Fundamentals of Radiation Therapy – Clinical Perspectives for Basic/Translational Science

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Outline

1) **Radiation Definition and History**
2) **Basic Radiobiology**
    - Radiation Induced DNA Damage
    - 4 R’s of Radiobiology
    - Assays for quantifying Radiation induced DNA Damage
    - Cell Survival
3) **Dose Fractionation**
4) **Radiation with chemotherapy**
    - Radiation sensitizing agents
    - Radioprotective agents
5) **Radiation with immunotherapy**
6) **Utilizing Radiation in the Clinic**
7) **Conclusions & Summary**

What is Radiotherapy?

Use of high energy radiation (X-rays, gamma rays, neutrons, protons, etc.) to kill cancer cells and tumors.

Goals:

Cure vs. Palliative.

Delivery of Radiotherapy

External Beam Radiotherapy: Machine generated X-rays delivered to tumor.

- Linac based radiotherapy
- Intraoperative radiotherapy
- Stereotactic radiotherapy
- Particle beam radiotherapy

Brachytherapy: Radioactive sources permanently or transiently placed inside body.

Radioisotope therapy: Liquid form of radiation administered via infusion or injection.

Basic Radiobiology

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Therapeutic ratio

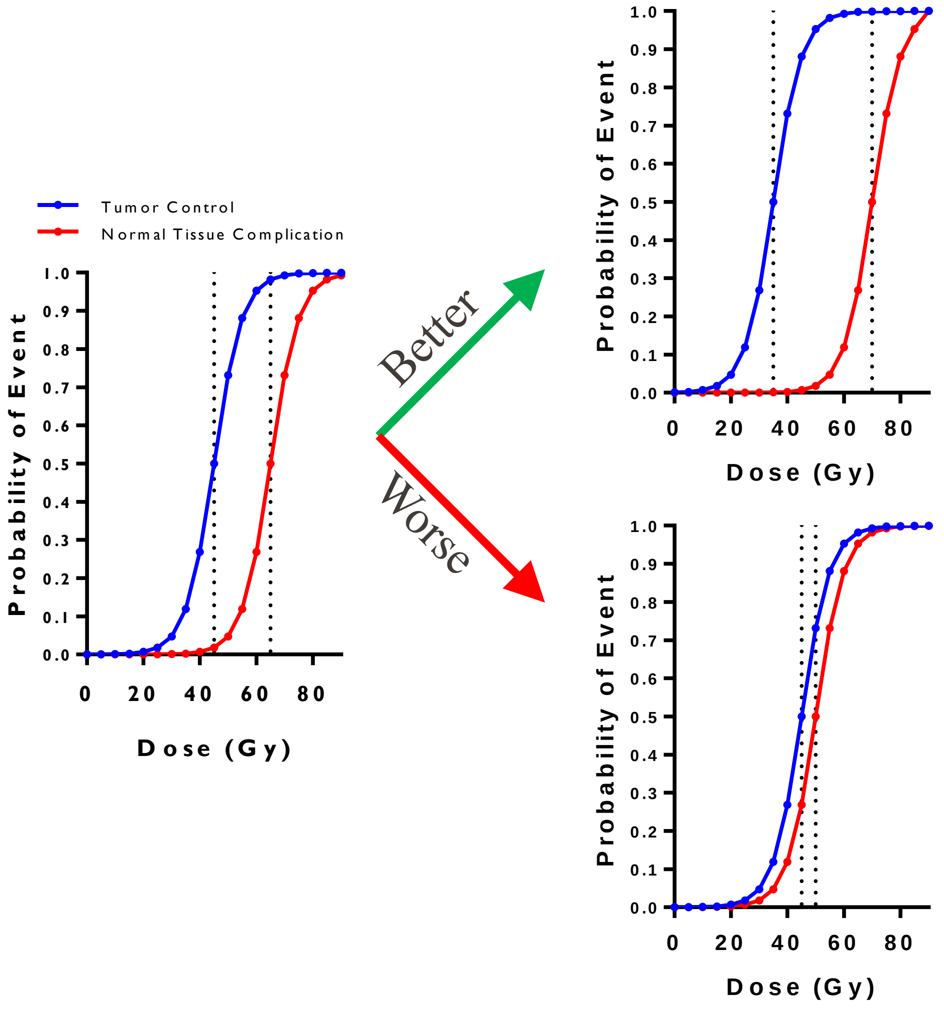
Radiotherapy damages malignant and normal tissue.

- For a given dose, there are discrete probabilities of both tumor control and normal tissue complications.
- The relationship between these doses defines the therapeutic ratio.

Various factors can influence the therapeutic ratio:

- Biology
  - Intrinsic radiosensitivity
  - Capacity for sublethal repair
- Anatomy
  - Organization of functional units with a tissue/organ
  - Proximity of tumor to critical structures

Altering properties of treatment can modify the therapeutic ratio and improve the likelihood of successful treatment.



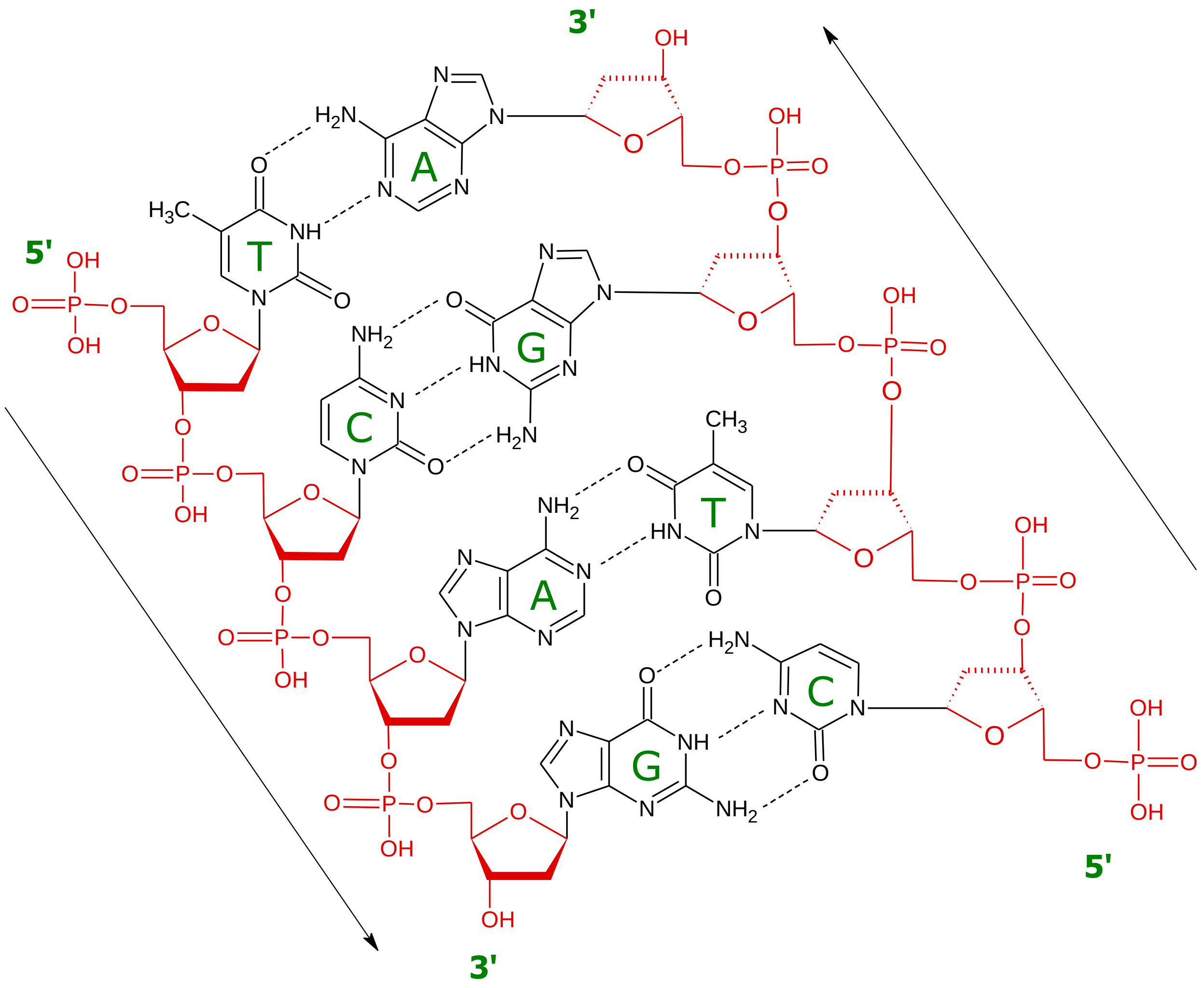
Hall, E. J. (2000). *Radiobiology for the radiologist*. Philadelphia, PA: Lippincott Williams & Wilkins.

Review of DNA structure

Each strand of DNA is a polymer constructed of deoxyribonucleotide subunits.

The subunits are joined by a phosphodiester bond formed between the 5’ phosphate group and a 3’ hydroxyl group.

Antiparallel strands interact via hydrogen bonds between nitrogenous purine/pyrimidine bases (A↔T, G↔C).



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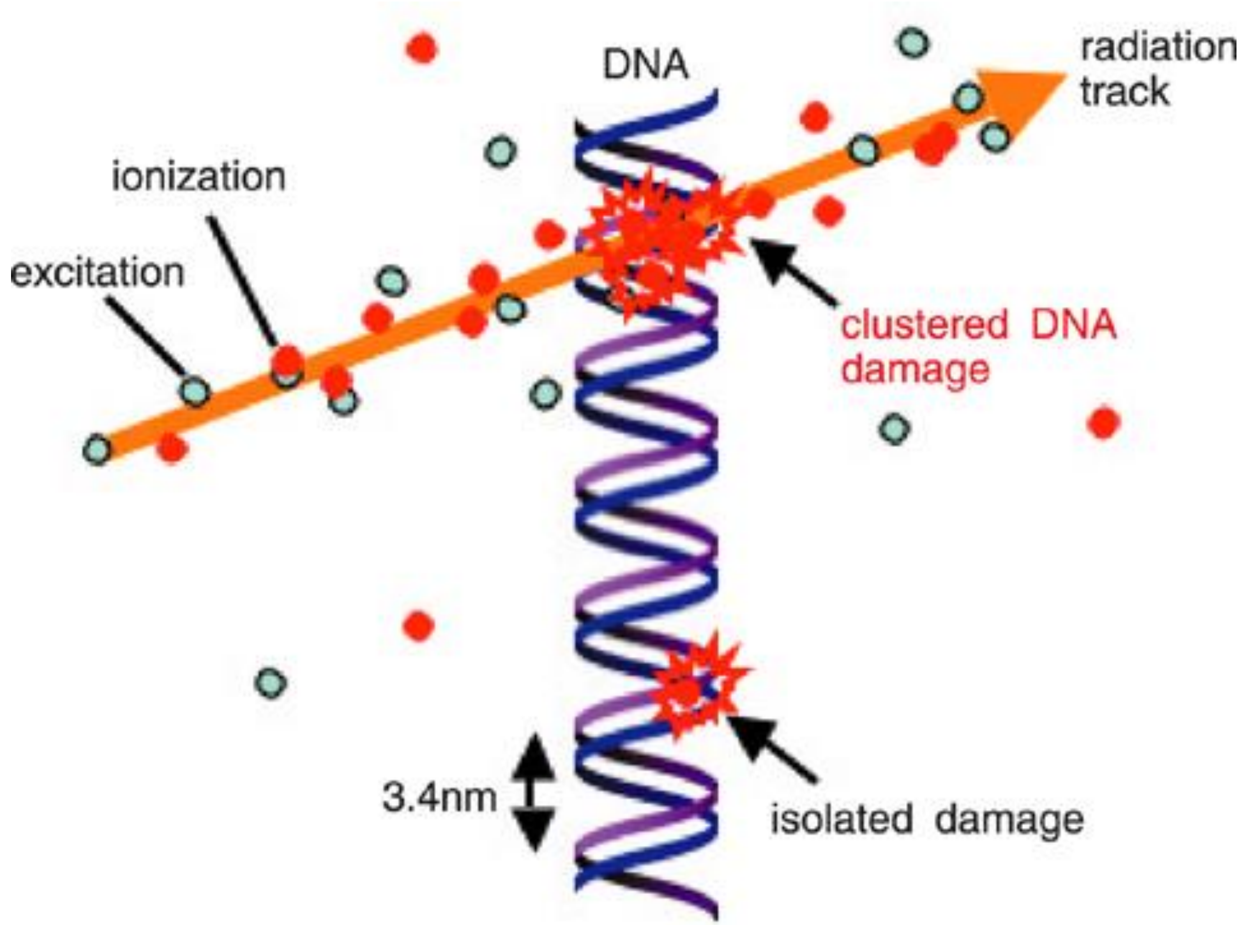
Overview of DNA damage

- When spurs or blobs of ionizations physically coincide with DNA, "multiply damaged sites" or "clustered lesions" occur.
  - These sites of complex damage typically span ~20 bp and include multiple types of DNA damage.



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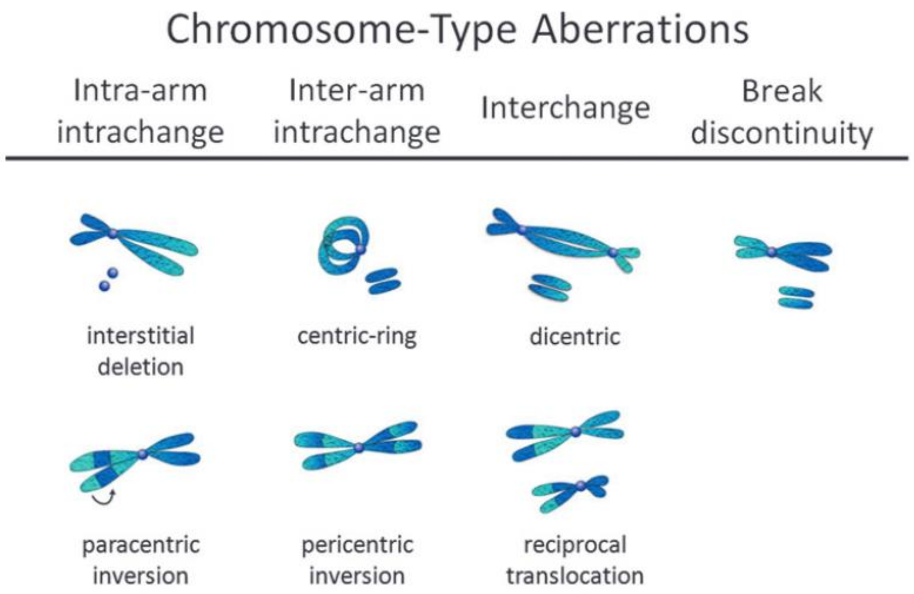
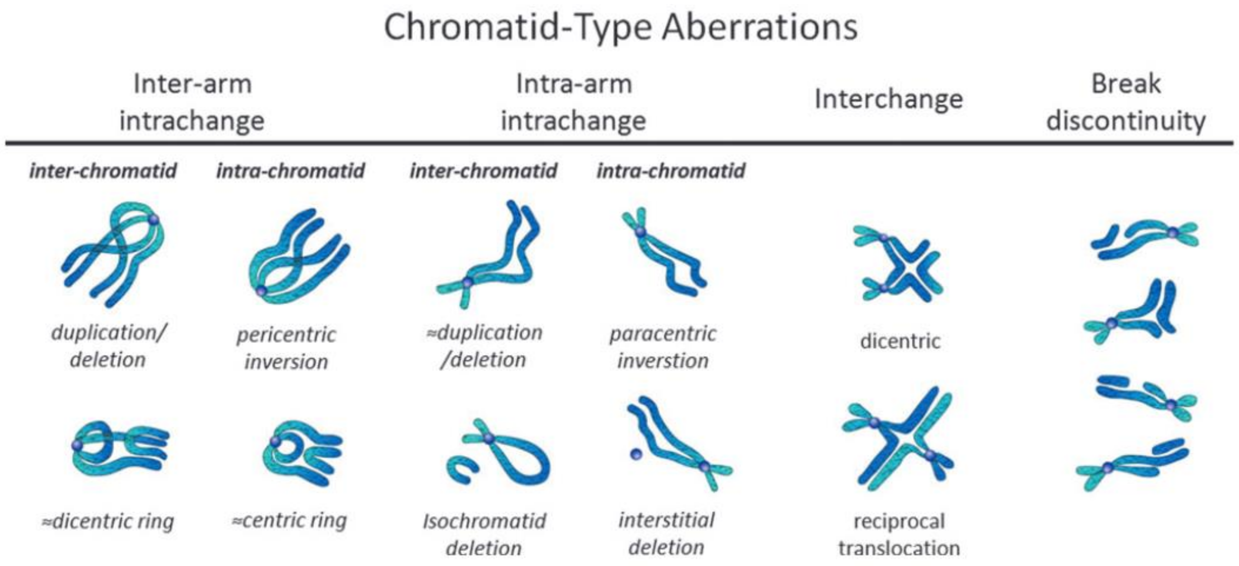
Overview of DNA damage

Event	Lesions / Cell / Gy
Ionizations	~100,000
Base Damage	>1,000
Single Strand Breaks	>1,000
Double Strand Breaks	~40
DNA Protein Crosslinks	~40

Chromosomal damage and radiation cytogenetics





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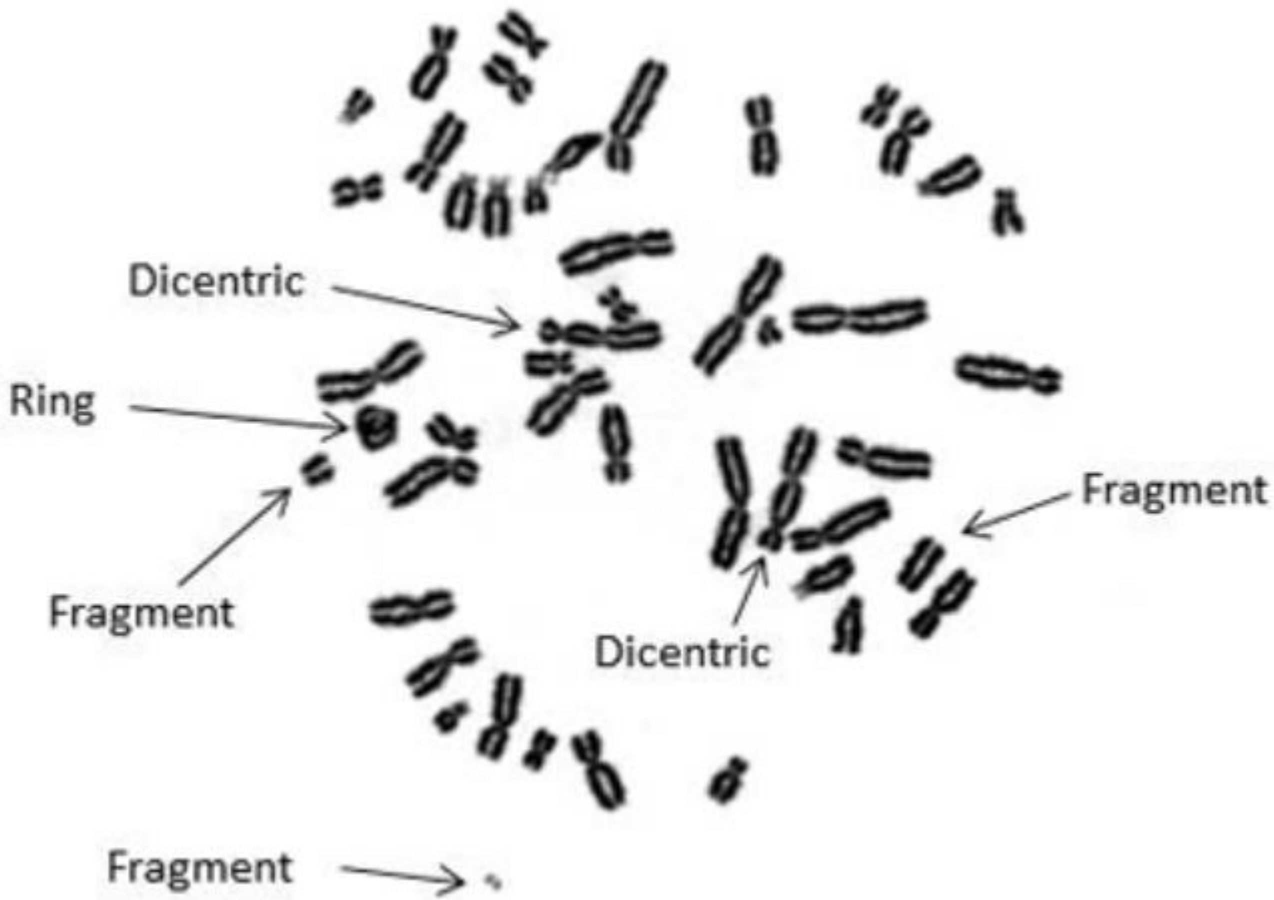
Chromosomal damage and radiation cytogenetics



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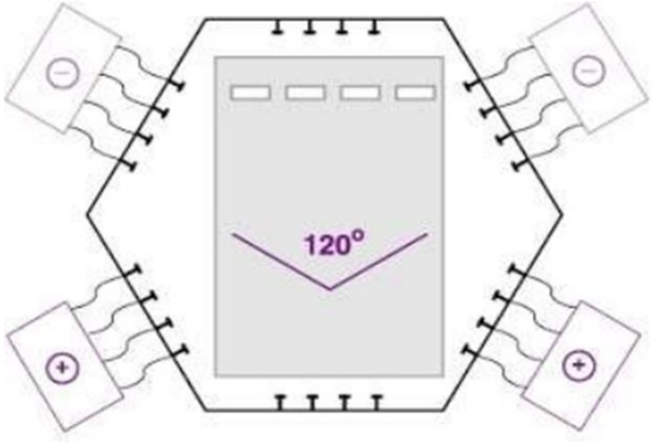
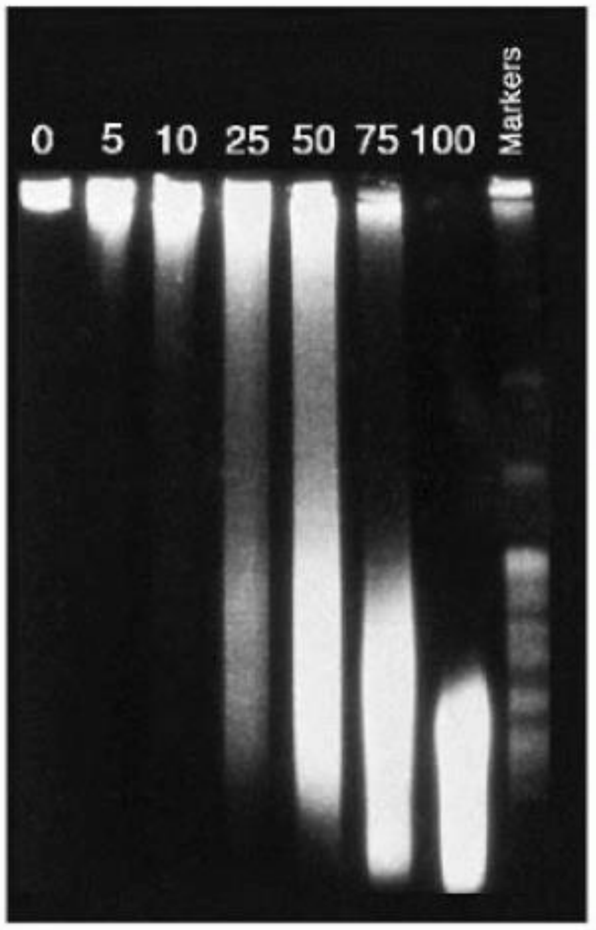
Methods for detecting and quantifying DNA damage

Pulse field gel electrophoresis (PFGE)

- Cells are irradiated, embedded in agarose plugs, and then lysed *in situ*.
- These plugs are then loaded into agarose gels and are subjected to electrophoresis.
  - Unlike normal electrophoresis, the direction of the electric field is cycled between -60°, 0°, +60°.
  - This increases the ability to resolve extremely large fragments of DNA.



Electric field alternates 120° every 90 seconds for 18 to 24 hours at 14° C

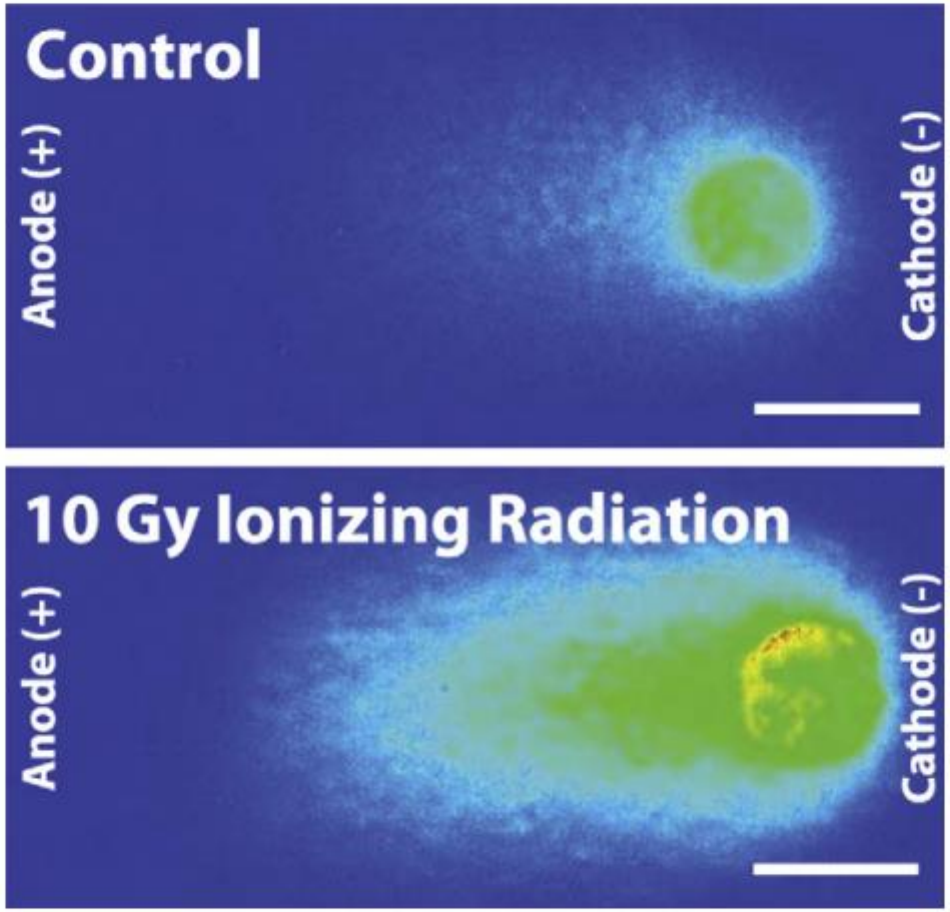


N. Denko, *OSU*

Methods for detecting and quantifying DNA damage

Comet assay

- Cells are irradiated, imbedded in agarose, lysed *in situ*, and then electrophoresed.
- The nature of the lysis and electrophoresis buffers allows differential resolution of specific types of DNA damage.
  - – Neutral comet assay – DSBs
  - – Alkaline comet assay – SSBs

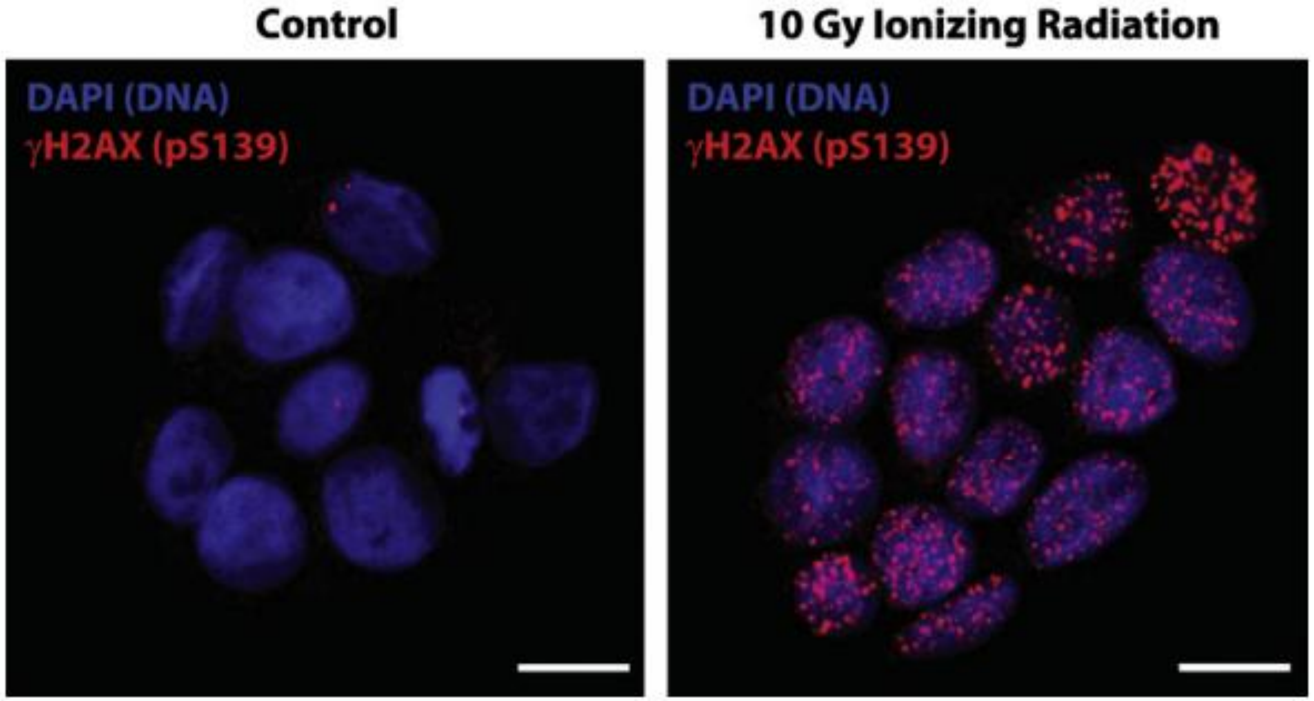


Stecklein and Jensen, *Translational Research*, 2012

Methods for detecting and quantifying DNA damage

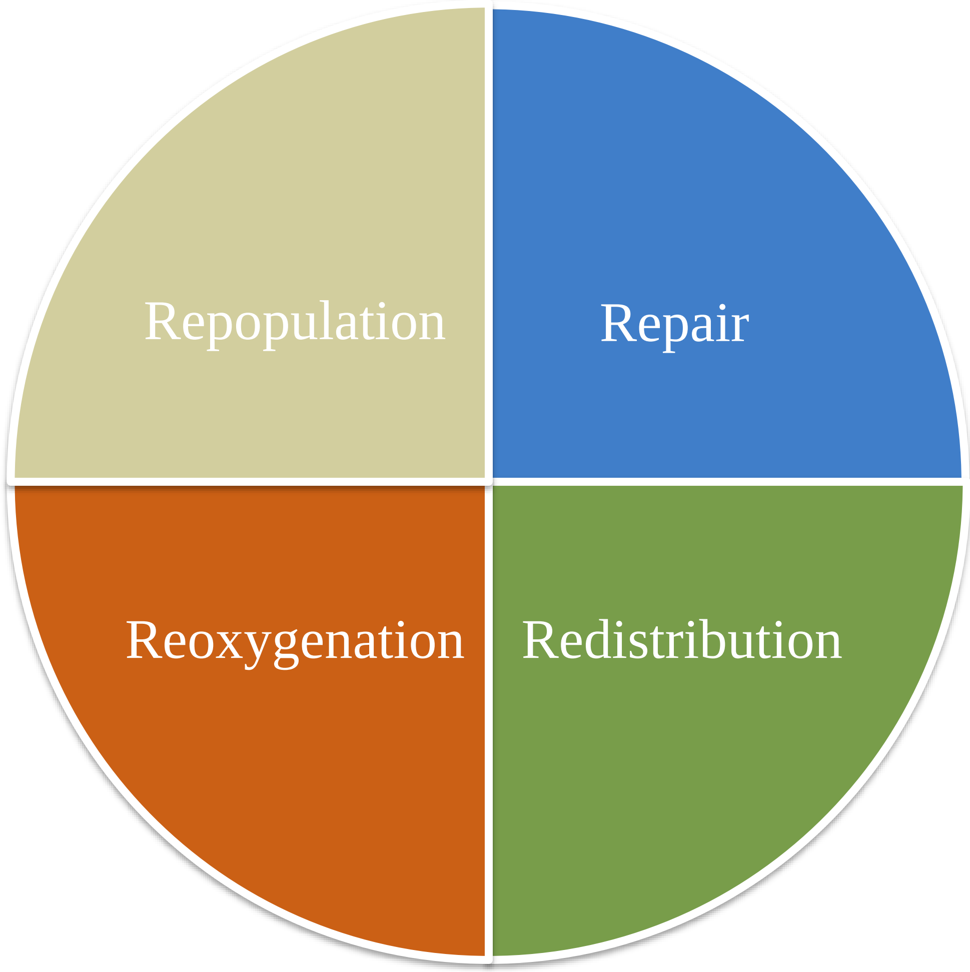
DNA damage-induced nuclear foci

- Cells are irradiated, fixed, and then immunofluorescence staining is used to detect nucleoproteins that localize to sites of DNA damage.



Stecklein and Jensen, *Translational Research*, 2012

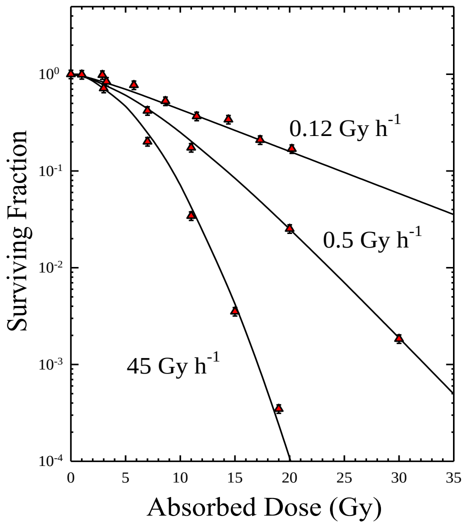
The four R's of radiobiology



The four R's of radiobiology

Repair

- **This is the most important rationale for fractionation.**
- There are three major categories of damage:
  – Lethal damage – Irreparable, irreversible damage. Inevitably leads to cell death or loss of reproductive capacity.
  – Sublethal damage – This damage can be repaired as long as additional sublethal damage is not incurred prior to complete repair.
  – Potentially lethal damage – Damage that may be resolved if cells are allowed to stay in a non-cycling state for a prolonged period of time.
- The initial shoulder in a cell survival curve reflects the ability of cells to accumulate sublethal (and potentially lethal?) damage.



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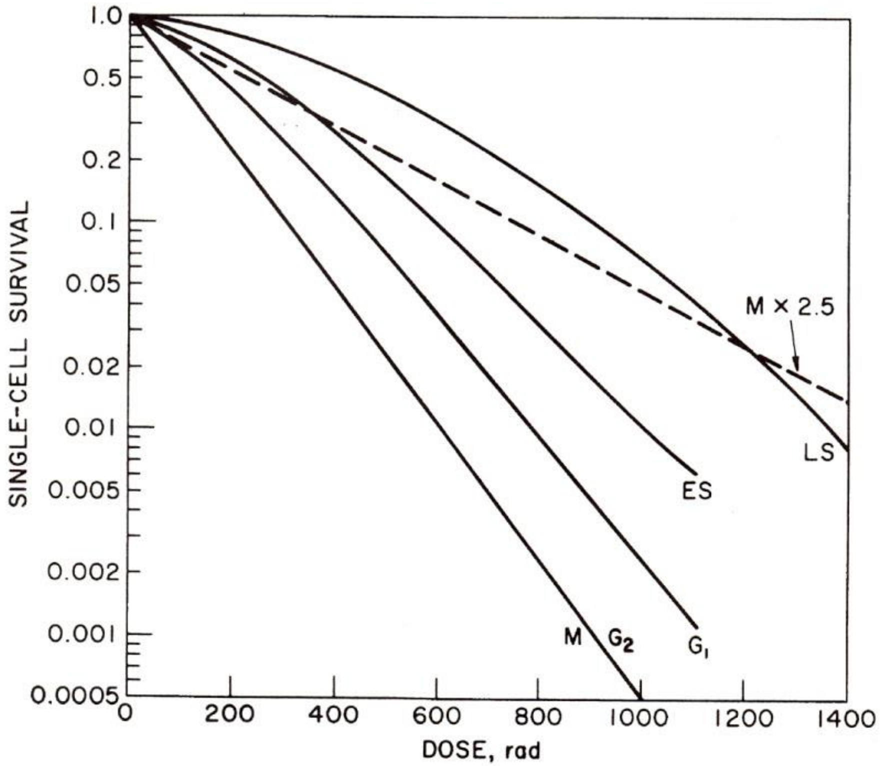
The four R's of radiobiology

Redistribution

- This reflects movement of cells from radioresistant to radiosensitive phases of the cell cycle between fractions.
- In asynchronous cell populations, late S-phase cells are the most likely to survive irradiation.
  – By **fractionating, some of these resistant cells will have moved into more radiosensitive phases and will be more sensitive at the next fraction.**



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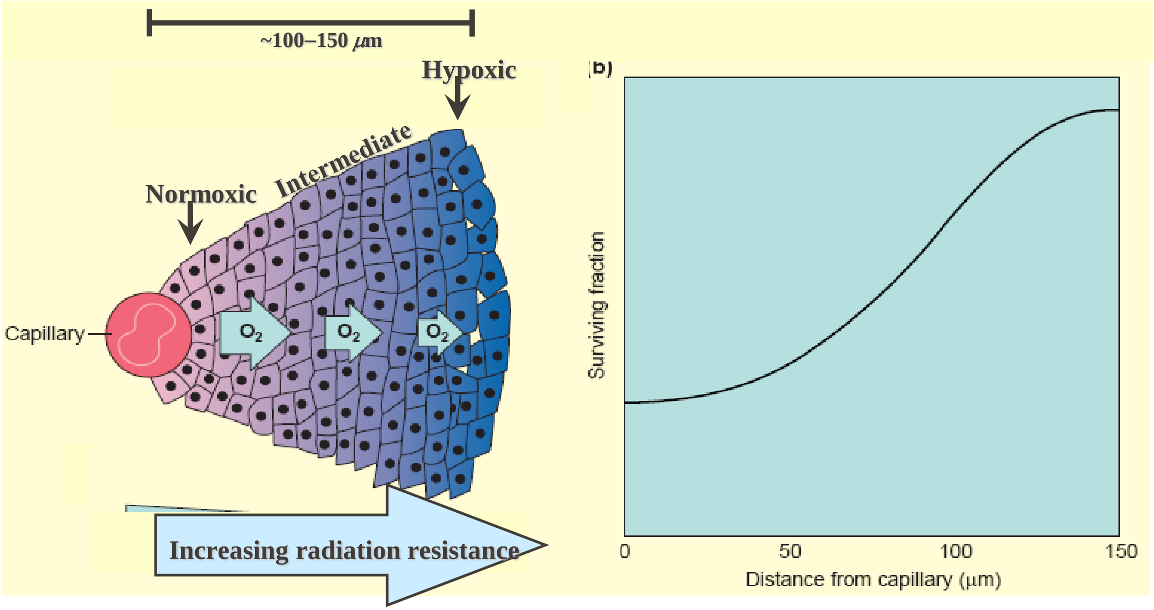
The four R's of radiobiology

Reoxygenation

- Acute and chronic hypoxia can both contribute to resistance to radiation therapy
- Fractionation can cause reversal of both acute and chronic hypoxia and increase radiosensitivity.



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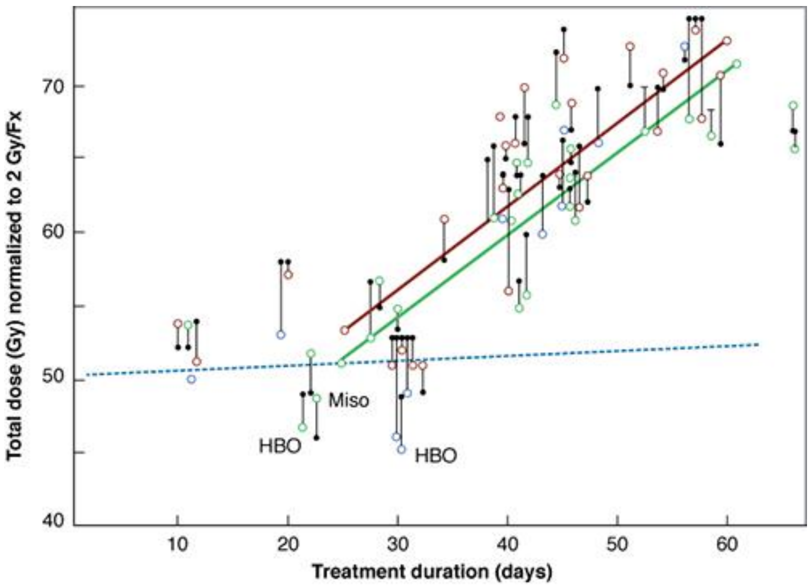
The four R's of radiobiology

Repopulation

- Both normal and malignant cells may divide in between fractions of radiotherapy.
- Extended treatment time may reduce the likelihood of tumor control because cell division during the course of treatment increases the number of clonogens that must be inactivated.
- **<u>In some tumors (e.g., head and neck, cervix), radiotherapy can trigger "accelerated repopulation"</u>**
  – Surviving clonogens divide more rapidly than normal, dramatically increasing the number of clonogens
  – Additional dose may be necessary to counteract the effect of accelerated repopulation



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Cell survival

The definition of cell survival is context dependent.

- Is the cell alive?
- Can the cell perform a specific function?
- Does the cell have the capacity to replicate indefinitely?

The dose of irradiation required to “kill” a cell may be markedly different depending on the endpoint.

- True cell death may require acute doses of 100s of Gy.
- Loss of specific functions may require acute doses of 10s of Gy.
- Loss of reproductive capacity may require only a few Gy.

Ovarian Tolerance

Endpoint	Dose
Necrosis	≅ 100 Gy
Loss of Endocrine Function	≅ 10 Gy
Loss of Gonadal Function	≅ 2 Gy

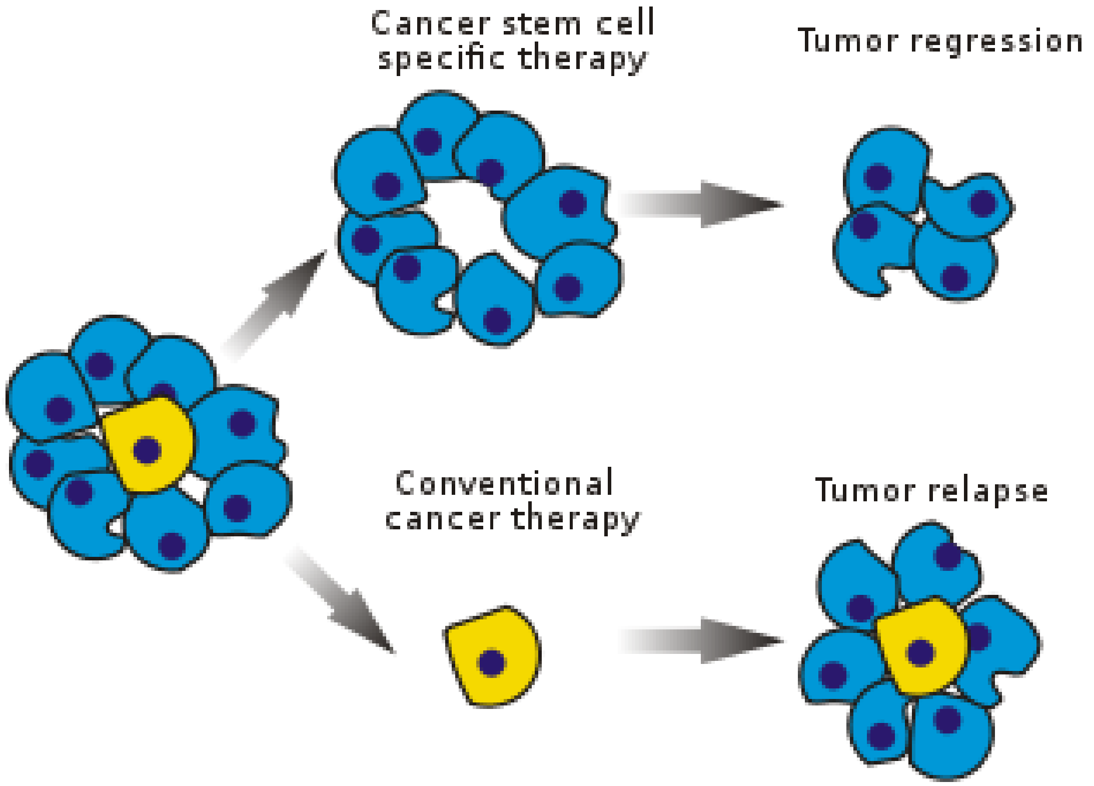
Clonogenic growth

A “clonogen” is a cell with limitless replicative potential.

Stem cells are by definition clonogens, with the additional ability to partially or fully recapitulate the cellular and functional capacity of a tissue or organ.

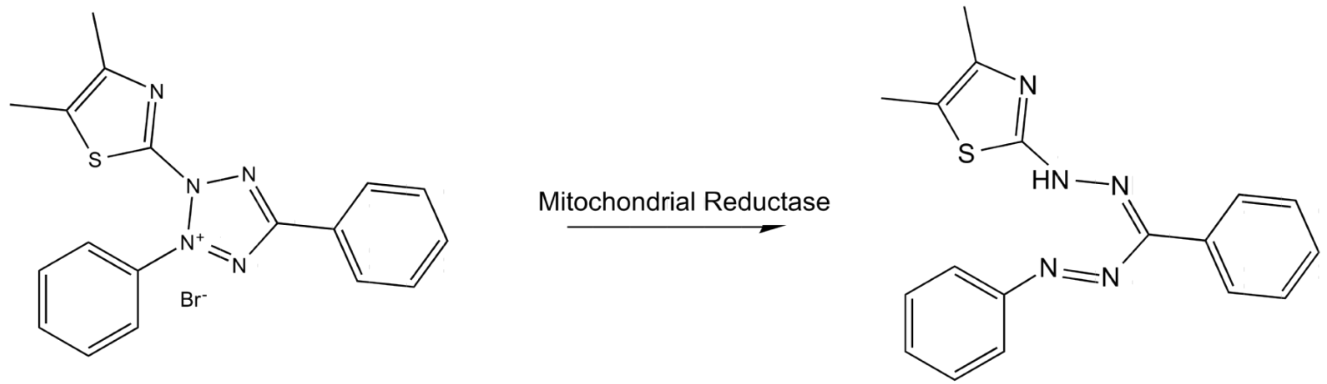
Cancer stem cells (CSCs) can be considered malignant clonogens.

Successful cancer therapy requires elimination of all malignant clonogens.



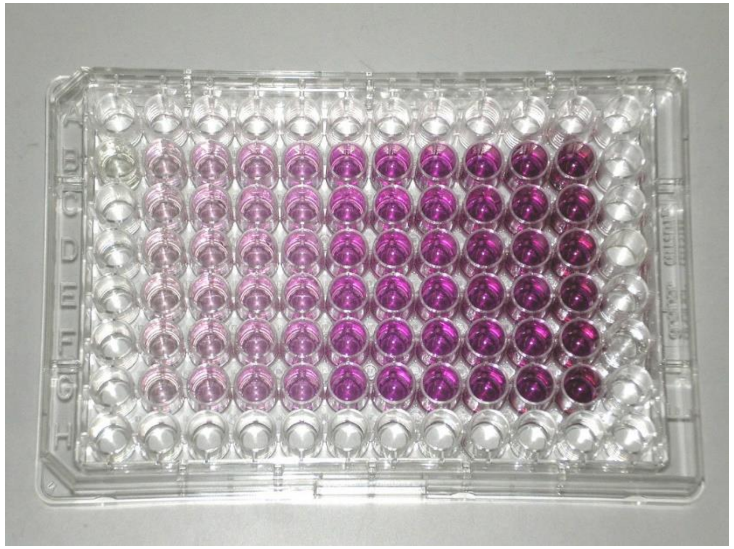
Clonogenic growth

- Viability assays that rely on metabolic conversion of a substrate (e.g., MTT) are generally not reliable measures of clonogenic growth.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(MTT)

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan
(Formazan)



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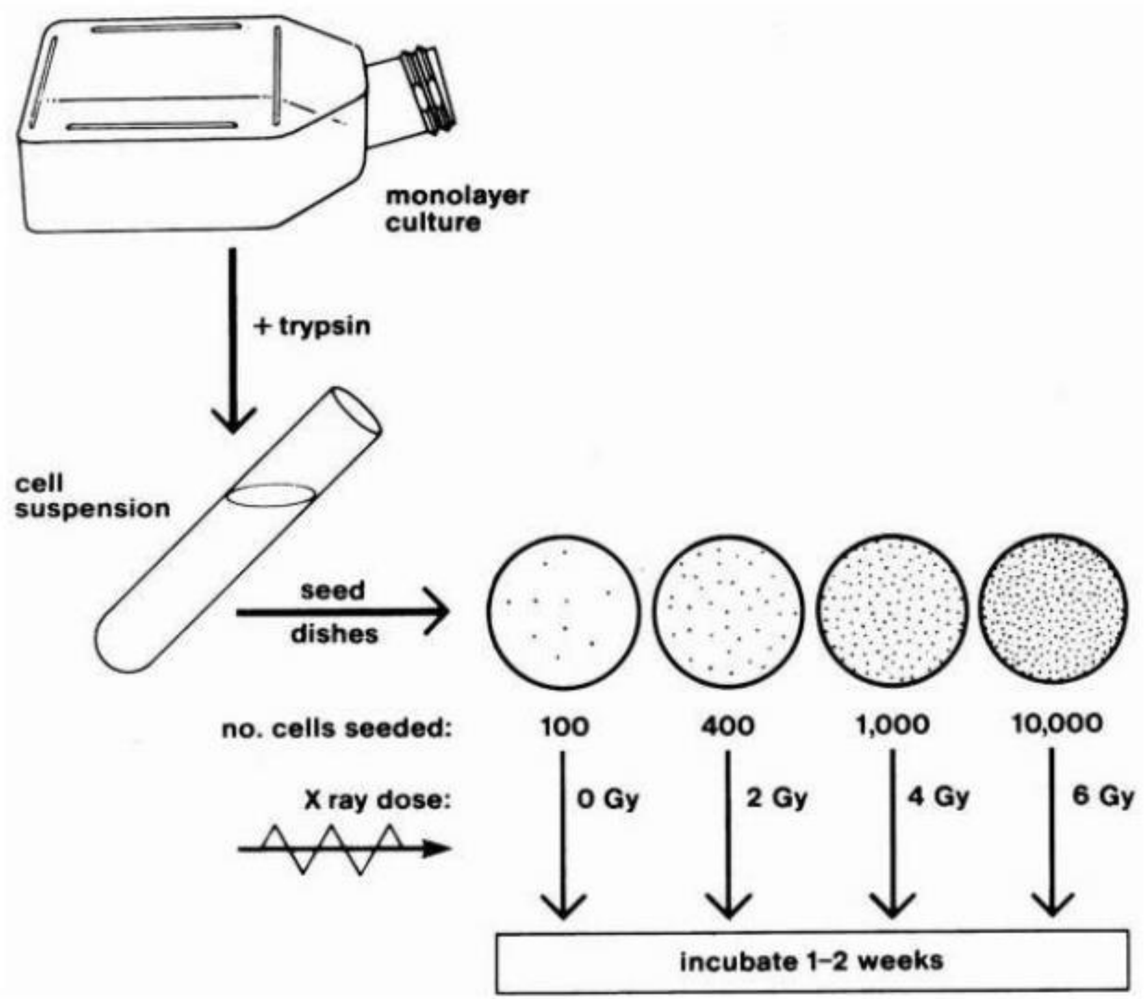
Clonogenic growth

The *in vitro* clonogenic survival assay is the gold standard for measuring loss of reproductive integrity in tumor biology.

Unlike metabolic assays, which measure viability of bulk tumor cells, this assay measures the ability of a single cell to regenerate a colony of >50 cells (~4-5 cell divisions).



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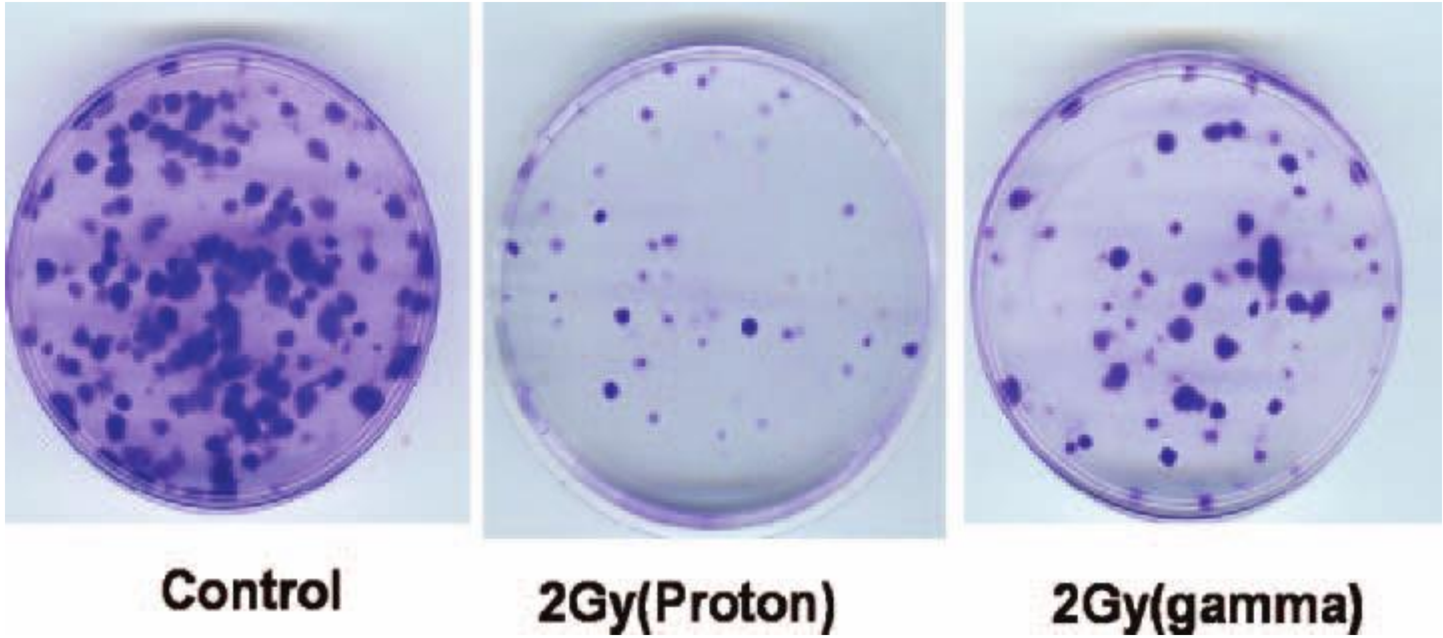
Clonogenic growth



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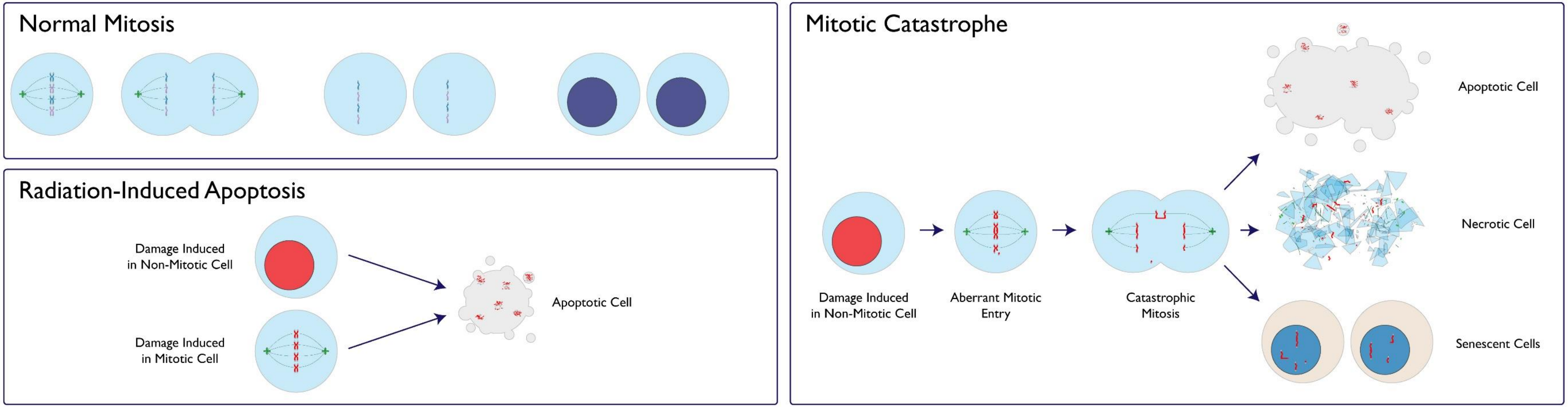
Mechanisms of cell death

In radiotherapy, mitotic catastrophe and apoptosis are the most relevant mechanisms of cell death.

- Hematopoietic (especially lymphoid) cells are prone to apoptosis after irradiation.
  - Likely p53-dependent.
  - p53-mutant lymphoid neoplasms tend to be relatively radioresistant.
- **The most common form of radiation-induced cell death in most tissues is mitotic catastrophe**.
  - Mitotic catastrophe may ultimately induce apoptosis, necrosis, or senescence.

Mechanisms of cell death



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Absorbed Radiation Dose

- Energy absorbed per unit mass of tissue.
- Measured in Gray (Gy).
- Gy = 1 Joule/kg = 100 rads.
- Equal doses of adsorbed energy do NOT equate to equal biological effects.

Biologically Effective Radiation Dose

Biologically effective dose (BED): compares fractionated radiation regimens are compared.

Used in photon radiotherapy.

Based on linear-quadratic equation.

Relative biological effectiveness (RBE): compares the biologic effectiveness of different types of ionizing radiation.

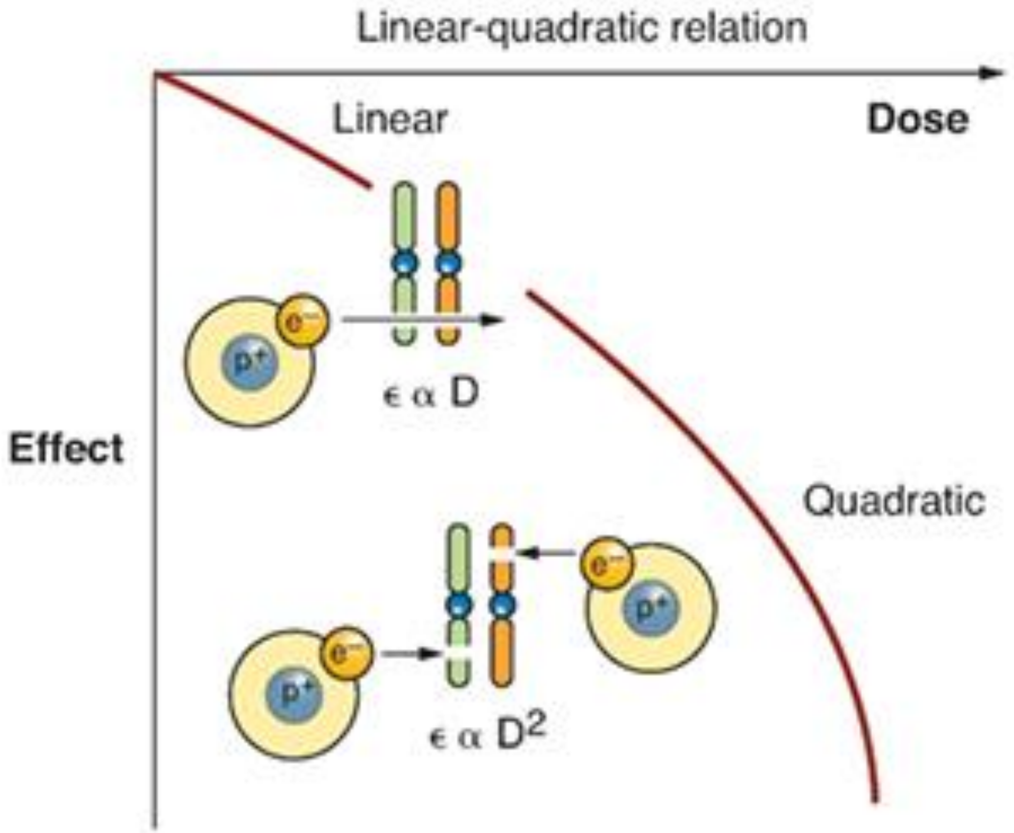
Used in particle (proton) radiotherapy.

Empiric measurement.

Depends on the biologic endpoint chosen.

Varies with radiation dose.

Linear Quadratic Equation



- Linear quadratic model nature of radiation survival curve: $S = e^{-\alpha D-\beta D^2}$
- α number of log cell kills per Gy for linear portion, β number of log cell kills per Gy in the quadratic portion.

Hall, E. J and Giaccia, A. J. (2019). *Radiobiology for the radiologist*. Philadelphia, PA: Wolters Kluwer. Figure 3.5.

Early Rationale for Fractionation

Multi-fractioned regimens a consequence of experiments from 1920s and 1930s

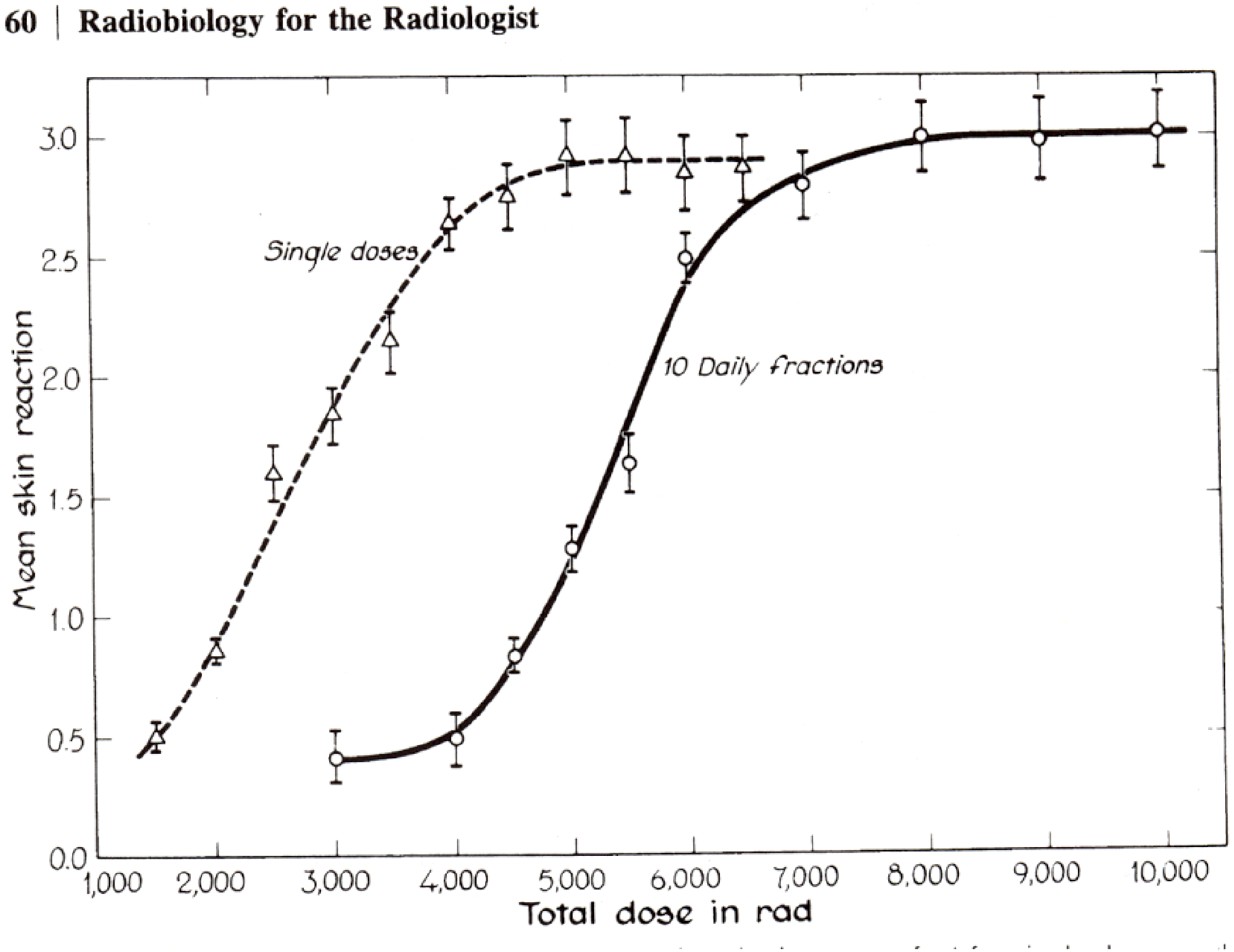
Single dose for sterilization caused extensive skin damage

If radiation was spread out over series of daily fractions, sterilization was possible without unacceptable skin damage

Conclusion: fractionation of dose produces better tumor control for given level of toxicity than single dose

Hall, E. J and Giaccia, A. J. (2019). *Radiobiology for the radiologist*. Philadelphia, PA: Wolters Kluwer. Figure 23.1.

Acute Skin Toxicity Major Driver of Fractionation



QUESTION: is skin a significant issue for high energy photon radiation therapy?

Hall, E. J. (2000). *Radiobiology for the radiologist*. Philadelphia, PA: Lippincott Williams & Wilkins.

Altered Fractionation

- **Large fraction sizes/Hypofractionation:** Increase late reactions. Similar early reaction when adjusting for dose.
- **Hyperfractionation:** Increase early reactions. Decrease late reactions.
- **Late effects:** Fraction size most important, overall treatment time has little influence.
- **Acute effects:** Fraction size and overall treatment time determine acute effects.

Reasons for Hypofractionation

- Tissues with low α/β (i.e. prostate) acts more similarly to late responding tissues.
  - Smaller number of higher dose fractions is better.

- SRS and SBRT: Drastically reduced margins minimizes amount of normal tissues exposed to radiation.
  - High LC rates that decrease with increasing tumor volume.
  - Rationale for including a hypoxic radiosensitizer.
  - Likely due to large BEDs result from few large dose fractions.

- Carbon ions/Particle therapy with different RBE: smaller number of large dose fractions.

Radiation with Systemic Therapy

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Interaction of chemotherapy and radiotherapy

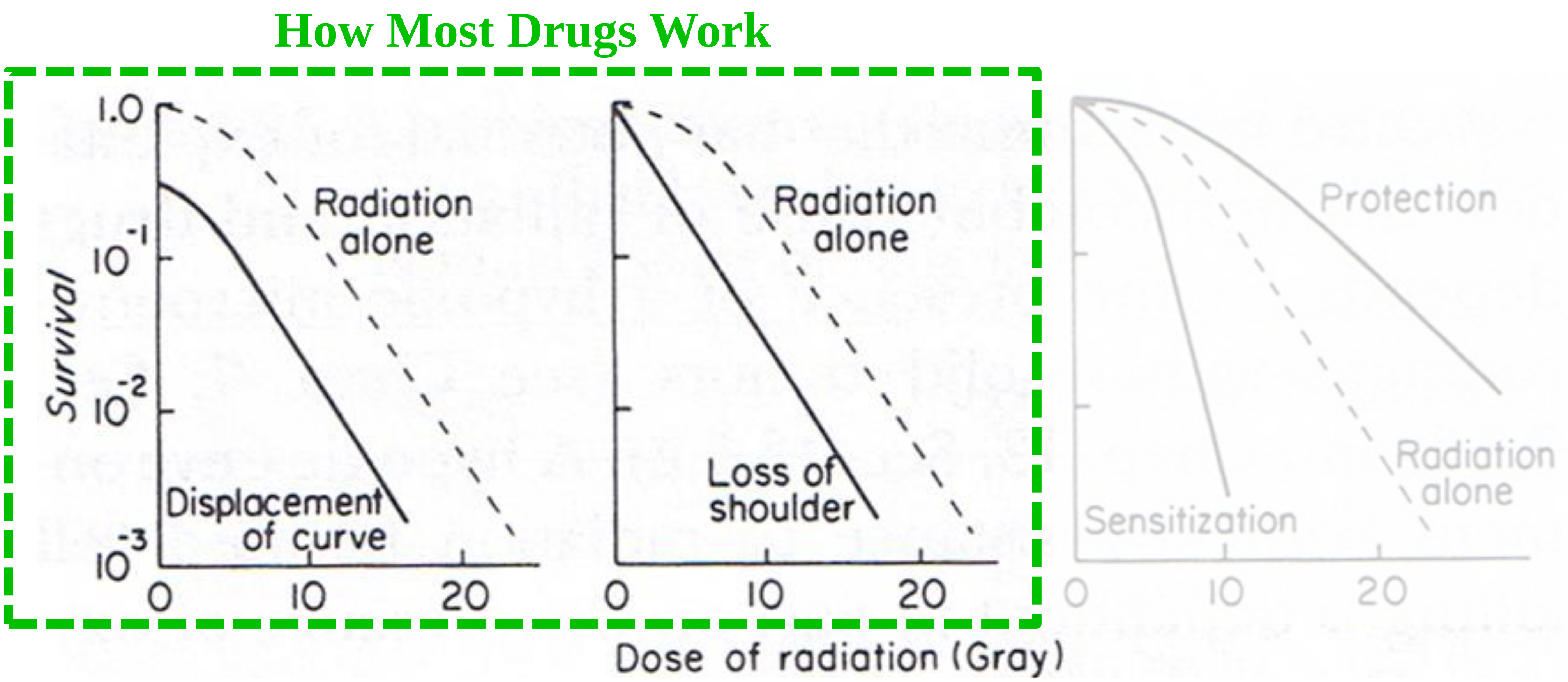
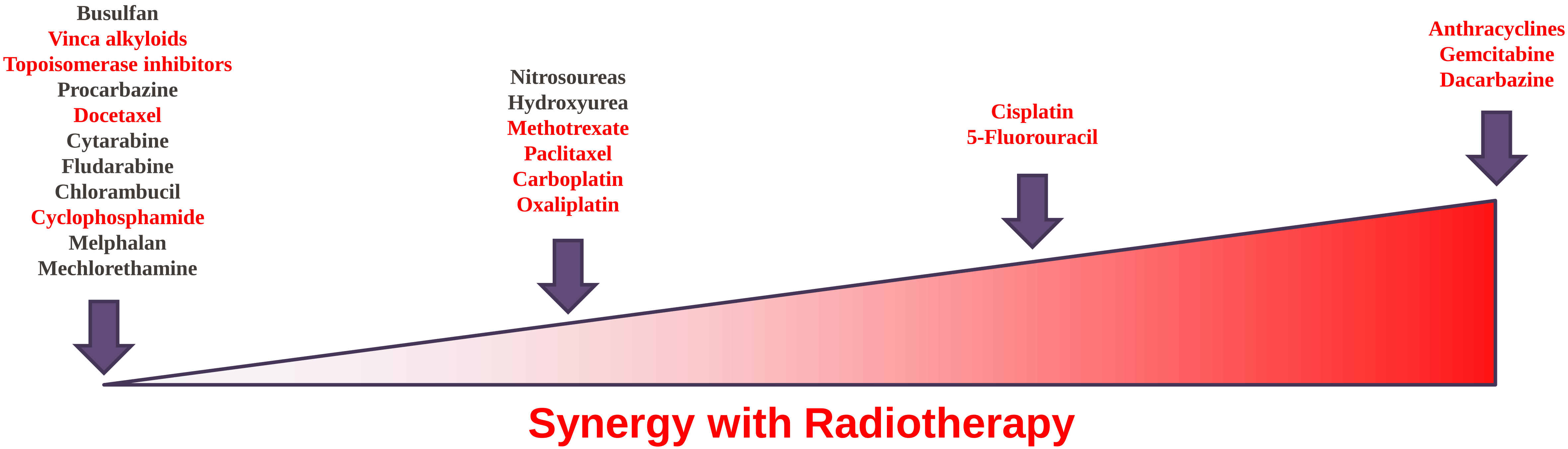


Figure from *Radiobiology for the Radiologist*, 6th Edition

Interaction of chemotherapy and radiotherapy



Radiation-modifying drugs

Potential mechanisms of action for radiosensitizers:

- ↑ DNA damage
- ↓ DNA repair
- Alter molecular response to radiotherapy
- Alter cell cycle distribution
- Induce alternative mechanisms of cell death

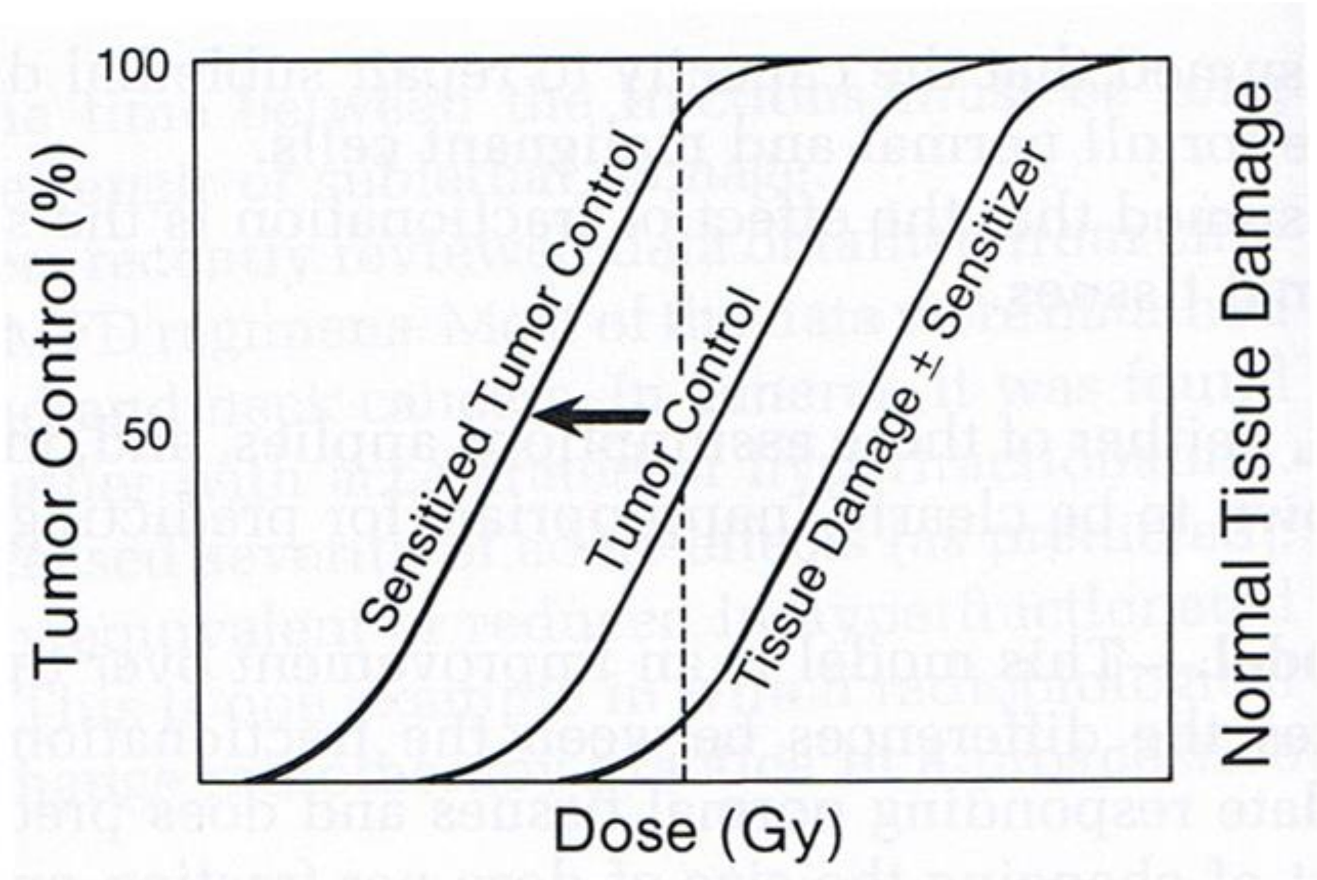


Figure from *Radiobiology for the Radiologist*, 6th Edition

Radiation-modifying drugs

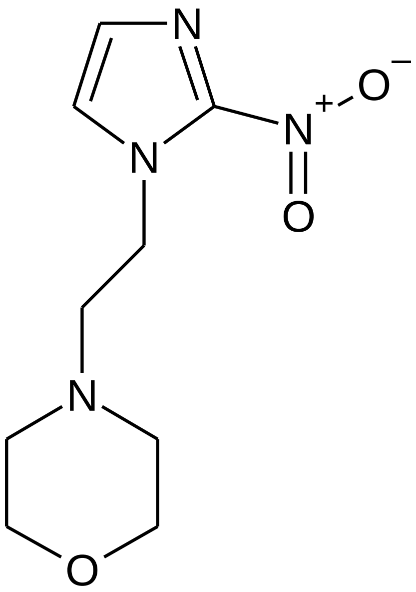
Nitroimidazoles

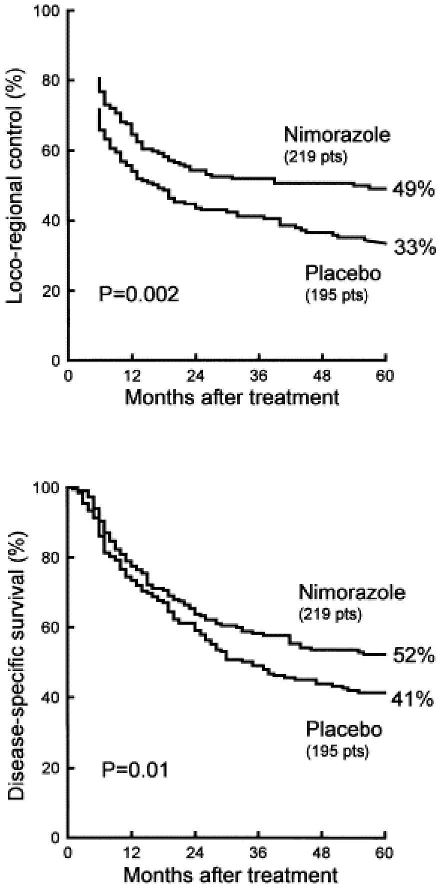
- Hypoxic cell sensitizers
- Greater diffusion potential than molecular O_2
- Sensitization is related to increased electron affinity

Multiple clinical trials:

- Misonidazole (20+ trials)
- Etanidazole
- Nimorazole
  - Improvement in LRC and DFS in Danish (DAHANCA 5) trial of supraglottic larynx and pharyngeal cancer
  - DAHANCA 28 (published Green Journal 2020): Phase I/II looked at HART-CN (hyperfx accel RT w/Cis+nimorazole) for HPVneg locally adv H&N ca
    - Showed “tolerable” but higher acute toxicity than Cis alone

Nimorazole





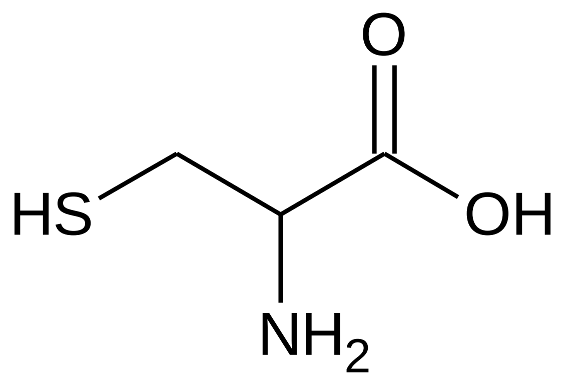
Overgaard et al., *Radiotherapy and Oncology*, 1998

Radiation-modifying drugs

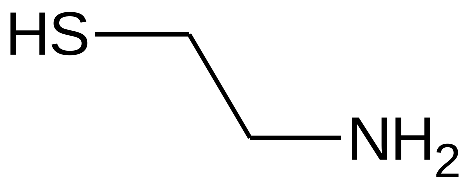
Sulfhydryl compounds are the major class of radioprotectors.

- They function by scavenging free radicals.
- Protection by these agents parallels the oxygen effect.
  - Highest level of protection with low LET radiation.
  - Less/minimal effect from high LET radiation.

The earliest sulfhydryl radioprotectors were discovered in 1948.



Cysteine



Cysteamine

Radiation-modifying drugs

The US military developed >4,000 sulfhydryl compounds in the 1950s during the Cold War.

These were primarily intended to be used by troops in the event of a nuclear event.

The most effective agent to be identified was amifostine (WR-2721).

Amifostine remains the only FDA-approved radioprotector.

- IV or IP administration is required, as it is inactivated by gastric acid.
- It is activated by alkaline phosphatase into the active metabolite WR-1065

To be effective, it must be administered immediately before exposure t

Amifostine

3-[(2-sulfanylethyl)amino]propan-1-aminium

Radiation-modifying drugs

Amifostine is currently only FDA approved for two indications:

- Xerostomia protection in post-operative head and neck cancer.
- Renal protection in cisplatin-treated patients.

Organ	DRF
Bone marrow	2.4-3.0
Liver	2.7
Skin	2.0-2.4
Testes	2.1
Salivary gland	2.0
Intestine	1.8-2.0
Lung	1.2-1.8
Kidney	1.5
Esophagus	1.4
Oral mucosa	1.0-1.2

Radiation with Immunotherapy

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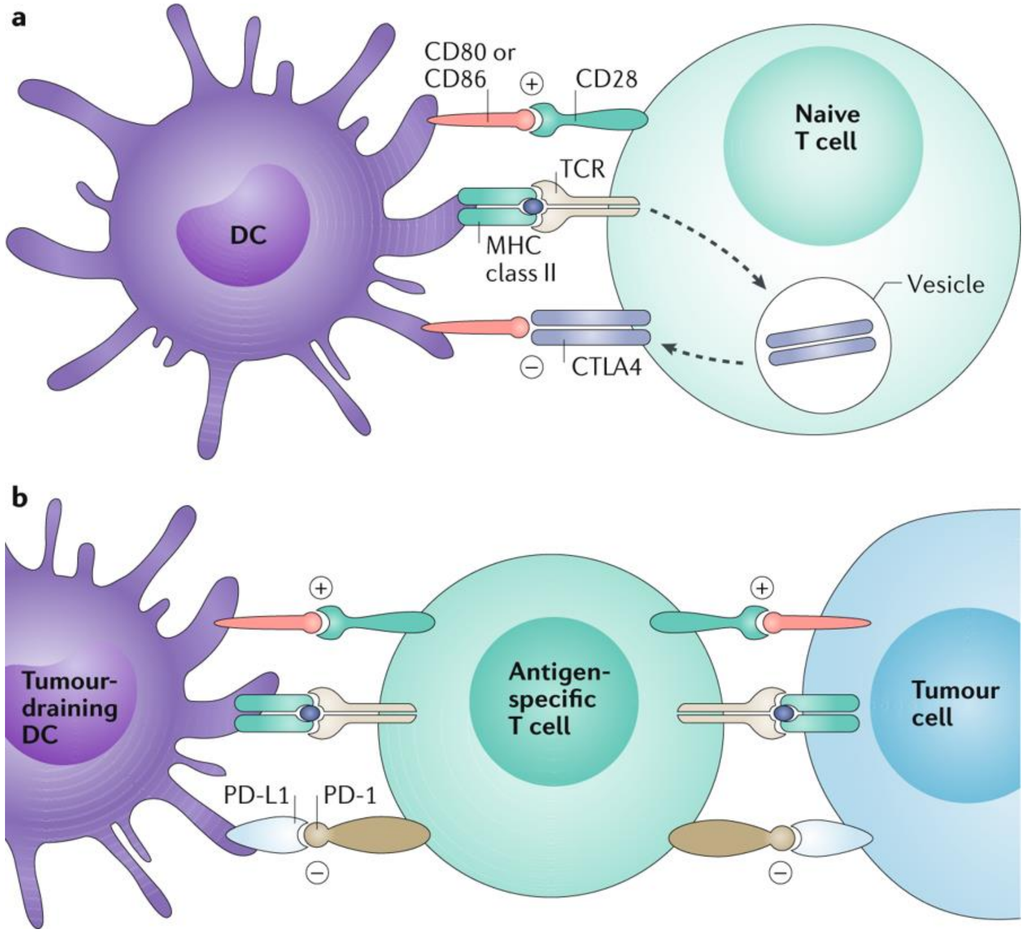
Cancer immunotherapy

Immune checkpoint blockade utilizes monoclonal antibodies directed at T-cell repressive surface molecules present on the surface of T-cells (CTLA4 and PD-1) or APCs and tumor cells (PD-L1 and PD-L2).

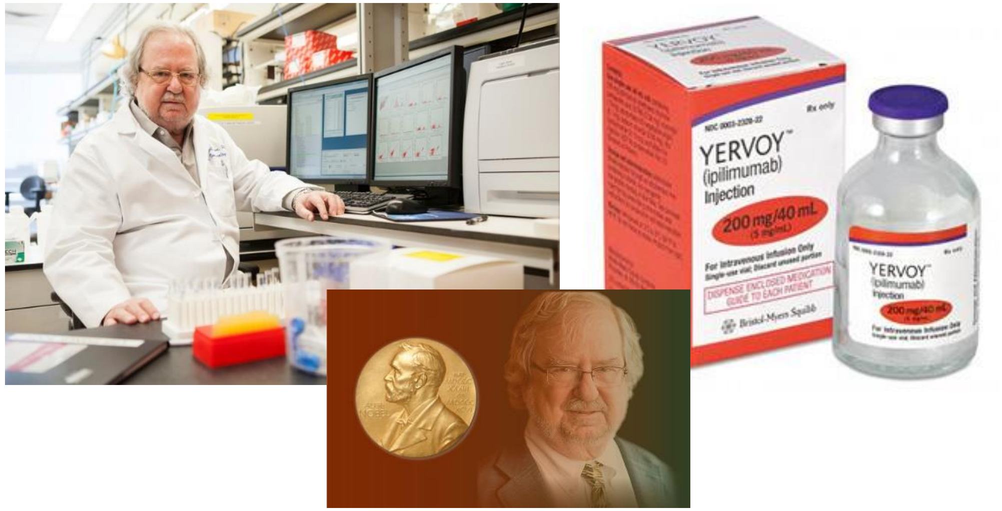
This upregulates the T-cell response and increases anti-tumor immunity.

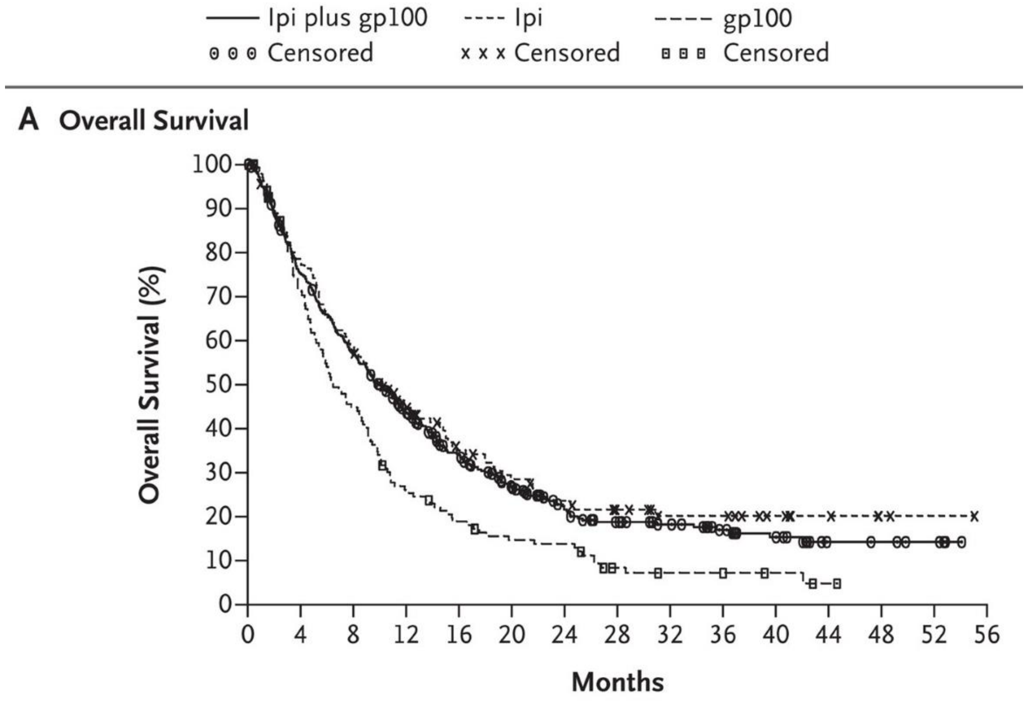


Tang et al., *Cancer Immunology Research*, 2014

Cancer immunotherapy

Ipilimumab (anti-CTLA4) was the first immune checkpoint blockade developed, and resulted in durable “cure” of patients with metastatic melanoma.





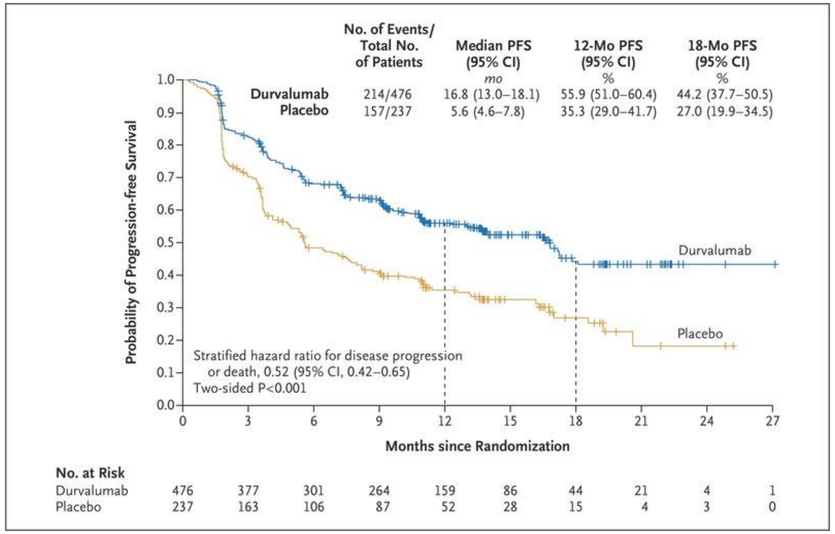
No. at Risk

Ipi plus gp100	403	297	223	163	115	81	54	42	33	24	17	7	6	4	0
Ipi	137	106	79	56	38	30	24	18	13	13	8	5	2	1	0
gp100	136	93	58	32	23	17	16	7	5	5	3	1	0	0	0

Hodi et al., *New England Journal of Medicine*, 2010

Cancer immunotherapy

Drug Name	Approval Date	Manufacturer	Antibody Name	Target
Yervoy	3/25/2011	BMS	Ipilimumab	CTLA4
Ketruda	9/4/2014	MSD	Pembrolizumab	PD-1
Opdivo	12/22/2014	BMS	Nivolumab	PD-1
Tecentiq	5/18/2016	Genetech	Atezolizumab	PD-L1
Imfinzi	2/16/18	AstraZeneca	Durvalumab	PD-L1

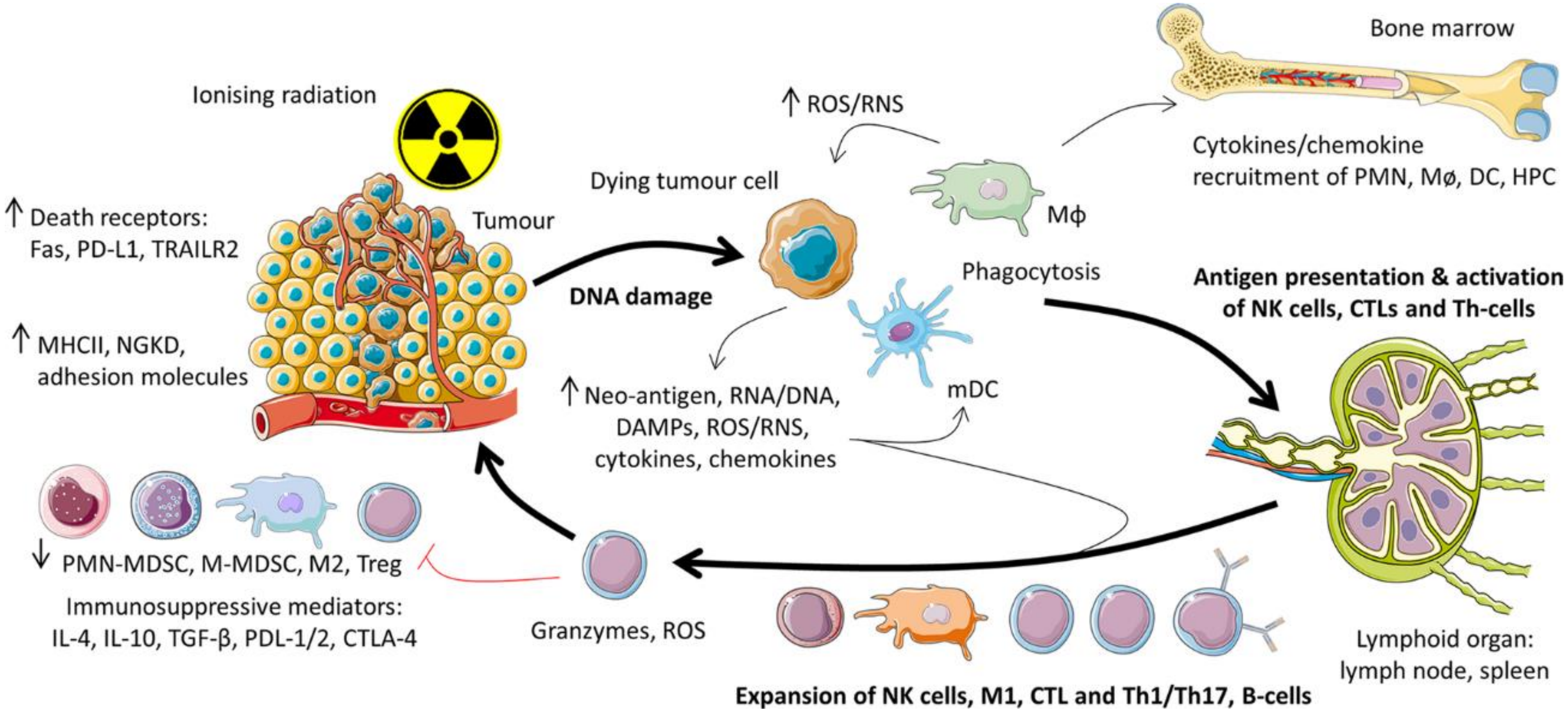


Antonia et al., *New England Journal of Medicine*, 2017

Radiotherapy and tumor immunology

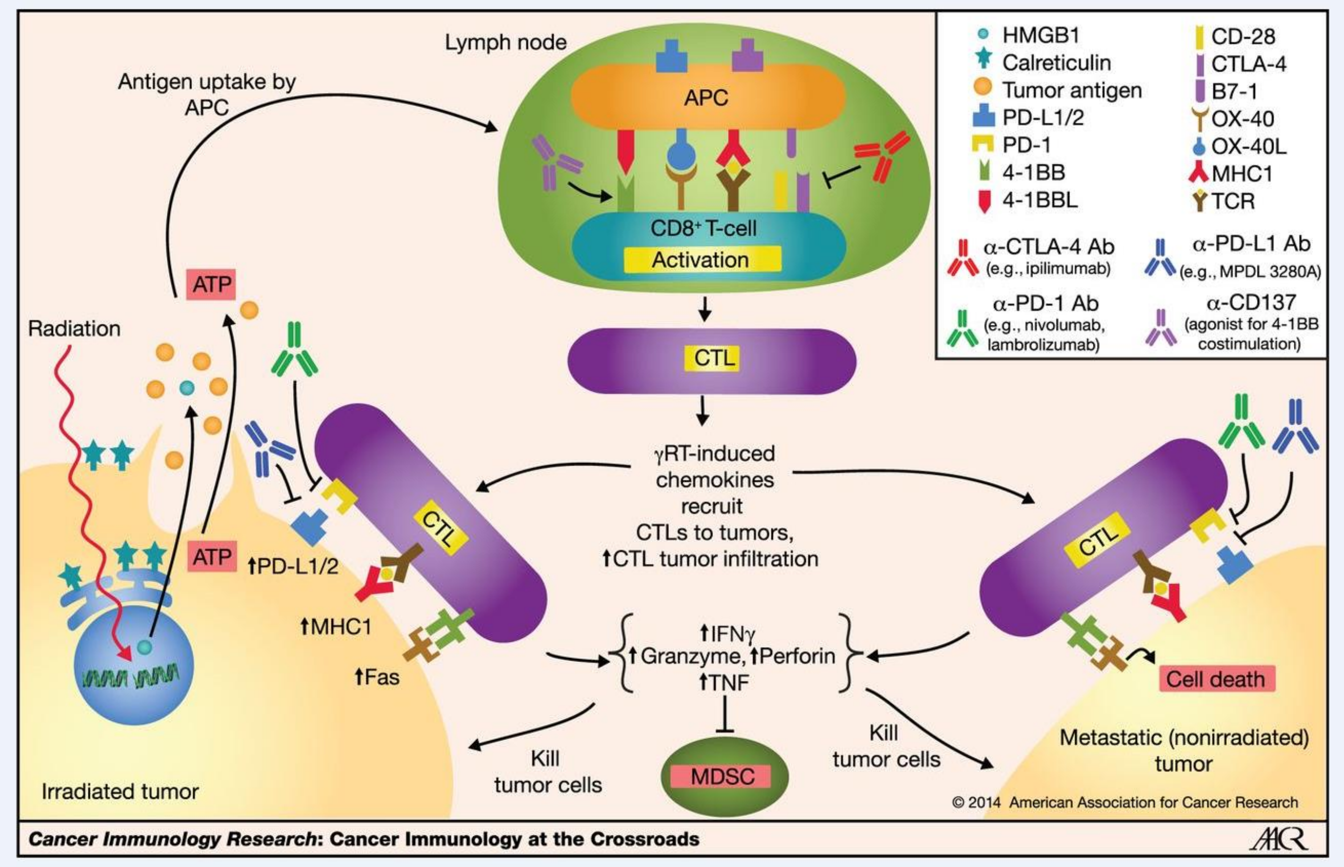
Radiotherapy has the potential to overcome or reverse multiple mechanisms of immune system evasion by tumor cells

Radiotherapy can be both highly immunogenic and highly immunosuppressive.



McKelvey et al., *Mammalian Genome*, 2018

Combined use of Radiotherapy and immunotherapy



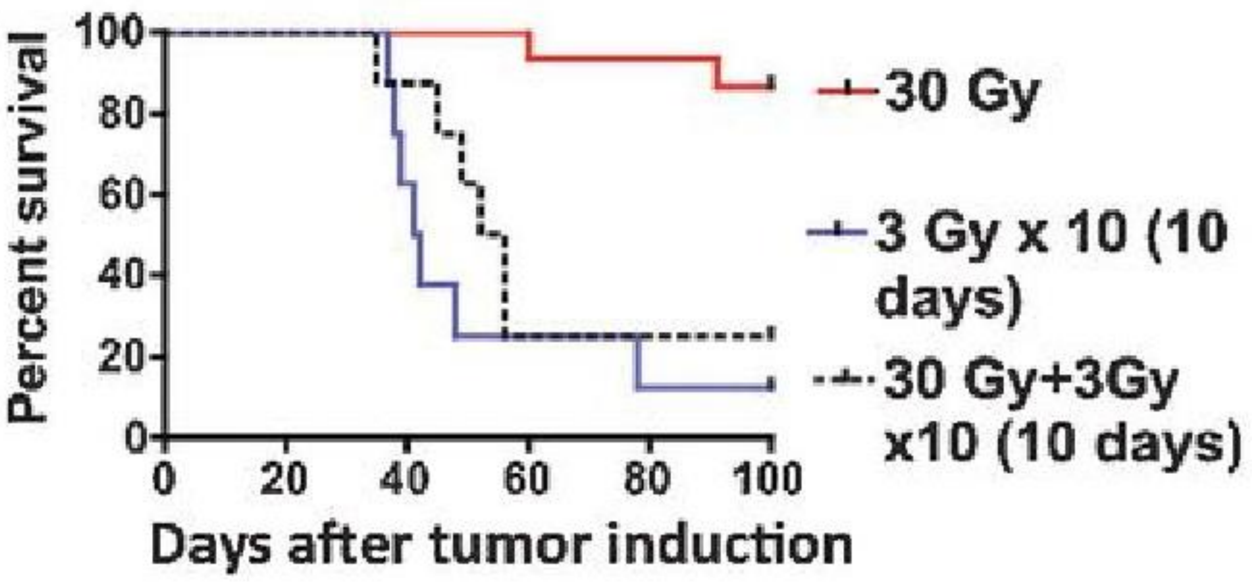
Tang et al., *Cancer Immunology Research*, 2014

Combined use of Radiotherapy and immunotherapy

Though radiotherapy can initiate and augment anti-tumor immunity, radiotherapy can also be strongly locally immunosuppressive.

- Daily fractionation is likely to kill the majority of effector lymphocytes that localize to the tumor.

What is the best dose and fractionation to induce anti-tumor immunity?



Filatenkov et al., *Clinical Cancer Research*, 2015

Utilizing Radiation in the Clinic

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Radioresistance vs Radiosensitive solid tumor histologies

Radiosensitive:

- **Lymphoma**
- **HPV+ Head and Neck and Gynecologic Malignancies (including Anal carcinoma)**

Intermediate:

- **Breast Cancer & Prostate Cancer**
- **Lung Cancer**
- **Colorectal Cancer**
- **Hepatocellular Cancer**

Radioresistant

- **Melanoma**
- **Sarcoma**
- **Glioblastoma**

Treatment Intent

- **When we see a patient in clinic, we always ask ourselves- what are the goals of care?**

Definitive Radiation – we are trying to ablate the tumor

Consolidative Radiation – we are trying to provide local control to an active tumor which has already been weakened by chemotherapy

Palliative Radiation – goal is to provide relief (typically pain) of symptoms

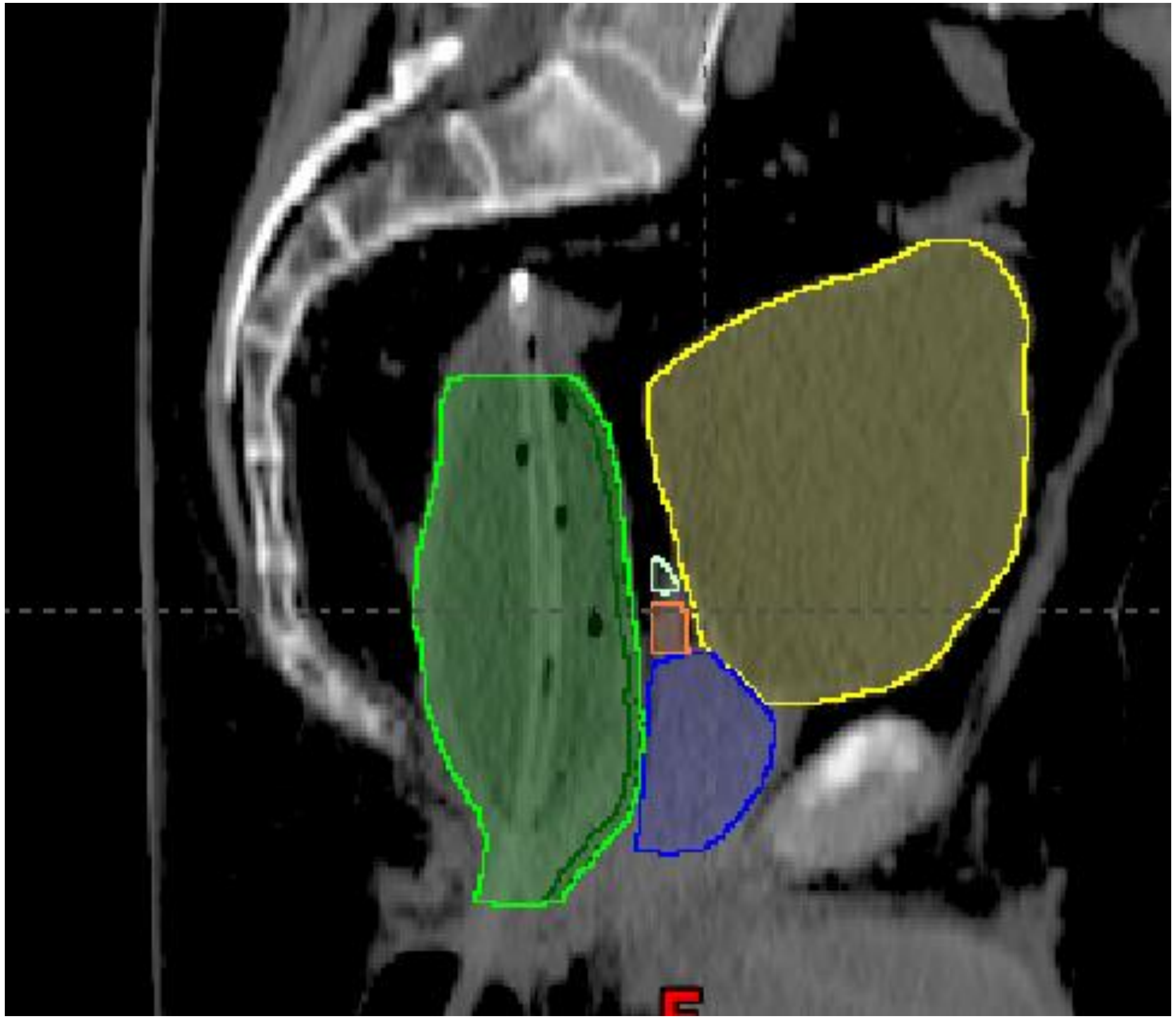
Adjuvant Radiation – treat after surgical resection to reduce risk of local recurrence

Preoperative Radiation – shrink the tumor so that surgery may become more manageable

Types of Radiation

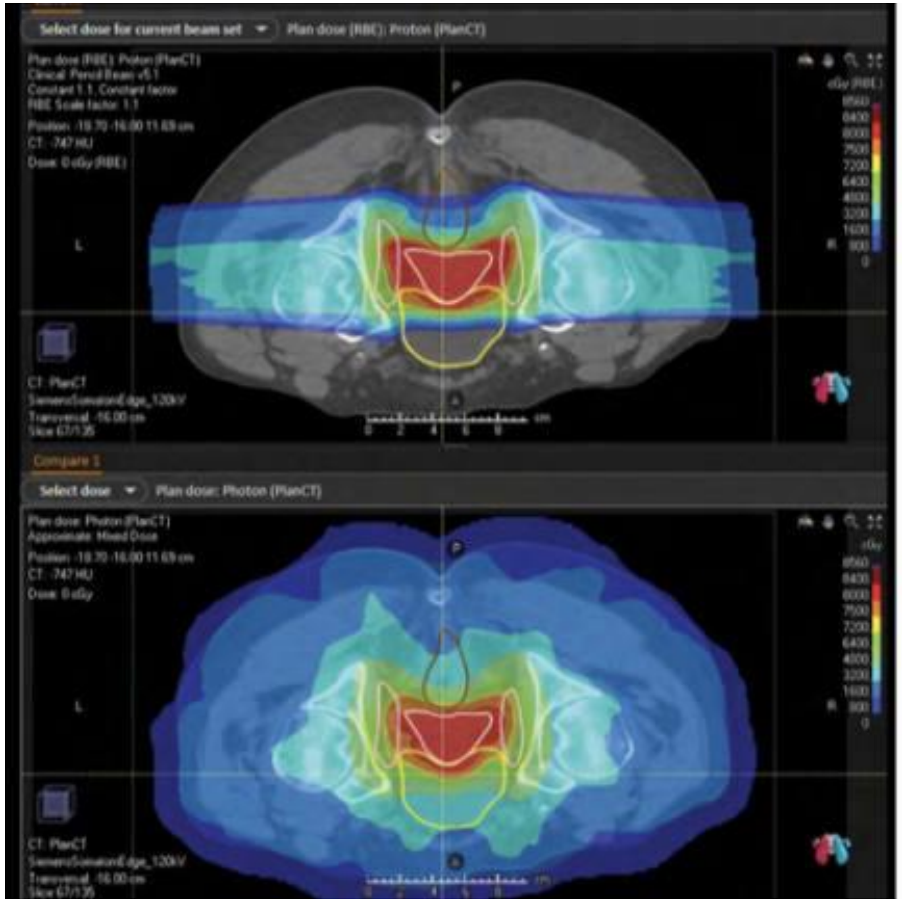
- External beam radiation therapy (EBRT) with photons
- Brachytherapy – utilizing radioactive seeds implanted permanently or temporarily (using catheter)
- Proton therapy
- Other heavy ions including Carbon, Neutron, Helium
- Stereotactic radiotherapy
- Gamma Knife radiotherapy using Cobalt-60 source

Examples – Prostate Cancer definitive radiation

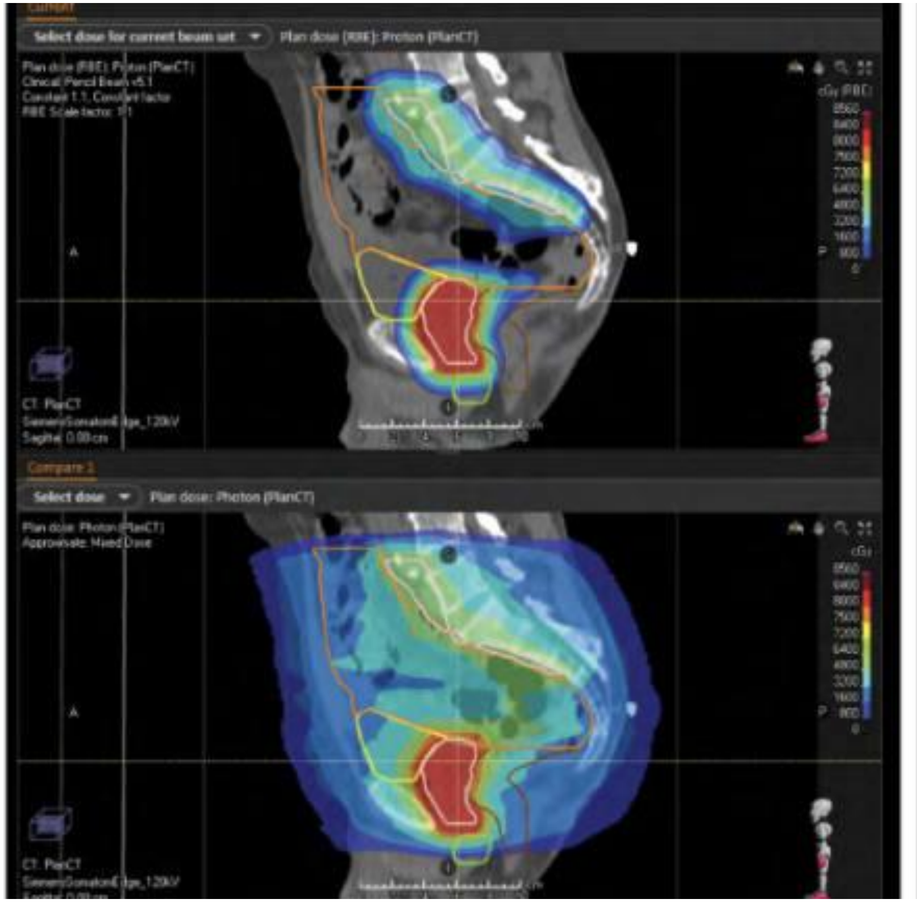


External Beam Radiation Techniques – Prostate Cancer

Proton Therapy

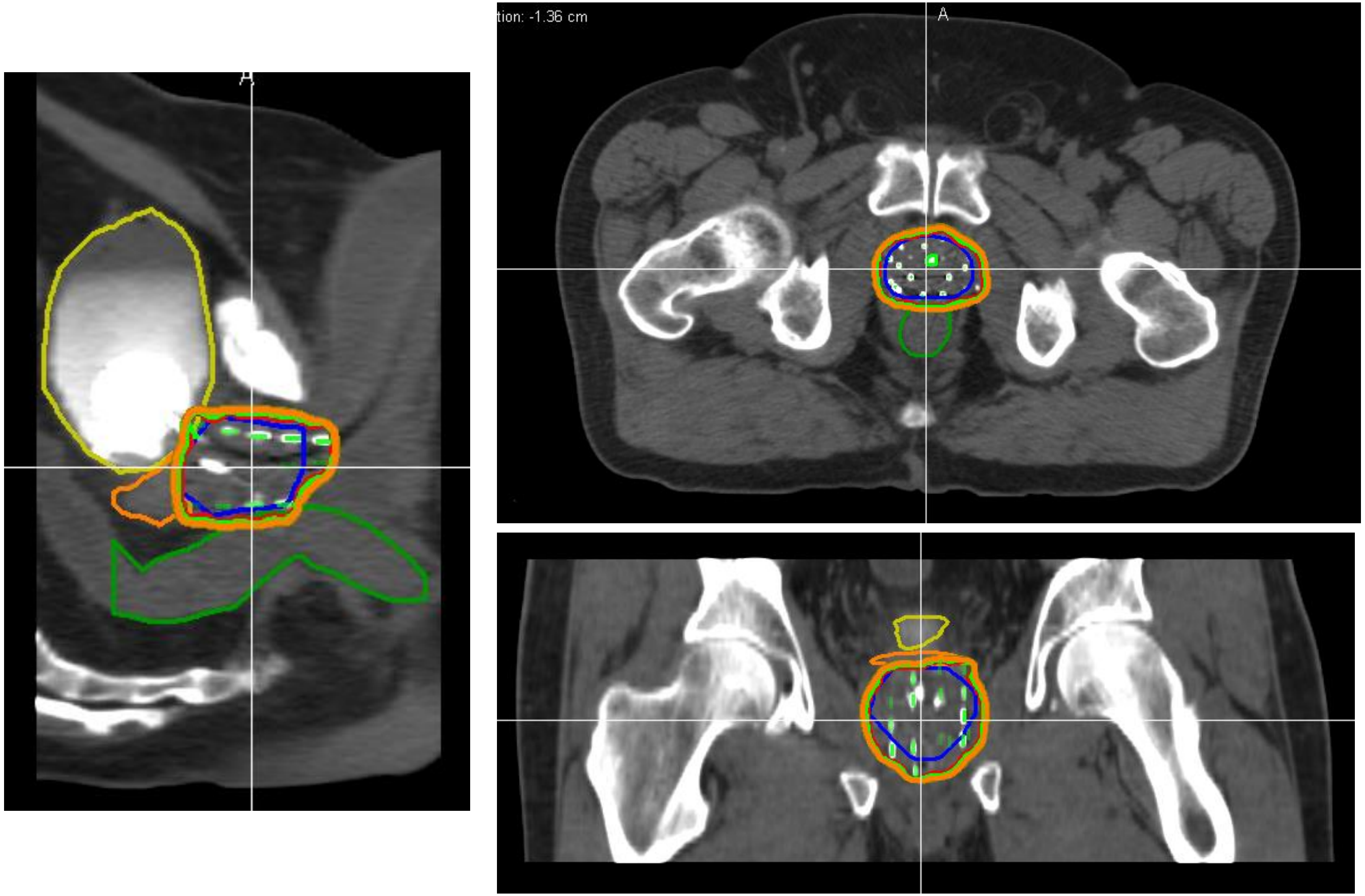


Photon Radiation



Courtesy of Dr. Frank

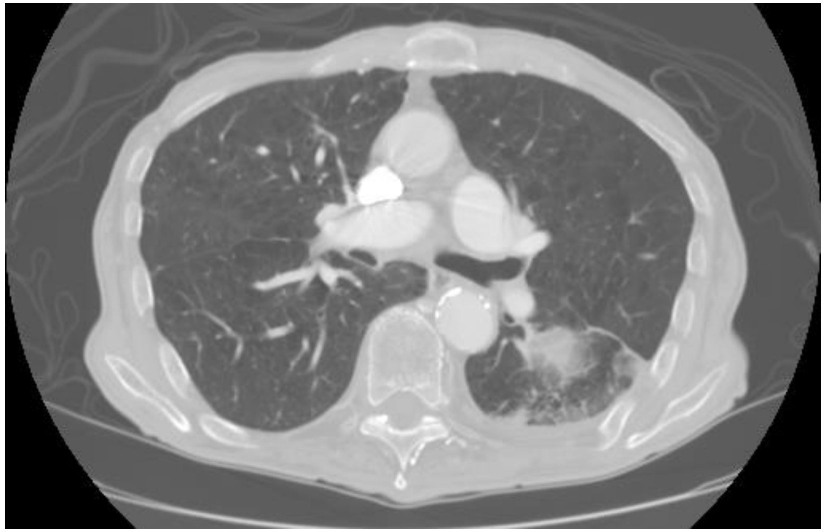
Examples – Prostate Cancer – Brachytherapy Seed Implant

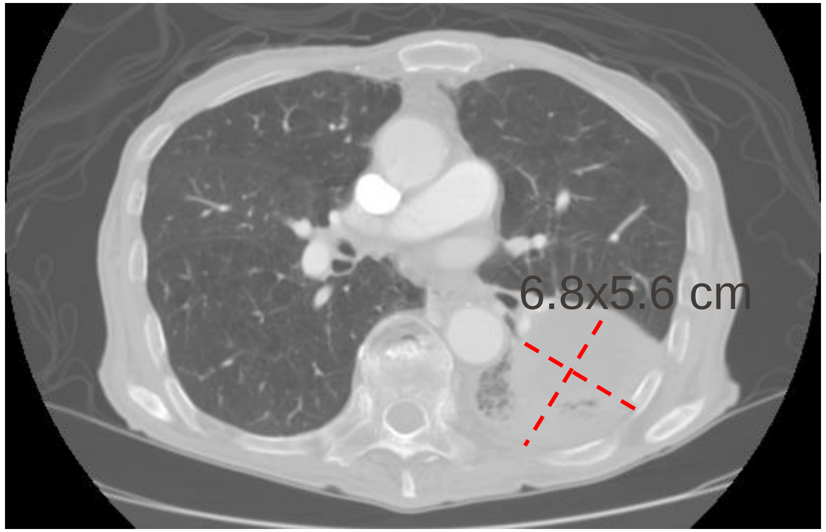


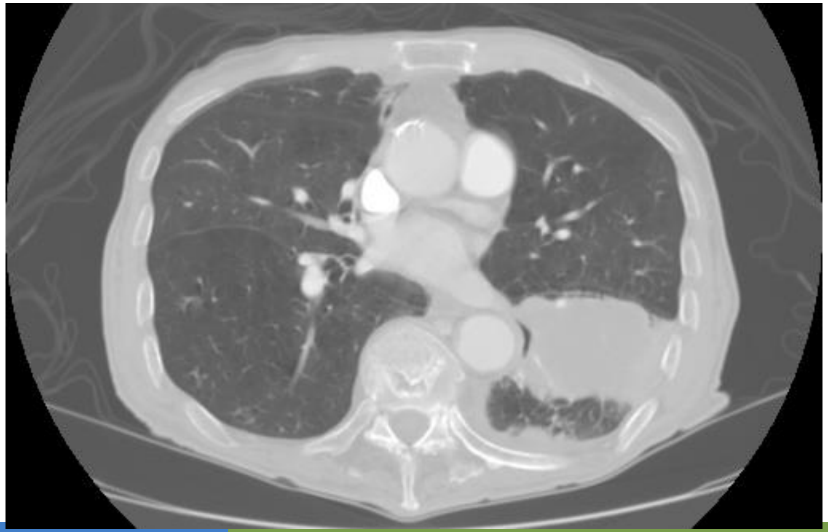
Courtesy of Dr. Frank

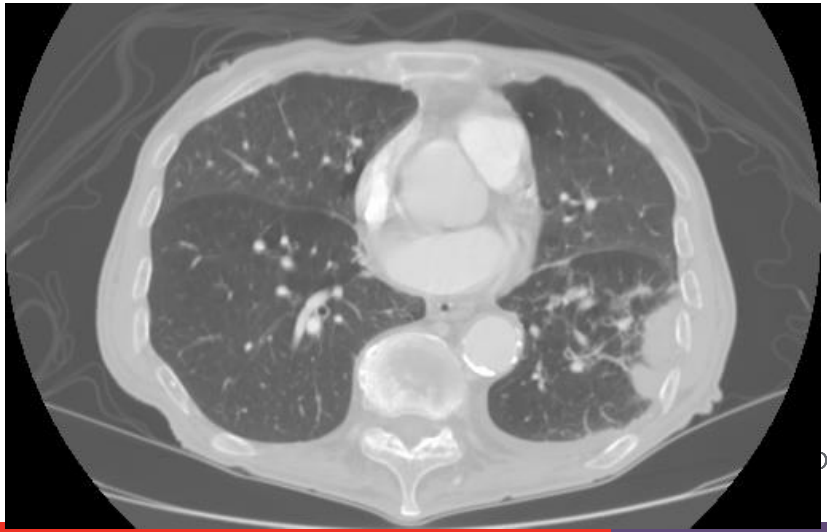
Example – Spinal metastases treated with Palliative Radiation

70 yo M with Lung Cancer

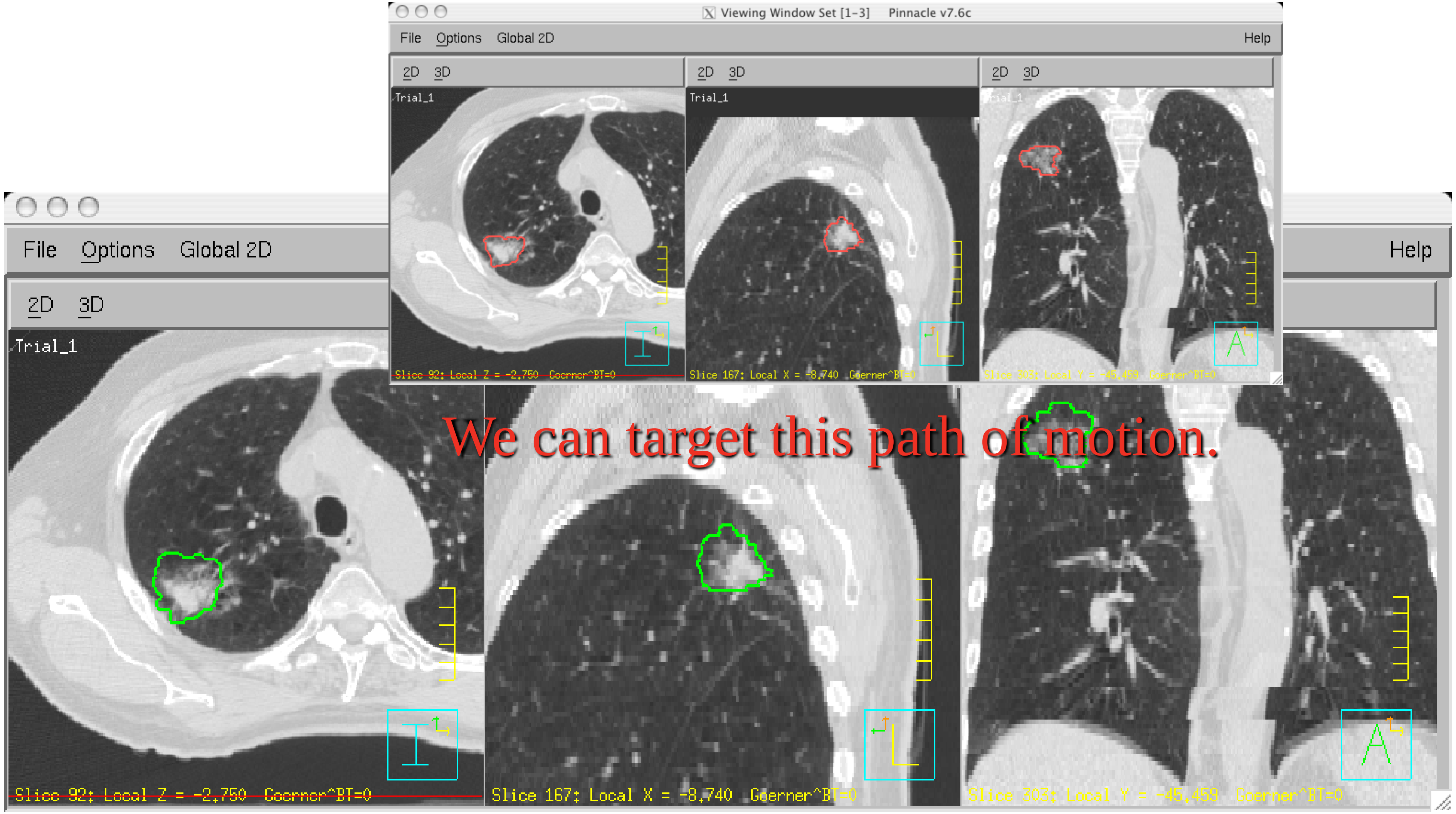






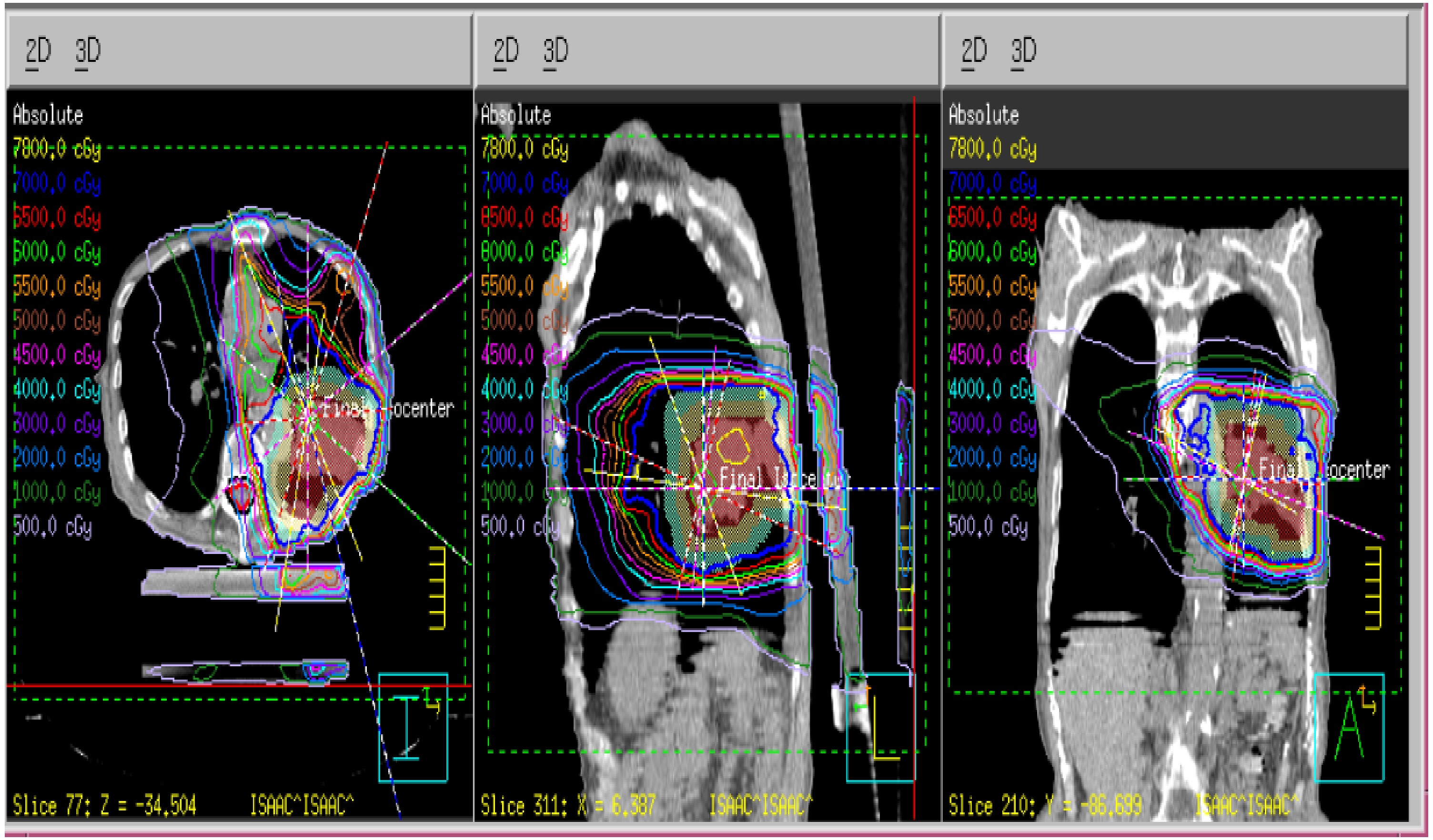


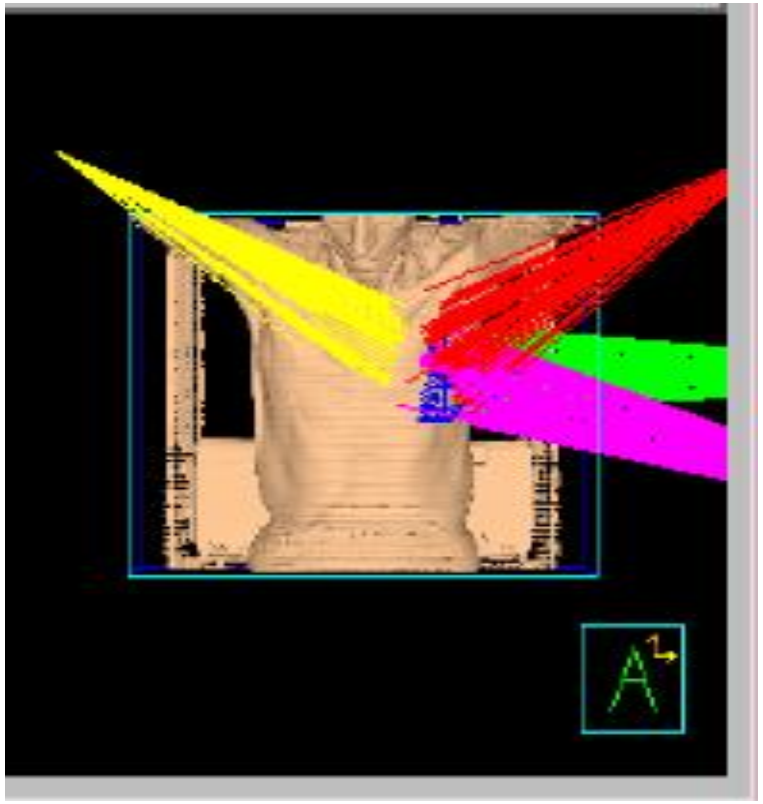
4DCT allows us to determine the path of the tumor with respiration.



Courtesy of Peter Balter

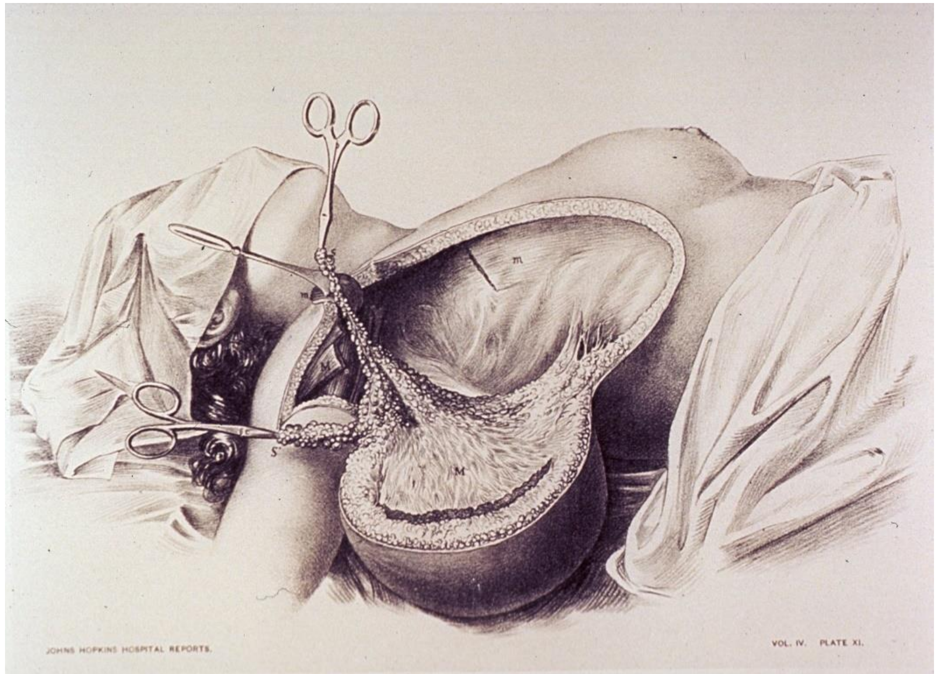
Radiation Treatment Plan





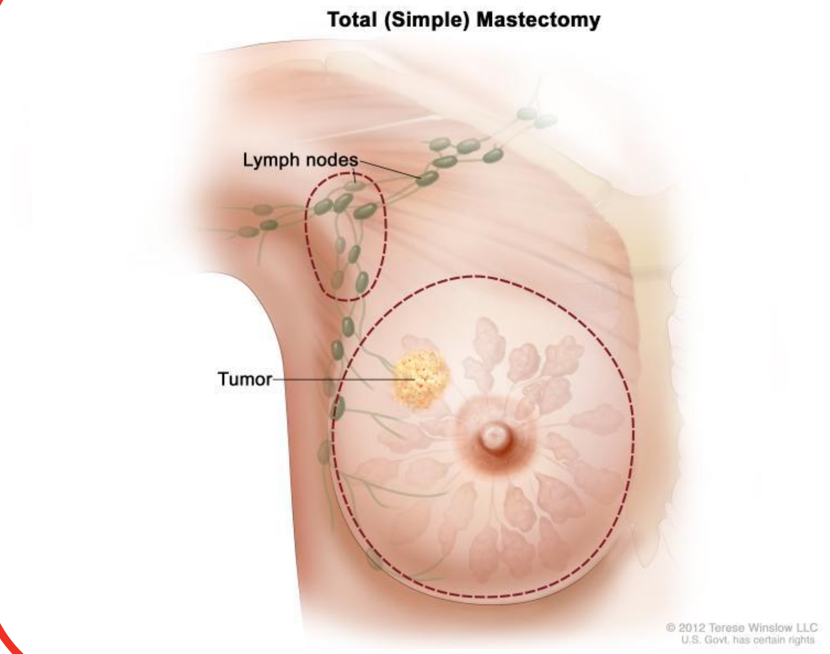
Example Breast Cancer – Adjuvant Radiation

Radical Mastectomy (no longer done)



Adapted from the National Cancer Institute

Surgery (now)



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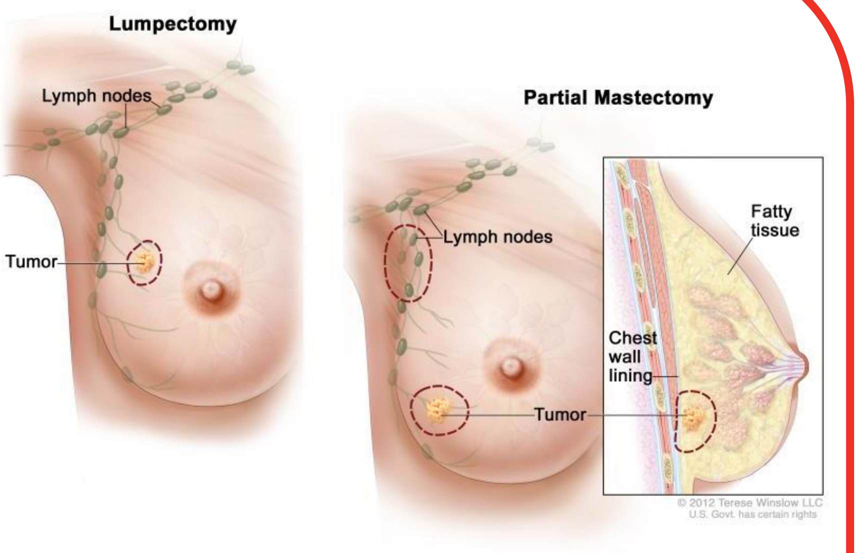

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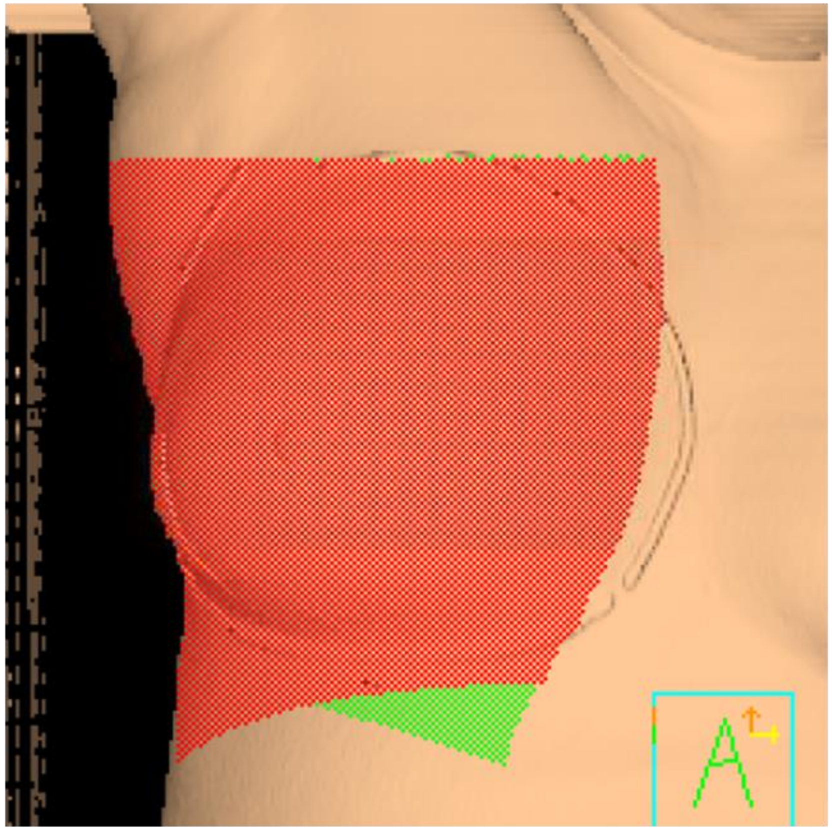
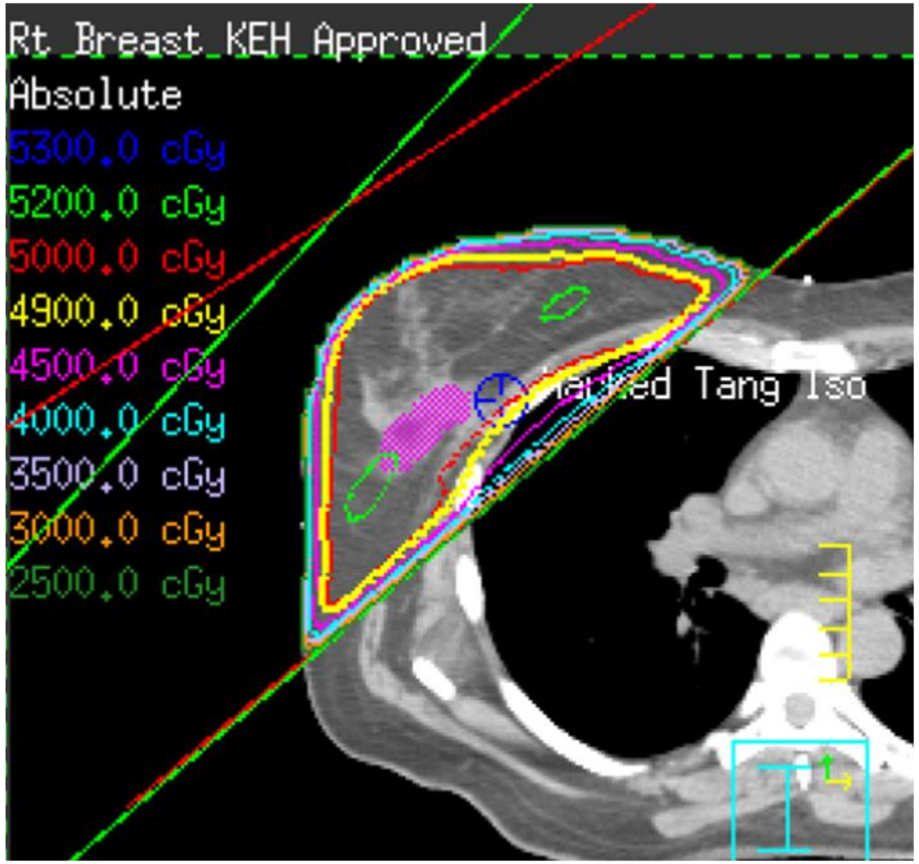
Radiation

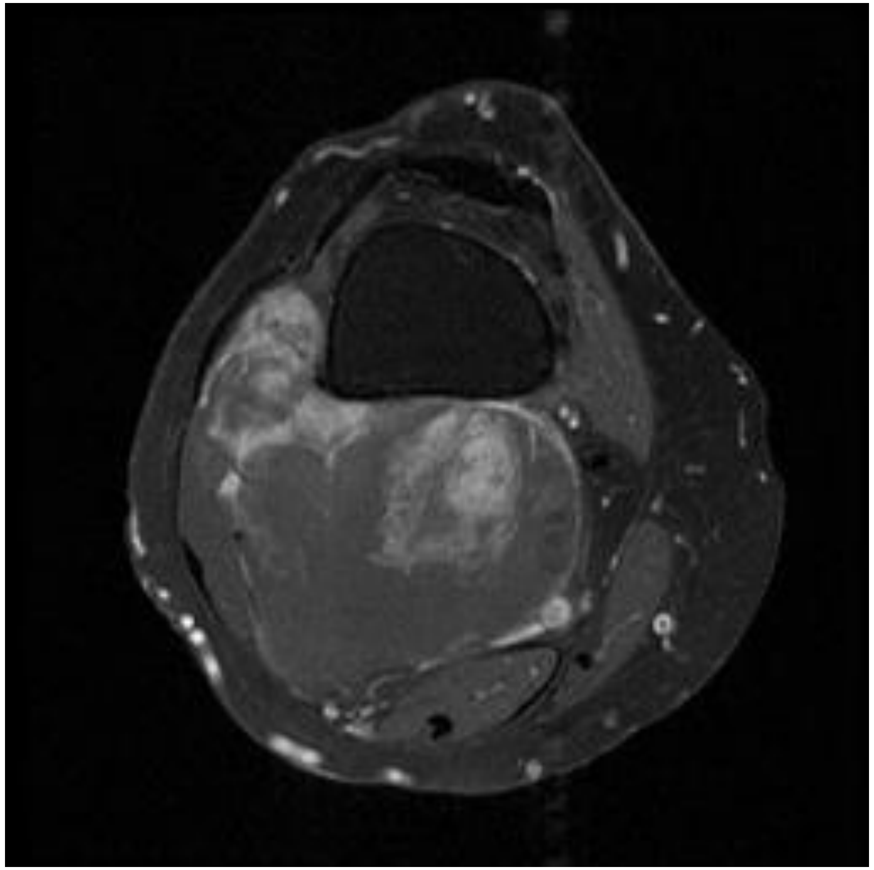
Adapted from the National Cancer Institute

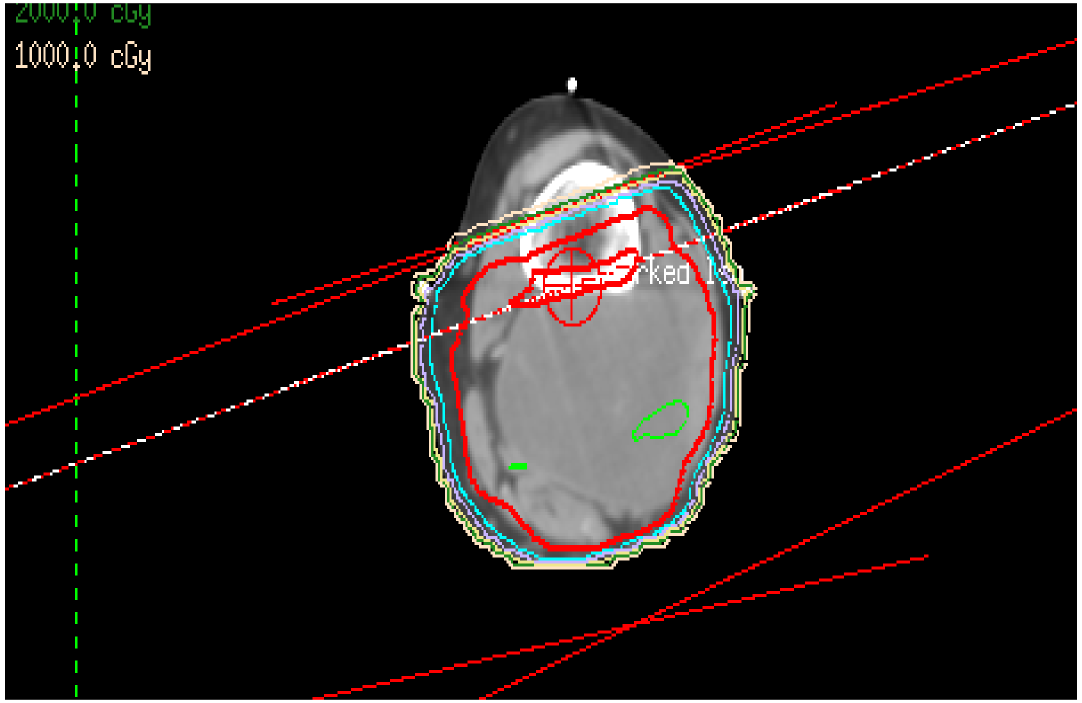
Whole Breast RT





Example sarcoma – preoperative radiation





Summary

- **Radiation has been used to treat cancer since the 1950s**
- **Radiation works by damaging the DNA of cancer cells**
- **Radiation can be combined with both various types of chemotherapy and immunotherapy to enhance the efficacy of our treatment**
- **Common myth that radiation is no longer going to be “needed” in the future**
- **Advances in our understanding of both radiation biology and radiation physics have allowed us to deliver ablative doses of radiation that were previously not technically feasible**
- **Radiation can be given in many ways – externally and internally**
- **Radiation can be used as the sole therapy for the management of a cancer (prostate cancer, H&N cancer, gynecologic cancer)**
- **Other times radiation is indicated to be given preoperatively (sarcomas) or post-operatively (breast cancer) in the adjuvant setting**

Primary References

1) Hall, Eric J. *Radiobiology for the Radiologist.* Hagerstown, Md. :Medical Dept., Harper & Row, 2018. 1st - 8th edition.
2) National Cancer Institute Website
3) Internal MD Anderson Resident Didactic Lecture Series

Thank you!

Special thanks to Dr. Shane Stecklein MD PhD and Dr. Spiotto MD PhD who I borrowed some slides from.

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Questions?