ICRP –1
DNA mutations and cancer

• Any mutation in a somatic cell (and particularly stem cells) can be step #1 toward cancer cell

• At least two mutations are needed to generate neoplastic phenotype and often more are needed.
Native DNA replication machinery is error prone

Table 1. Model parameters. These parameters were used for the algebraic model to see how colorectal cancer incidence scales with body size. Parameter values were taken from [7]. The mutation rate assumes that there are three genes (1 kb each) per pathway and a background mutation rate of $10^{-9}$ mutations per base pair per cell division.

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
<th>definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$u$</td>
<td>$3 \times 10^{-6}$</td>
<td>mutations/oncogenic pathway/cell division</td>
</tr>
<tr>
<td>$d$</td>
<td>$\text{age(days)}/4$</td>
<td>divisions since birth (rate = 1 div./4 days)</td>
</tr>
<tr>
<td>$k$</td>
<td>6</td>
<td>rate limiting mutations required for cancer</td>
</tr>
<tr>
<td>$N$</td>
<td>8</td>
<td>effective stem cells per crypt</td>
</tr>
<tr>
<td>$m$</td>
<td>$(1.5 \times 10^{-3} - 1.5 \times 10^{10})$</td>
<td>crypts per colon</td>
</tr>
</tbody>
</table>

Native DNA replication machinery is error prone – evidence from increased numbers of new mutations in children as parents as they age

![Figure 3](image_url)

**Figure 3** | Scatter plots with linear regression line on parental ages and their respective number of de novo mutations in the 61 trios with Illumina sequencing data. (a) The number of DNMs of paternal origin is plotted against the father’s age (in years). The blue line shows the linear fit (estimate of the slope $= 0.31, P = 5.15 \times 10^{-5}$) and the grey band represents the 95% confidence interval. (b) The number of DNMs of maternal origin is plotted against the mother’s age (in years), the blue line shows the linear fit (estimate of slope $= 0.12, P = 0.02$), and the grey band represents the 95% confidence interval.

Native DNA replication machinery is error prone – many cancers are spontaneous (at least initially).

Native DNA replication machinery AND numbers of stem cells may be inversely correlated to enable longevity.

**Figure 2.** Estimated somatic mutation rates scaling with size. Mutation rate estimates show that a 3.2-fold decrease enables an animal that is $1000 \times$ larger (and so with $1000 \times$ more stem cells) than a human to have the same cancer risk. The mutation rates shown in the plot resulted in cancer risk predictions for the given number of cells that best matched the estimates for human (i.e. $1.2 \times 10^8$ colonic stem cells) using the Calabrese–Shibata algebraic model [7].

Stem cells

Embryonic Stem Cells

- In vivo fertilized egg
- 8 cell embryo
- Cultured undifferentiated stem cells
- Pluripotent
- Blastocyst
- Neural cells
- Cardiac muscle
- Blood cells

Adult Stem Cells

- Brain
- Heart
- Bone marrow
- Neural cells
- Cardiac muscle
- Blood cells

- Long-Term repopulating potential (LTRP)
- Self renewal
- Multi-lineage differentiation capacity
Cancer stem cells
- form in-vitro Sarcospheres
- highly tumorigenic after injection in recipient mice

(Red Fluorescence: Osteosarcoma cells were stably labelled with Cherry-Fluorescence-Protein)
Multistep-Model of Carcinogenesis

Initiation
 Activation of Oncogenes

Hyperplasia

Promotion
 Mutation in Tumor-Suppressor-Genes

Carcinoma in situ

Progression
 Activation of genes that promote metastasis, invasion, angiogenesis immunological tolerance etc.

malignant Tumor
FIGURE 22.11 Illustrating a typical growth curve for an animal tumor, which is best fitted by a Gompertz function. When a tumor is composed of only a few cells, it may grow exponentially, but when it gets larger, the growth rate slows as the supply of oxygen and nutrients are outgrown.

E. J. Hall and A. J. Giaccia, “Radiobiology for the Radiologist,” 8th Edition, Lippincott Williams & Wilkins, Philadelphia, 2018. pg. 411. Fig. 22.11
DNA mutations and radiation

• Ionizing radiation is one of possible sources of DNA mutations
• Ionizing radiation is considered a weak mutagen compared to many others
• At high enough doses, radiation can induce cell death as well
Figure 1.9  Illustration of the generally accepted sequence of events from the absorption of radiation to the expression of the various forms of biological damage. (Developed in collaboration with Dr. Noelle Metting, U.S. Department of Energy.)
DNA and Ionizing Radiation

For Low LET radiation, 67% of damage is indirect action.

For High LET radiation, most (all?) of the damage is direct action.

Critical distance of indirect IR action is within 2nm radius from DNA.

(From Hall and Giaccia) Figure 1.8 Direct and indirect actions of radiation. The structure of DNA is shown schematically. In direct action, a secondary electron resulting from absorption of an x-ray photon interacts with the DNA to produce an effect. In indirect action, the secondary electron interacts with, for example, a water molecule to produce a hydroxyl radical (OH·), which in turn produces the damage to the DNA. The DNA helix has a diameter of about 20 Å (2 nm). It is estimated that free radicals produced in a cylinder with a diameter double that of the DNA helix can affect the DNA. Indirect action is dominant for sparsely ionizing radiation, such as x-rays. S, sugar; P, phosphorus; A, adenine; T, thymine; G, guanine; C, cytosine.
Low vs. High LET Ionization Pattern

Proton and carbon ion tracks are compared microscopically to an illustration of a DNA molecule before, in and behind the Bragg maximum, for the same energy [41].

Sylvester CB, Abe JI, Patel ZS, Grande-Allen KJ. Radiation-Induced Cardiovascular Disease: Mechanisms and Importance of Linear Energy Transfer. Front Cardiovasc Med. 2018 Jan 31;5:5.

Fokas et al. Biochimica et Biophysica Acta 1796: 219
DNA Damage Induced by high and low LET

Repair of simple and complex DNA lesions induced by low- and high-LET radiation exposure. A majority of the DNA lesions induced by low-LET irradiation are simple lesions and are repaired within hours of induction via NHEJ- and HR-mediated repair pathways, with pathway preference dependent on cell cycle. On the other hand, a majority of the high-LET radiation-induced DNA damages are clustered lesions, which may impede DNA repair pathways, causing damage to remain unrepaired for longer periods (days to weeks). In addition to radiation-induced ROS, unrepaired DNA lesions may also increase the ROS levels in cells, causing further generation of simple to complex DNA lesions. Unrepaired/misrepaired lesions in mitochondrial or nuclear DNA (dotted line) may also further enhance and perpetuate ROS levels. Ultimately, the unrepaired/ misrepaired DNA lesions may promote genomic instability, leading to initiation of carcinogenesis.

RBE versus LET from published experiments on in vitro cell lines. RBE is calculated at 10% survival, LET values are given in keV/µm in water. Different colours indicate different ions, from protons to heavy ions. Data points are extracted from the Particle Radiation Data Ensemble (PIDE) database, which currently includes 855 survival curves for cells exposed to photons (α/β ratio ranging 1–30) and ions. Abbreviations: LET, linear energy transfer; RBE, relative biological effectiveness.
Cell and Tissue Damage

Schematic representation of known mechanistic links between biomarkers that define cell fates, which promote or protect from cancer risk.

Sources of Ionizing Radiation

Annual risk (Americans, excluding radiation therapy)

- Radon, 'natural', but preventable, causes ~10% of all lung cancer, according to BEIR VI
- Medical imaging procedures contribute almost 50% of total exposure, which would contribute ~50K fatal cancers, however much of this is delivered to fatally ill patients.
- Occupational exposure only causes ~100 cancers per year, but this is concentrated in a small cohort -- consisting mostly of aviation and medical workers.

Hall 2012 figure 16.6
Sources of Ionizing Radiation

**FIGURE 15.6** Percentage contribution of the various sources of exposure to the US population (in 2006) and the UK population (in 2010). The average annual effective dose per individual in the United States is more than double that in the United Kingdom (6.2 mSv compared with 2.7 mSv). There is not much difference in the contribution from natural background radiation between the two countries but the contribution from medical radiation is 7 times larger in the United States than in the United Kingdom (US data from National Council on Radiation Protection and Measurements; Ionizing Radiation Exposure of the Population of the United States, Bethesda, MD: National Council on Radiation Protection and Measurements; 2009. Report 160; UK data from Public Health England, 2016.)

E. J. Hall and A. J. Giaccia, “Radiobiology for the Radiologist,” 8th Edition, Lippincott Williams & Wilkins, Philadelphia, 2018. pg. 211. Fig. 15.6
Exceptional acute and chronic radiation exposures
Acute radiation effects at high doses are separated into distinct syndromes.

**FIGURE 9.2** Showing the result of an experiment in which nonhuman primates were given the granulocyte colony-stimulating factor (G-CSF, filgrastim [Neupogen]) 24 hours after a total-body irradiation (TBI) of 7.5 Gy. Eighty percent of the animals survived compared with 40% in controls that did not receive the G-CSF. In other experiments (results not shown), the agent proved to be ineffective if administration was delayed to 48 hours postirradiation. (Adapted from Farese AM, Cohen MV, Katz BP, et al. Filgrastim improves survival in lethally irradiated nonhuman primates. *Radiat Res.* 2013;179:89–100; and Farese AM, Brown CR, Smith CP, et al. The ability of filgrastim to mitigate mortality following LD_{50/60} total-body irradiation is administration time-dependent. *Health Phys.* 2014;106[1]:39–47.)

Post radiation events include different types of cancer—numbers of stem cells affected are important when cancer type is to be considered.

**FIGURE 10.17** Relative risk of leukemia, excluding chronic lymphocytic leukemia, associated with 2-year lagged cumulative red bone marrow dose. The lines are the fitted linear dose-response model for different dose ranges, whereas the shaded areas represent the 90% CIs. (Adapted from Leuraud K, Richardson DB, Cardis E, et al. Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers [INWORKS]: an international cohort study. Lancet Haematol. 2015;2:e276–e281.)

E. J. Hall and A. J. Giaccia, “Radiobiology for the Radiologist,” 8th Edition, Lippincott Williams & Wilkins, Philadelphia, 2018. pg. 154 Fig. 10.17
**FIGURE 13.2** Clinical appearance of a typical radiation cataract in the posterior scapular region in an interventional cardiologist with 22 years of occupational radiation exposure. **A:** Retroillumination image (i.e., using the light that is reflected by the retina back through the lens). **B:** Conventional slit lamp imaging (i.e., where an optical section of the lens is directly visualized). In both cases, the position of the opacity is indicated by an arrow.
(Courtesy of Dr. Norman Kleiman.)
Radiation uses in therapy

• High doses – low volume of tissue
Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer

Model for non-redundant mechanisms and resistance to RT and immune checkpoint blockade.

Anticancer immunotherapy. Several anticancer immunotherapeutics have been developed during the last three decades, including tumor-targeting and immunomodulatory monoclonal antibodies (mAbs); dendritic cell (DC)-, peptide- and DNA-based anticancer vaccines; oncolytic viruses; pattern recognition receptor (PRR) agonists; immunostimulatory cytokines; immunogenic cell death inducers; inhibitors of immunosuppressive metabolism; and adoptive cell transfer. 1MT, 1-methyltryptophan; APC, antigen-presenting cell; IDO, indoleamine 2,3-dioxigenase; IFN, interferon; IL, interleukin; IMiD, immunomodulatory drug; NLR, NOD-like receptor; TLR, Toll-like receptor.

Ionizing radiation induces immunogenic cell death of tumors, which facilitates cross-priming of CTLs. Ionizing radiation induces translocation of calreticulin (CRT) to the tumor cell membrane, which acts as an “eat me” signal to dendritic cells (DCs), facilitating receptor mediated endocytosis through CD91. This makes tumor antigens available for cross-presentation on MHC-I for priming of tumor-specific T-cells. Radiotherapy also induces the release of danger associated molecular patterns (DAMPs), such as ATP and HMGB-1, which are endogenous immune adjuvants that stimulate DC activation, inducing DCs to provide co-stimulatory signals to naïve T-cells, facilitating cross-priming of CTLs. Together, these processes constitute immunogenic cell death of tumor cells.

LNT model ... or not

FIGURE 10.19 Illustrating the controversy of how to extrapolate cancer risks from high doses, for which there are epidemiologic data, to low doses characteristic of the radiation protection scenario. Line B illustrates the linear no-threshold hypothesis, favored by BEIR, UNSCEAR, ICRP, and NCRP. Line A assumes that risks are higher at low doses than would be predicted from a linear extrapolation. This might, for example, be a consequence of the bystander effect. Line C assumes that there is a threshold in dose, below which there are no deleterious biologic effects. Line D illustrates the hypothesis that low levels of radiation are beneficial, activating repair mechanisms that protect against disease; this is known as hormesis.