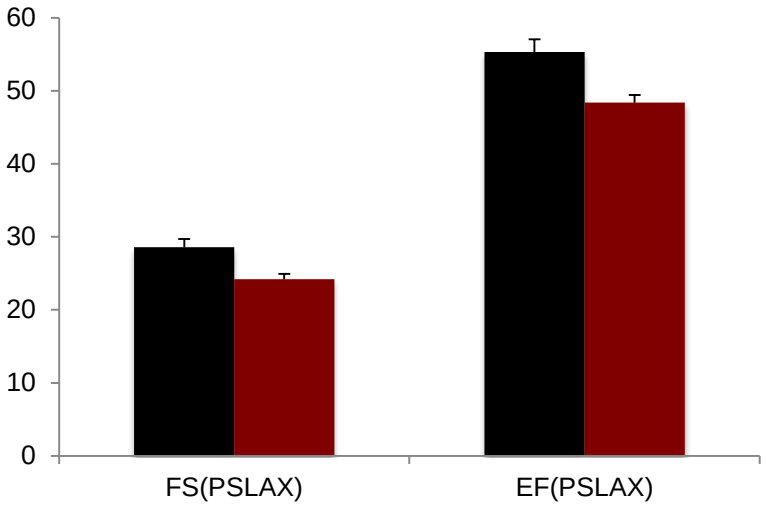
In general, 60% of mice had some lesions,
33% involving >1 organ

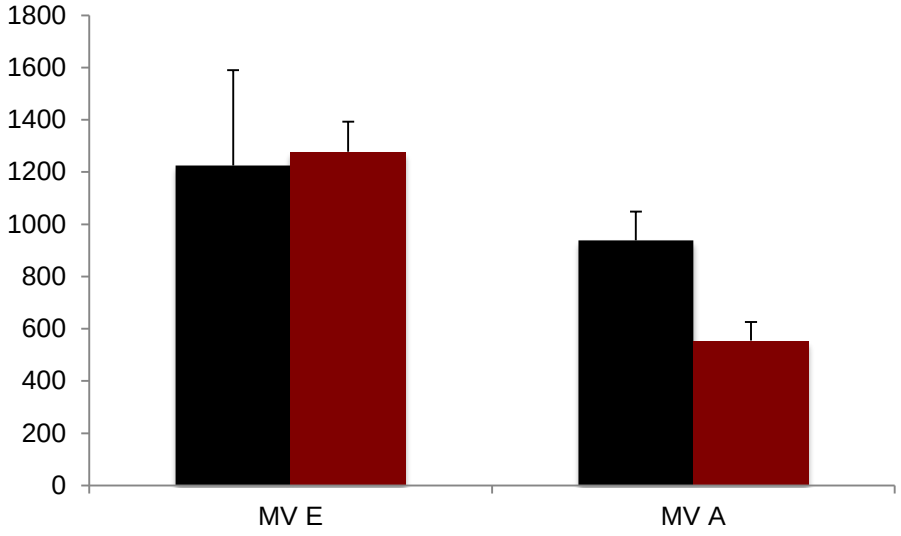


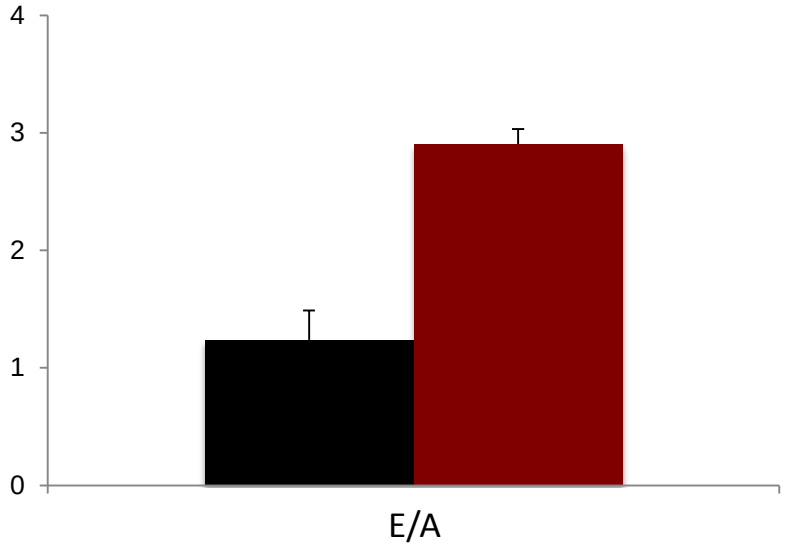


Echocardiography on mice 3 months post WBI

Control
WBI

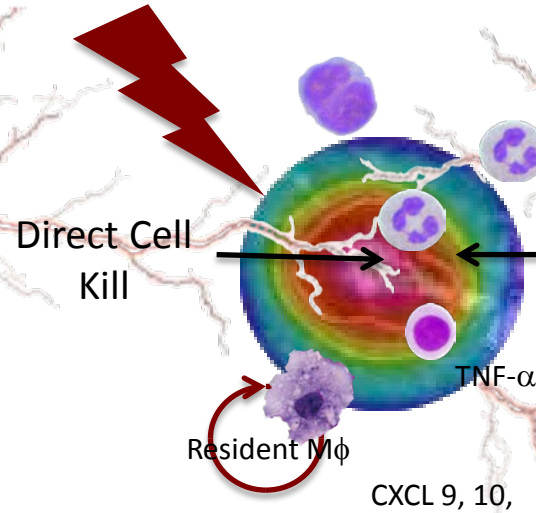


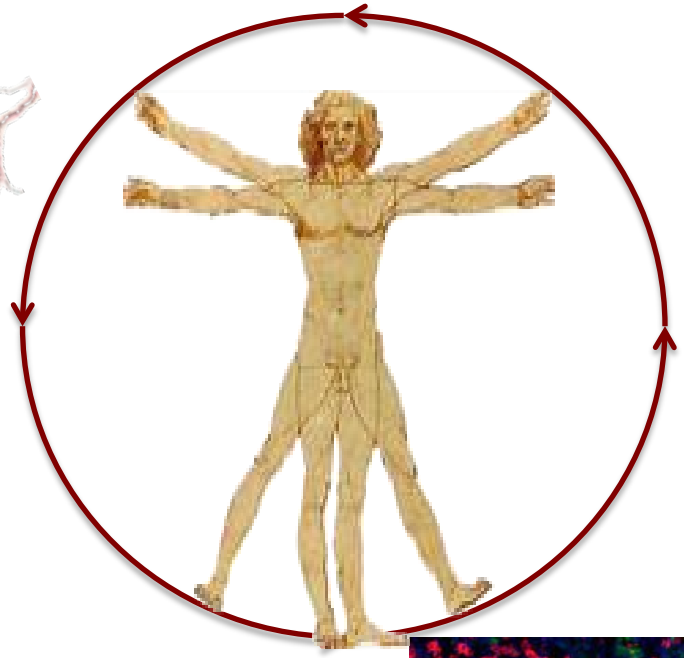


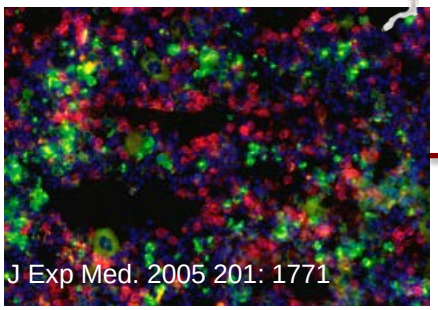


FS = fractional shortening
EF = Ejection fraction
MV = mitral valve
E = early wave; A = atrial wave

Indicative of hypertension, left ventricular hypertrophy, or diastolic dysfunction



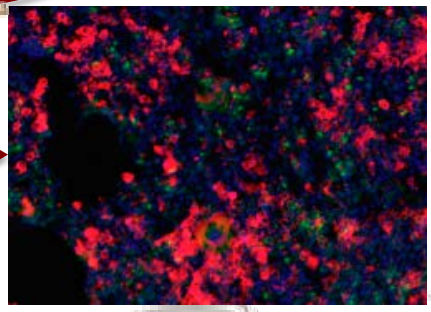




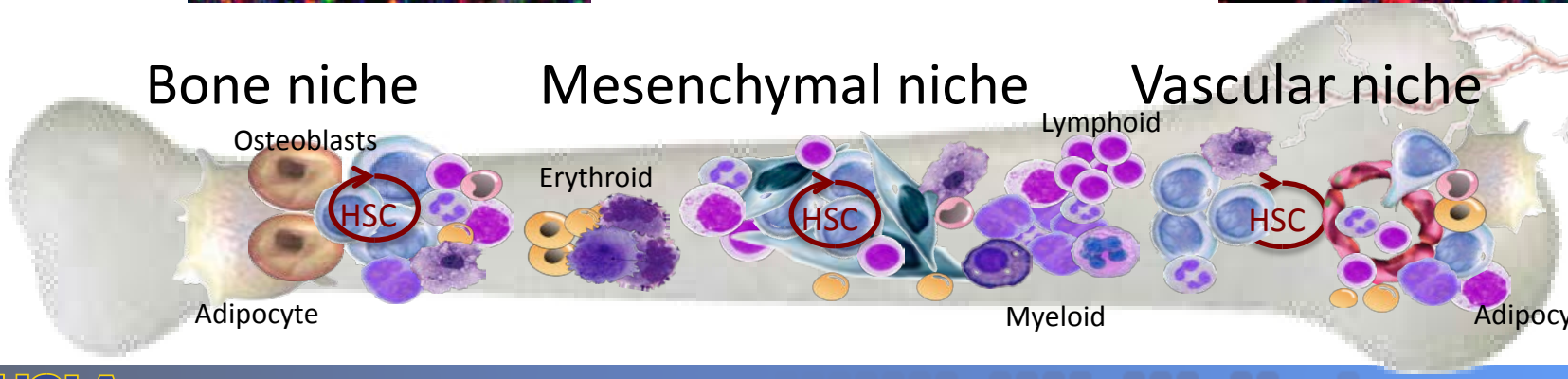
CXCL12
CXCR4
CCL2

Inflammation

Lymphocytes



Gr1+



Summary

- The time to a complication depends on the normal tissue turnover.
- Tissue failure occurs when there is a lack of timely regeneration.
- The tolerated dose is determined by the structural organization of the tissue and the time to and extent of regeneration, in addition to intrinsic radiosensitivity.
- Acute and late lethality can be mitigated against.
- Acute and late effects generate acute and chronic inflammation
- Late effects are complex:
  - Some late radiation complications are independent of acute damage.
  - Some are a consequence of acute tissue damage.
  - Late RI-MODS after acute high dose exposure are likely due to cytokine "storms" resulting from dysregulated wound healing/immune processes
  - Late non-cancer effects after low dose exposure may be due to similar imbalances and may be precipitated by pro-inflammatory stimuli, infection, genetic factors. These complications should be amenable to intervention.

Questions and Answers

U.S. Department of Health and Human Services
National Institutes of Health | National Cancer Institute
www.dceg.cancer.gov/RadEpiCourse
1-800-4-CANCER
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