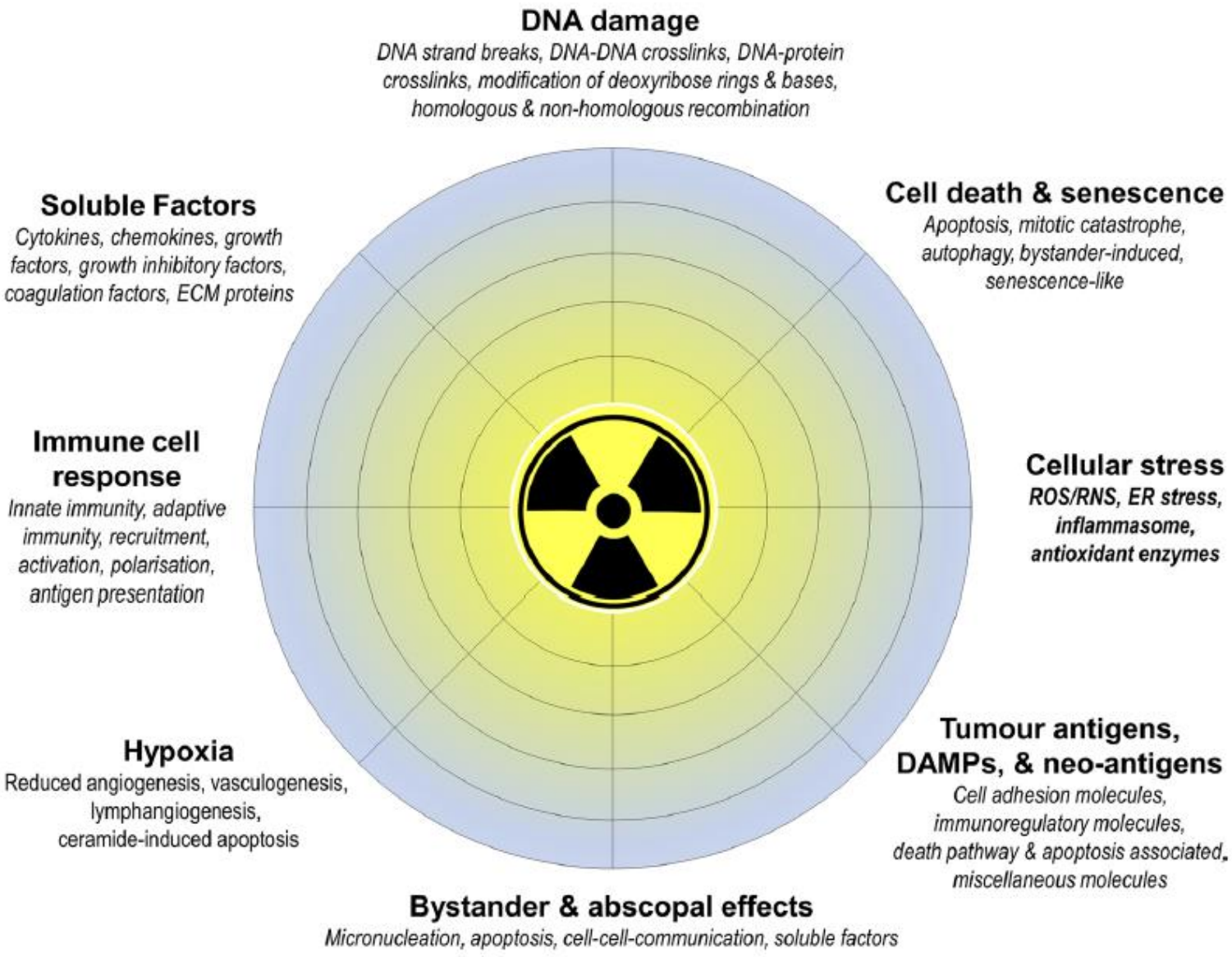
Radiation Therapy in Oncology

Simon N. Powell MD PhD

Memorial Sloan-Kettering Cancer Center
Radiation Oncology Department
& Molecular Biology Program

Radiation-induced Effects on Cells

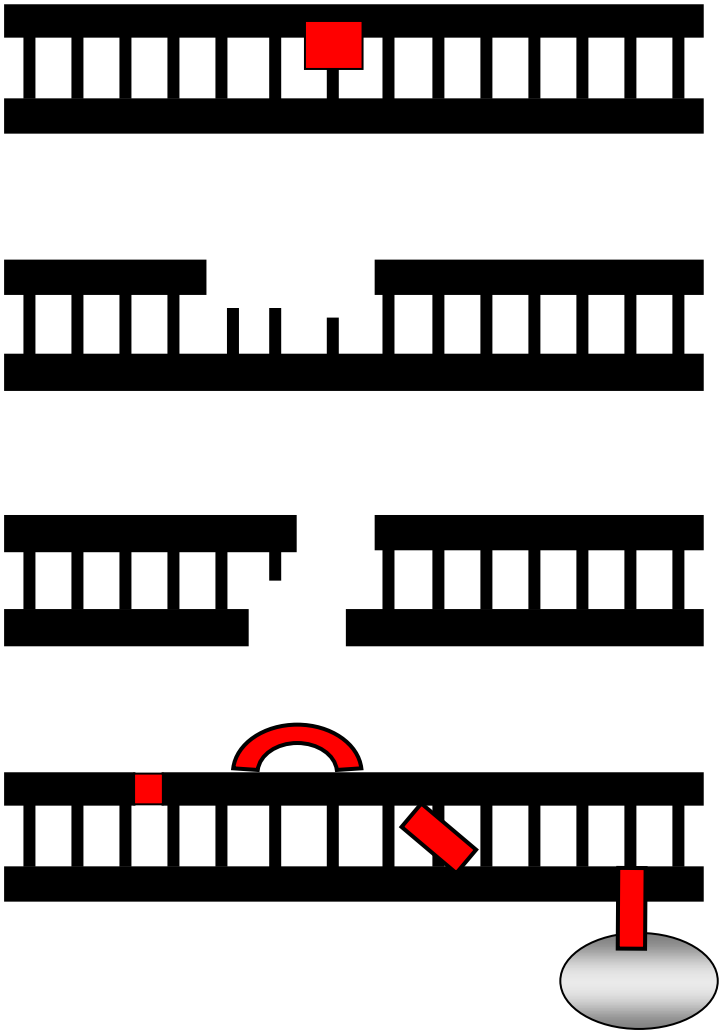


Radiation-induced Effects on Cells

Multiple cellular stress responses:

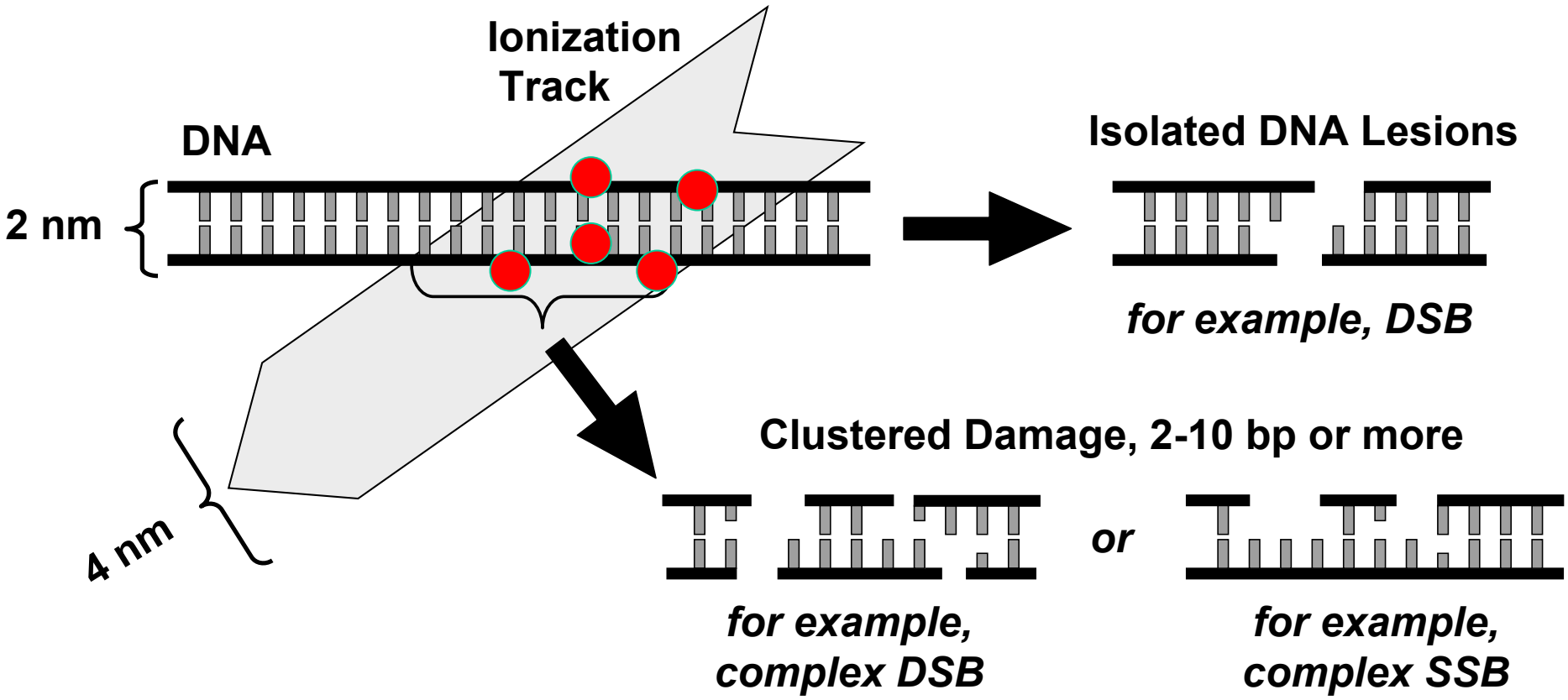
- DNA damage: wide variety of types
- Altered metabolism: oxygen, acidity, Warburg
- Free Radical Stress
- Cytosolic DNA (including as micronuclei)
- Cytokine release from cells: IFN signaling
- Inflammatory responses
- Immune infiltrates: suppressive vs anti-tumor

Ionizing Radiation (IR) induced DNA Damage



Damage type	No./Gy/cell
base damage	> 1000
single-strand break (SSB)	500-1000
double-strand break (DSB)	~ 40
sugar damage, DNA-DNA and DNA-protein cross links	*various*

IR Causes Clustered DNA Damages



IR typically induces oxidized purines and pyrimidines, abasic (AP) sites and SSB.

DSB may be clustered or non-clustered or arise during BER repair.

DNA Repair Pathways: IR Sensitivity

DNA Damage Response

Damage Sensing:
ATM/ATR/DNA-PK
DNA damage response (DDR)
ATM defective cancers
Replications Stressed cancers

Single-strand damage

Base damage
Base Excision Repair (BER)

Single-strand breaks
Single-Strand Break Repair (SSBR)

Intra-strand adducts or photo-products
Nucleotide Excision Repair (NER)



Replication Fidelity

Base damage for replication forks: daughter strand gaps
Translesion Synthesis (TLS)

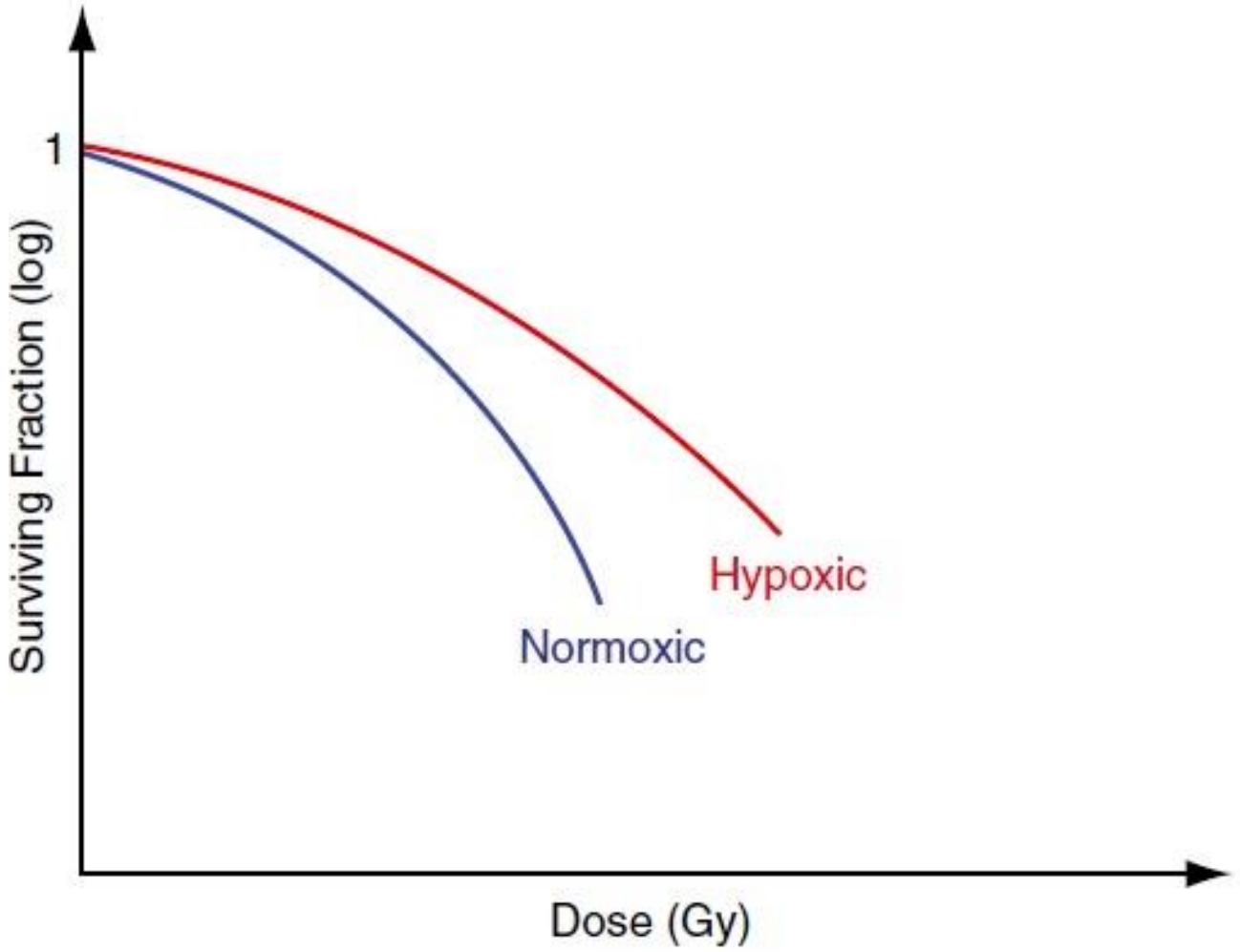
Mismatched bases or template slippage
Mismatch Repair (MMR)

Double-strand damage

DNA double-strand breaks or broken replication forks
Homologous Recombination(HR)

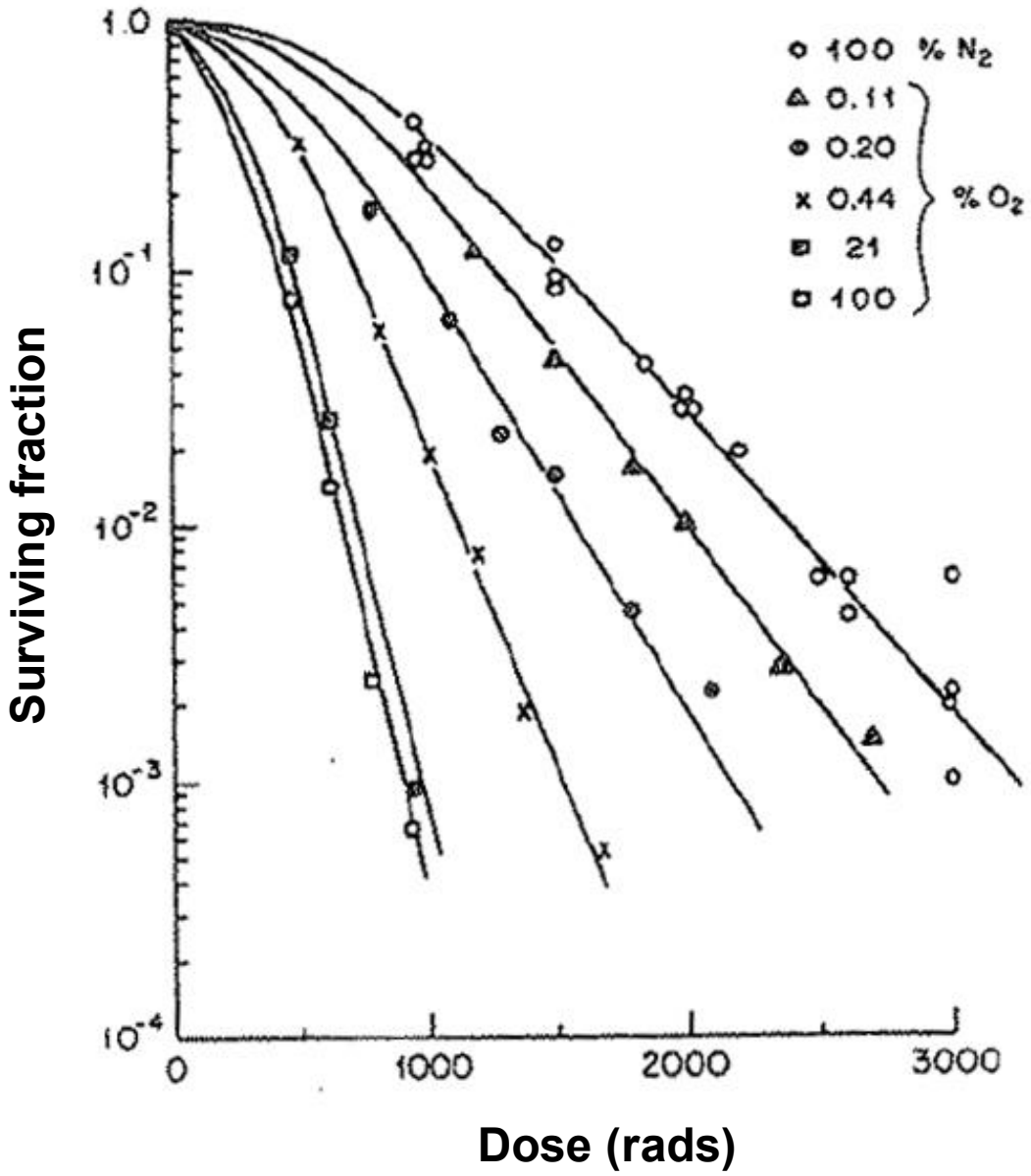
DNA double-strand breaks
Non-Homologous End-Joining (NHEJ)

Oxygen Sensitizes Cells to Radiation

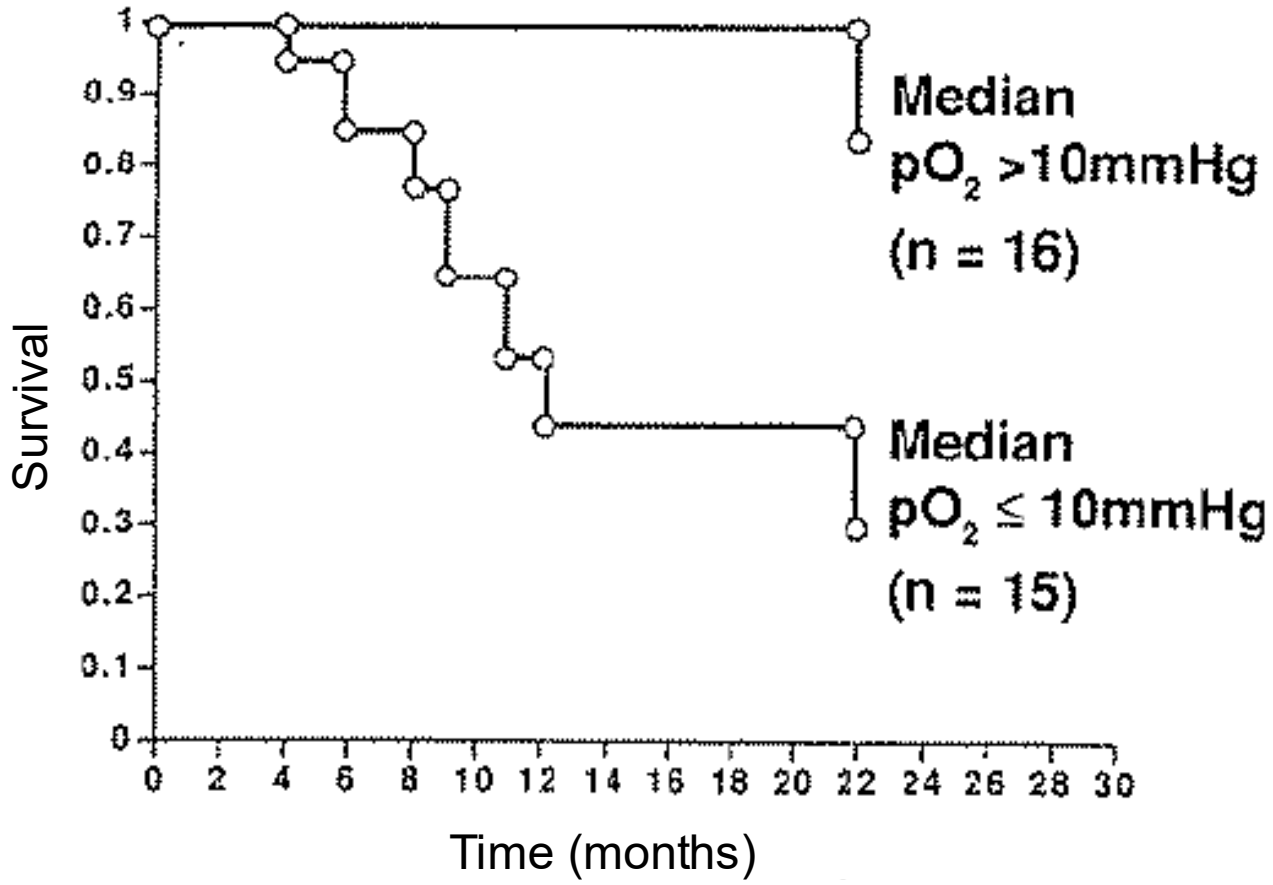


The effect of oxygen on the surviving fraction after radiation. Note that the effect of oxygen is generally greater at higher doses.

IR Exposure and the Oxygen Effect

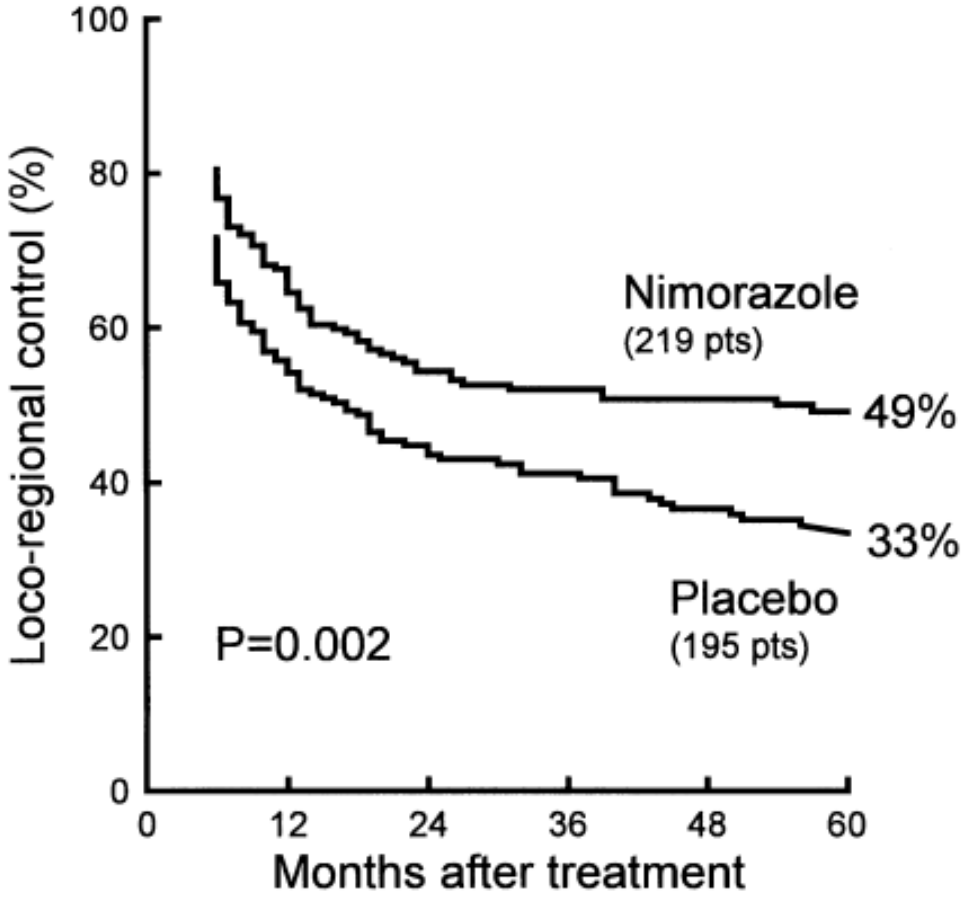


Oxygen Effect has a Clinical Impact



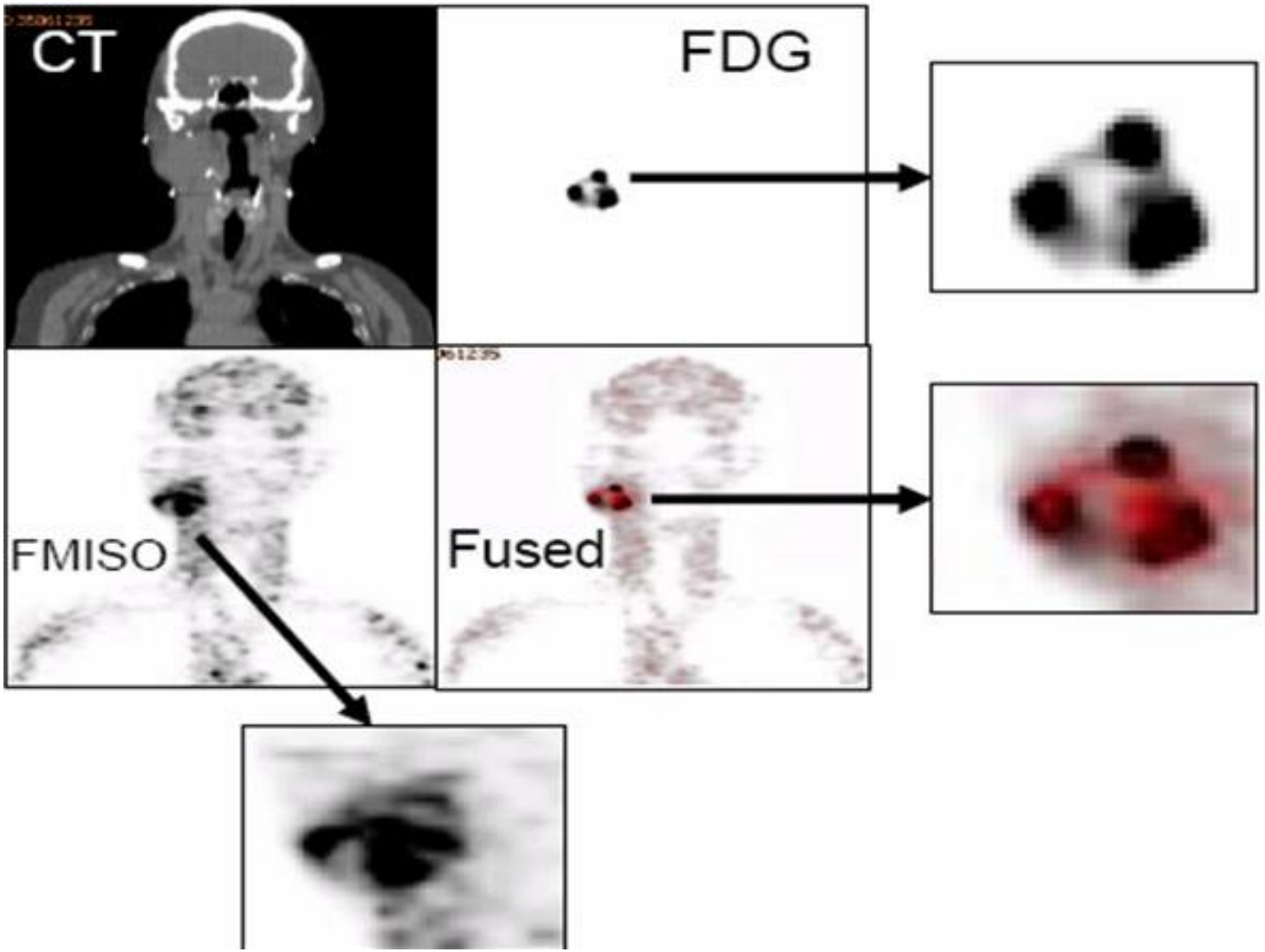
Recurrence-free survival in patients with advanced cervical carcinoma treated by a combination of external beam radiation therapy and brachytherapy. The patients were stratified on the basis of pretreatment oxygen probe measurements as indicated on the graph. (Reproduced from Hockel et al)

Oxygen Effect can also be mitigated by Drugs

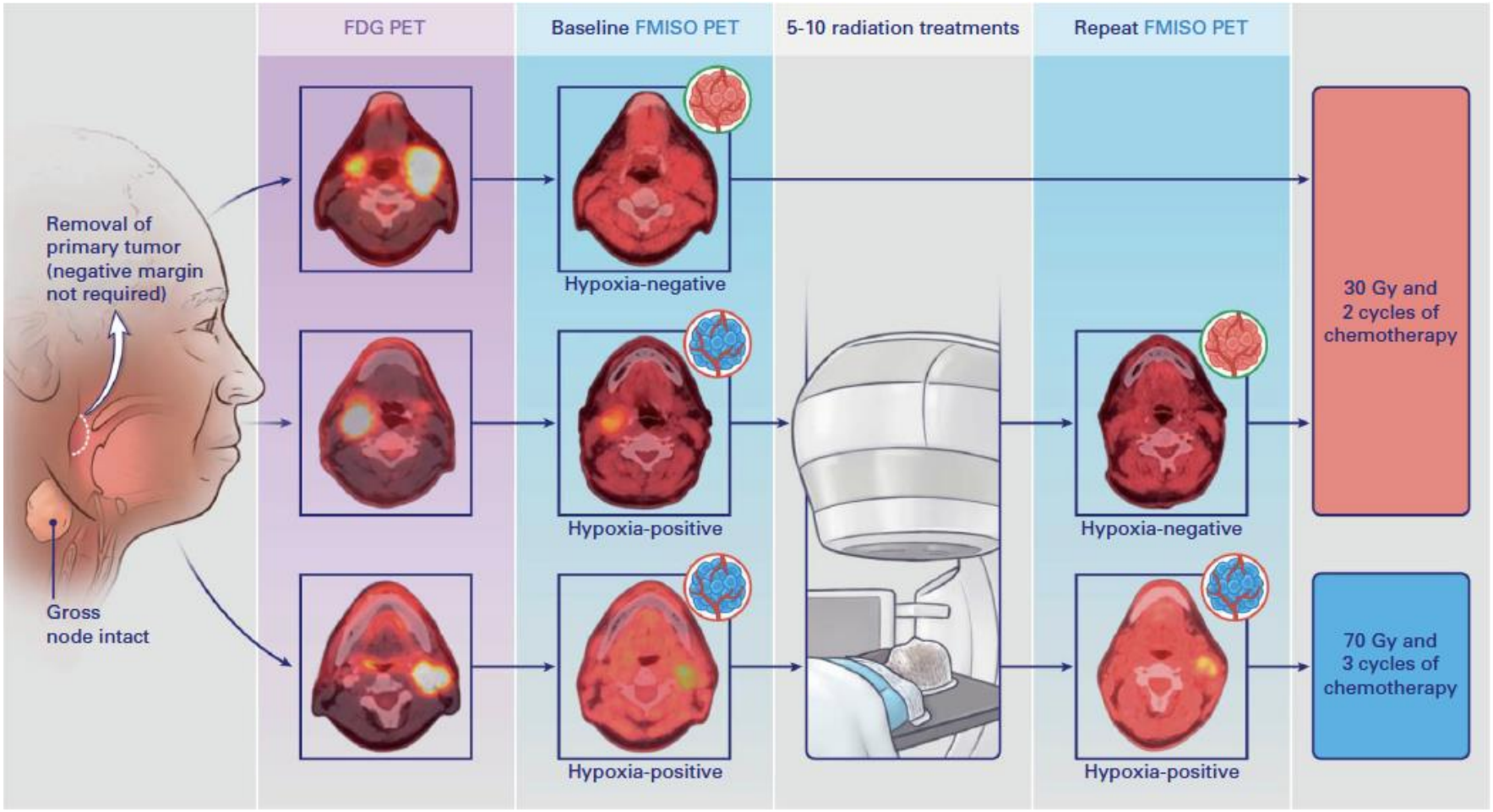


Actuarial loco-regional tumor control in patients randomized to receive nimorazole or placebo in conjunction with conventional radiotherapy for carcinoma of the pharynx and supraglottic larynx.

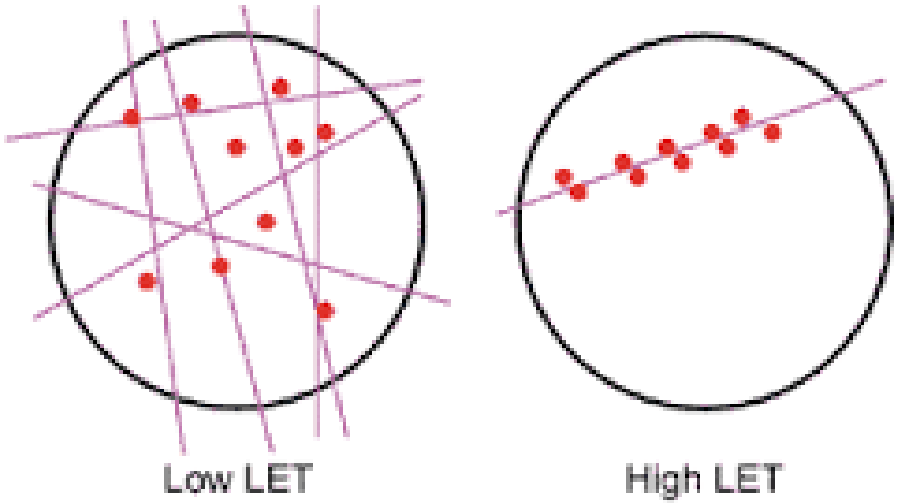
Hypoxic Tumors Can be Found in Many Different Tumors and Sizes

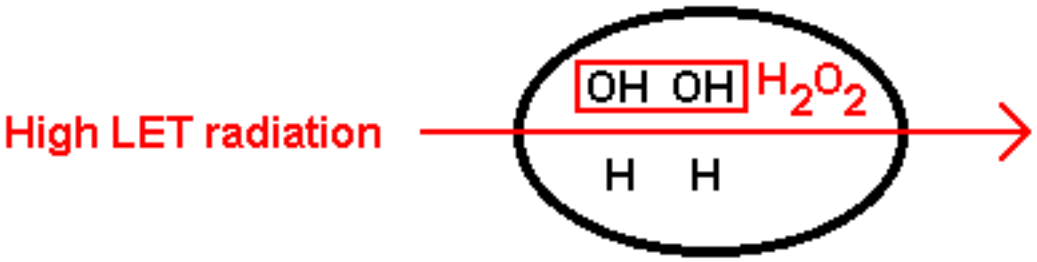


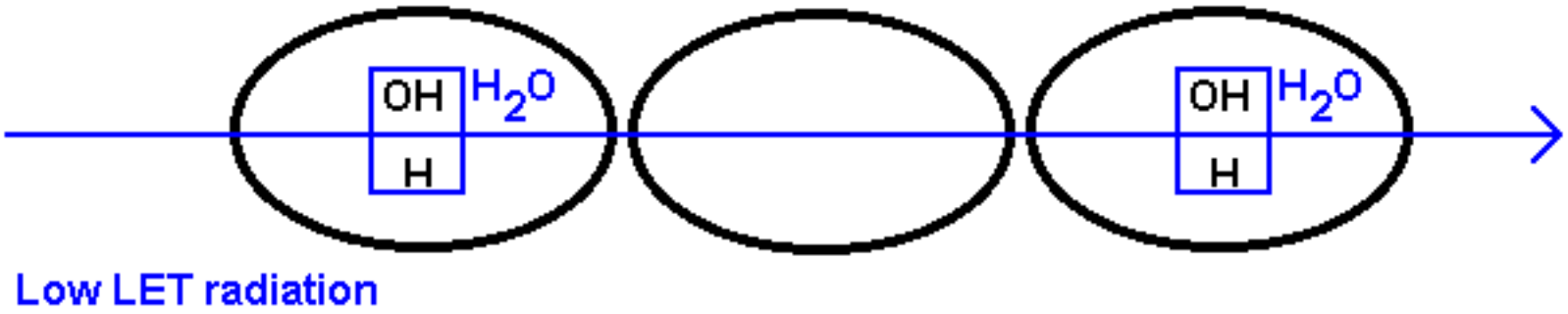
Hypoxia can be used to customize Precision Radiation Oncology



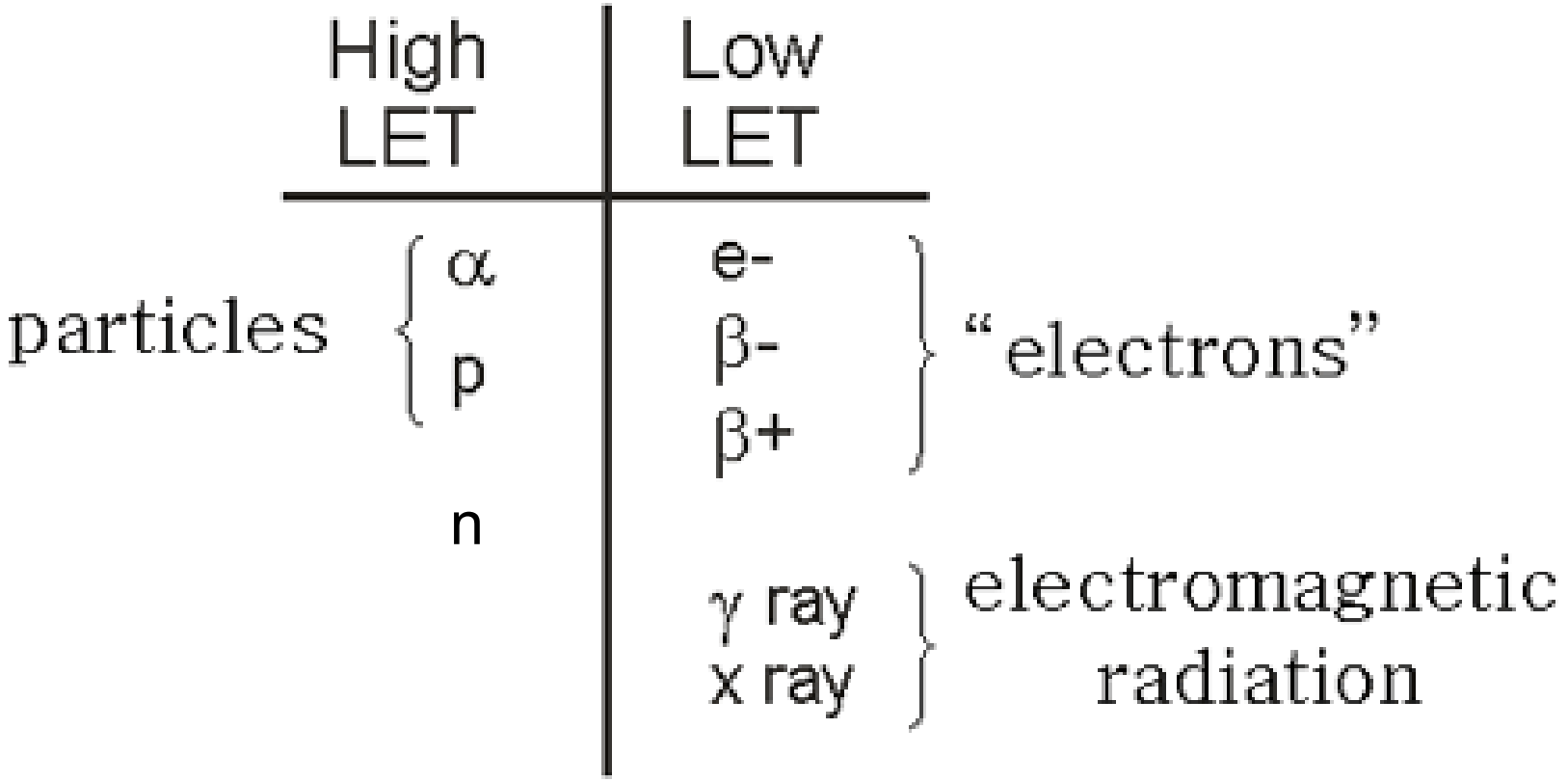
Linear Energy Transfer





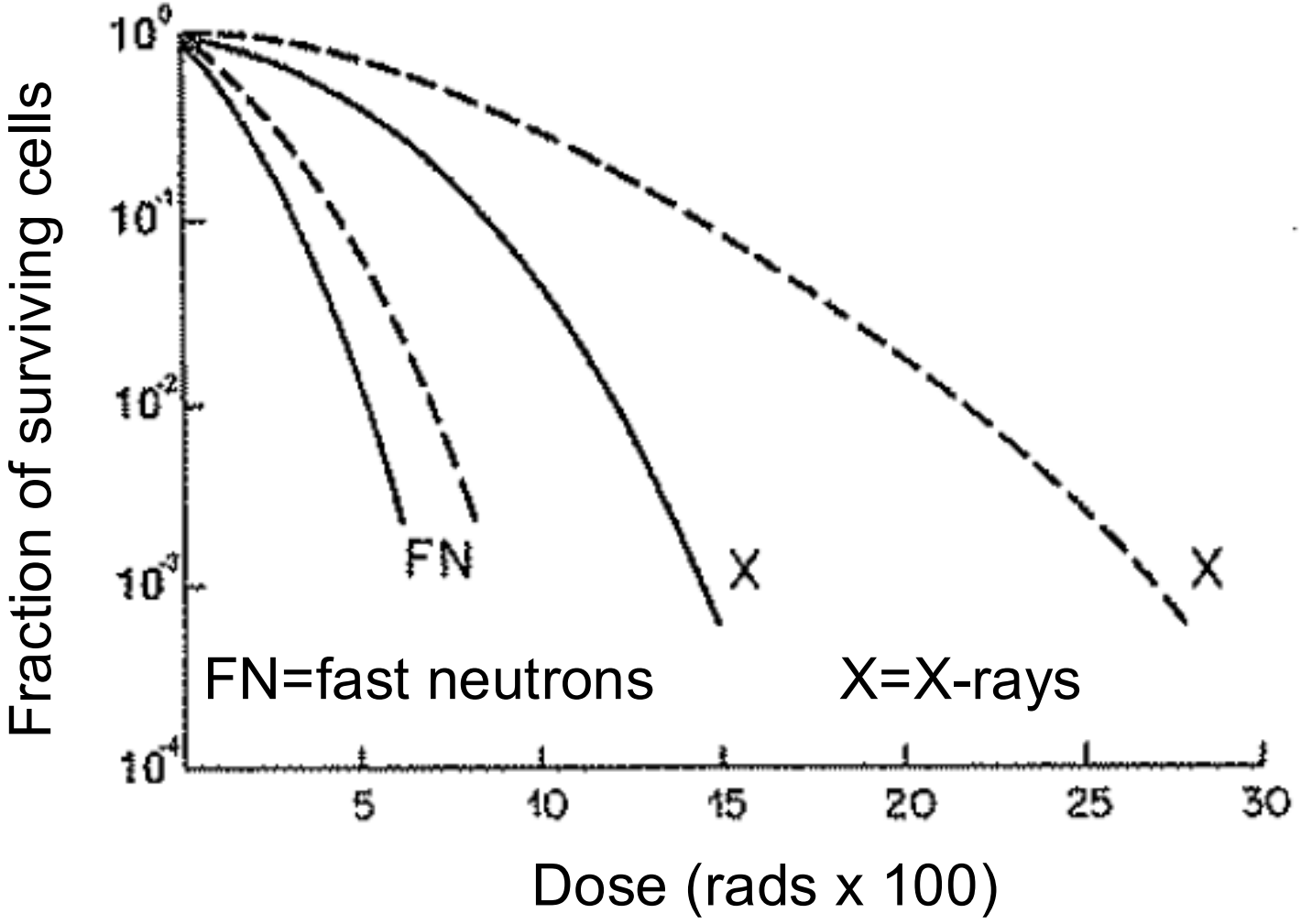


Linear Energy Transfer

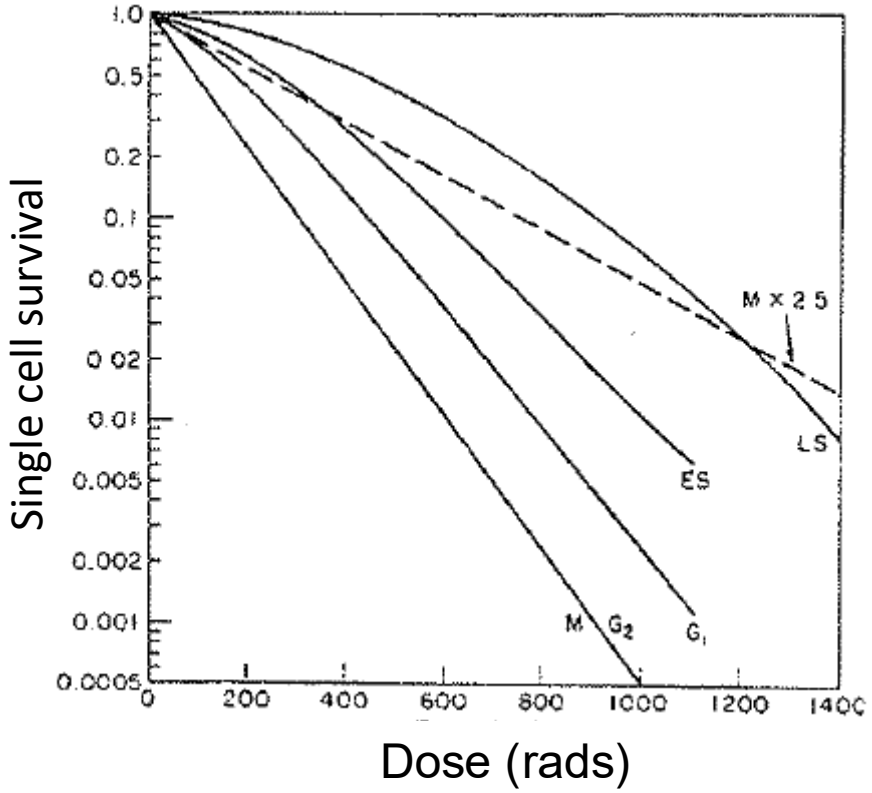


Linear Energy Transfer and RBE

Oxic vs Hypoxic

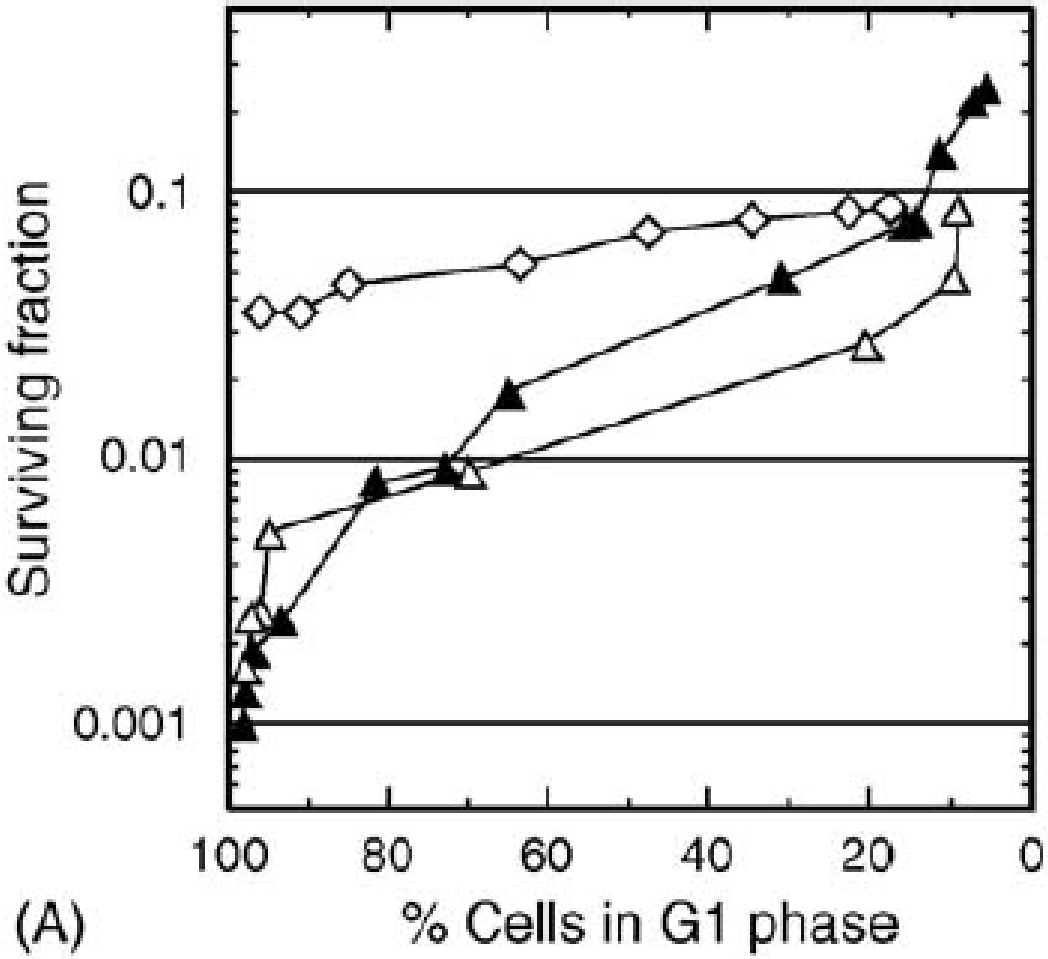


Cell Cycle Phase and Radiation Survival



Cell survival curves for Chinese hamster cells irradiated (single dose) in the M, G1, early S, and late S phases of the cell cycle.

cNHEJ-defective cells are most to IR in G1



Symbols: V3 (▲, Δ); V3-155 (◊).

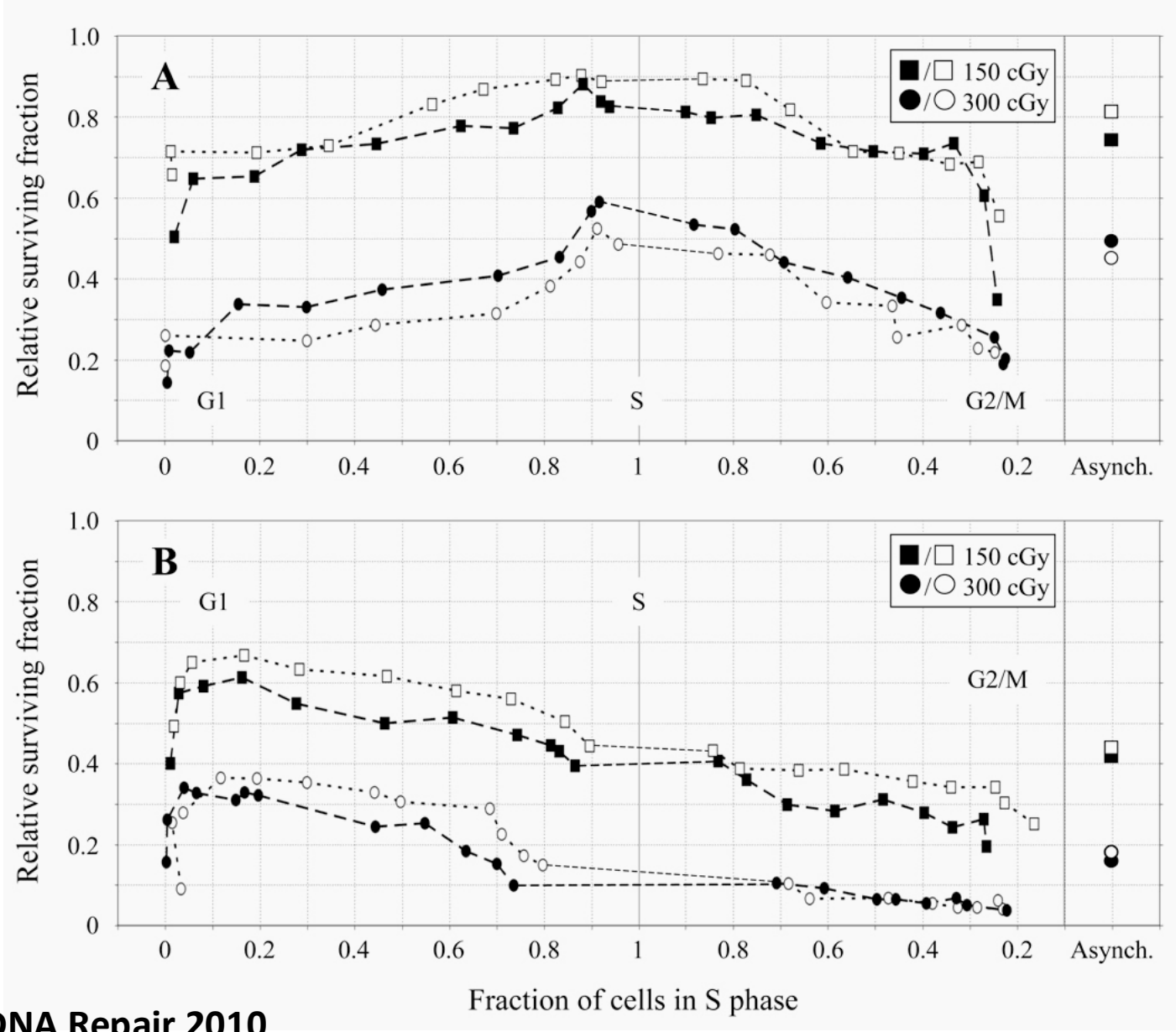
- **V3 cells: DNA-PKcs -/-**
- **Clone V3-155: complemented with DNA-PKcs**
- **Cells separated by centrifugal elutriation, irradiated, and assessed for colony formation.**

Hinz J & Thompson LH, DNA Repair 2005

In S/G2, HR DNA Repair ➔ Radiation Resistance

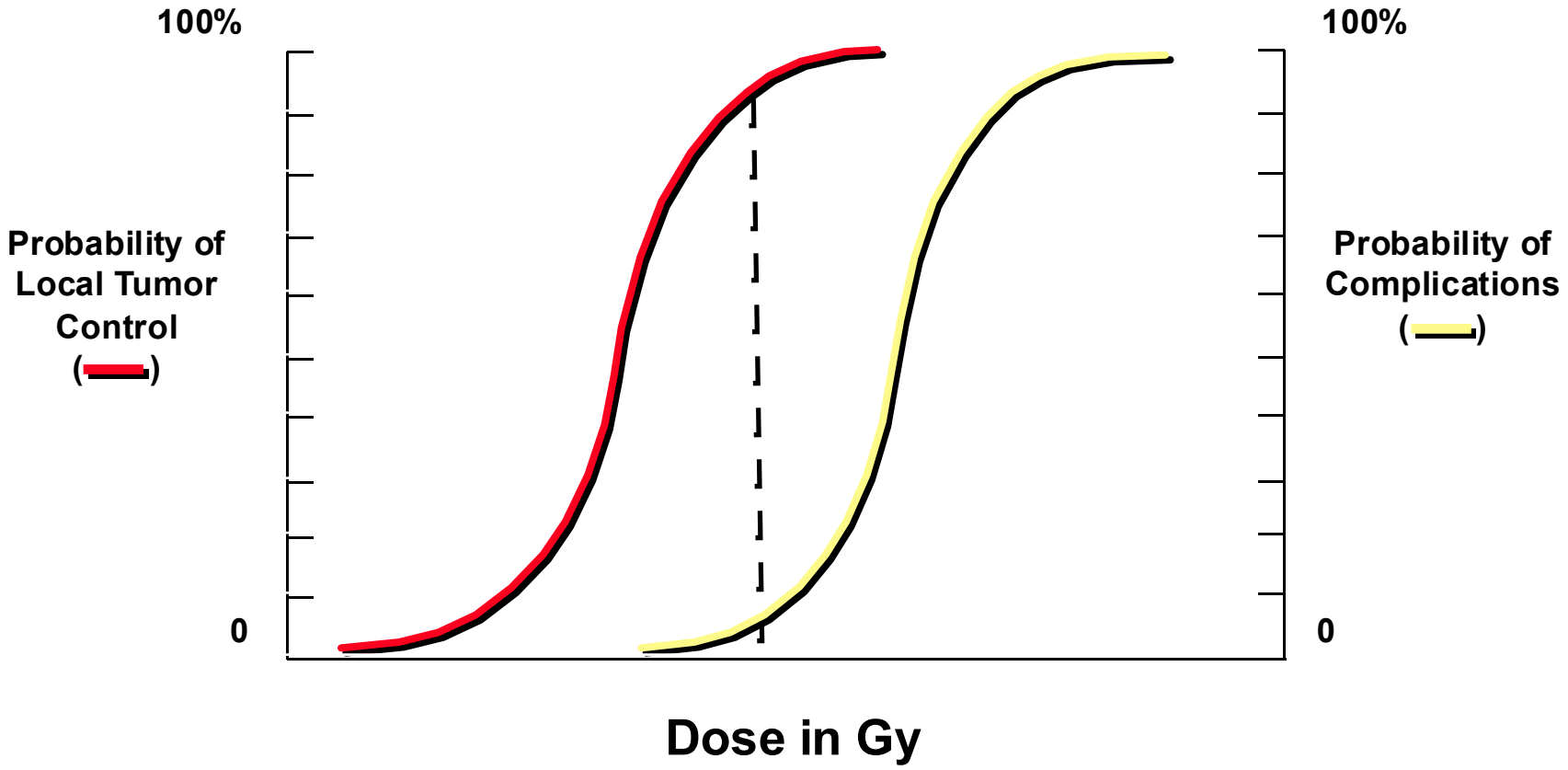
RAD51D corrected

RAD51D deficient

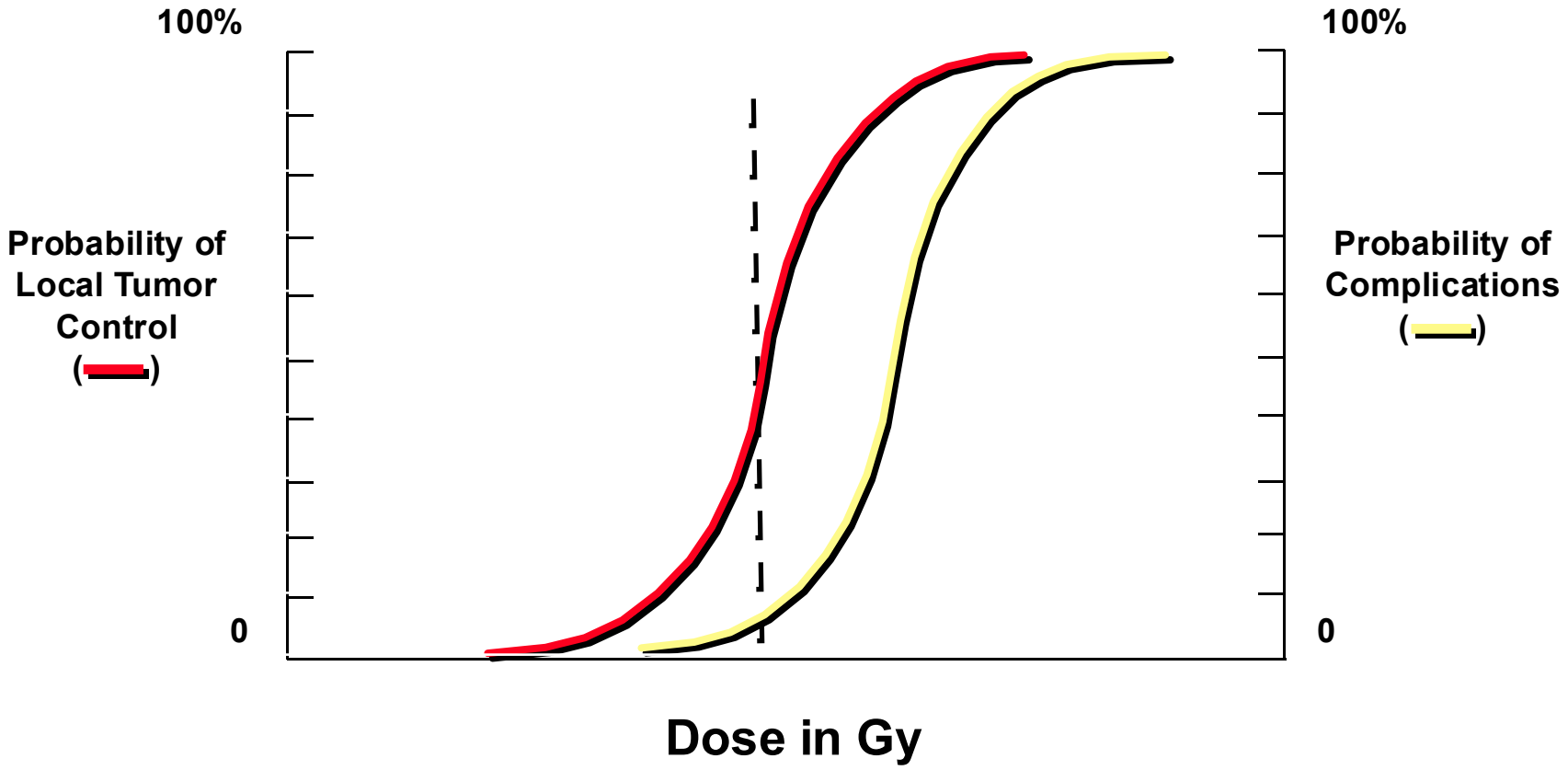


Wilson et al., DNA Repair 2010

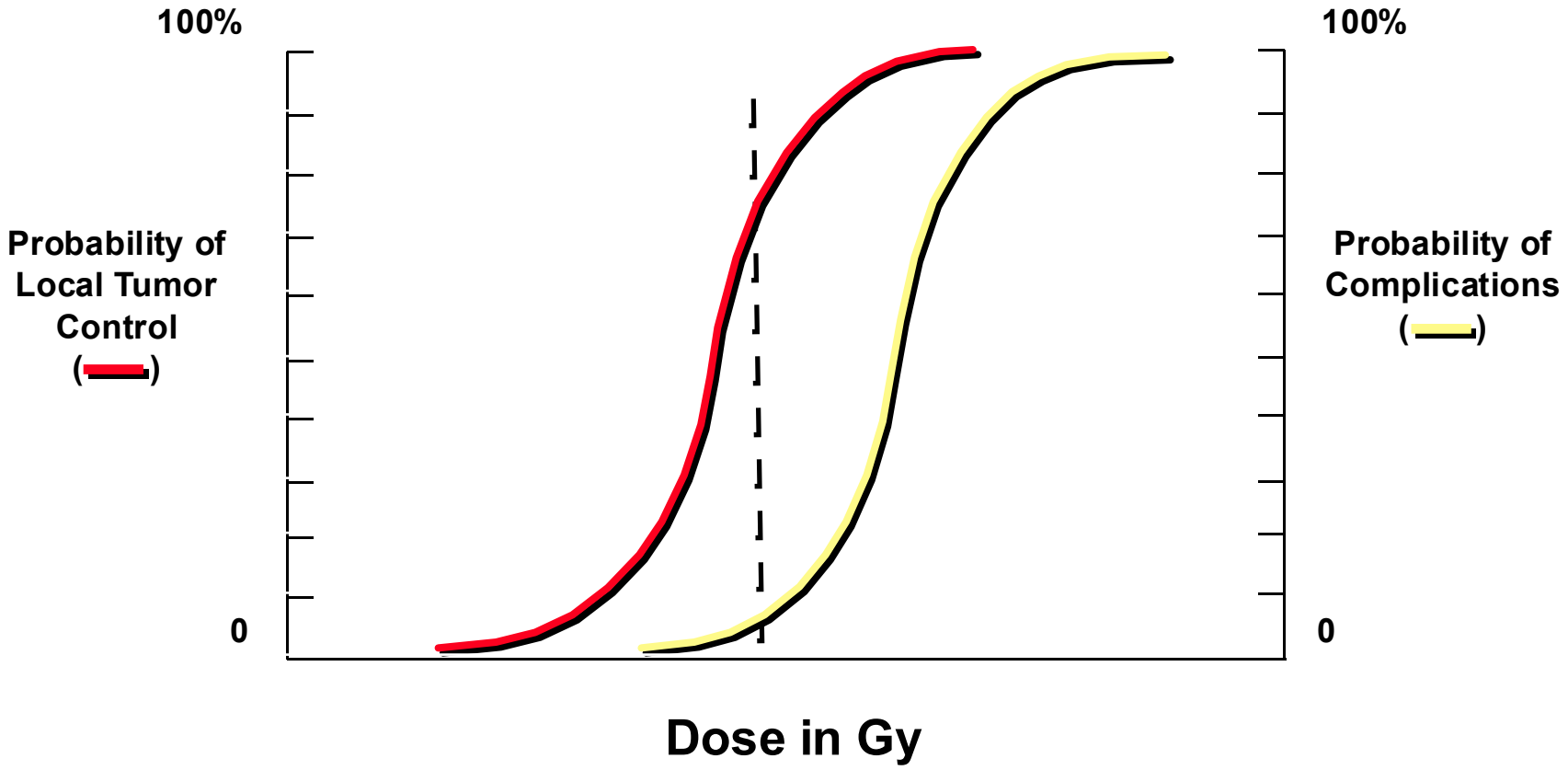
Therapeutic Ratio



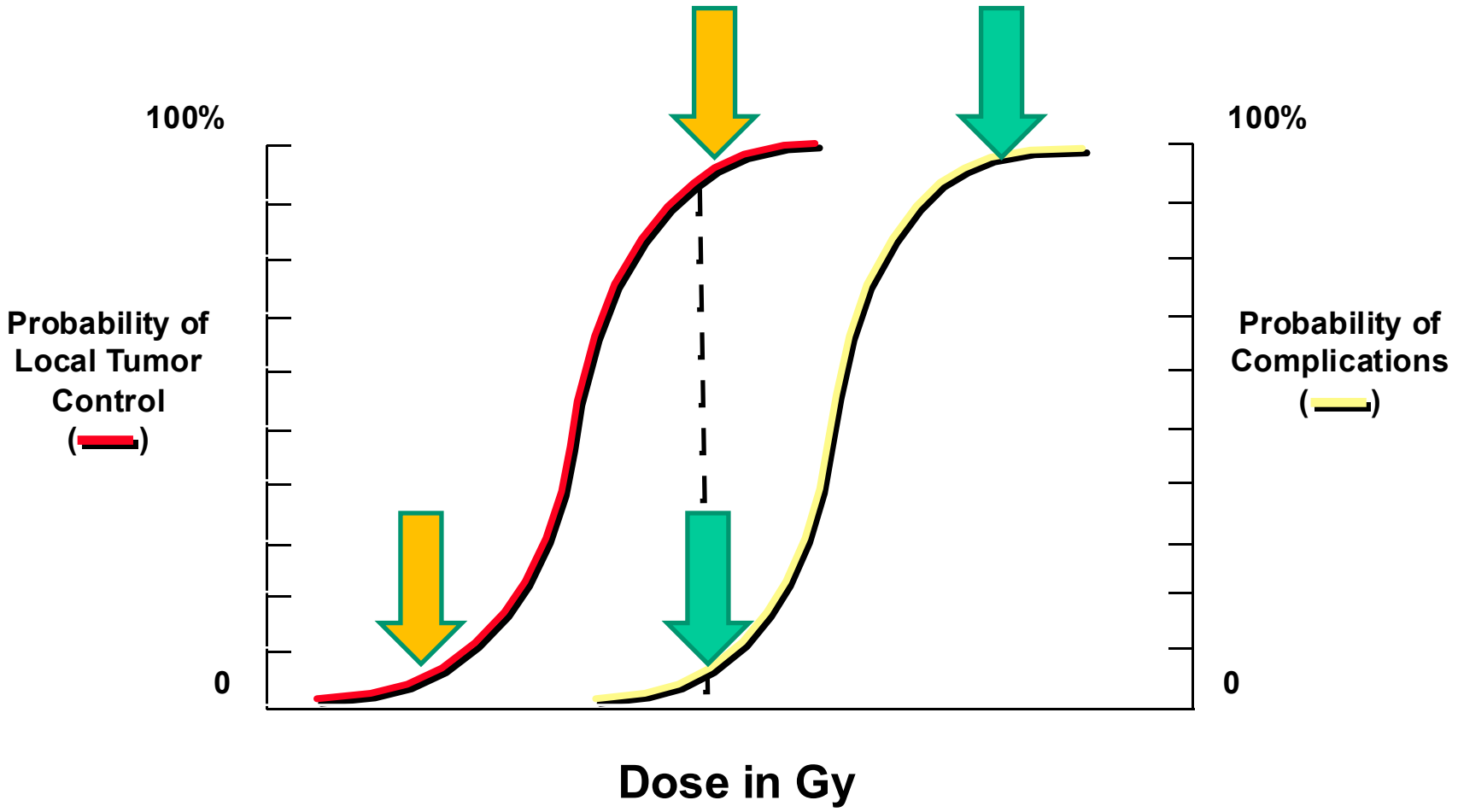
Tumor-Specific Radiosensitization



Therapeutic Ratio Balance



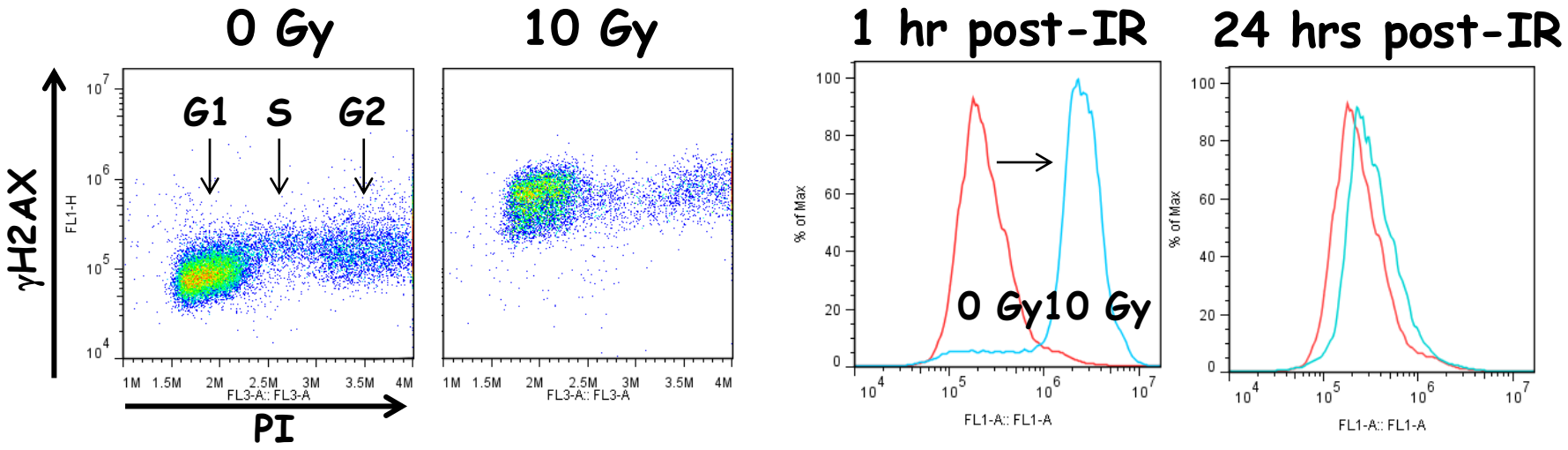
Sub-populations to be defined?

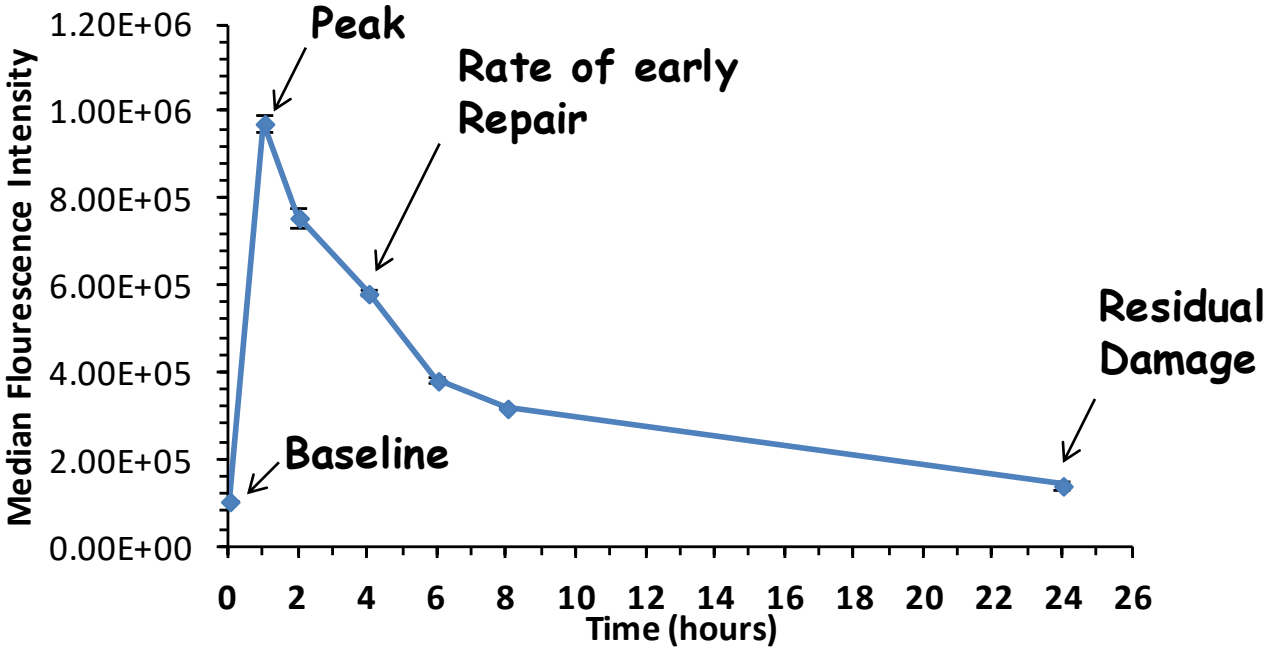


Normal Tissue Genetics

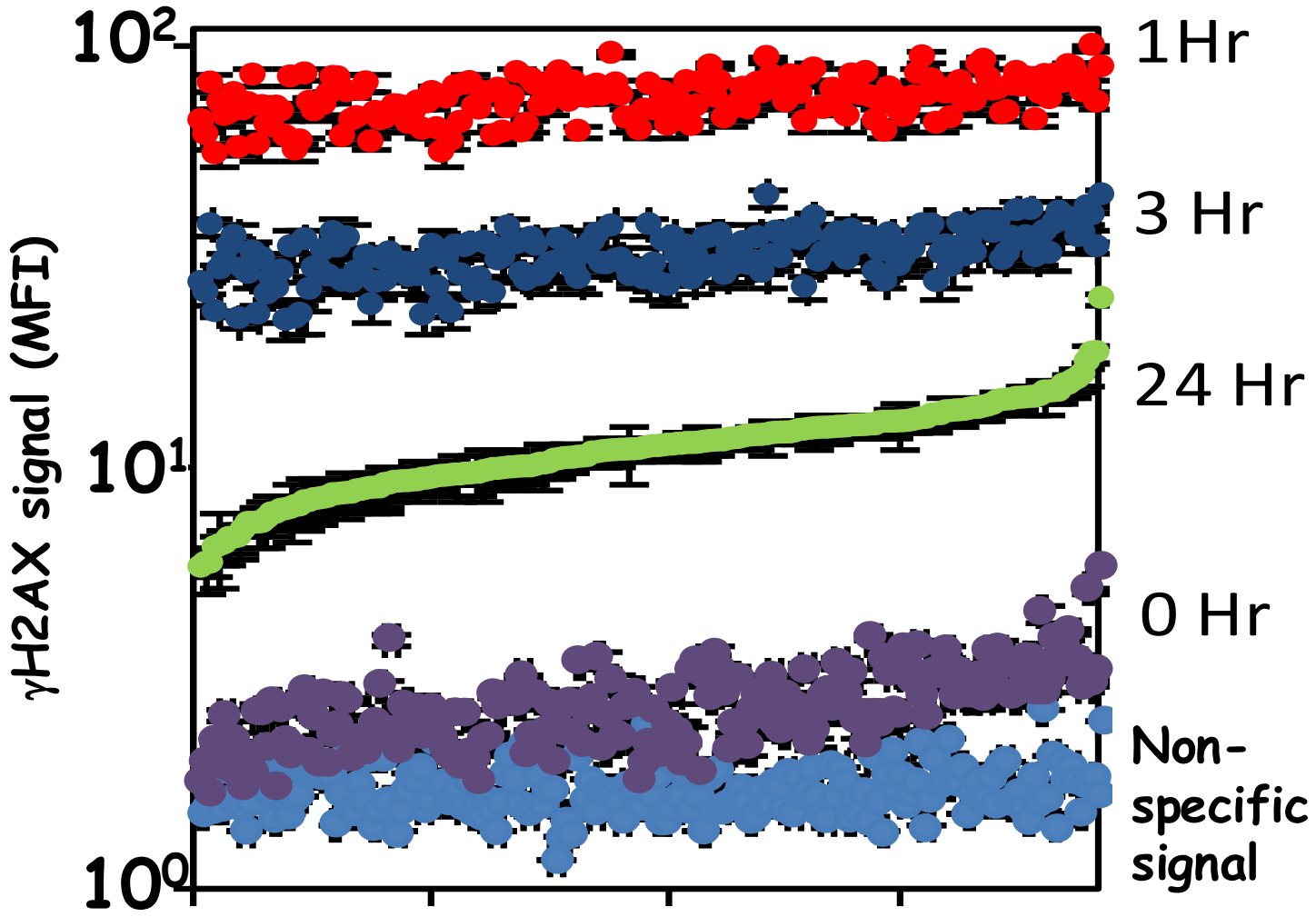
- DNA repair variants exist within the normal population
- Methods to detect population variables
- Genome-wide Association Studies
- Validation
- Allele testing and gene editing
- CRISPR screens for radiosensitivity

High throughput γH2AX flow cytometry assay

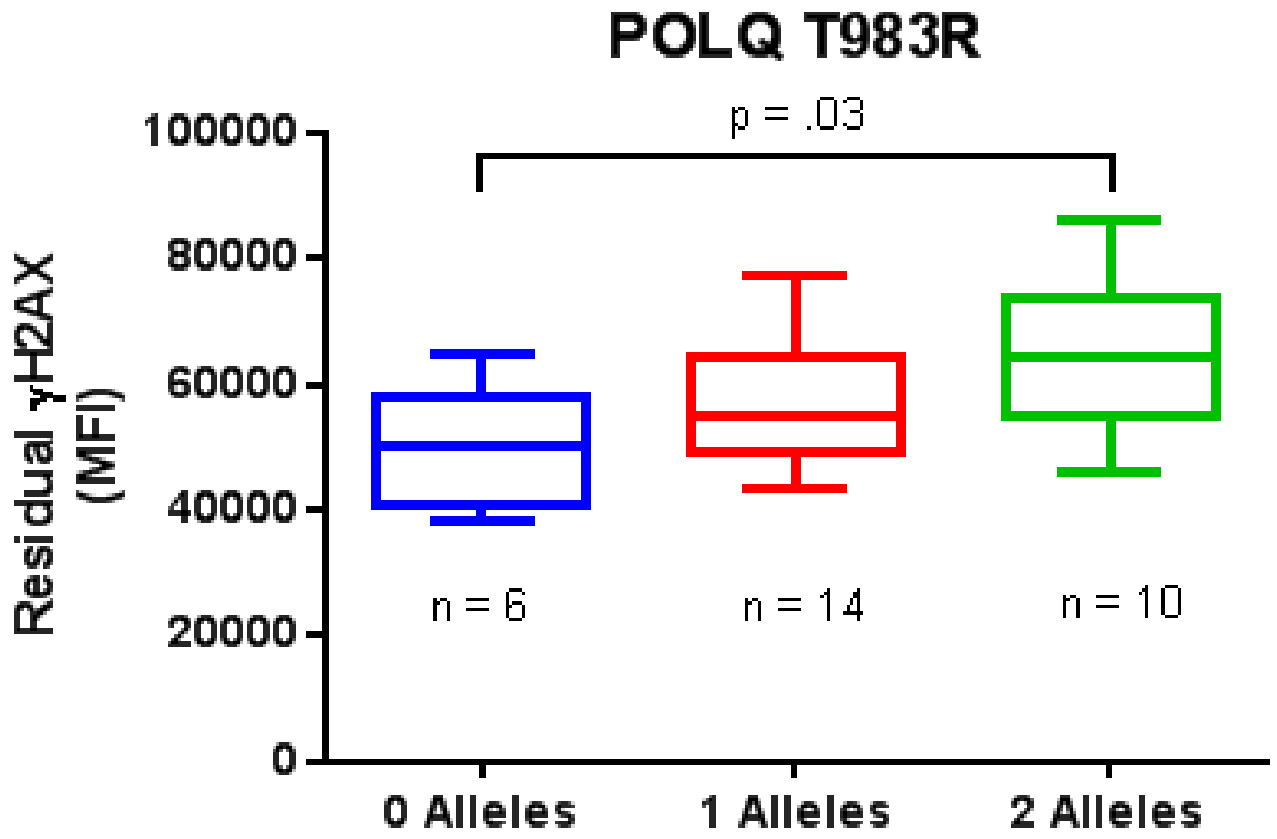




DNA damage and repair: 192 normal cell lines



POLQ loci validates in replication set

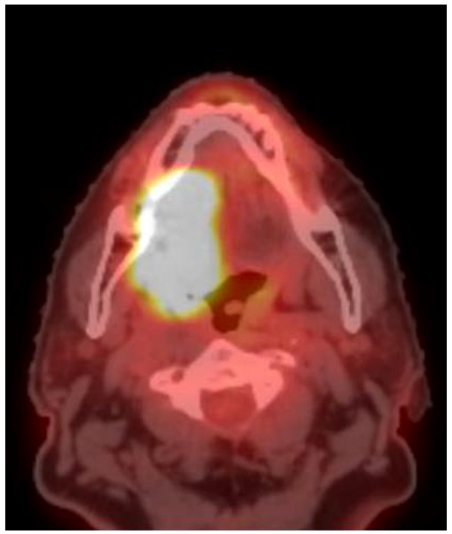


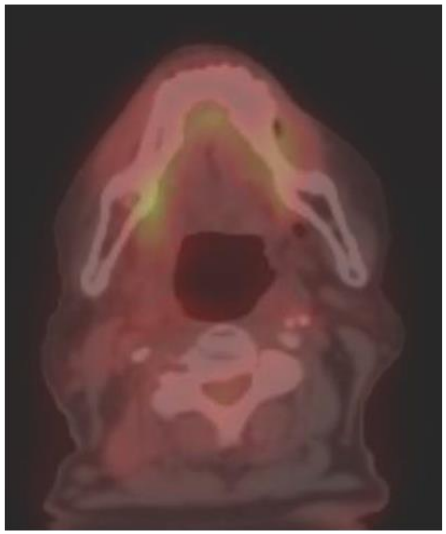
30 LCLs, independent dataset from primary screen

Cancer Genetics and DNA Repair

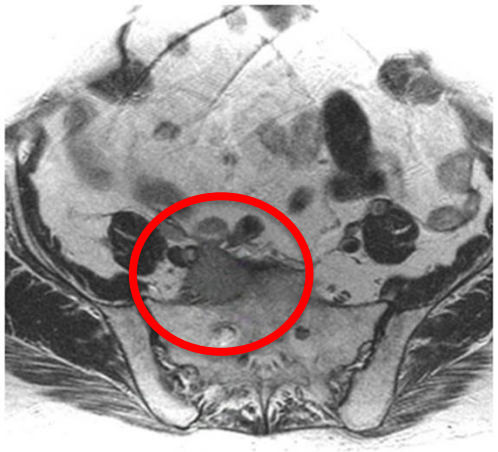
- DNA Repair abnormalities in cancer cells
- Mutational signatures observed in TCGA
  - Which are relevant for Radiation Therapy?
- Methods to detect HR-Deficiency (HRD)
  - Functional Assessment
  - Structural Variation
  - Mutational Signature
- HRD found outside of Breast/Ovarian Cancer
- Expanding the ability to deduce genomic landscapes from limited sequencing data
  e.g. MSKCC IMPACT data

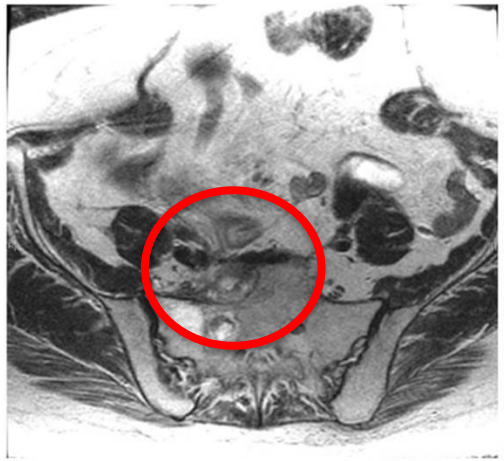
Exceptional Responders to RT: truncating ATM mutations





96 year old with Oral Cavity SCC
CR after 44.4 Gy. A biopsy one year after therapy was negative. Now 5 years post-therapy and NED.





68 year old with stage IB endometrial ca s/p TAH/BSO with pre-sacral lymph node recurrence 2 years later, s/p palliative RT to 36Gy. Now 4 years later and NED.

Ma, et. al., Oncotarget 2017

Defective HR (HRD): Therapeutic Strategies

Cisplatin, better with ionizing radiation?

Crosslinking agents: MMC +/-radiation

PARP inhibitors, combined with radiation?

Depletion of nucleotide pools, MTH1 inhibition?

Topoisomerase poisons: I vs II; combination with PARP inhibitors are toxic

Reactive Oxidizing Species: produce S-phase breaks

Back-up repair pathways:

Pol-theta, RAD52 inhibitors, TLS-pols inhibitors

MSK-15-032: HRD status determines response to Pt+IR

- Opened May 2015 for Triple Negative Breast Cancer (often 4th/5th/ or 6th line of treatment)
  - local recurrence or metastasis
- 49 patients accrued:
  - 42 have HRD status determined
- 23 are HRD, 19 are HR-proficient
  - Using RAD51 foci formation
  - Whole Genome Sequencing
- Response assessment by PET/CT

Repair Status	Response in RT region	Response outside RT
HRD	**78% (18/23)**	**24% (5/21)**
HRP	**21% (4/19)**	**6% (1/15)**

MGMT methylation predicts for response to treatment in GBM patients

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma

Monika E. Hegi, Ph.D., Annie-Claire Diserens, M.Sc., Thierry Gorlia, M.Sc., Marie-France Hamou, Nicolas de Tribolet, M.D., Michael Weller, M.D., Johan M. Kros, M.D., Johannes A. Hainfellner, M.D., Warren Mason, M.D., Luigi Mariani, M.D., Jacoline E.C. Bromberg, M.D., Peter Hau, M.D., René O. Mirimanoff, M.D., J. Gregory Cairncross, M.D., Robert C. Janzer, M.D., and Roger Stupp, M.D.

O6-methylguanine DNA methyltransferase
-DNA repair enzyme
-Reverses alkylator damage to DNA

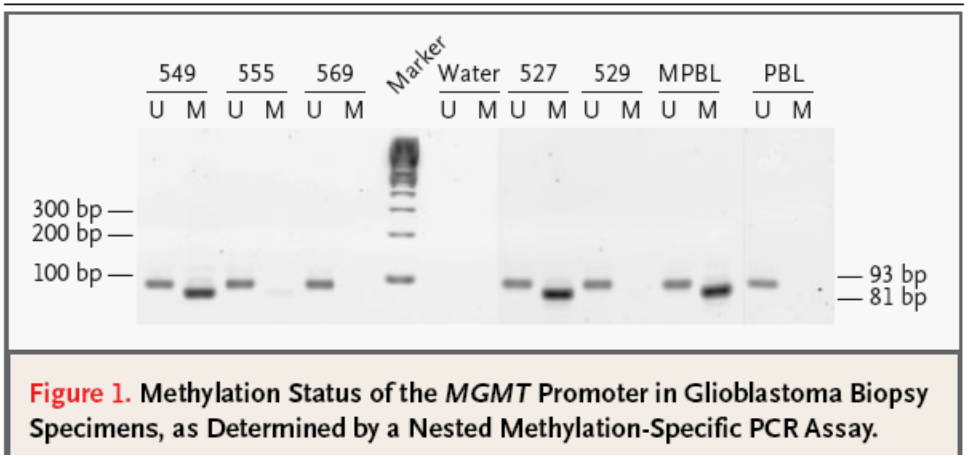
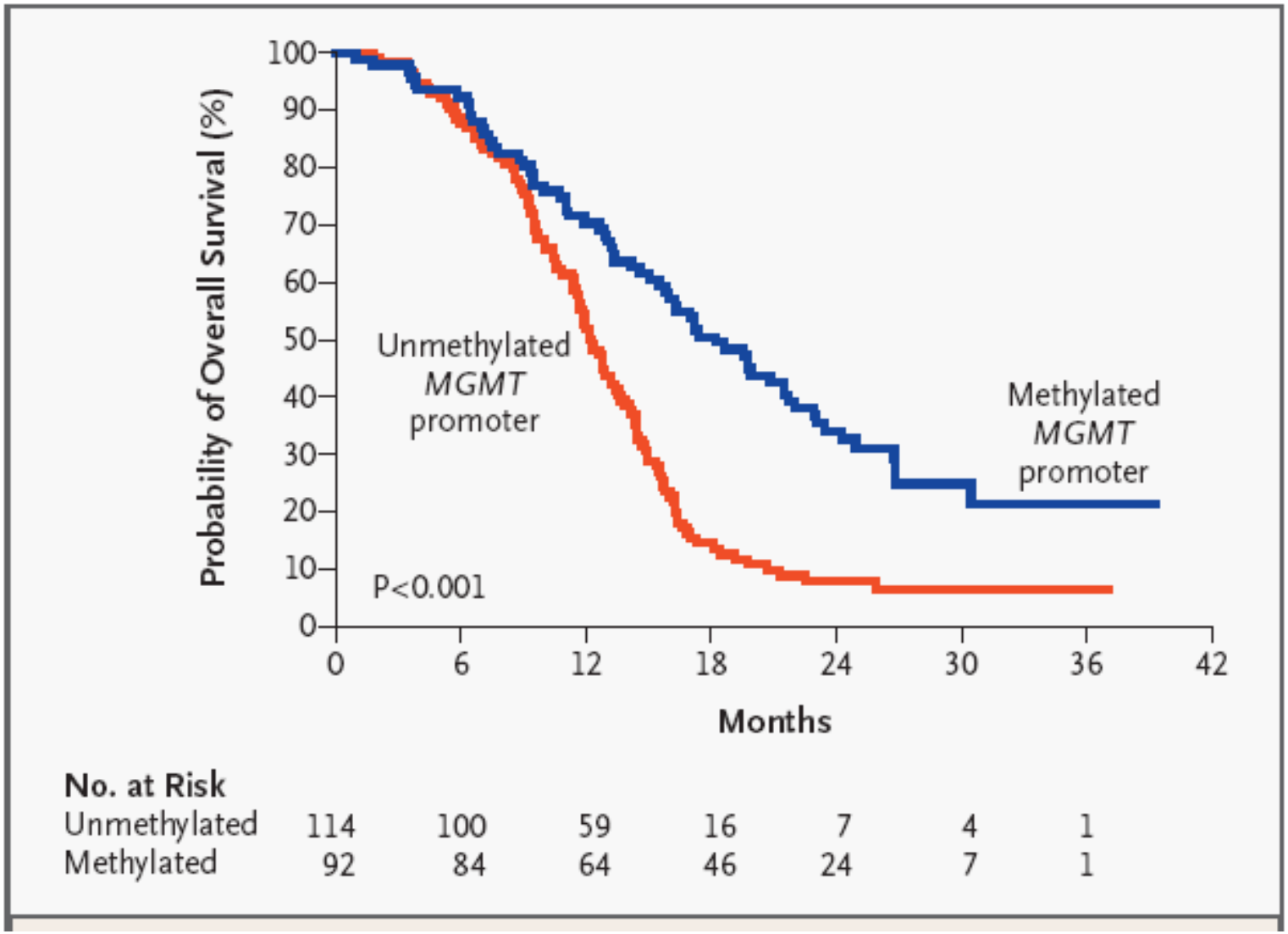


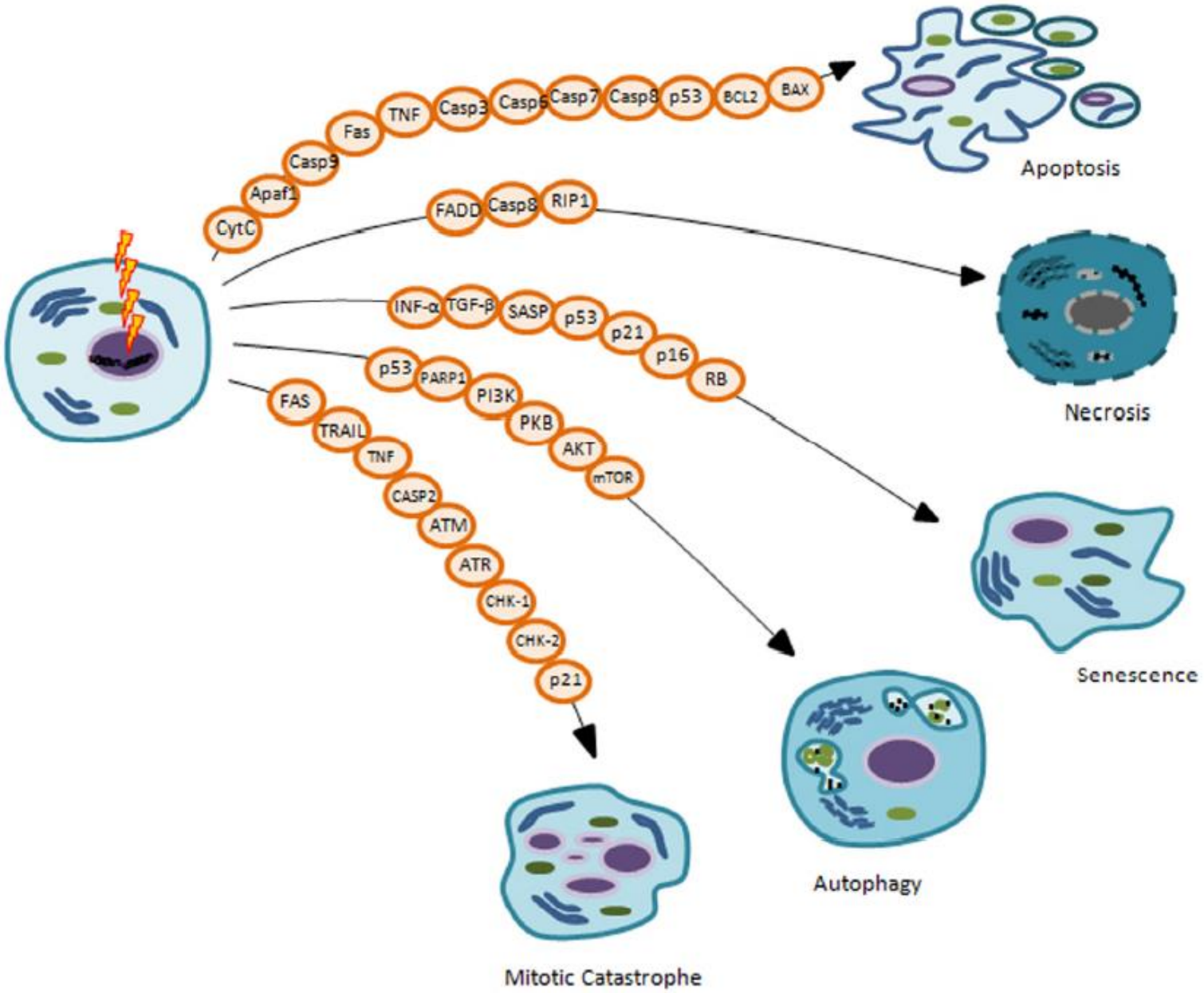
Figure 1. Methylation Status of the *MGMT* Promoter in Glioblastoma Biopsy Specimens, as Determined by a Nested Methylation-Specific PCR Assay.

Hegi et al. NEJM 2005

Overall survival by MGMT promoter methylation status



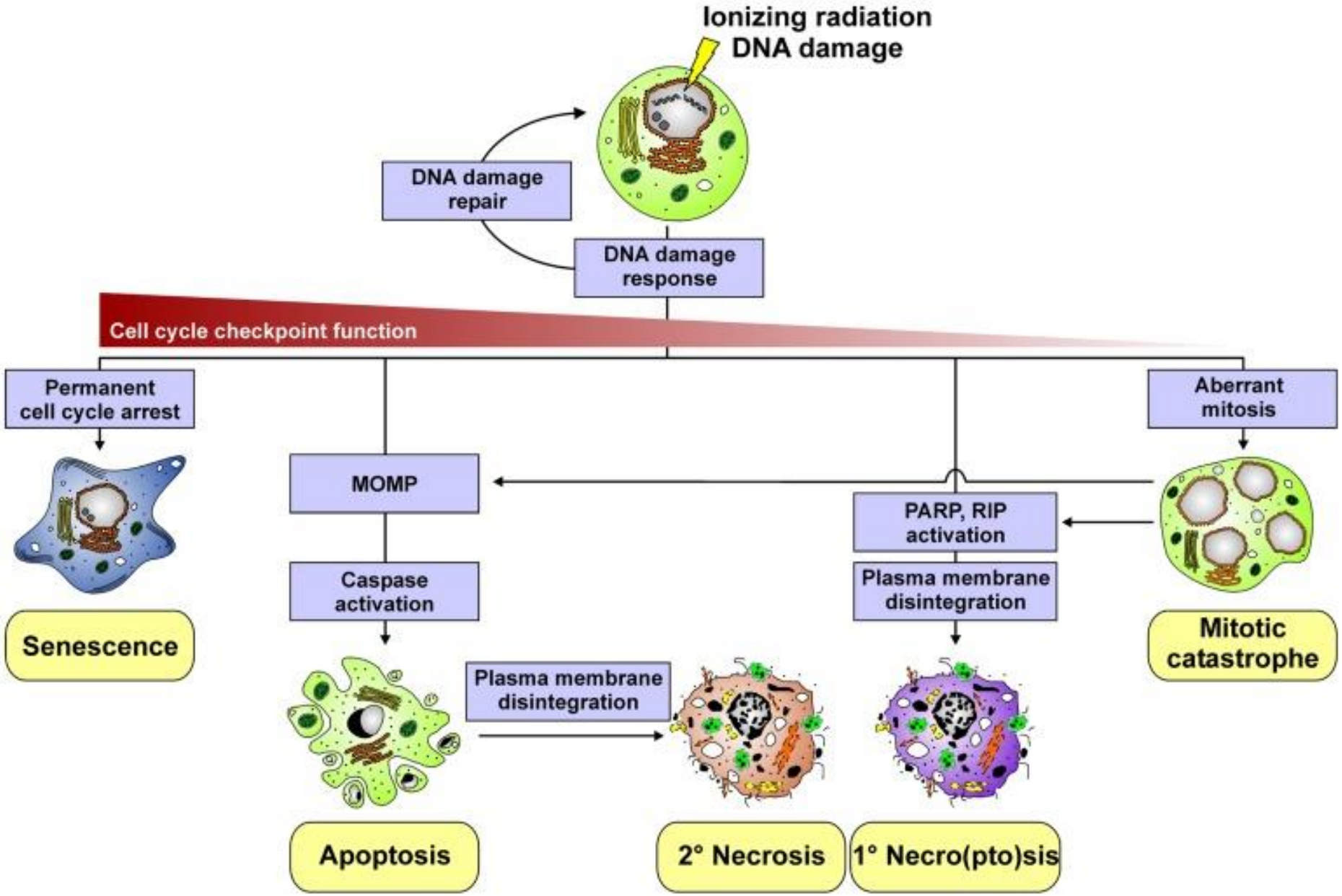
Cell Death from Radiation



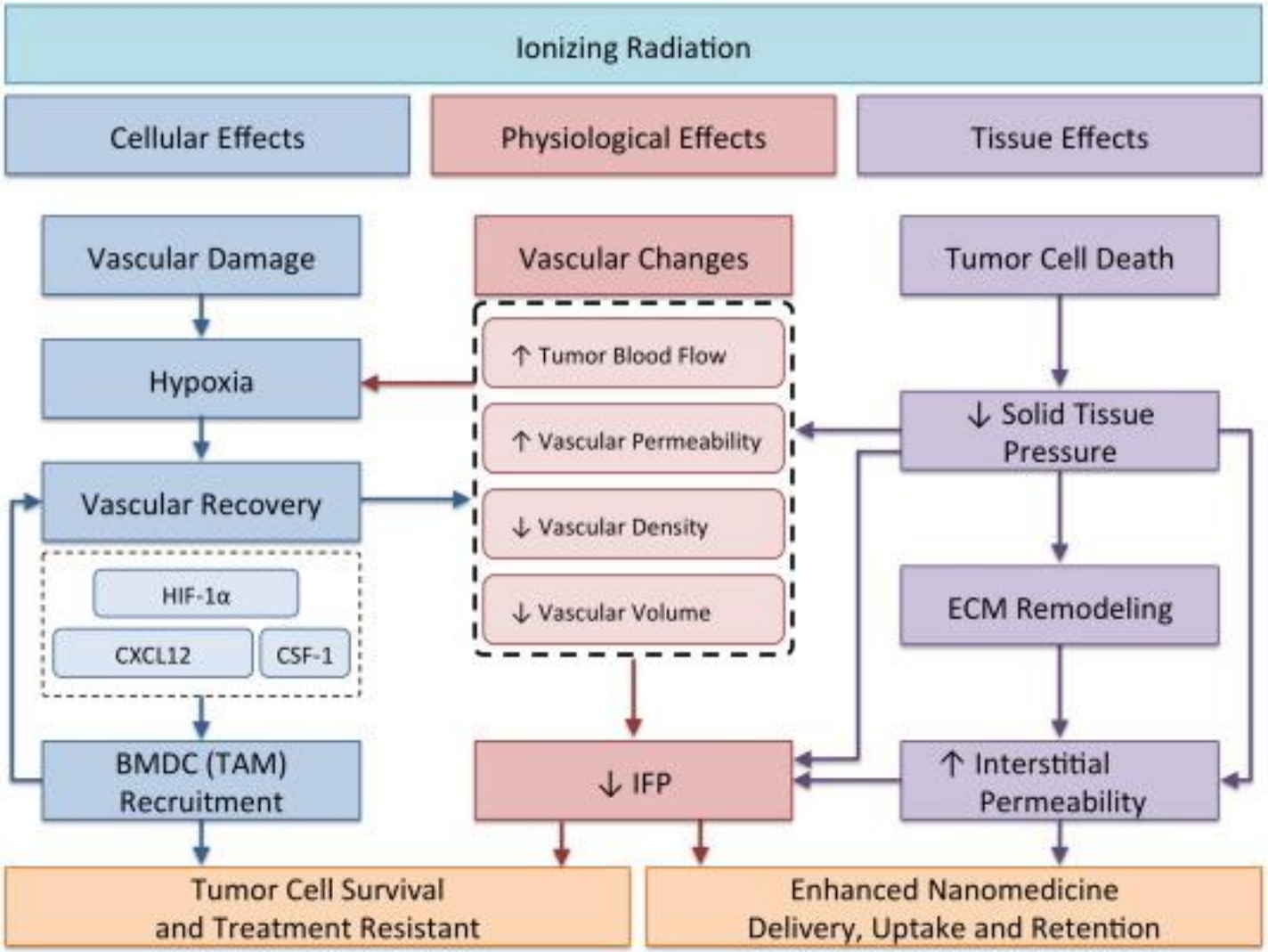
Cell Death from Radiation

- Interphase Apoptosis
- Autophagy
- Necrosis
- Senescence
- Post-mitotic cell death (mitotic catastrophe)
- Others
  - Necroptosis
  - Immune mediated death

Cell Death from Radiation



Radiation-induced Effects on Tissues/Tumors



Tolerance Doses of Tissues

Organ	Injury	TD5%/5yr	TD50%/5yr
Bone marrow	aplasia	2.5	4.5
Intestine	perforation	40	55
Liver	hepatitis	30	40
Brain	necrosis	45	60
Lung	pneumonitis	17	24
Kidney	nephrosclerosis	23	28
Skin	dermatitis	55	65
Rectum	ulcer, fistula	60	80
Saliv. glands	xerostomia	32	46
Testes	sterilization	1	2
Ovaries	sterilization	2-3	6-12
Bone (child)	growth arrest	10	30
Bone (adult)	necrosis	60	100
P. nerves	neuritis	60	100
Muscle	fibrosis	60	80
Breast	atrophy	50	100

Why Do Tolerance Doses Vary So Much?

Latency

- The time a tissue takes to express damage (latency)
  – depends on its turnover time

- On this basis, normal tissues can be divided into

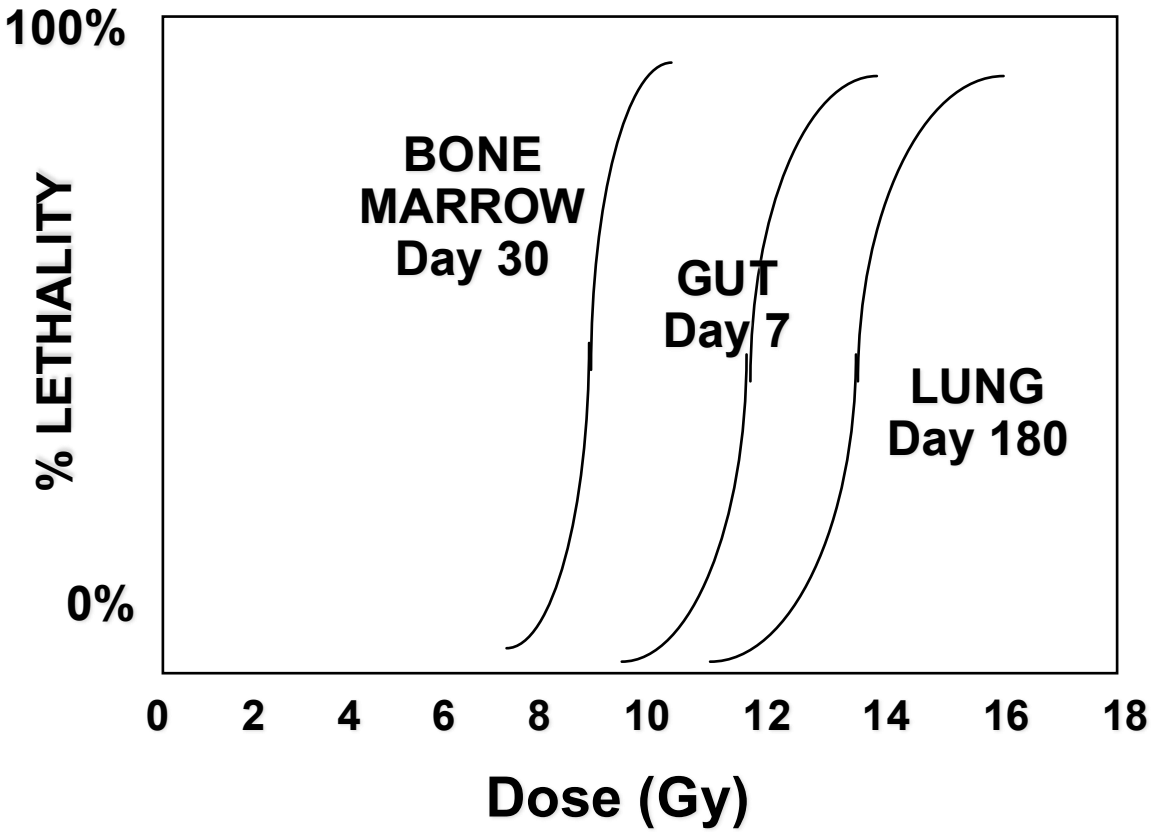
- ACUTE RESPONDING

- Gut, Skin, Bone Marrow, Mucosa

- LATE RESPONDING

- Brain, Spinal Cord, Kidney, Lung, Bladder, Bone

Latency and Tolerance



Different tissues have different tolerances to irradiation and fail at different times (latency) after irradiation.

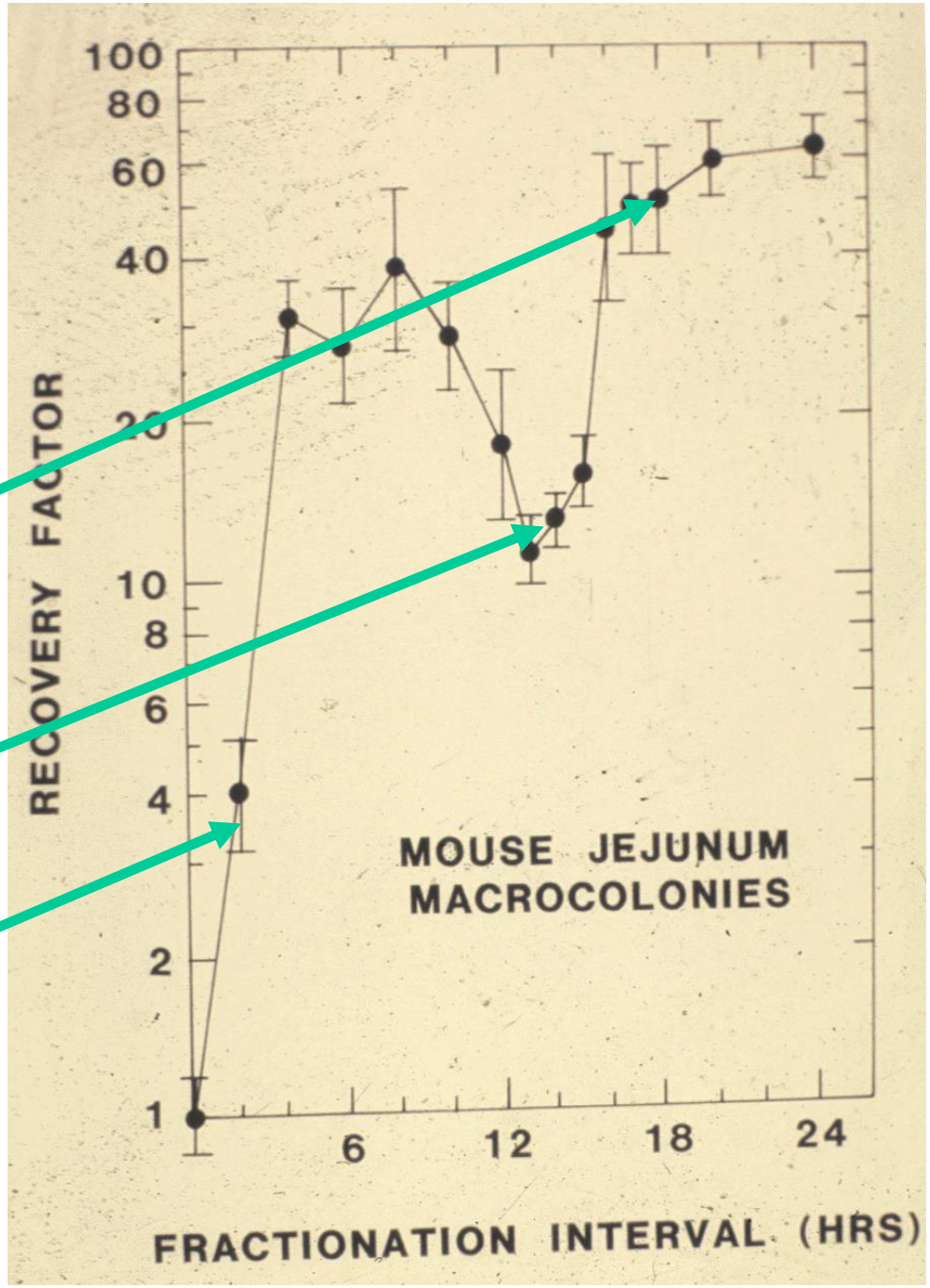
In these experiments, mice were given varying doses of whole body, abdominal, or thoracic irradiation, and the dose and time to failure assessed.

Effect of Dose + Fractionation on Clonogen Survival in Jejunum

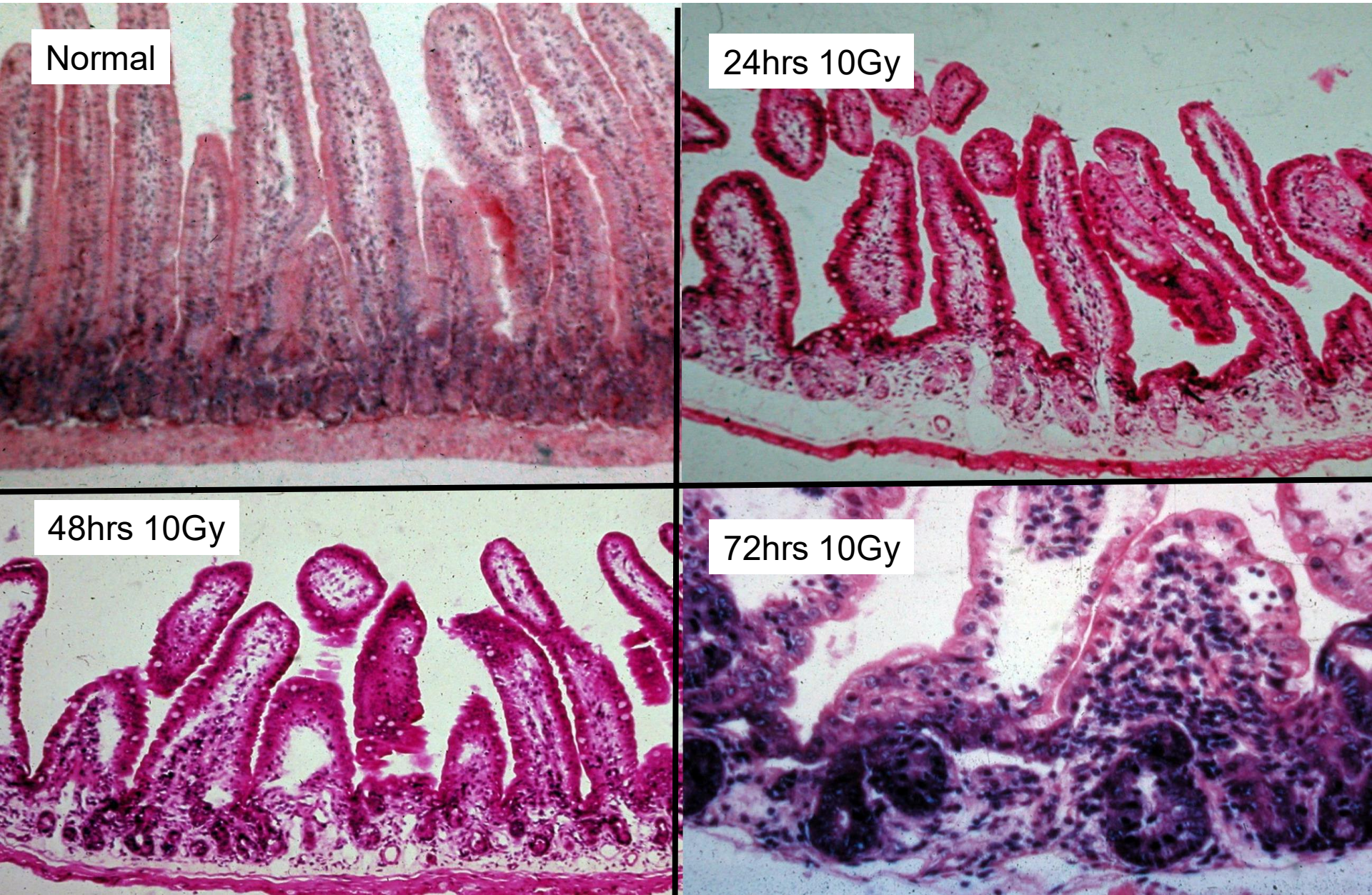
Repopulation

Redistribution

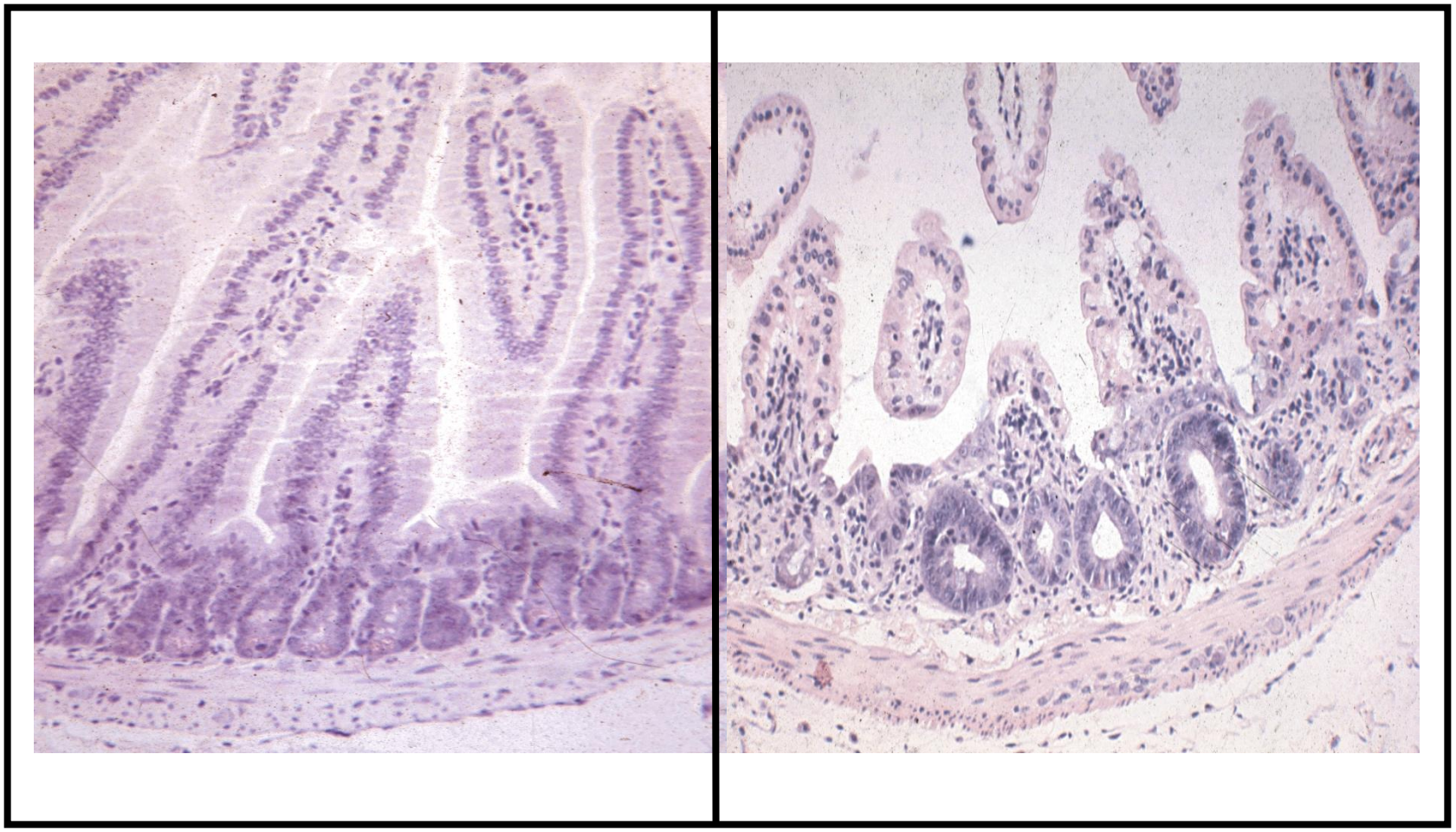
Repair



Murine Small Intestine

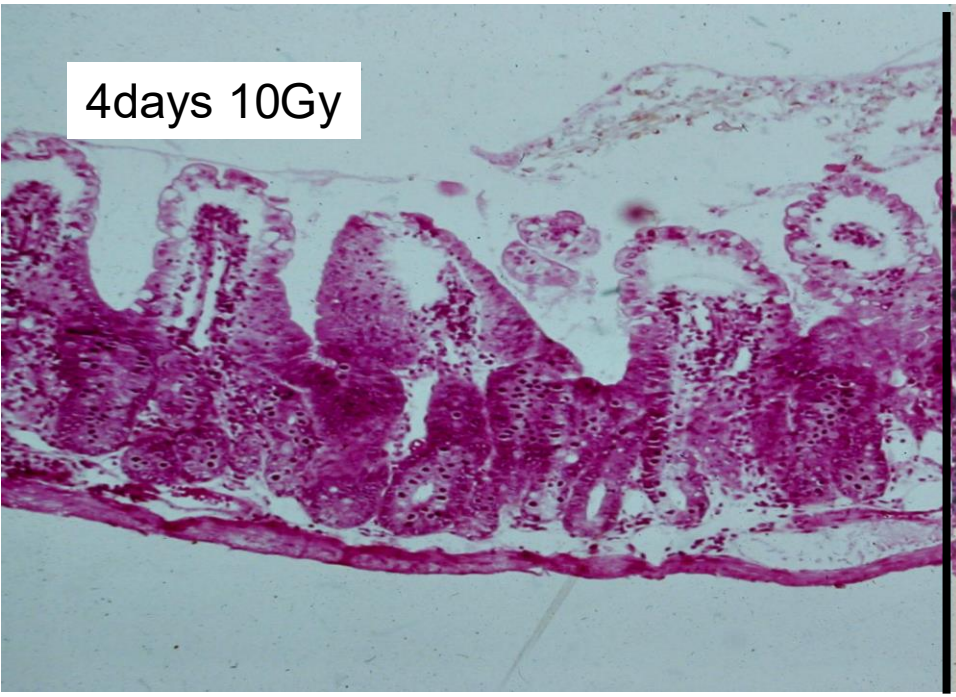


Micro-colonies after Gut Irradiation

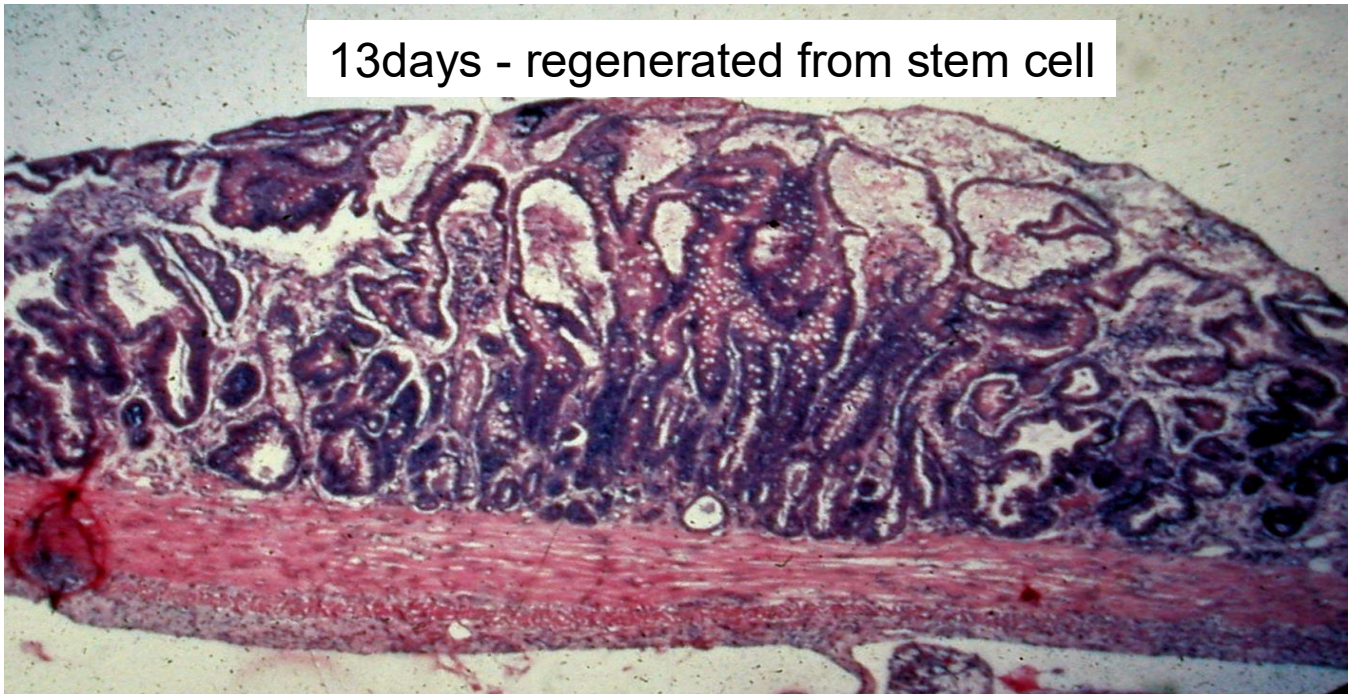


Control

Day 2.5 after 13 Gy Irradiation


4days 10Gy


5days 10Gy

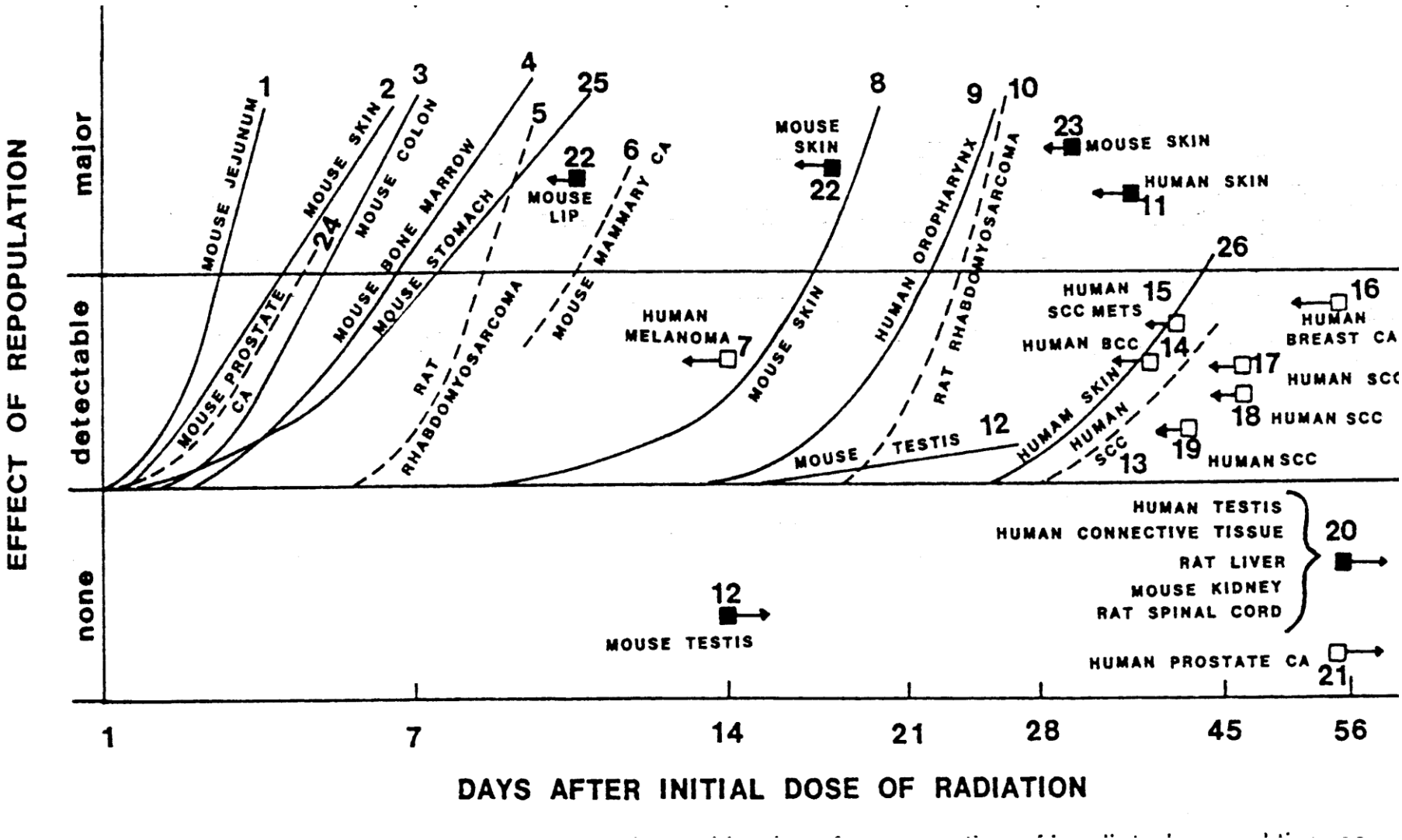

13days - regenerated from stem cell

Clinical Relevance

- Mucositis appears 2 to 3 weeks after the start of a standard RT course.

- Regeneration begins at about 10 to 12 days, before evident mucositis. This can increase the tolerance of the mucosa by about 1Gy/day, which is equivalent to clonogenic cell number doubling every 2 days.

Time to Regeneration



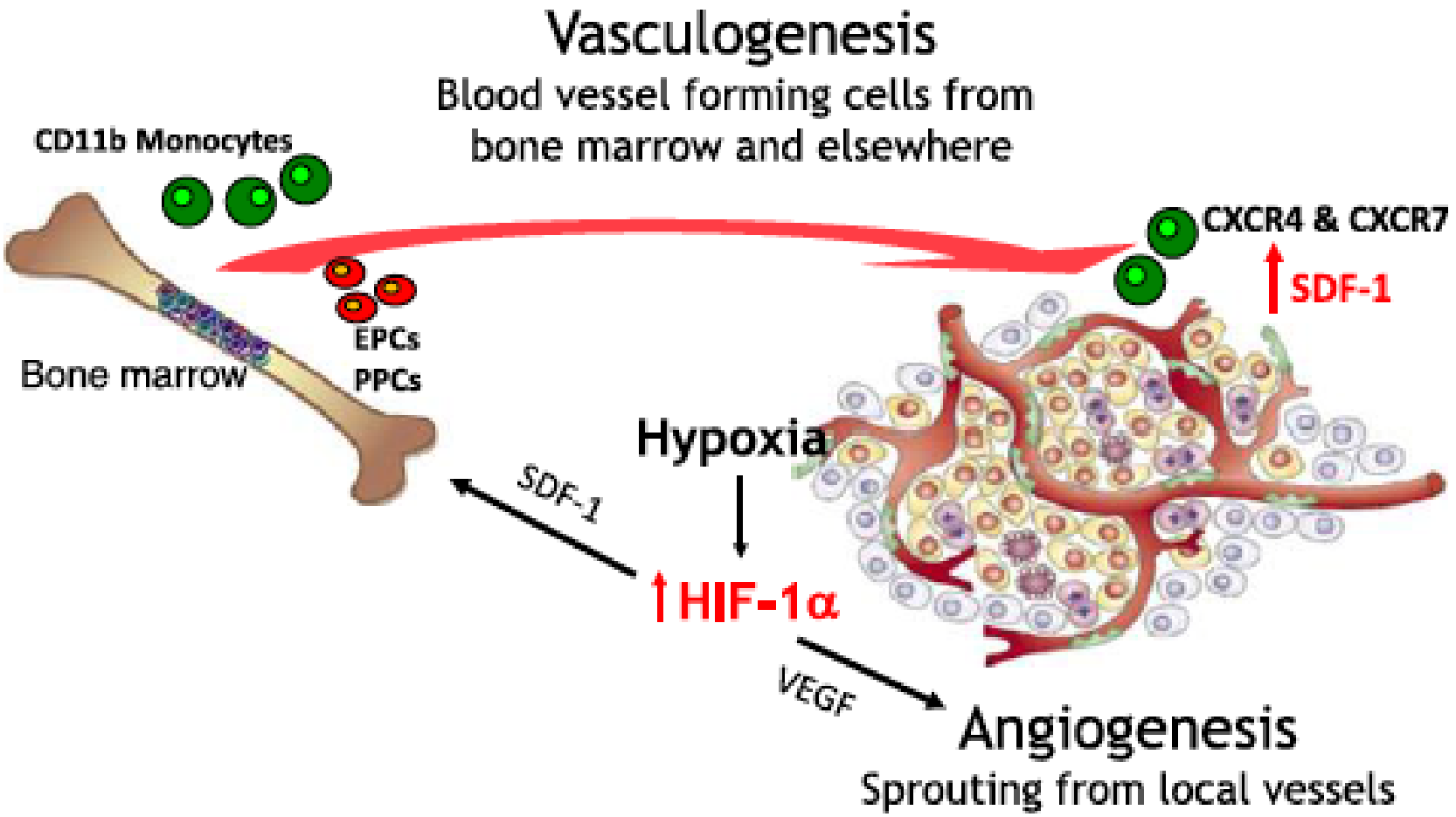
Kinetics of tissue regeneration. Withers and McBride, Perez.

Clinical Relevance

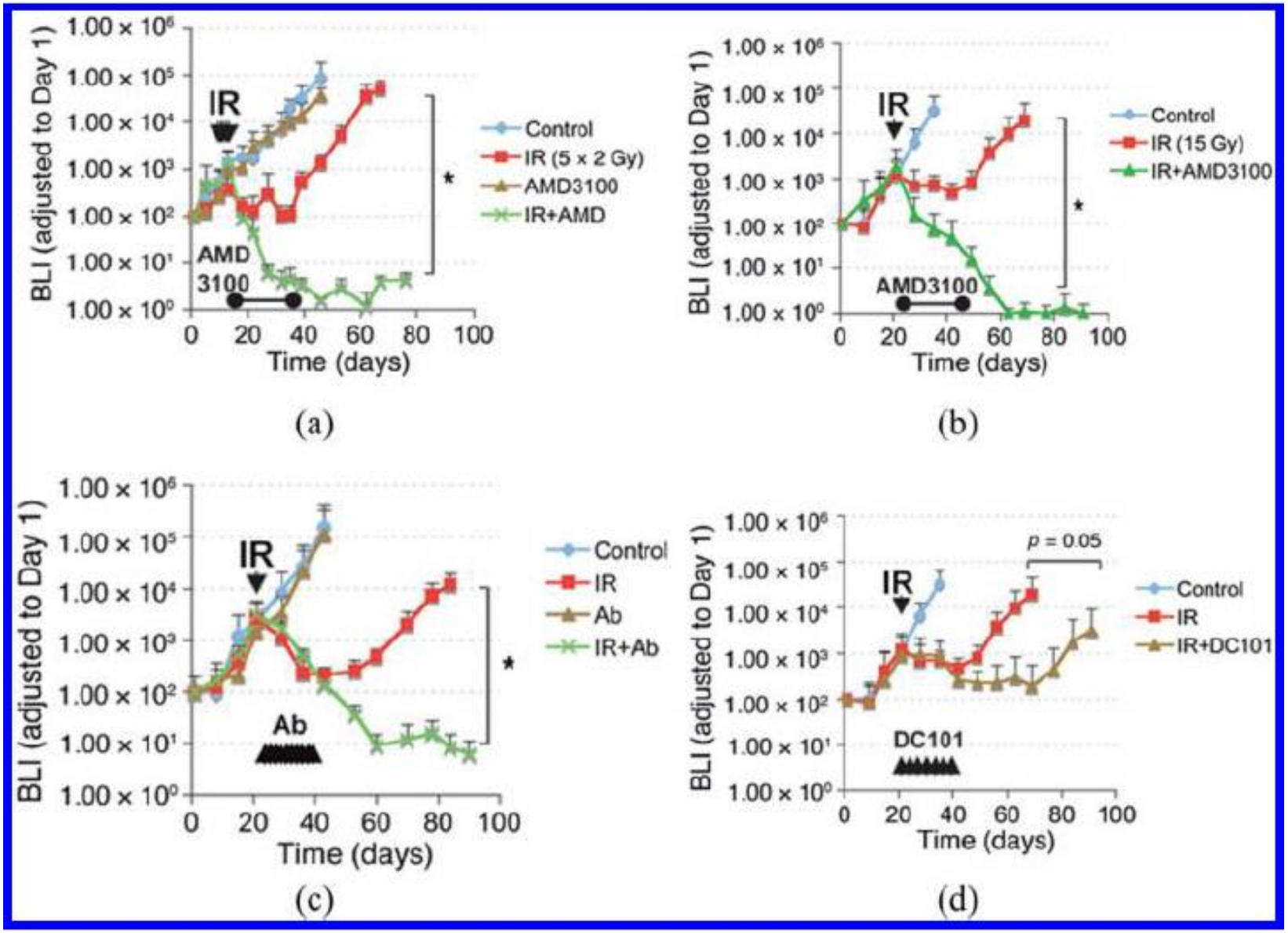
- Because of regeneration/repopulation in acute, but not late, normal tissue reactions occurs during treatment:
  - –protracting overall treatment time beyond the conventional 6 weeks may result in sparing (but tumors may also be spared)
  - –decreasing overall treatment time to less than the conventional 6 weeks may result in more acute normal tissue reactions
  - –treatment time has relatively little effect on responses in late effects tissues

Regeneration/repopulation in late, normal tissue reactions after treatment, may allow recovery and make retreatment possible

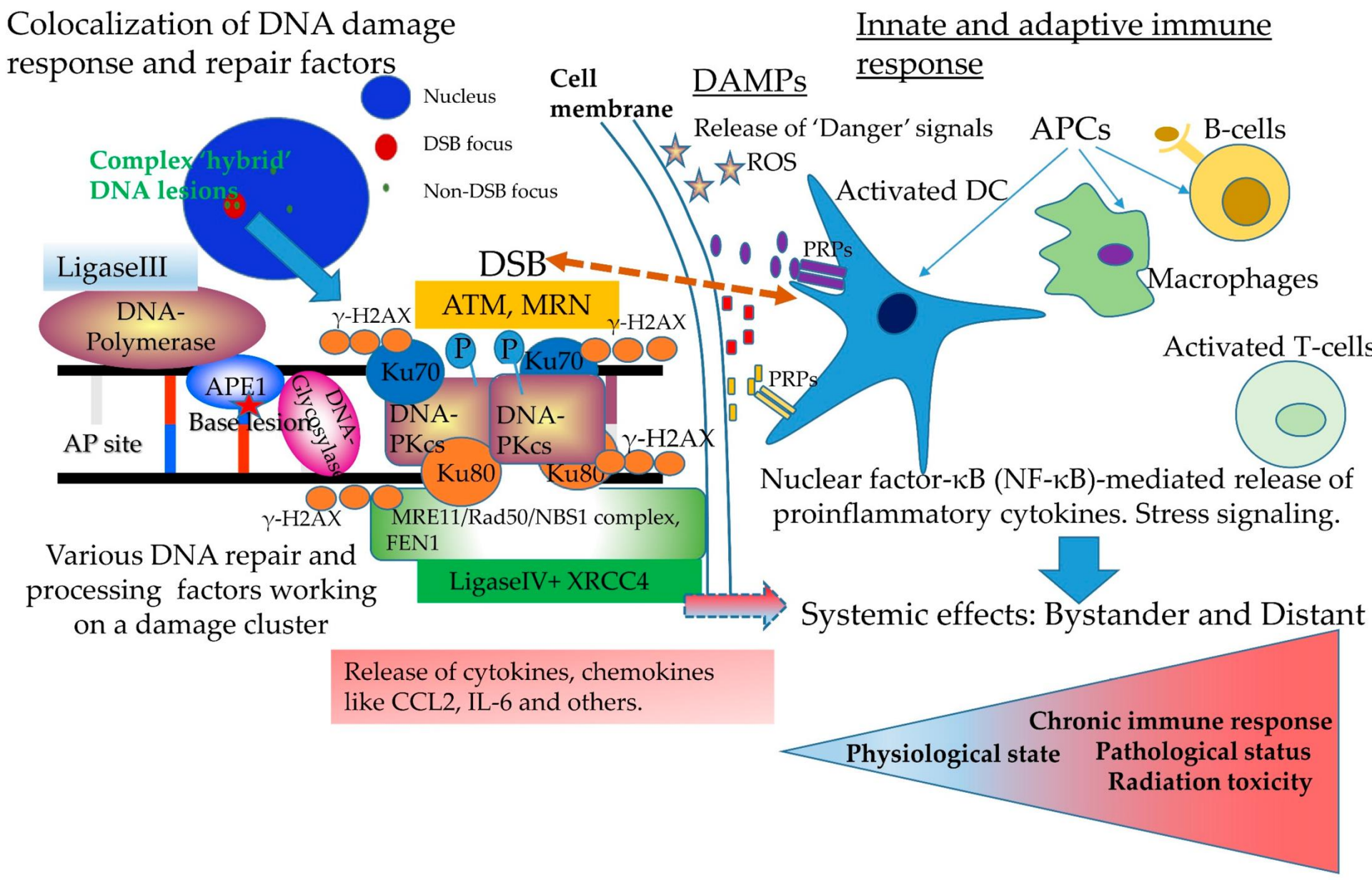
Cellular Recovery: Angiogenesis and Vasculogenesis



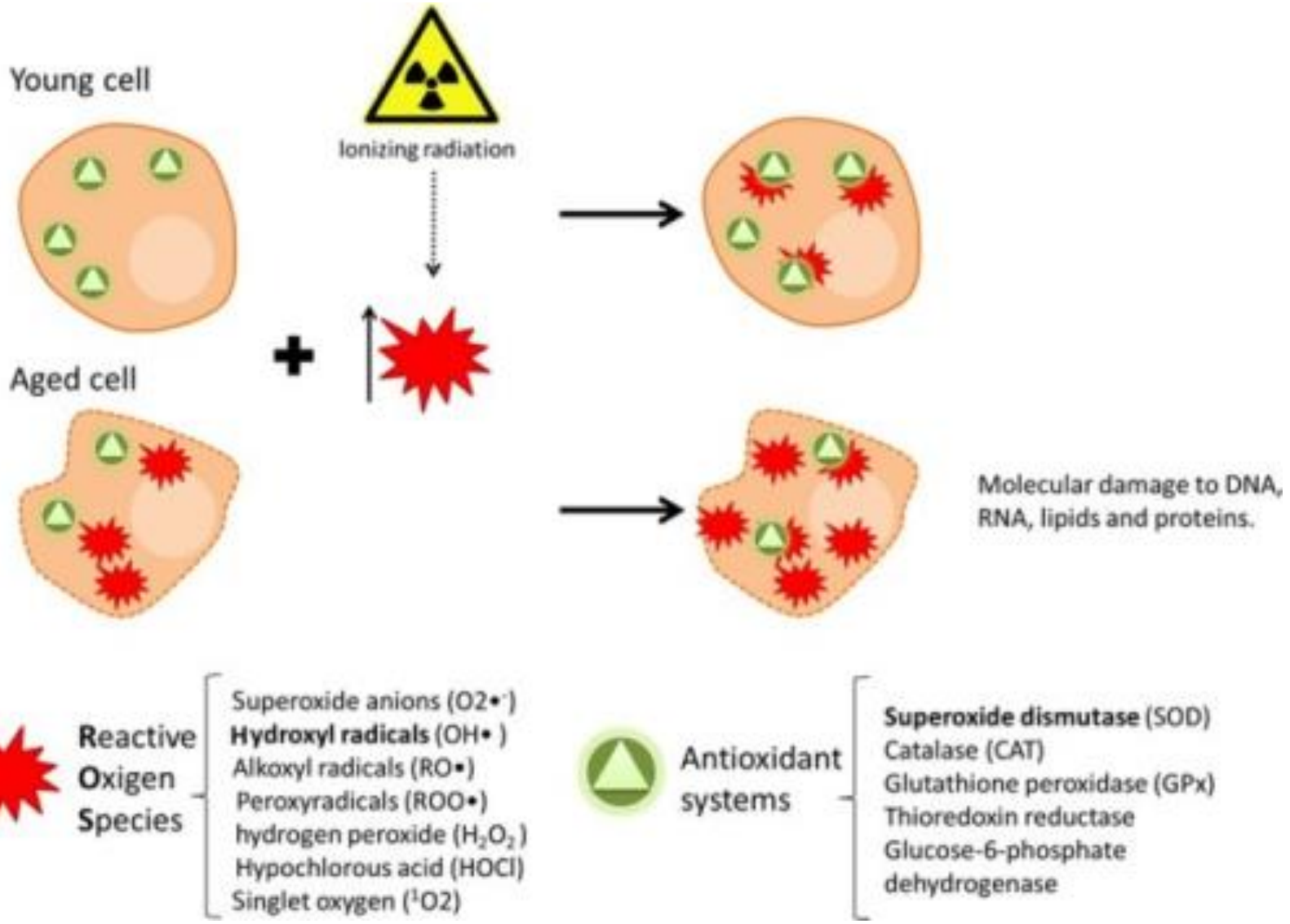
Vasculogenesis affects Tumor Control Dose



Radiation-induced Effects on Cells



Radiation Effects by ROS



Cytokines and Growth Factors

- Inflammatory Cytokines
  - Tumor Necrosis Factor (TNF-α), Interleukin-1 (IL-1)
- Angiogenesis
  - Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF), TNF-α
- Immune Cytokines
  - IL-2 and IL-4
- Fibrotic Cytokines
  - Transforming Growth Factor beta (TGF-β), bFGF, IL-6, CTGF
- Growth Factors
  - Colony Stimulating Factors - G-CSF, GM-CSF, IL-3, EPO, SCF
  - Epidermal Growth Factor (EGF), TGF-α, bFGF

Cytokines and Growth Factors

- Cytokines are expressed in tissues within hours of irradiation
- Over subsequent weeks and months there is a "cyclical cascade" of cytokines released that is aimed at tissue regeneration.
- Complications such as fibrosis arise when the regenerative process is unsuccessful.

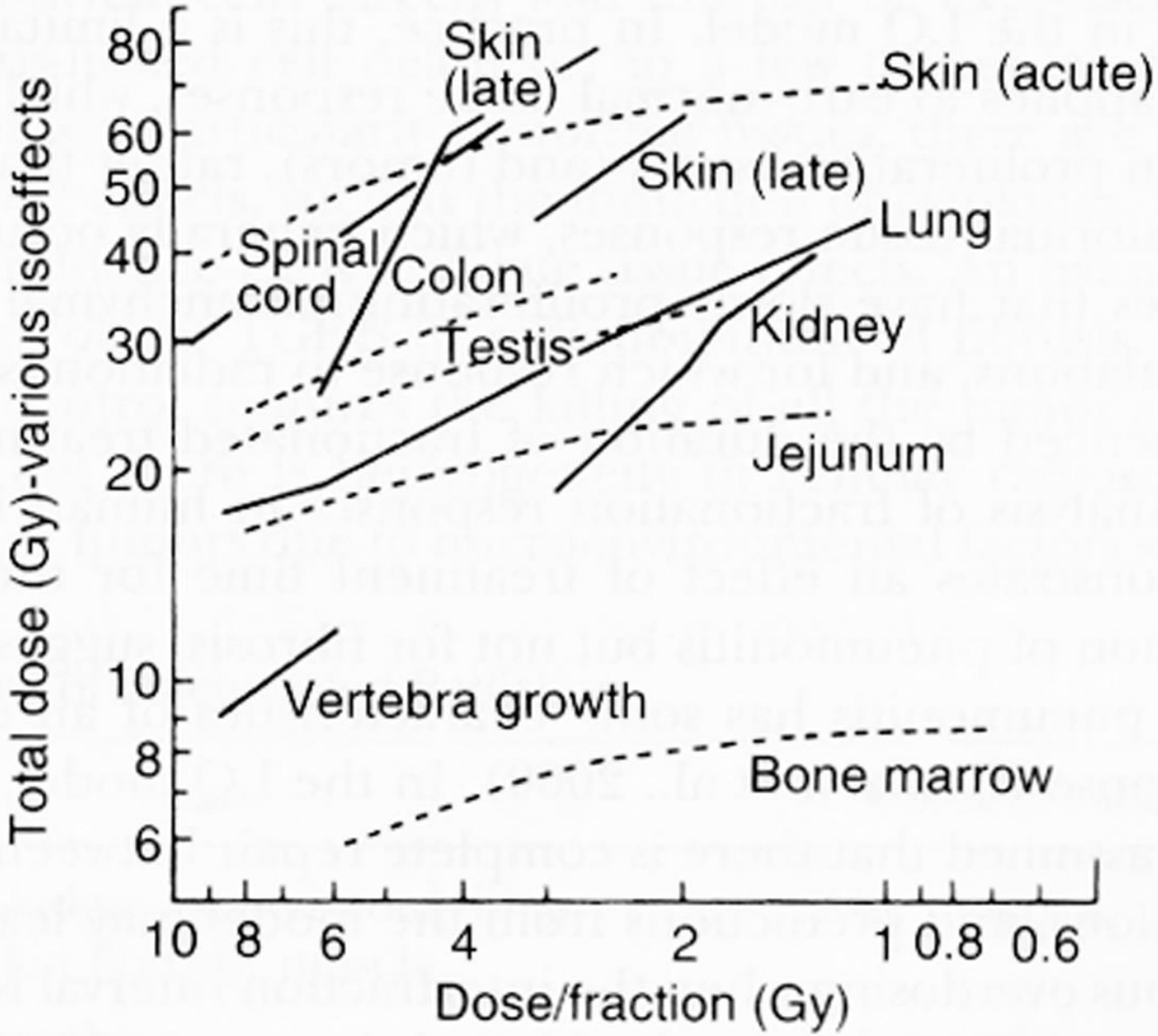
Late Effects Involve Wound Healing

- Wound healing involves cytokines and growth factors that mediate cellular responses including tissue regeneration

- They may also contribute to the pathogenesis of complications and may be responsible for some side effects of irradiation, such as
  - Fatigue
  - Edema
  - Erythema
  - Somnolence
  - Nausea

Late Effects Involve Fibrosis

- Fibrosis is a common complication of radiation exposure that is a response to cell loss.
- TGF-β and its target genes are involved in promoting tissue fibrosis.
- TGF-β induces fibroblast differentiation, senescence, and collagen production.
- Patients with persistent high serum levels of TGF-β, before and during a course of therapy for lung cancer have a higher risk of developing pneumonitis.

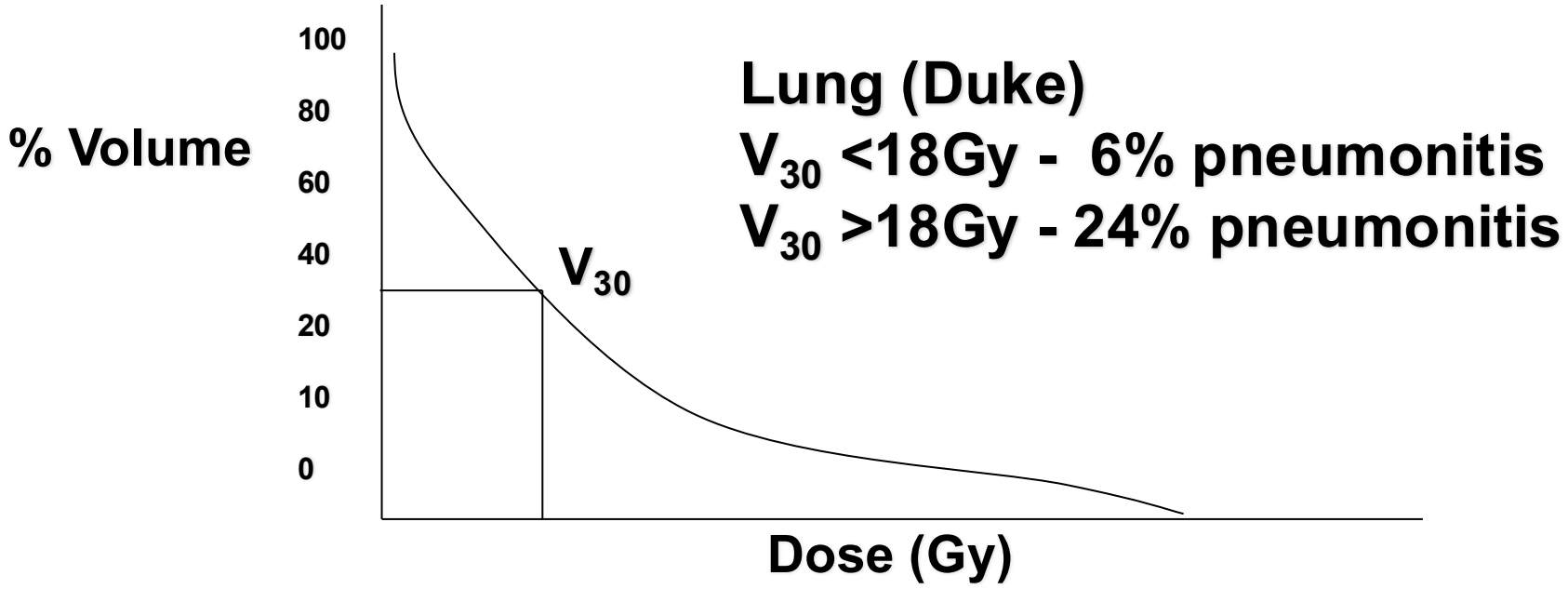


Late effects: steeper
As fraction size↓, total dose for isoeffect ↑

Fowler, Brock et al., 2005

Dose Volume Histograms

- The dose of radiation delivered can be distributed in a 3-D volume with smaller subunits each receiving different doses of radiation.
- May be a useful guide in comparing treatment plans to predict development of normal tissue complications, but there are potential pitfalls



DVH Analyses

- May hide “hot spots” resulting from dose heterogeneity
- Ignores structural heterogeneity within tissues
- Varies with endpoint
- The absolute volume is important rather than the area e.g. bladder surface
- The effects of changes in DVH will vary with the tissue, depending on FSU structure (series/parallel), physiological reserve, etc.

Modifying Fractionation

- Hyper-fractionation

  Increased total dose, same (or slightly longer) treatment time, more fractions. Reduces late effects.

  Typical schedule: 80.5 Gy in 70 fractions (1.15 Gy bid) over 7 weeks

- Accelerated treatment

  Reduced total dose, reduced treatment time, fractions? Reduces repopulation in tumors. Increase in late effects.

  Very accelerated: 54 Gy in 36 fx (1.6 Gy tid) over 12 days; moderately accelerated - 72 Gy in 42 fx (concomitant boost)

- Hypo-fractionation

  Reduced total dose, but larger fraction doses. Tumor has low $\alpha/ß$ ratio. Increases late effects (requires coning down of tumor and treatment volumes).

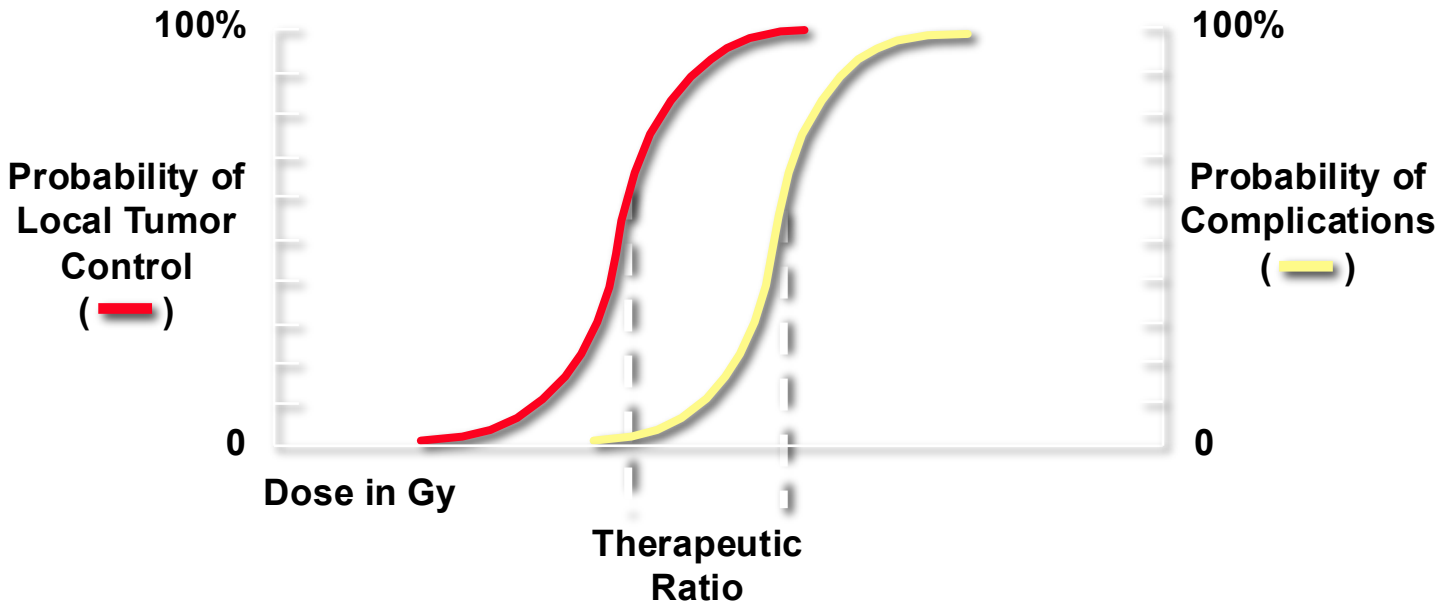
Conclusions to Conventional Fractionation

- Altered fractionation can be of benefit
- Addition of chemotherapy or biological therapies requires re-adjustment of radiotherapy schedule
- In principle, tumors should be treated for an overall treatment time that is as short as possible consistent with acceptable acute morbidity, but with a dose per fraction that does not compromise late responding normal tissues, or total dose.
- Avoid treatment breaks and treatment prolongation

New Modalities

- Intensity Modulated RT:
  - dose-volume effects
- Charged Particles
- SBRT
- Brachytherapy
- SDRT: engages a new biology

Therapeutic Ratio



Sensitization

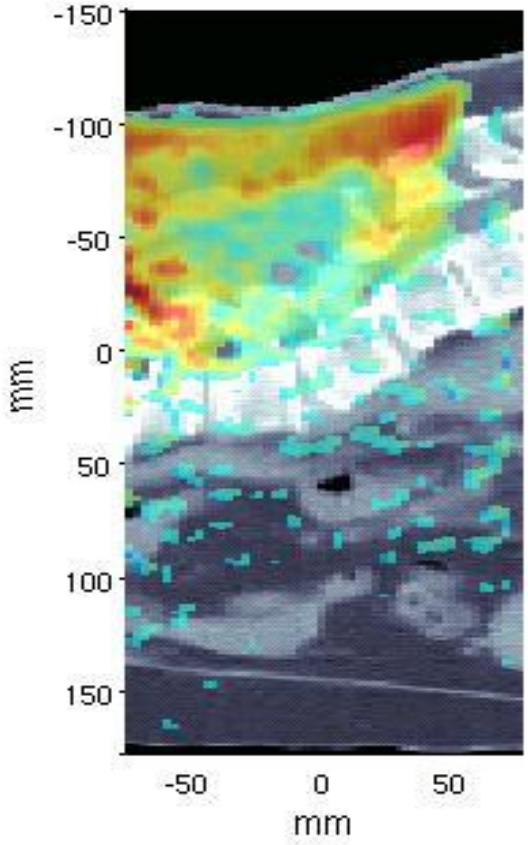
- Chemotherapy
- Oxygen
- Hypoxic cell sensitizers
- Biological Response Modifiers

Protection

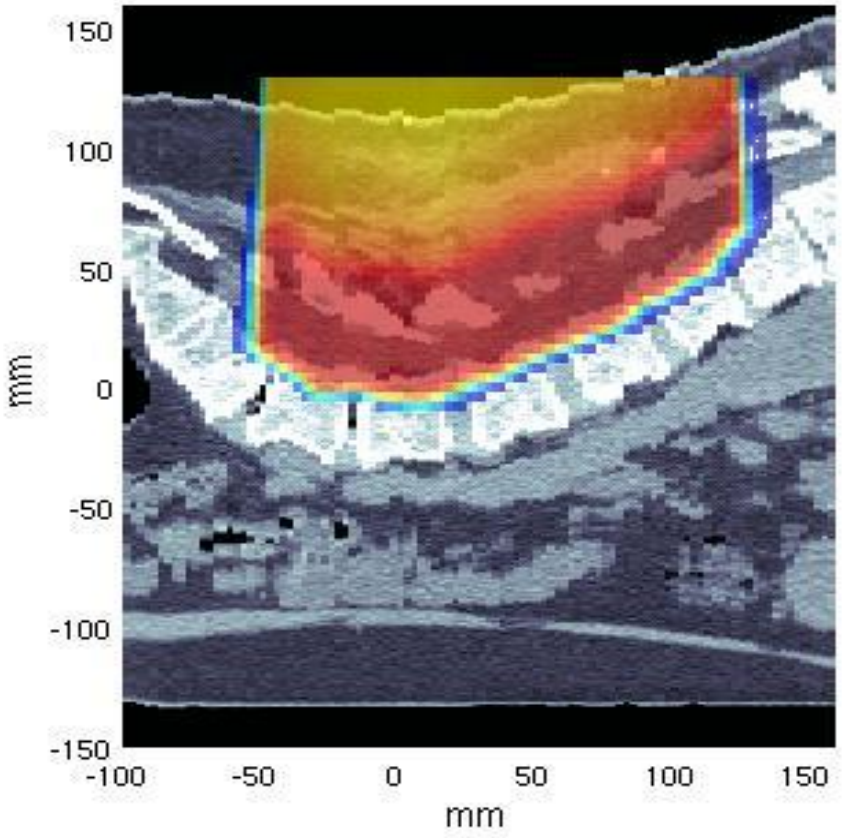
- 3-D Conformal radiation therapy
- Intensity modulated radiation therapy
- Protons
- Radioprotectors (Amifostine)

Chordoma: PET Verification of Proton Dose Distribution

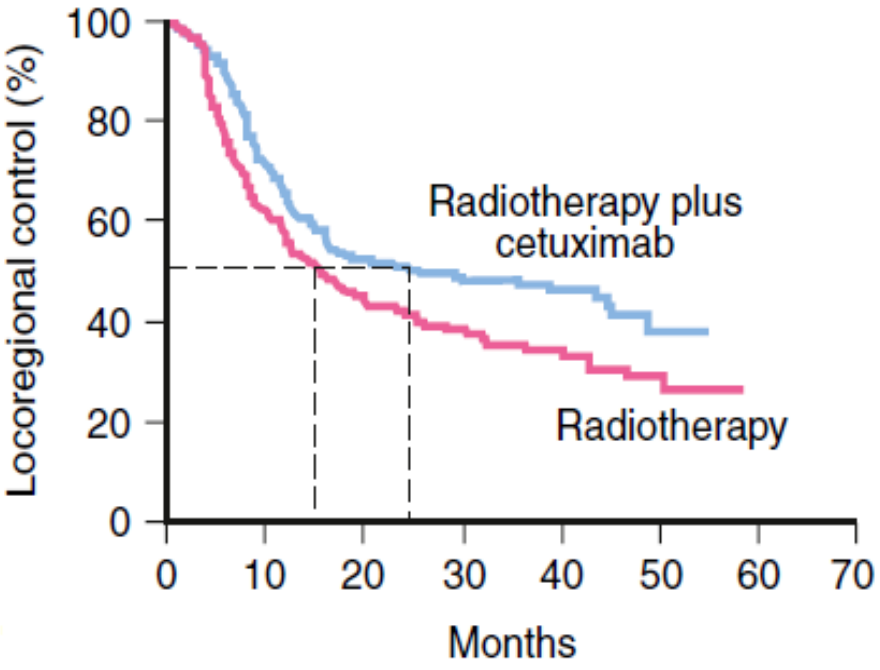
PET/CT measurement

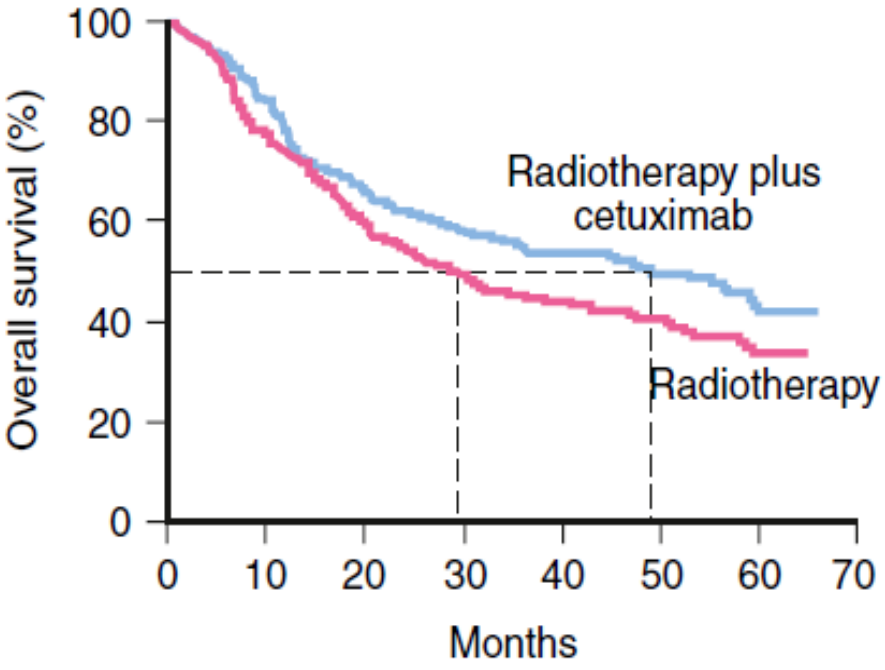


Planned dose

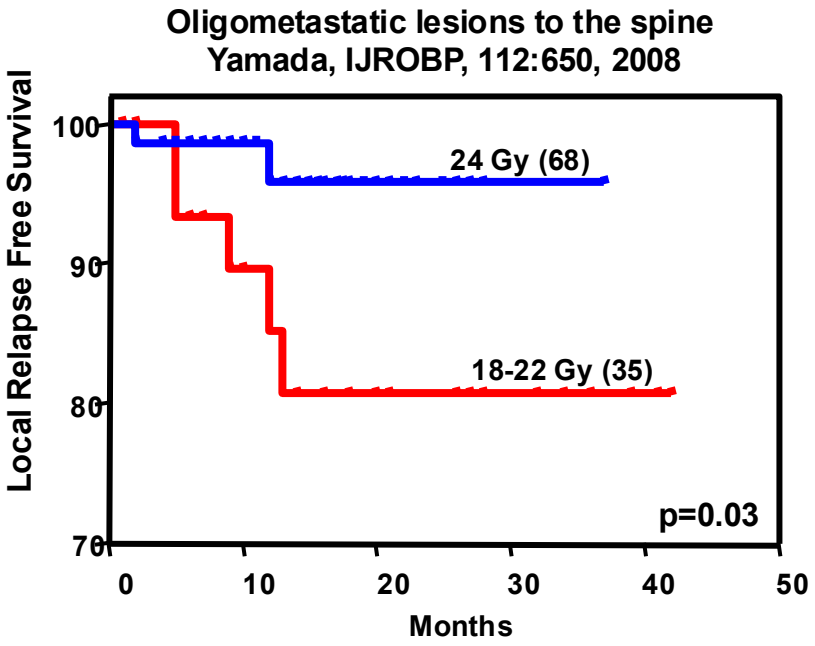


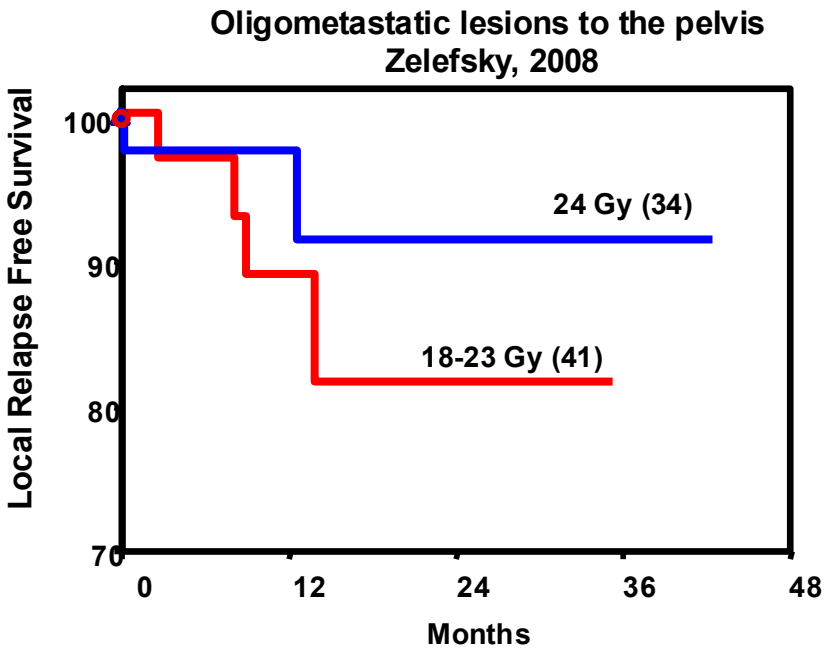
Targeting EGFR in Head and Neck Cancer





Local Control Oligometastatic Tumors The MSKCC Series of SD-IGRT, 2008





- Patients with metastatic breast, colorectal, lung, head and neck, liver, pancreatic, sarcoma, melanoma and other tumors

Single Dose Radiotherapy:

tumor and vasculature

asmsae$^{-/-}$ (microvascular-resistant host)

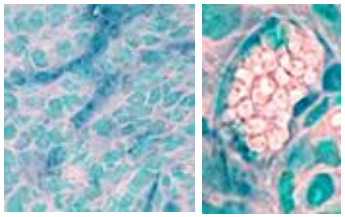
Single Dose Radiation (≥10Gy)

Tumor Cell DNA DSB

Tumor Cell DSB Repair

SCC Survive

Radioresistant Phenotype



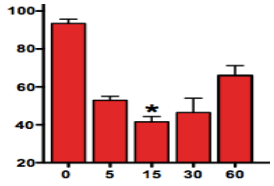
Intact Endothelium

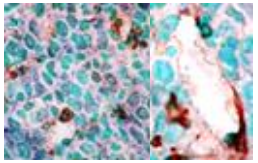
asmase+/+ (microvascular-sensitive host)

Single Dose Radiation (≥10Gy)

Tumor Cell DNA DSB

Endothelial ASMase Activation



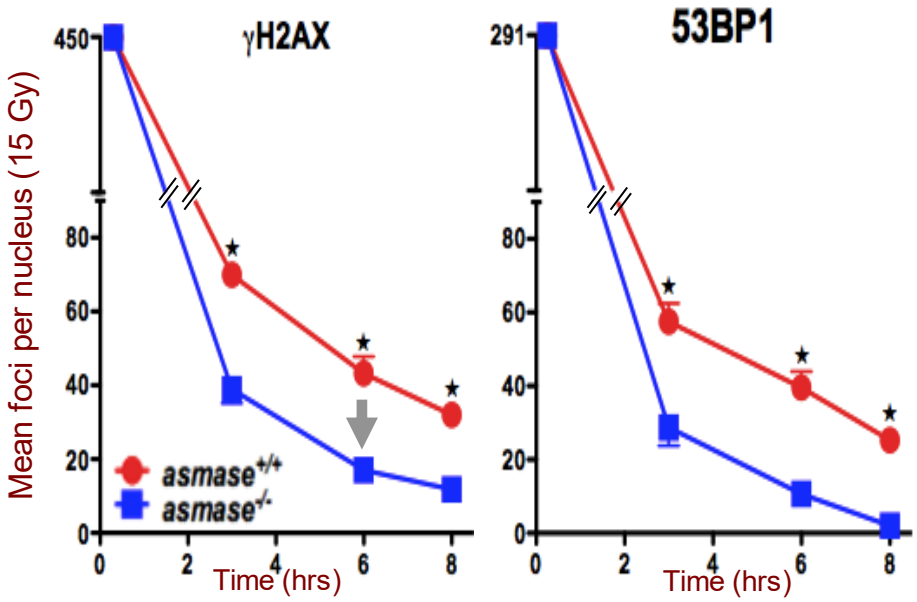


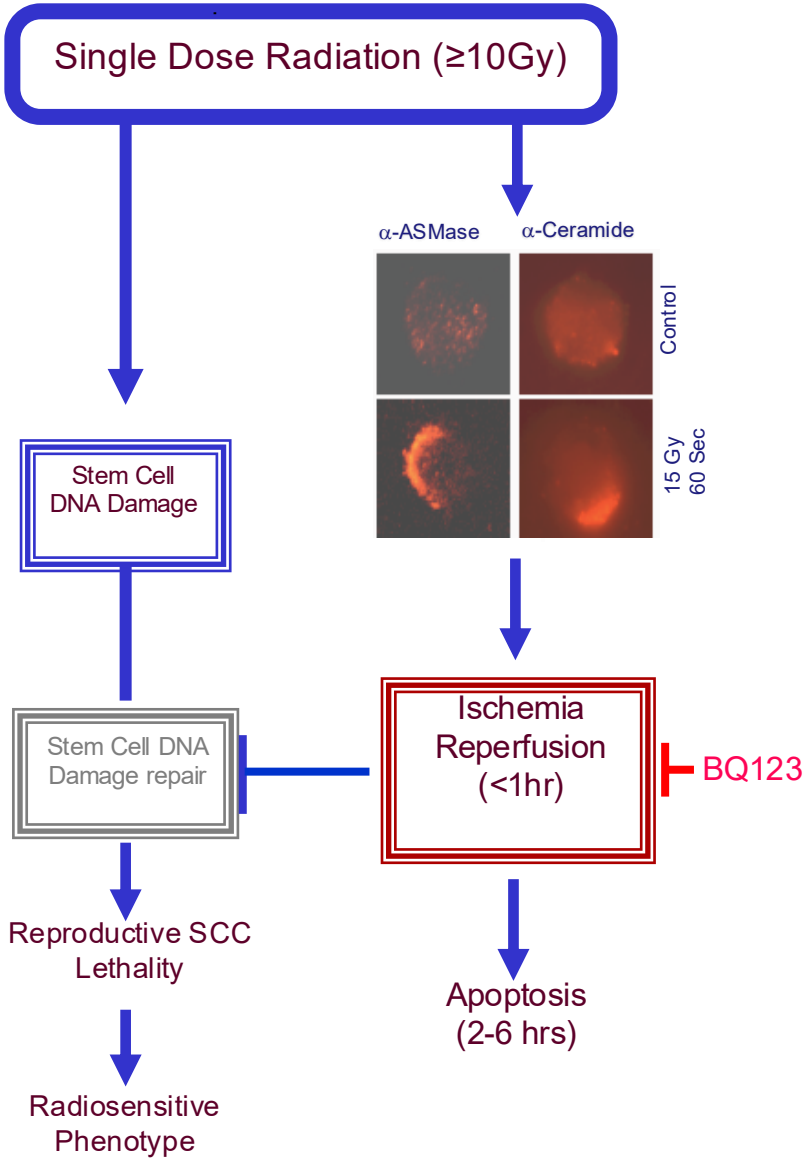
Tumor Cell DSB Repair

Tumor SCC Lethality

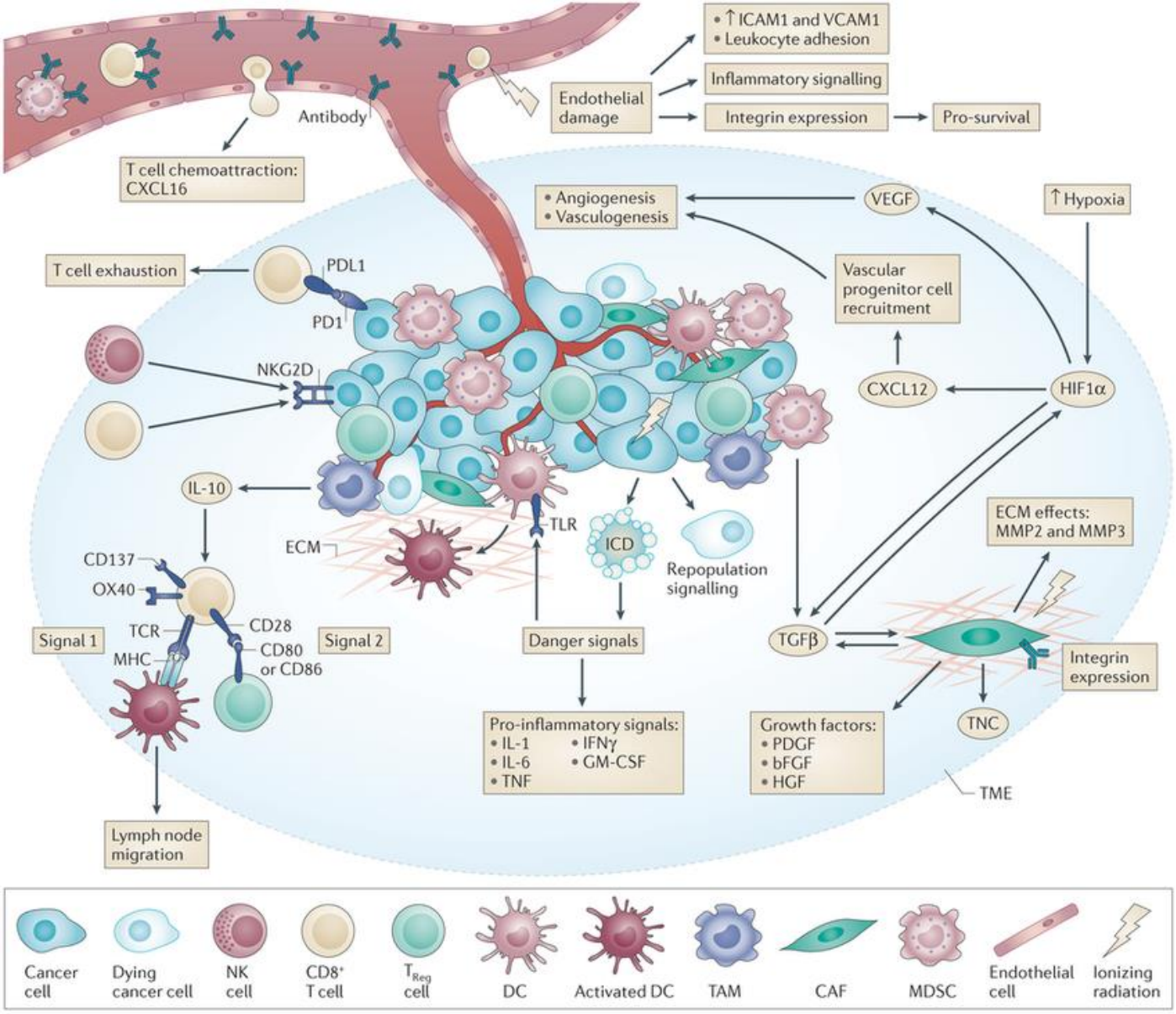
Radiosensitive Phenotype

Microvascular dysfunction inhibits DSB repair



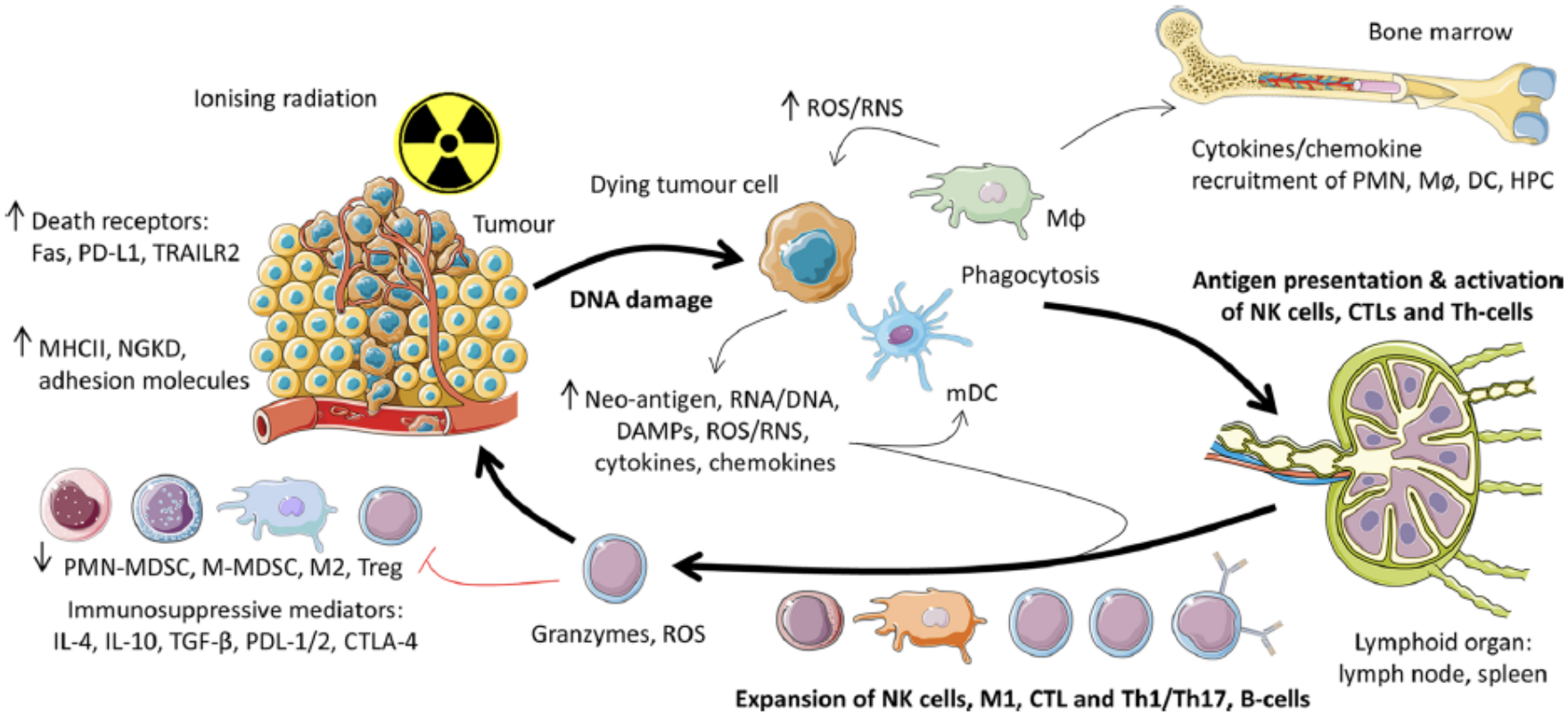


Reactions to Radiation Effects on Tumors

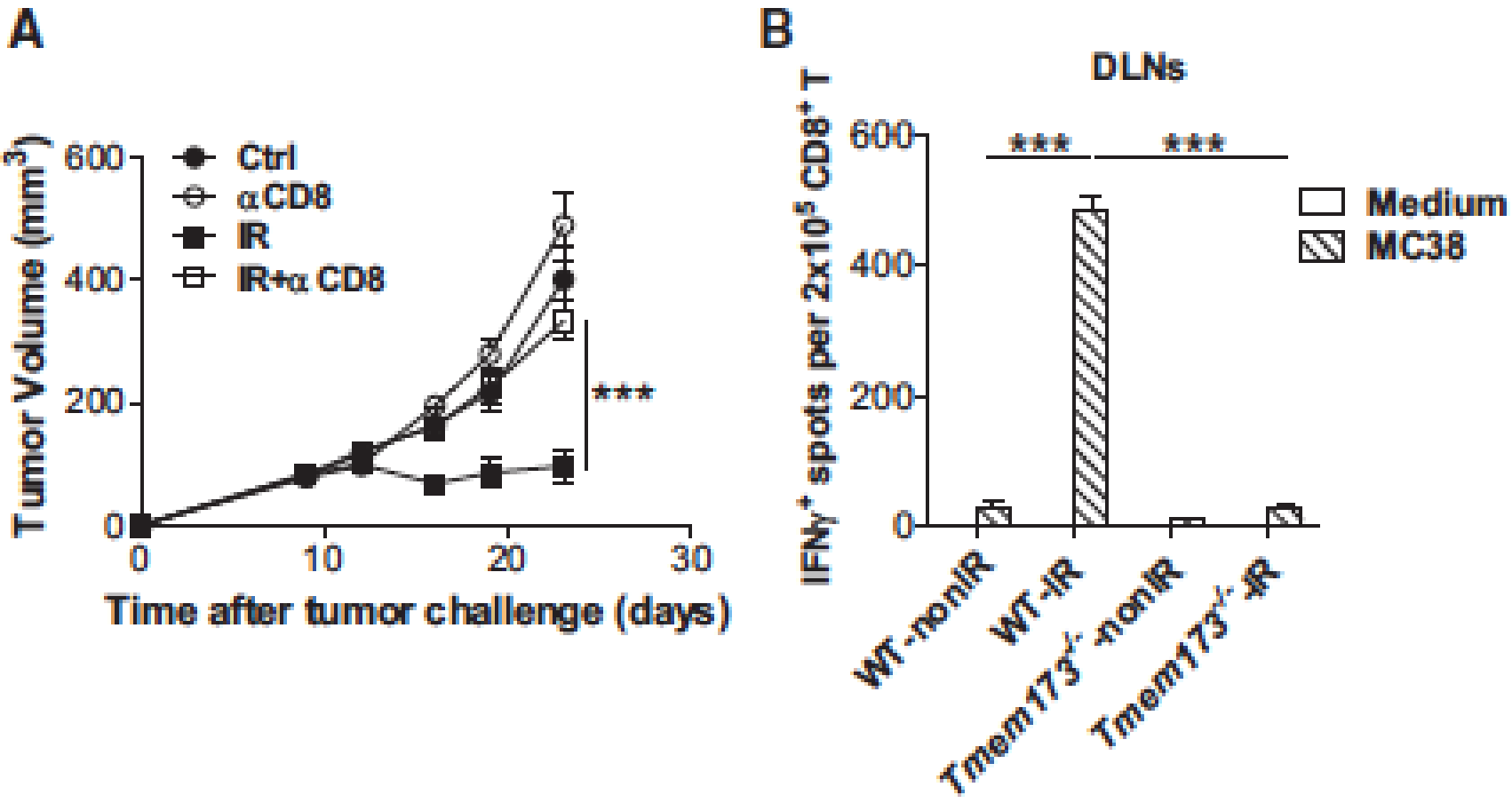


Nature Reviews | Cancer

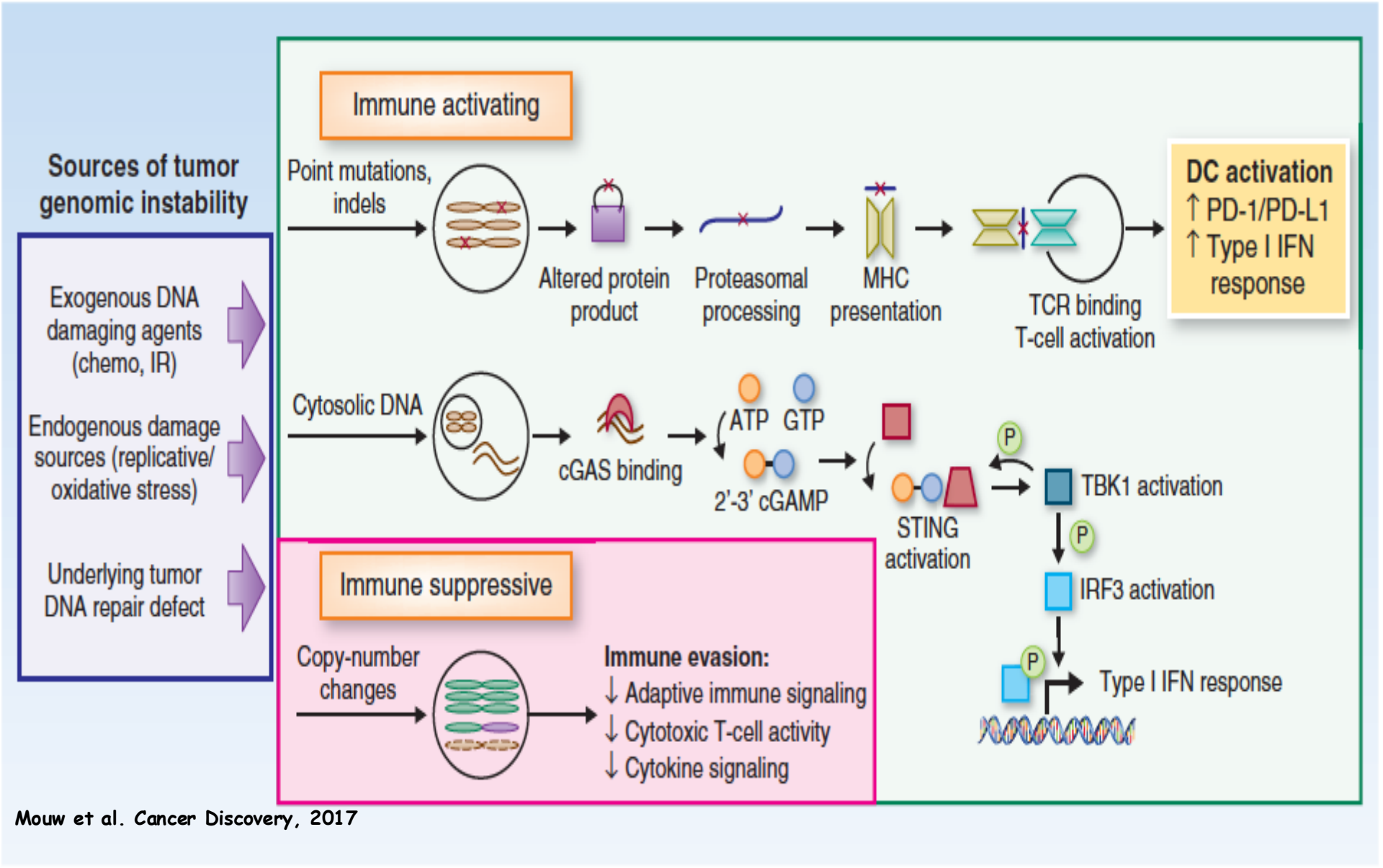
Immune Response to Ionizing Radiation



Radiation Tumor Control needs Immune Response



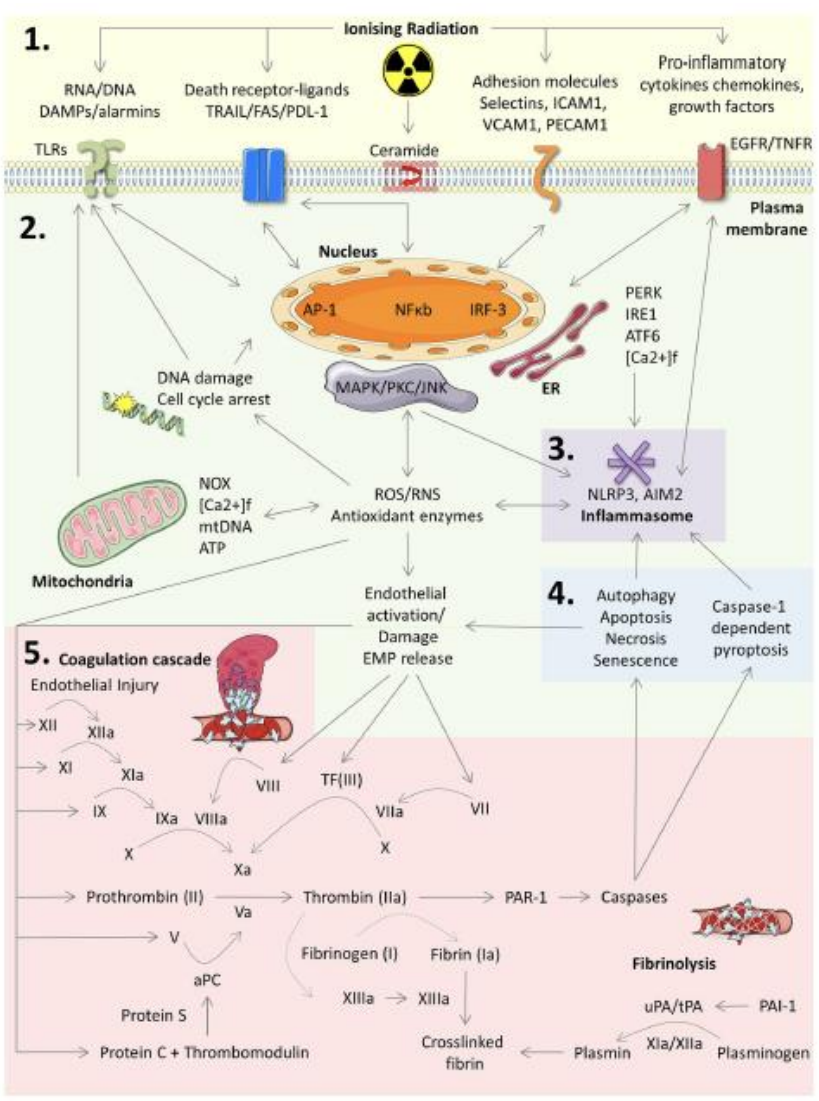
(Deng, Weischelbaum et al., Immunity, 2014)

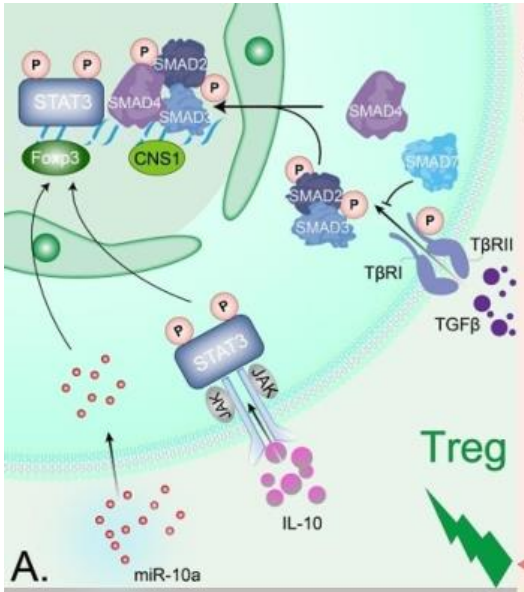


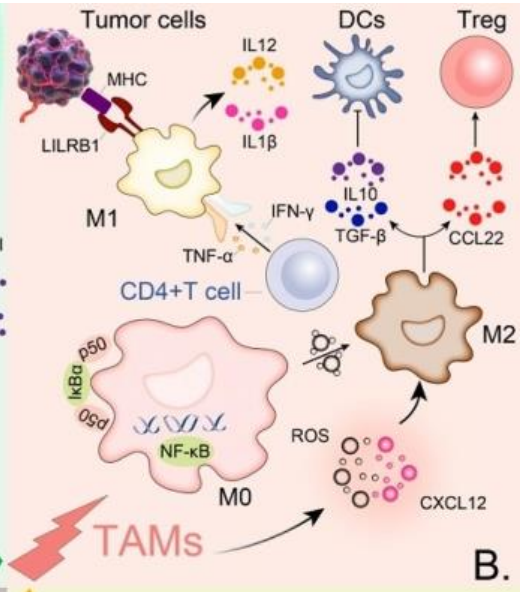
Mouw et al. Cancer Discovery, 2017

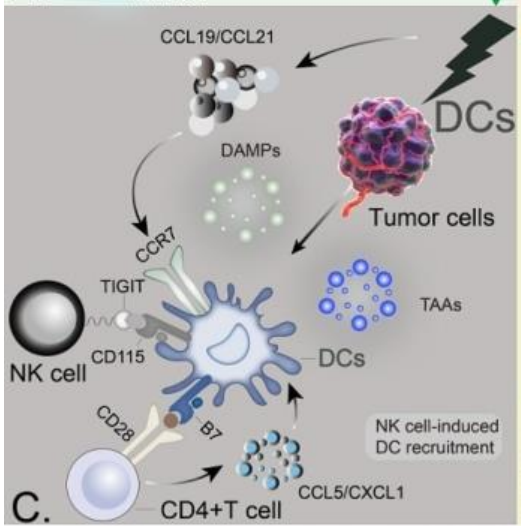
From Genomic Instability to Immune Response

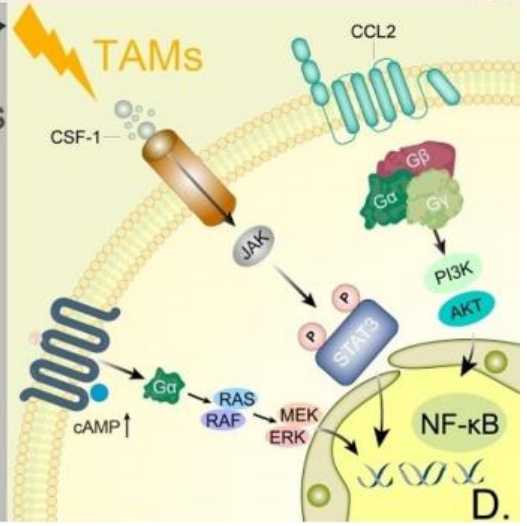
Immune Reaction to Radiation Effects on Tumors



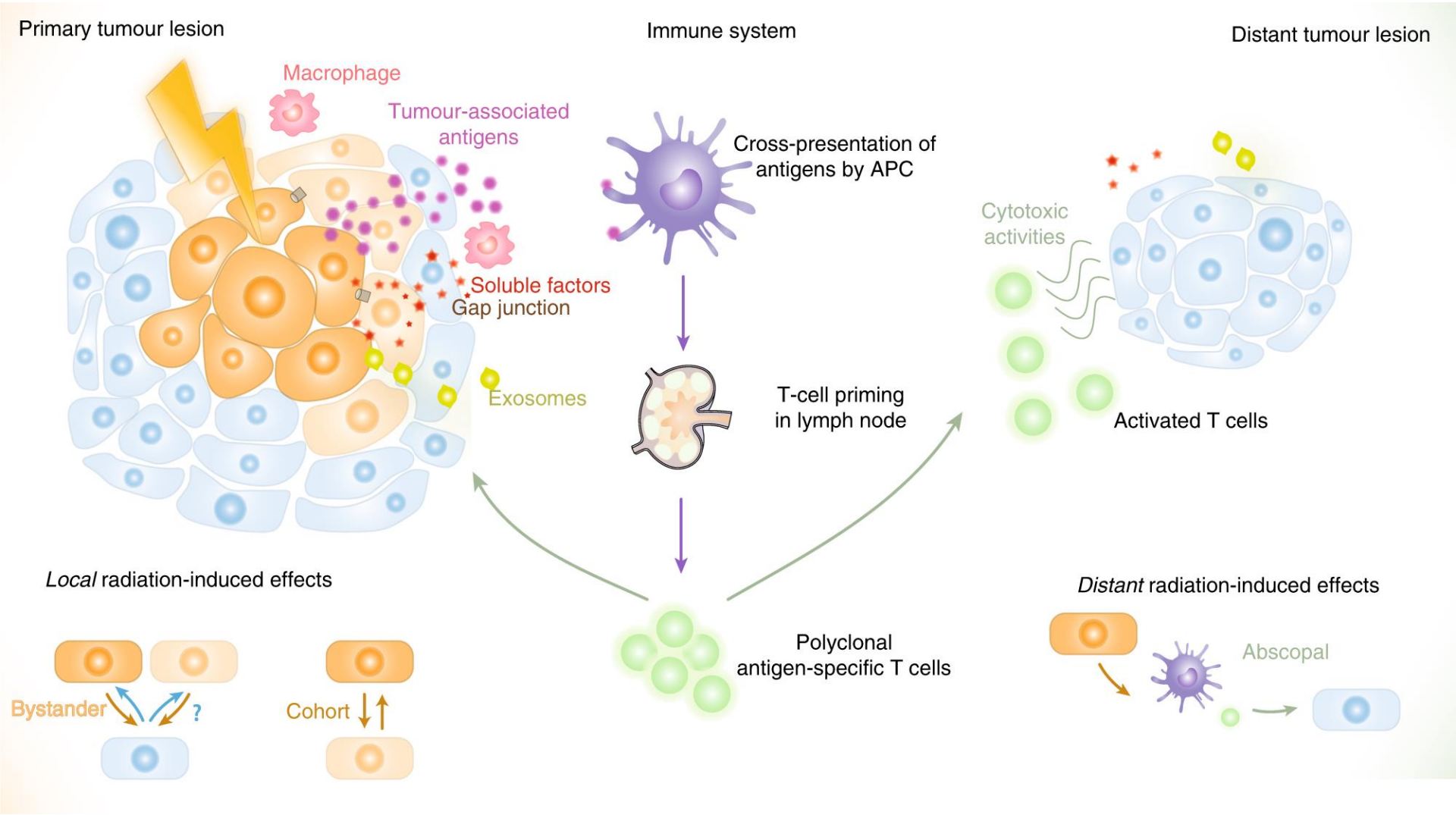








Systemic Immune Effects of Radiotherapy…



Conclusions

- Radiation induces DNA damage, which is modified by oxygen
- Type of radiation alters the linear deposition of ionization
- In oncology, the goal is to increase the therapeutic ratio
- Tissue tolerance depends not only on intrinsic radiosensitivity of clonogens, but also on organization of tissues
- The time to a normal tissue complication depends on the tissue turnover time. The extent of regeneration in normal tissues also determines how much dose can be tolerated
- Late effects are complex: the balance of cytokines and growth factors determine both regeneration, immune responses, vascular effects and normal tissue complications
- Volume effects are complex: goal is to put high dose in the tumor and spare dose to the surrounding normal tissues
- The dose to control a tumor depends on intrinsic tumor cell killing, but also vascular, stromal and immune effects of the tumor microenvironment