

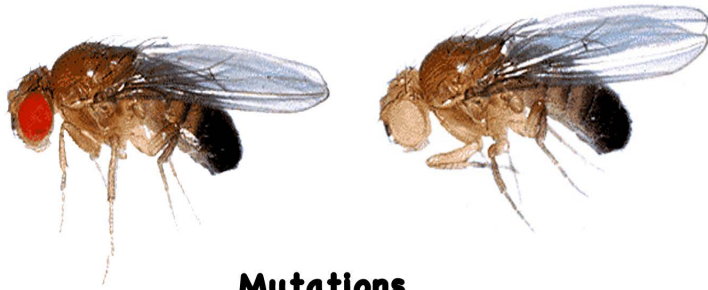
# WHAT IS RADIOBIOLOGY?

“THE STUDY OF THE EFFECTS  
OF ELECTROMAGNETIC  
RADIATION ON  
BIOLOGICAL SYSTEMS.”

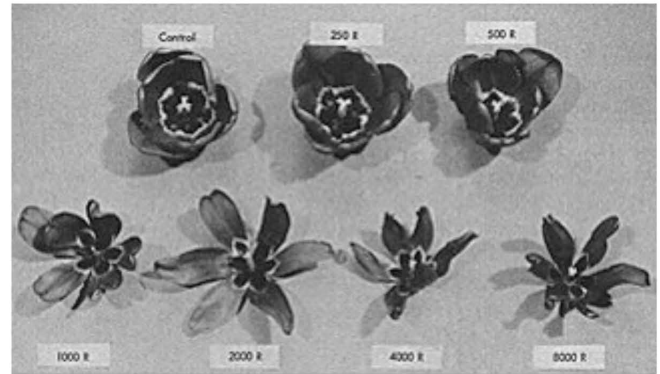
# EFFECTS

Red Eye (Wild Type)

White Eye (Mutant)



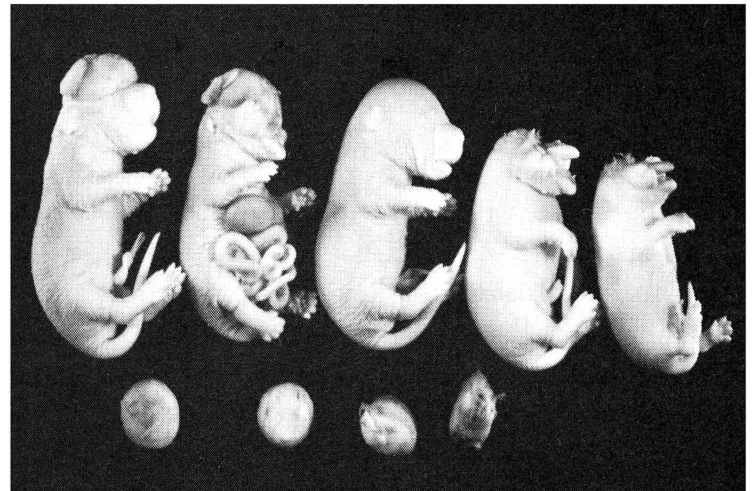
**Mutations**



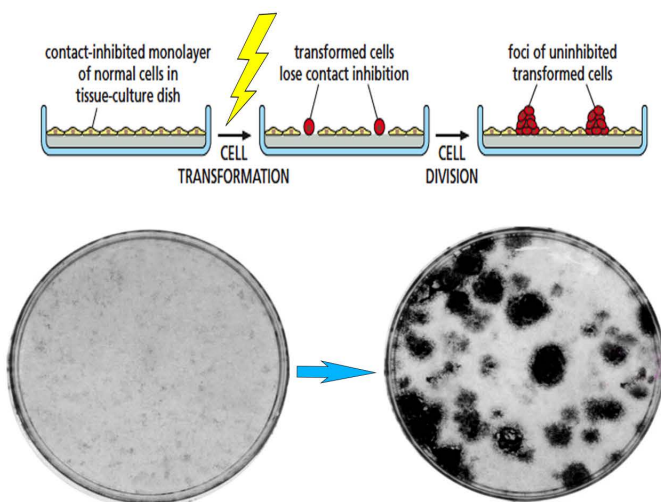
**Growth Delay/Disturbance**



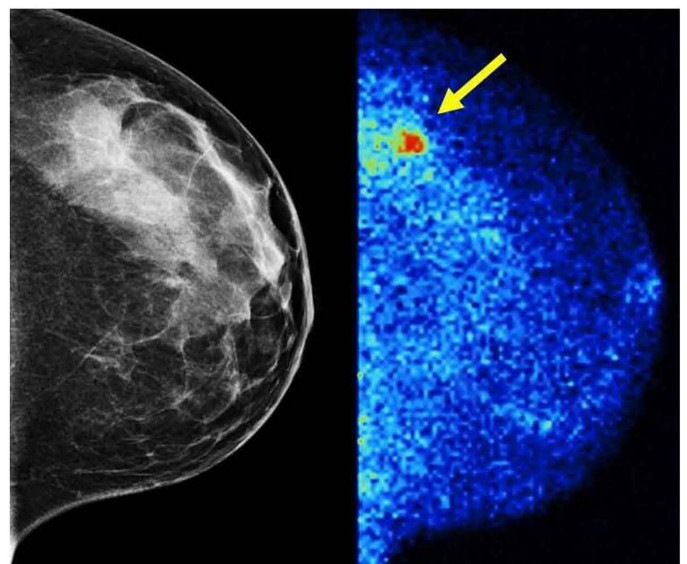
**Whole-Body  
Radiation Syndromes**



**Teratogenesis**

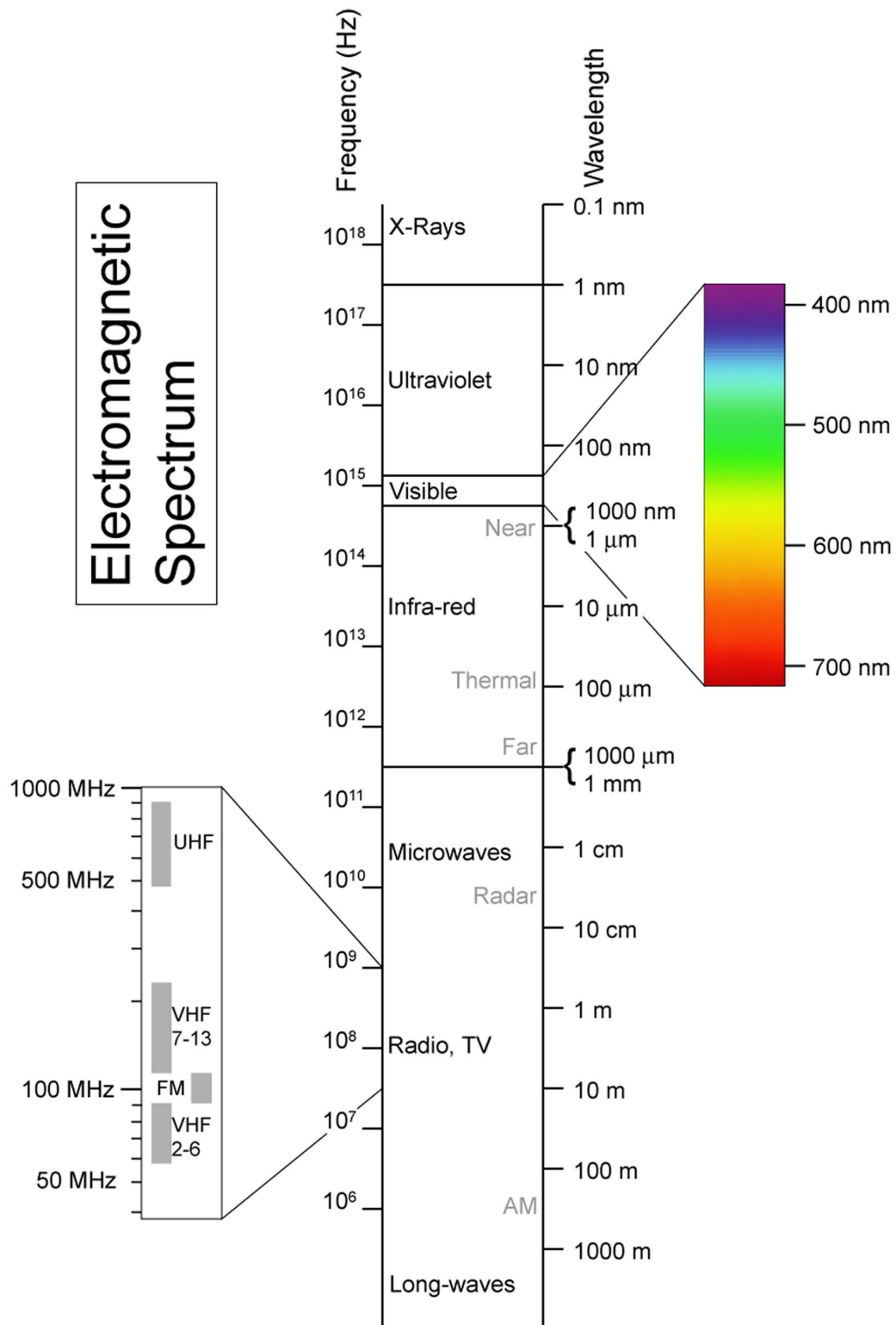


**Neoplastic Transformation**



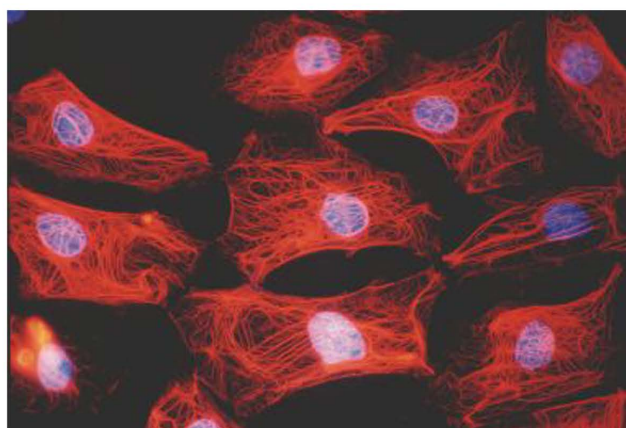
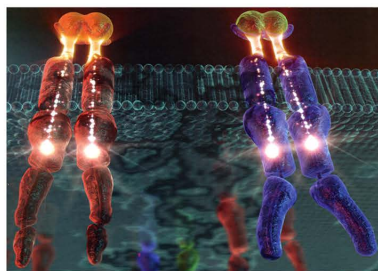
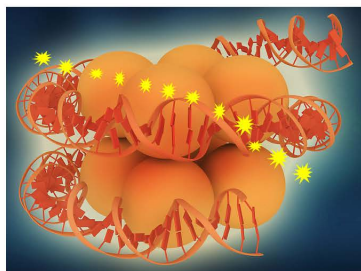
**Carcinogenesis**

# ELECTROMAGNETIC RADIATION

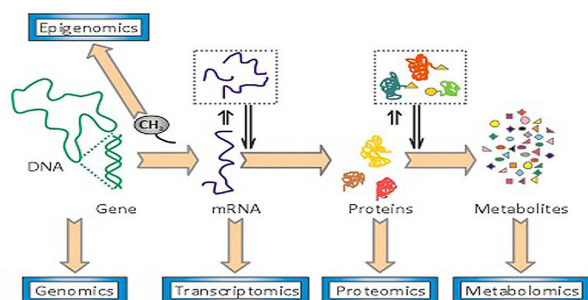




# BIOLOGICAL SYSTEMS



Cells in Culture

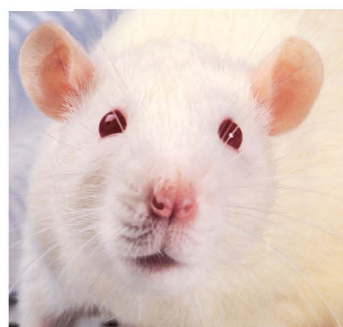


## Subcellular

(e.g., DNA damage and repair, signal transduction, 'omics, etc.)



Fruit Fly



Laboratory Rodents



C. elegans



Zebrafish



Arabidopsis



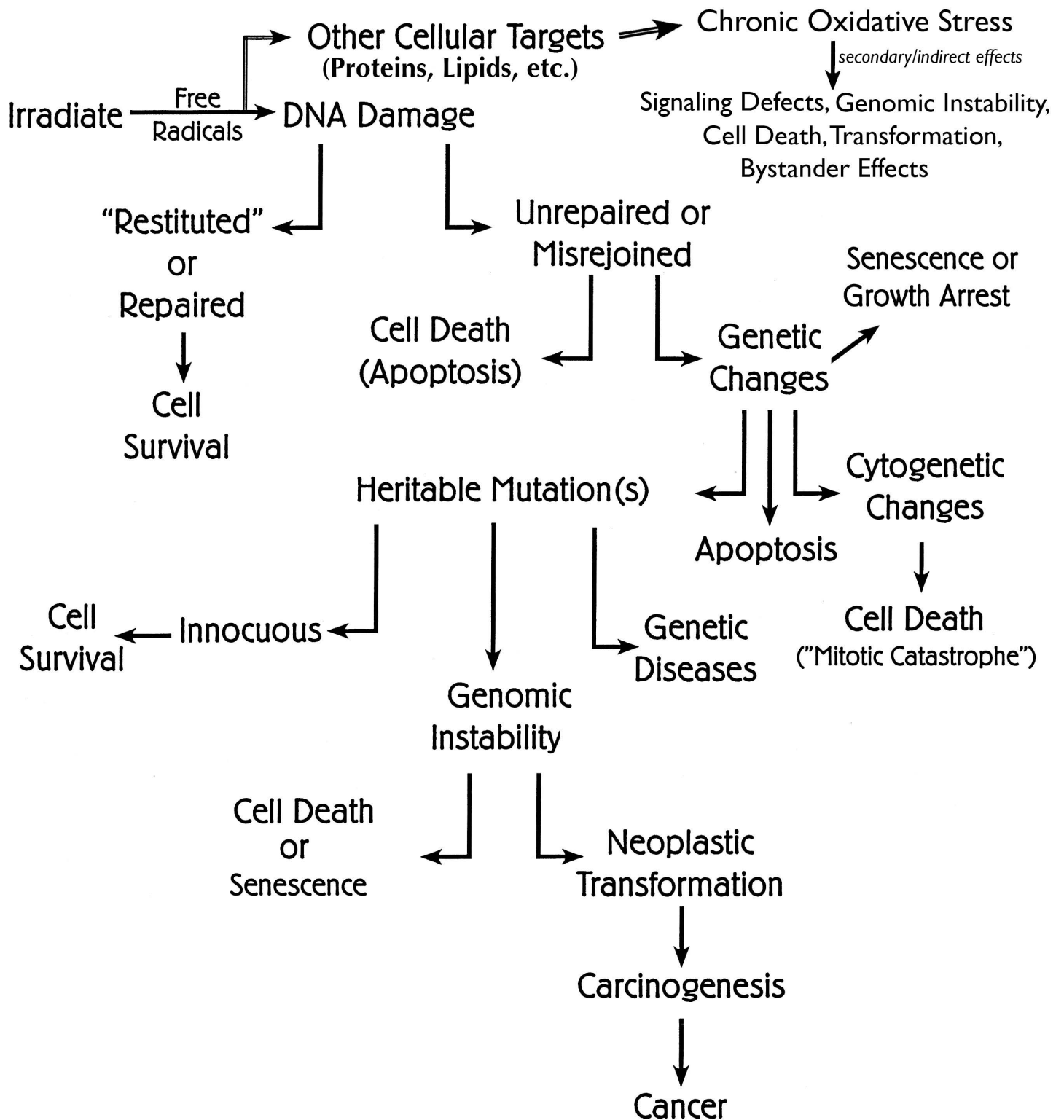
Us  
(Japanese A-Bomb Survivors)



Ecosystems  
(Bikini Islands)



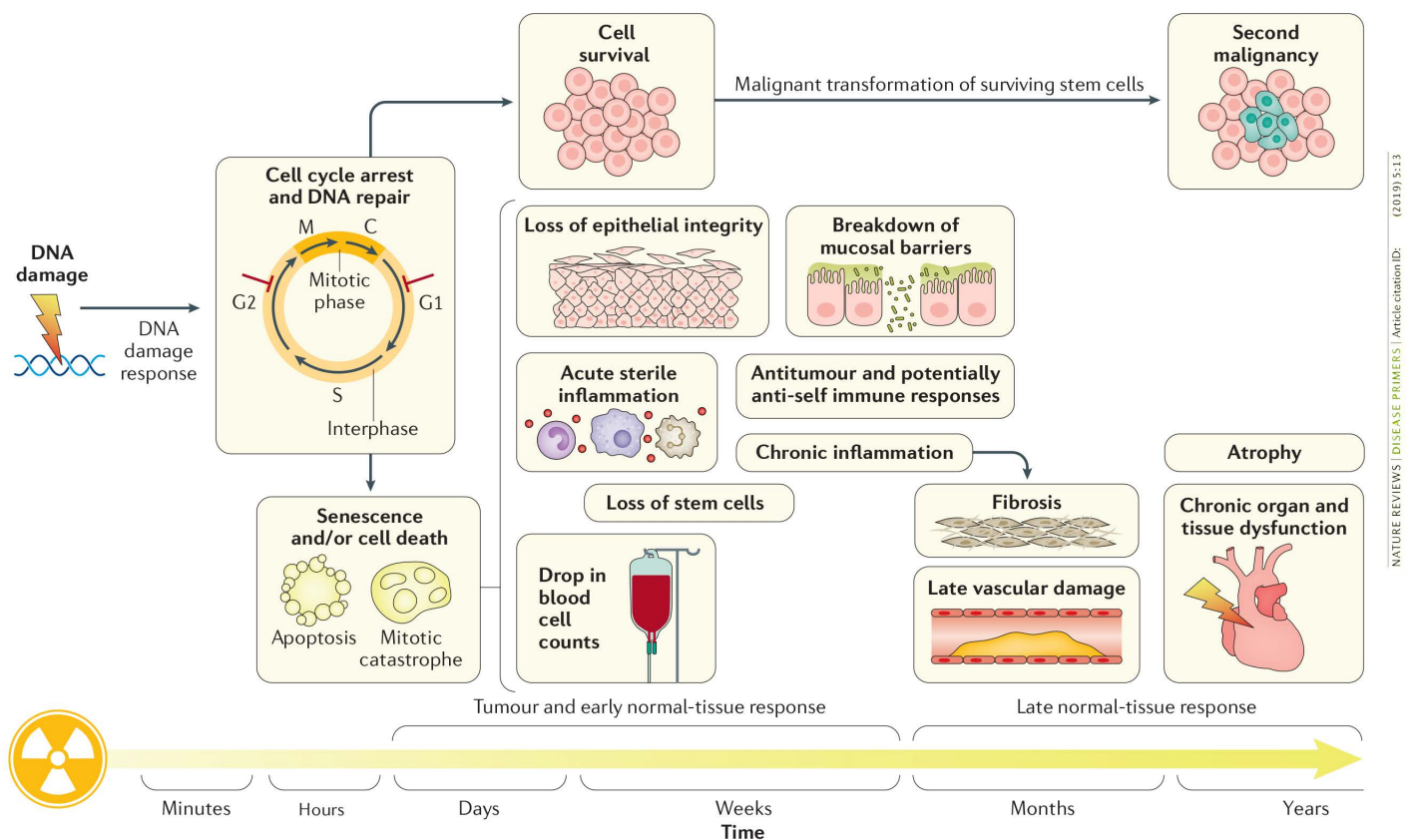
# The Flow Chart of Radiobiology



# The Flow Chart of Radiobiology

## Part II

The flow chart of radiobiology only considers *cellular* responses, but what happens at the tissue and organ levels once cells are damaged or killed?



The loss of cells precipitates repeat cycles of inflammation and the generation of reactive oxygen species which, when combined with damage to the tissue's vasculature, lead to organ dysfunction, ultimately resulting in the kinds of clinical toxicities observed during and after radiation therapy.

These tissue-level toxicities (in both tumors and normal tissues) develop over time as part of a complicated process that depends critically on interactions between different types of cells in the tissue and the tissue's microenvironment in general.

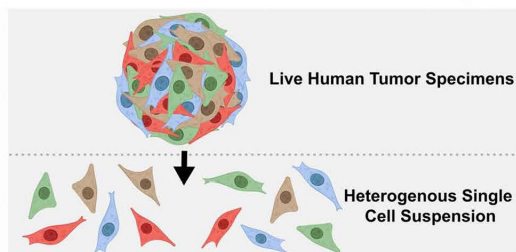
# “Modifiers” of the Radiobiology Flow Chart:

## *Tumor Heterogeneity*

Cancer is a heterogeneous and continuously evolving disease. When pathologists first looked at human cancers under the microscope, they saw that differing histologic appearances could define distinct subtypes of cancers from the same primary site of origin. These histology-based definitions of cancer subtypes have been modified and refined over time to elucidate both prognosis and prediction of response to specific treatments. Molecular data have revealed how radically different cancers from one primary site can be, and using this information, cancer classification systems have been revolutionized.

However, heterogeneity in cancer is not limited to differences between different patients, but also occurs within a single patient. This heterogeneity at the molecular, cellular, tissue, and organ levels bedevils clinical cancer care because it allows cancers to evolve and evade available therapeutics.

**Multi-omics**, the process of collecting and analyzing a combination of data that describes cells, provides a way to study tumor cell heterogeneity, and can be performed at even the single-cell level.

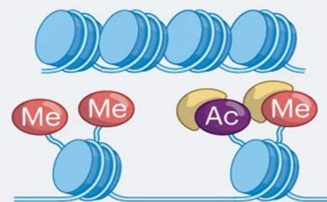


**While tumor cells can be from the same general lineage and have some common molecular features, thanks to tumor heterogeneity, no two tumor cells are ever *exactly* alike.**

It is the tools of “omics” that give us a feel for just how heterogeneous tumors can be, and armed with this information, the hope is that cancer treatment can become more and more personalized



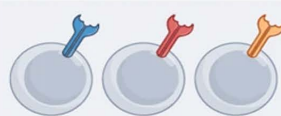
Genome



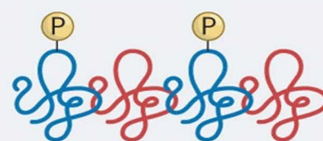
Epigenome



Transcriptome



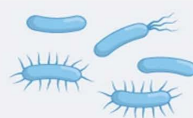
TCR repertoire



Proteome



Metabolome



Microbiome

What's the main reason we'd like to be able to “personalize” cancer treatment?

So that we don't:

- under-treat aggressive cancers

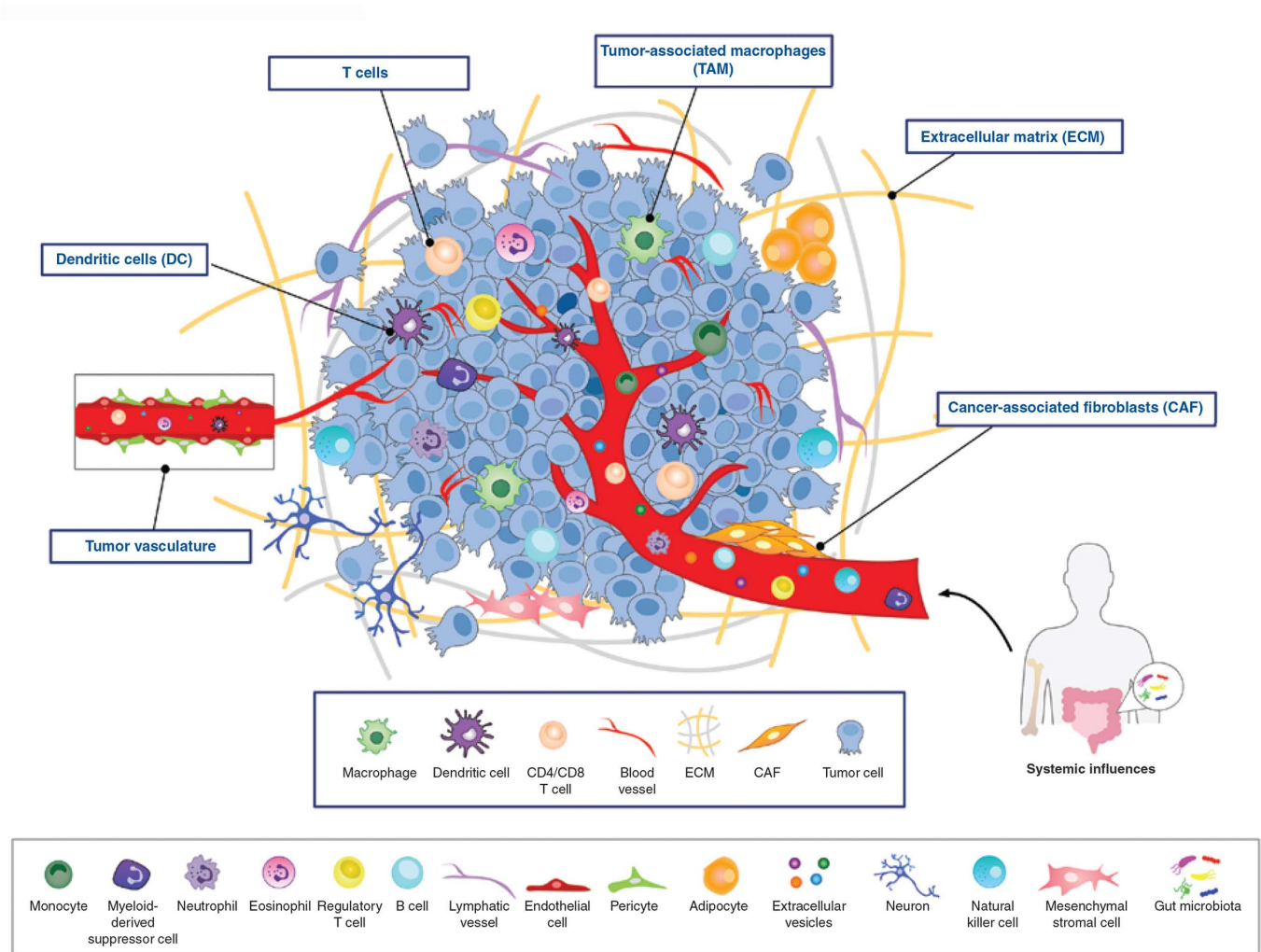
or

- over-treat indolent cancers



# “Modifiers” of the Radiobiology Flow Chart:

## *The Tumor Microenvironment*



Cancer Discov 2021;11:933–959

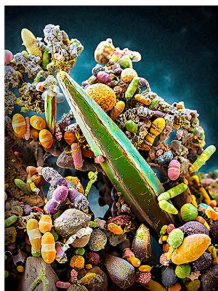
**THE PRIMARY TUMOR ENVIRONMENT** Cells within a tumor are supported by a complex and dynamic microenvironment composed of multiple infiltrating cell types, including endothelial cells (which line blood and lymphatic vessels), cancer-associated fibroblasts (CAFs), and a variety of bone marrow-derived cells (BMDCs). Infiltrating BMDCs mediate inflammatory responses during cancer progression and can have negative or positive consequences.

Major BMDCs within the tumor niche include tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), TIE2-expressing monocytes (TEMs), mesenchymal stem cells (MSCs), and various other cell types from lymphocyte and monocyte lineages. This tumor-associated cellular cocktail largely dictates the evolution of the surrounding environment and, ultimately, the outcome of disease.

# “Modifiers” of the Radiobiology Flow Chart:

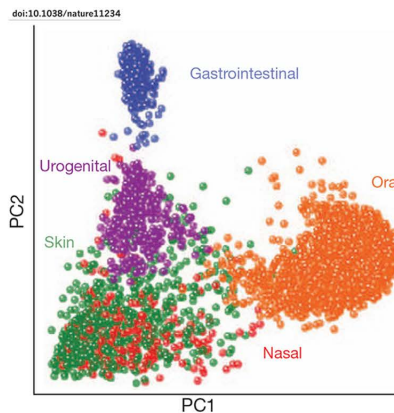
## *The Microbiome*

***The human microbiome includes all microbiota that reside on or within human tissues and biofluids, and is categorized according to the anatomical sites in which they reside. Types of human microbiota include bacteria, archaea, fungi, protists and viruses.***

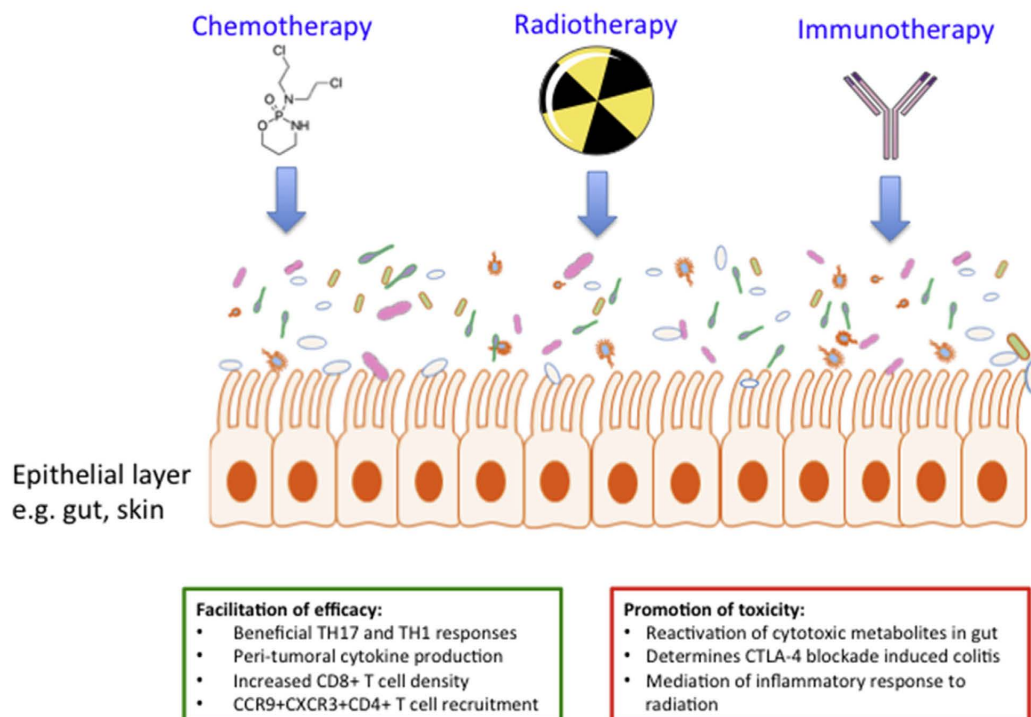


Microbiota

A “principal coordinates plot” shows the clustering of families of microorganisms by body area. There is some - but not a lot - of overlap between areas.



Emerging science suggests that the microbiome can not only influence the development of cancer (e.g., increasing inflammation, altering metabolism), but also affect response to cancer therapies, immunotherapies in particular. Altering the microbiome “mix” could have clinical implications – good or bad.

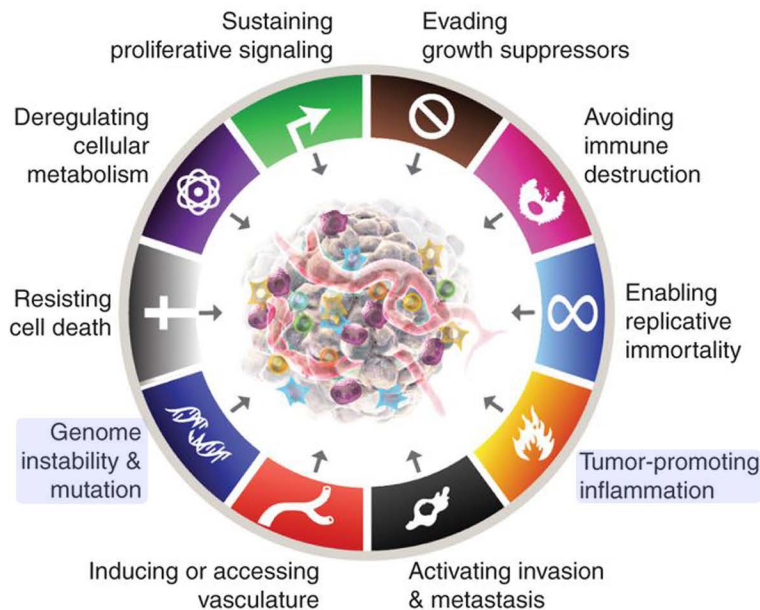


The microbiota are at the interface between cancer therapy and host responses.

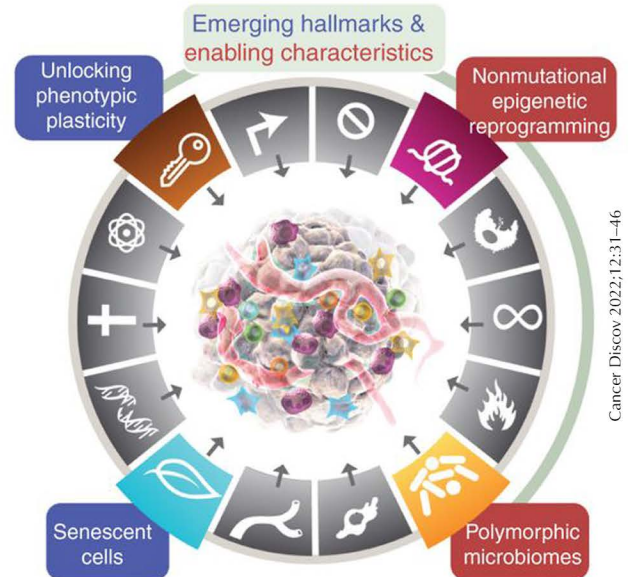


Always keep in mind your “Hallmarks of Cancer”...and how they relate to both radiobiology and radiotherapy.

### 2011 Version



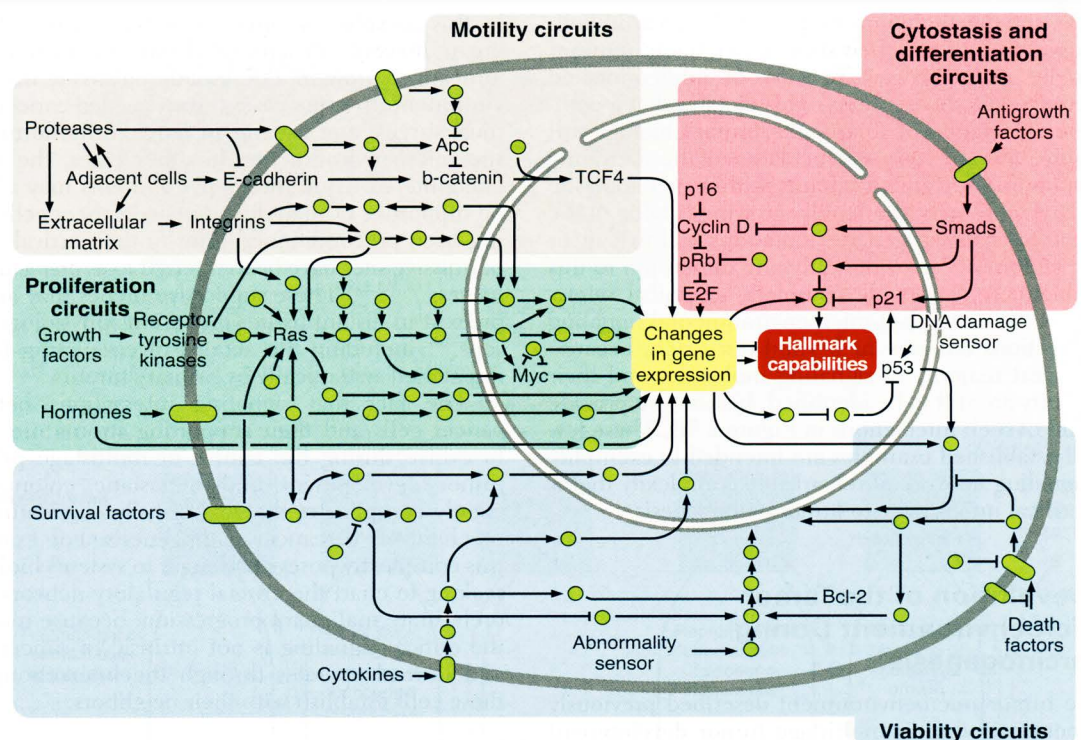
### 2022 Version



Cancer Discov 2022;12:31–46

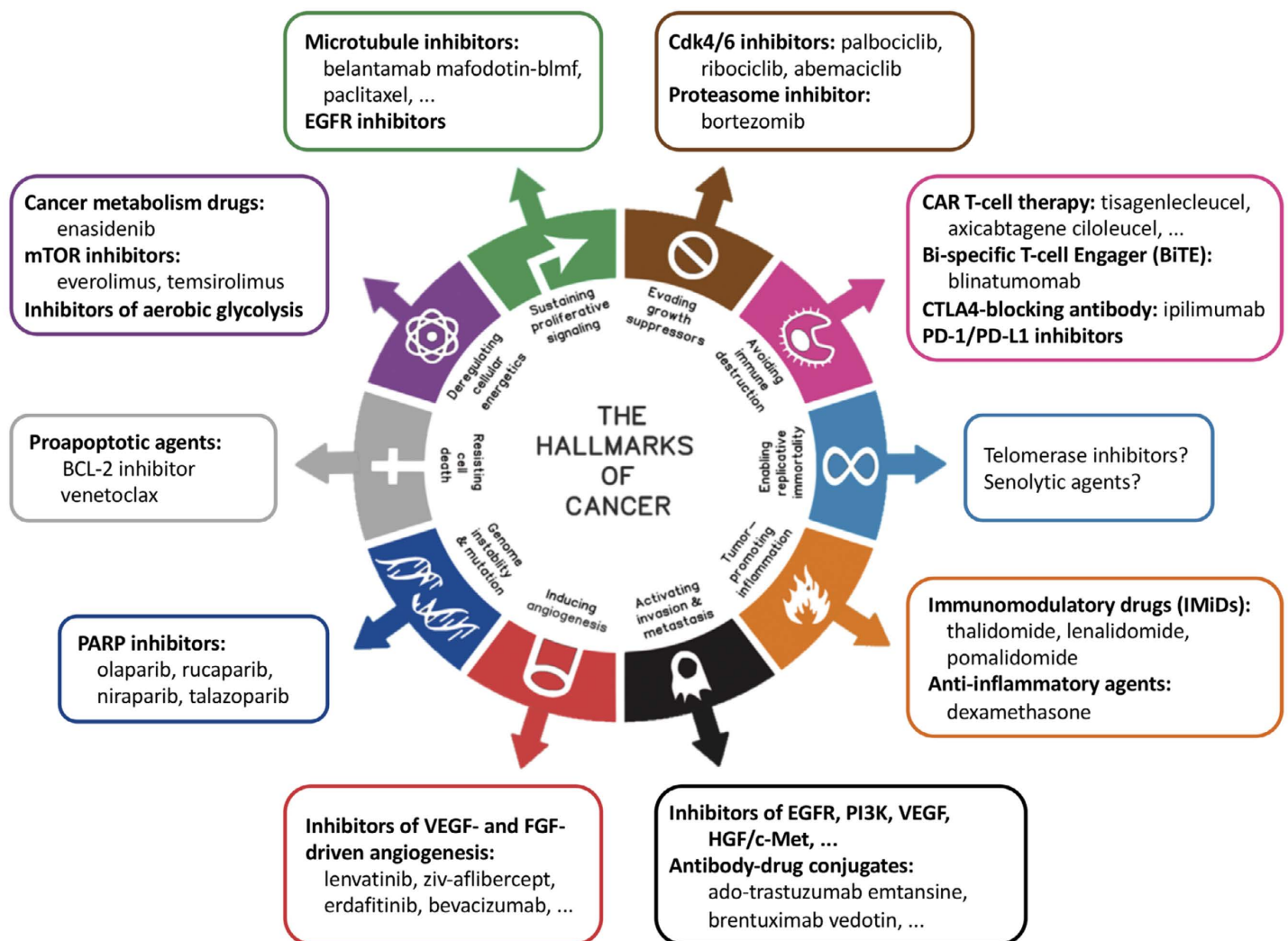
Updated cancer hallmarks for 2022: “Unlocking phenotypic plasticity” = ability of tumor cells to avoid terminal differentiation (by blocking differentiation, dedifferentiating and/or transdifferentiating); “Nonmutagenic epigenetic reprogramming” = that tumor cells can change gene expression epigenetically (i.e., without needing changes to DNA itself), typically in response to cues from the tumor microenvironment; “Polymorphic microbiomes” = ability of certain tissue-resident microorganisms to exert protective or deleterious effects on carcinogenesis, tumor progression and/or response to treatment, and; “Senescent cells” = that in certain situations, the presence of senescent cells can *enhance* rather than inhibit acquisition of the cancer hallmarks

Defects in key genetic “circuits” are responsible for cells acquiring the various hallmarks of cancer





AND how the various hallmarks are driving new cancer therapy paradigms (including with radiation) and the search for new molecular targets for anti-cancer drug development



### Targets of approved small molecule inhibitors

Targets are mapped to the hallmarks of cancer

# Personalized Cancer Therapy

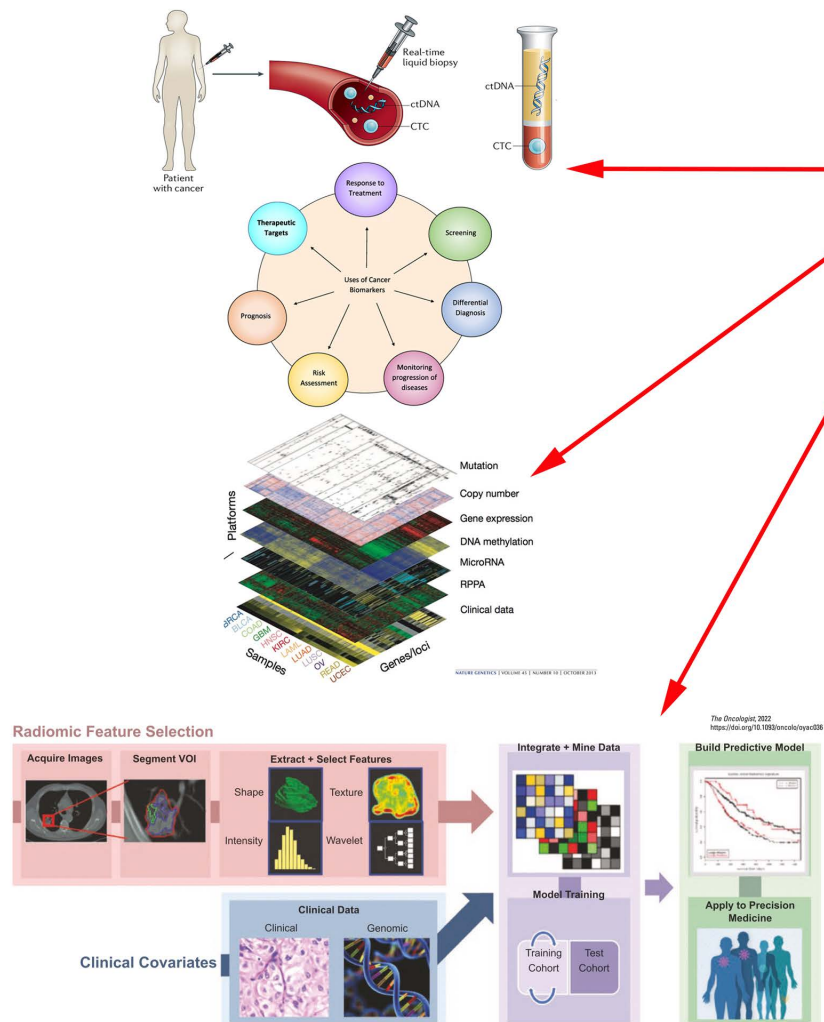


**Biomarkers** are cellular and molecular (including genetic and epigenetic) characteristics by which normal and/or abnormal processes can be recognized and/or monitored. They are measurable in biological materials such as tissues, cells, and/or bodily fluids.

...is the ultimate goal, given everything we've learned, and continue to learn, about cancer biology.

However, **none of this will be possible without robust biomarkers** that allow us to match patients to particular therapies, monitor their progress during treatment (and make changes, if necessary), and continue with followup looking for evidence of recurrence.

Some biomarkers are also useful in diagnosis as well as therapy.

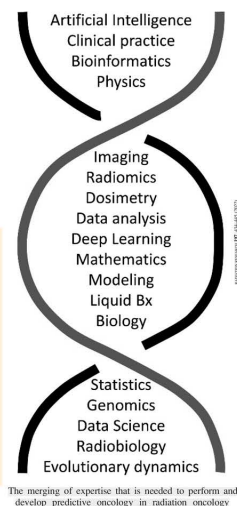


Two technologies currently being used in the quest for biomarkers include **liquid biopsy** (e.g., circulating tumor DNA or cells) and **molecular profiling** (e.g., next generation sequencing).

The newest kid on the block is **radiomics**, which involves extracting physical properties of tissues from medical images, combining this data with existing clinical and genomic data, and trying to come up with a patient-specific treatment plan or prediction of outcomes.

The amount of “big data” generated in these studies requires a ton of computing power (and the use of AI).

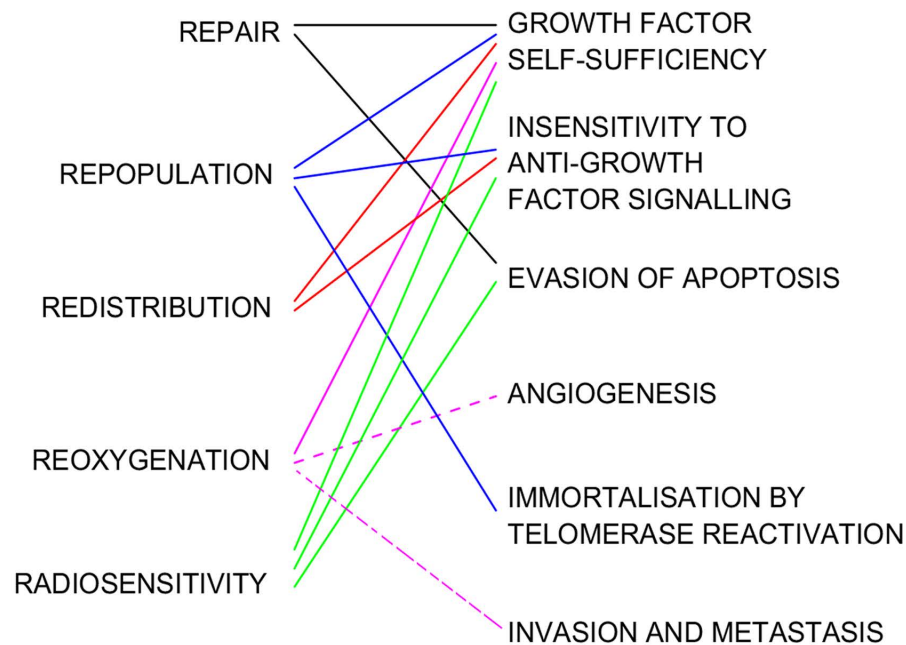
In addition, any candidate biomarkers that are identified will need to be validated through functional testing in model systems.



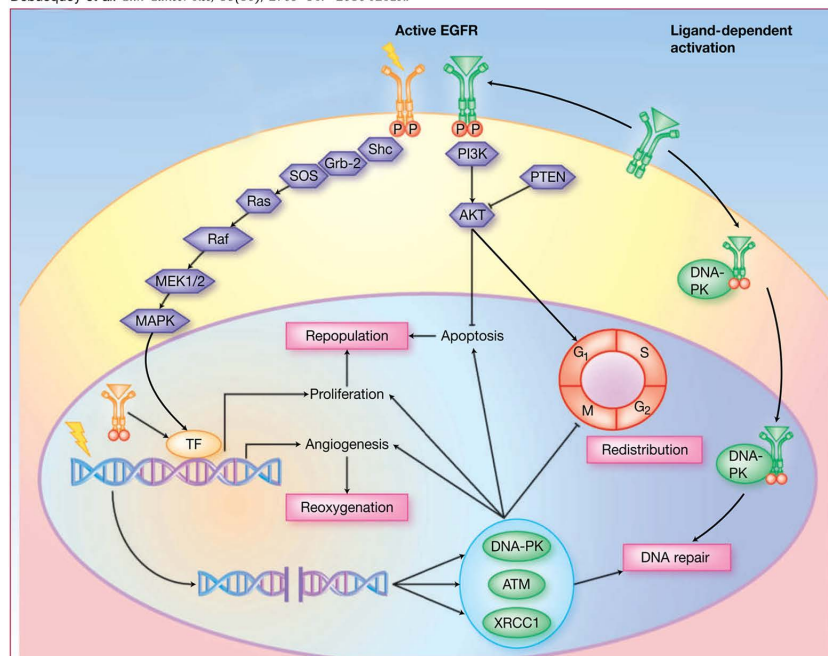
## Where does “foundational” radiobiology fit into the bigger cancer biology picture?

Technically, radiation biology is a sub-specialty within cancer biology, although historically, the field tended to keep to itself (to its detriment in many respects).

The challenge is to re-frame old school radiobiology concepts – elucidated, in most cases, decades before the modern era of molecular biology and biotechnology – in the context of modern cancer biology. For example:



Debucquoy et al. *Clin Cancer Res*; 16(10): 2709–14. ©2010 AACR.

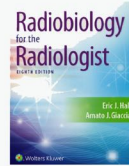




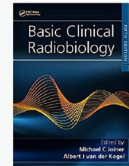
## Recommended Reading for Radiation and Cancer Biology

### Best two textbooks:

EJ Hall and AJ Giaccia. *Radiobiology for the Radiologist, Eighth Edition*. Wolters Kluwer, Philadelphia, 2019. ISBN: 978-1496335418.



M Joiner and A van der Kogel, Editors. *Basic Clinical Radiobiology, Fifth Edition*. Taylor & Francis (CRC Press), London, 2019. ISBN: 978-1444179637.



### Useful textbooks vis-à-vis radiation effects in humans:

FA Mettler and AC Upton. *Medical Effects of Ionizing Radiation, 3rd Edition*. Saunders-Elsevier, Philadelphia, 2008. ISBN: 978-0-7216-0200-4.

DC Shrieve and JS Loeffler. *Human Radiation Injury*. Lippincott Williams & Wilkins, Philadelphia, 2011. ISBN: 798-1-60547-011-5.

### Best textbook for general cancer biology:

**(Long-Awaited) New Edition!** L Harrington, I Tannock, R Hill and D Cescon. *The Basic Science of Oncology, 6th Edition*. McGraw Hill / Medical, New York, 2021. ISBN: 978-1-259-86207-6.

RA Weinberg. *The Biology of Cancer, 2<sup>nd</sup> Edition*. Garland Science, Taylor & Francis Group, LLC, New York, 2014. ISBN: 0-8153-4078-8.

### ABR's list of "secondary references":

Radiation-induced cell death mechanisms.

Eriksson D, Stigbrand T.

Tumour Biol. 2010 Aug;31(4):363-372. doi: 10.1007/s13277-010-0042-8. PMID: 20490962

[The hallmarks of cancer and the radiation oncologist: updating the 5Rs of radiobiology.](#)

Good JS, Harrington KJ.

Clin Oncol (R Coll Radiol). 2013 Oct;25(10):569-577. doi: 10.1016/j.clon.2013.06.009. PMID: 23850153

[The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence.](#)

Barker HE, Paget JT, Khan AA, Harrington KJ.

Nat Rev Cancer. 2015 Jul;15(7):409-425. doi: 10.1038/nrc3958. PMID: 26105538

[Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability.](#)

Bristow RG, Hill RP.

Nat Rev Cancer. 2008 Mar;8(3):180-192. doi: 10.1038/nrc2344. PMID: 18273037

[Hallmarks of cancer: the next generation.](#)

Hanahan D, Weinberg RA.

Cell. 2011 Mar 4;144(5):646-674. doi: 10.1016/j.cell.2011.02.013. PMID: 21376230

[Common markers of proliferation.](#)

Whitfield ML, George LK, Grant GD, Perou CM.

Nat Rev Cancer. 2006 Feb;6(2):99-106. doi: 10.1038/nrc1802. PMID: 16491069

[Opportunities and challenges of radiotherapy for treating cancer.](#)

Schaue D, McBride WH.

Nat Rev Clin Oncol. 2015 Sep;12(9):527-540. doi: 10.1038/nrclinonc.2015.120. PMID: 26122185

[Radiation resistance of cancer stem cells: the 4 R's of radiobiology revisited.](#)

Pajonk F, Vlashi E, McBride WH.

Stem Cells. 2010 Apr;28(4):639-648. doi: 10.1002/stem.318. PMID: 20135685.

[Cell cycle proteins as promising targets in cancer therapy.](#)

Otto T, Sicinski P.

Nat Rev Cancer. 2017 Jan 27;17(2):93-115. doi: 10.1038/nrc.2016.138. PMID: 28127048

[Molecularly targeted cancer therapy: some lessons from the past decade.](#)

Huang M, Shen A, Ding J, Geng M.

Trends Pharmacol Sci. 2014 Jan;35(1):41-50. doi: 10.1016/j.tips.2013.11.004. PMID: 24361003

[Role of local radiation therapy in cancer immunotherapy.](#)

Demaria S, Golden EB, Formenti SC.

JAMA Oncol. 2015 Dec;1(9):1325-1332. doi: 10.1001/jamaoncol.2015.2756. PMID: 26270858



## Radiation Oncology

### Initial Certification Qualifying (Computer-based) Exam: Study Guide for Radiation and Cancer Biology

This exam tests your knowledge of the principles of radiation and cancer biology underlying the practice of radiation oncology. Included are questions on the general domains listed below. Exam performance will be reported to you based on an overall pass/fail grade, with specific information provided regarding quintile performance in the 10 individual domains. Because of the nature of scientific knowledge and subcategories, there may be some overlap of items across domains. Each exam will include items from every domain, but individual subtopics may not be included in every exam and the number of items per domain depends on the domain.

I.	Interaction of radiation with matter	1% to 4%
II.	Molecular and cellular damage and repair	13% to 17%
III.	Cellular response to radiation	7% to 10%
IV.	Linear energy transfer (LET) and oxygen effect	3% to 5%
V.	Tumor biology and microenvironment	3% to 5%
VI.	Cancer biology	19% to 23%
VII.	Radiobiology of normal tissues	9% to 12%
VIII.	Dose delivery	11% to 15%
IX.	Combined modality therapy	11% to 15%
X.	Late effects and radiation protection	6% to 9%

The ranges above are those generally in effect for the exam to be administered in 2020 and are intended only for guidance in candidate preparation. They do not necessarily represent a precise number of scorable items.

- 
- I. Interaction of radiation with matter**
    - a. Definition of ionizing radiation, free radicals, and radical damage
    - b. Direct and indirect action of radiation, numbers and types of DNA lesions
    - c. Consequences of unrepaired DNA DSB
  - II. Molecular and cellular damage and repair**
    - a. Molecular mechanisms of DNA damage
      - i. Assays for measuring DNA damage and repair
      - ii. Single lethal hits, accumulated damage, and multiple damaged sites
    - b. Molecular mechanisms of DNA repair
      - i. Repair of base damage, single-strand and double-strand breaks



- ii. DSB repair: Homologous recombination and non-homologous end joining
  - iii. Molecular mechanisms of DNA DSB damage recognition and damage signaling to initiate repair
- c. Cellular recovery
  - i. Repair at the cellular level
  - ii. Sublethal damage repair
  - iii. Dose-rate effects and repair
  - iv. Dose-fractionation effects and repair
- d. Chromosome and chromatid damage
  - i. Assays for measuring chromosome damage – Giemsa to FISH
  - ii. Dose-response relationships
  - iii. Use of peripheral blood lymphocytes in *in vivo* dosimetry
  - iv. Human genetic diseases that affect DNA repair, fragility, and radiosensitivity
  - v. Stable and unstable chromatid and chromosome aberrations

### III. Cellular responses to radiation

- a. Mechanisms of cell death
  - i. Mechanisms and major characteristics of pathways of radiation-induced apoptosis, necrosis, autophagy, and senescence
  - ii. Mitotic-linked cell death and chromosome aberrations
  - iii. Cell division post-radiation and time to clonogen death
- b. Cell and tissue survival assays: measurement of response
  - i. In vitro clonogenic assays - effects of dose and dose rate
  - ii. In vivo clonogenic assays - bone marrow stem cell assays, jejunal crypt stem cell assay, skin clones, and kidney tubules
- c. Models of cell survival
  - i. Random nature of cell killing and Poisson statistics
  - ii. Single hit, multitarget models of cell survival – survival curve descriptors
  - iii. Linear-quadratic models: definition of  $\alpha/\beta$  ratio
  - iv. Calculations of cell survival with dose and dose rate
  - v. Shapes of the dose-response curves for early and late responding tissues
  - vi. Isoeffect curves and impact of changing fraction size and number on survival and LQ parameters

### IV. Linear energy transfer (LET) and oxygen effect

- a. Linear energy transfer
  - i. Definition of LET and quality of radiation
  - ii. RBE defined
  - iii. RBE as a function of LET in cells and tissues
  - iv. Effect on RBE on change in fractionation
- b. Oxygen Effect
  - i. Definition of OER
  - ii. Dose or dose per fraction effects
  - iii. OER vs LET
  - iv. Impact of  $O_2$  concentration
  - v. Mechanisms of oxygen effect

### V. Tumor biology and microenvironment

- a. Solid tumor assay systems
  - i. Concept of xenograft and syngeneic tumor models

- ii. Assay of tumor response to treatment– growth delay
- iii. TCD50 tumor control assay
- b. Tumor microenvironment
  - i. Characteristics of tumor vasculature and microenvironment; effect of radiation on them
  - ii. How tumor microenvironment can regulate tumor growth and vasculature
  - iii. Angiogenesis and neovasculogenesis
  - iv. Clinical consequence and relevance of hypoxia in tumors and tumor progressions
  - v. Reoxygenation after irradiation
  - vi. Cellular and molecular responses to hypoxia and hypoxia-induced signal transduction
  - vii. Cellular composition of tumors
  - viii. Immune microenvironment and role of inflammation

## VI. Cancer biology

- a. Cell and tissue kinetics
  - i. Methods to assess cell cycle kinetics
  - ii. Proteins involved in cell cycle control and checkpoint initiation (e.g., CDKs, cyclins, CDK inhibitors)
  - iii. Phases of cell cycle and radiation sensitivity
  - iv. Cell cycle arrest and redistribution after irradiation
- b. Molecular signaling
  - i. Main signaling pathways and critical proteins involved (e.g., PI3K/AKT, RAS/ERK, TGF- $\beta$ , Wnt, Notch, NFkB)
    - a) Receptors/ligand (e.g., EGFR, VEGFR, c-MET, HER2, FGFR, ALK)
    - b) Kinases
      - 1). Definition of kinases (e.g., STKs, TKs/RTKs, DSKs)
      - 2). Common kinases in cancer (e.g., ATM, ATR, Chk1, Chk2, PI3K, MAPK) and corresponding phosphatases (e.g., PTEN)
  - ii. Molecular signaling pathways activated by IR
  - iii. Transcription factors involved in cancer regulation (e.g., MYC, TP53 and associated proteins)
  - iv. Cell death pathways and main associated players
    - a). Intrinsic vs extrinsic apoptosis (caspases)
    - b). *BCL-2* family member proteins (pro- vs anti-apoptotic)
- c. Mechanisms of cancer development
  - i. Hallmarks of cancer and how they could affect 4/5 Rs of radiobiology
  - ii. Common oncogenes (e.g., *HER2/neu*, *Ras*, *Myc*) & tumor suppressors (*Rb*, *p16*, *p53*, *BRCA1/BRCA2*, *APC*, *NF1*)
  - iii. Telomeres and pathways in cancer to overcome telomere shortening (e.g., TERT promoter mutations and alternative lengthening of telomeres [ALT])
  - iv. Signaling abnormalities and association with treatment response
  - iv. Cancer as a genetic disease
  - v. Multistep nature of carcinogenesis
  - vi. Signaling abnormalities in carcinogenesis
  - vii. Prognostic and therapeutic significance of tumor characteristics
- d. Cancer genetics/genomics
  - i. Types of epigenetic regulation (e.g., DNA methylation (DNMTs/TETs), histone modifications (e.g. HDACs/HATs), chromatin remodelers)
  - ii. Main epigenetic alterations (e.g., CpG island methylator phenotype [CIMP]) in cancer
    - a). *IDH1/2* mutations in glioma and AML
    - b). *TET2* mutations in AML

- iii. Epigenetic targets in cancer (DNMTi, HDACi, IDHi, EZH2i)
- iv. Omics approaches in cancer (next-gen sequencing/arrays) and newer methods (ctDNA)
- v. Biomarkers in cancer (e.g., BCR-ABL, EGFR, ALK)
- vi. Molecular profiling of cancer

## **VII. Radiobiology of normal tissues**

- a. Clinically relevant normal tissue responses to radiation
  - i. Responses in early versus late responding tissues
  - ii. Reirradiation
- b. Mechanisms of normal tissue radiation responses
  - i. Molecular and cellular responses in slowly and rapidly proliferating tissues
  - ii. Mechanisms underlying clinical symptoms
  - iii. Tissue kinetics
- c. Total body irradiation
  - i. Prodromal radiation syndrome
  - ii. Acute radiation syndromes
  - iii. Mean lethal dose and dose/time responses
  - iv. Immunological effects
  - v. Assessment and treatment of radiation accidents
  - vi. Bone marrow transplantation

## **VIII. Dose delivery**

- a. Therapeutic ratio
  - i. Tumor control probability (TCP) curves
  - ii. Normal tissue complication probability (NTCP) curves
  - iii. Causes of treatment failure
- b. Time, dose, and fractionation
  - i. The four R's of fractionation
  - ii. Radiobiological rationale behind dose fractionation
  - iii. Effect of tissue/tumor type on the response to dose fractionation ( $\alpha/\beta$  ratios)
  - iv. Quantitation of multifraction survival curves
  - v. BED and isoeffect dose calculations
  - vi. Hypofractionation
- c. Brachytherapy
  - i. Dose-rate effects (HDR and LDR)
  - ii. Choice of isotopes
  - iii. Radiolabeled antibodies and other ligands
- d. Radiobiological aspects of different radiation modalities
  - i. Protons, high LET sources
  - ii. Stereotactic radiosurgery/radiotherapy, IMRT, IORT, and systemic radionuclides
  - iii. Dose distributions and dose heterogeneity

## **IX. Combined modality therapy**

- a. Chemotherapeutic agents and radiation therapy
  - i. Classes of chemotherapy agents
  - ii. Mechanisms of action
  - iii. Oxygen effect on radiation therapy and chemotherapy
  - iv. Main drug resistance mechanisms (e.g., MDR genes)
  - v. Interactions/synergism of chemotherapy with radiation therapy
  - vi. Targeted therapeutic agents



- b. Radiosensitizers, bioreductive drugs, and radioprotectors
  - i. Definition of therapeutic window
  - ii. Tumor radiosensitizers (e.g., oxygen) and mimics (e.g., nitromidazole)
  - iii. Normal tissue radioprotectors (e.g., amifostine)
  - iv. Biological response modifiers (e.g., IL-2 and IFN)
  - v. DNA repair inhibitors (e.g., PARPi, ATMi, ATRi, Chk1/2i)
- c. Immune therapeutics
  - i. Types of immunotherapy treatments in oncology
    - a) Monoclonal antibodies (MABs)
    - b) Checkpoint inhibitors
    - c) Cytokines
    - d) Vaccines
    - e) Adoptive cell transfer types (chimeric antigen receptors [CARs], tumor infiltrating lymphocytes [TILs], and T cell receptors [TCRs])
  - ii. Combination of immune therapies and radiation
    - a) Recently published trials (e.g., PACIFIC, KEYNOTE)
    - b) Known predictors of response/biomarkers
- d. Hyperthermia

## **X. Late effects and radiation protection**

- a. Radiation carcinogenesis
  - i. Dose response for radiation-induced cancers
  - ii. Importance of age at exposure, time since exposure, sex, and tissue
  - iii. Second tumors in radiation therapy patients
  - iv. Risk estimates in humans
- b. Heritable effects of radiation
  - i. Relative vs absolute mutation risk
  - ii. Doubling dose
  - iii. Heritable effects in humans
  - iv. Risk estimates for heritable effects
- c. Radiation effects in the developing embryo
  - i. Dependence of abnormalities and death on dose and gestational stage
  - ii. Microcephaly, intellectual disabilities
- d. Radiation protection
  - i. Stochastic effects and tissue reactions
  - ii. Tissue and radiation weighting factors
  - iii. Equivalent dose, effective dose, committed dose
  - iv. Dose limits for occupational and public exposure

**NOTE:** This study guide is subject to future revision as feedback is received on both content and clarity.