3 classes of genes:

(1) Oncogenes (dominant pattern)
(2) Tumor suppressor genes (recessive pattern)
(3) DNA stability genes

4 mechanisms of activation of proto-oncogene:

(1) Retroviral activation eg c-src gene (rat sarcoma) [no human examples]
(2) Gene deletion/ point mutation, eg N-ras (Melanoma), K-ras (Colon cancer),neu (neuoblastoma)
(3) Gene amplification, eg cerbB2 (breast cancer), N-myc (neuroblastoma), L-myc (lung cancer), EGFR (squamous cell carcinoma)
(4) Chromosomal translocation, eg bcr-abl [(t9;22)] (CML),
c-myc [(8;14)} & [t(2;8)] (Burkitt's lymphoma),
blc-2 [(t14;18)] (DLBCL)

Tumor suppressor genes:

Tumor-Suppressor Gene	Syndrome	Tumor
Rb	Retinoblastoma	Retinoblastoma
WT1	Familial Wilms' tumor	Wilms' tumor
NF1	Neurofibromatosis type 1	Neurofibroma, sarcoma
NF2	Neurofibromatosis type 2	Schwannoma, meningioma
APC	Familial adenomatosis polyposis	Tumor of colon, stomach, intestine
p53	Li-Fraumeni Syndrome	Breast, lung, brain tumors, sarcoma
VHL	von Hippel-Lindau disease	Tumor of kidney, adrenal
E-CAD	Familial gastric cancer	Tumor of stomach, breast
PTCH	Gorlin syndrome	Basal cell carcinoma
PTEN	Cowden syndrome	Hamartoma
MEN1	Multiple endocrine neoplasia	Tumor of pituitary, pancreas, parathyroid

Somatic homozygosity:

Both copies of a suppressor gene in the sporadic form of retinoblastoma and other solid tumors may result from two independent allelic mutations, but in practice, it occurs more often by the process of somatic homozygosity. The steps appear to be as follows: One

chromosome of a pair is lost, a deletion occurs in the remaining chromosome, and the chromosome with the deletion replicates. Instead of having each of the two alleles contributed by different parents, the cell has both alleles from the same parent, with loss of a vital piece containing the tumor-suppressor gene .This process has been documented for chromosome 13 in the case of retinoblastoma, chromosome 11 in Wilms' tumor, chromosome 3 for small-cell lung cancer, and chromosome 5 for colon cancer.

Development of malignancy:

(1) Deregulated proliferation
(2) Failure to respond to growth restrictive signals
(3) Failure to undergo apoptosis
(4) Escaping senescence
(5) Angiogenesis
(6) Invasion & metastasis

Deregulated proliferation:
Due to overexpression of growth factor receptors, eg EGFR,PDGFR.
Growth-factor receptors can structurally be divided into extracellular ligand-binding domains, transmembrane-spanning domains, and intracellular kinase domains.
There are numerous intracellular circuits that transduce the signal from the cell surface to the nucleus of the cell. The Src, Ras and Abl proteins are all members of this group. By and large, most members of this group are tyrosine-kinases or serine/threonine-kinases.
Src and **Abl** are tyrosine-kinases located on the **cytoplasmic** side of the cell membrane. **H-ras**, **K-ras** and **N-ras** are a family of GTP-binding proteins also located on the **cytoplasmic** side of the cell membrane and are the most frequently mutated oncogene family in human cancers.

Failure to respond to growth restrictive signals:
Loss of Rb or failure to produce p21 or lack of NF1protein.
When Rb protein is in a hypophosphorylated state, Rb protein blocks progression into S phase by sequestering E2F transcription factors that regulate the expression of genes that are essential for the transition from G_1 to S phase.

Failure to undergo apoptosis:
Caspases are responsible for apoptosis. Pro-apoptotic: p53, bax, bid genes
Anti-apoptotic: bcl-2, myc genes

Escaping senescence:
Loss of p53 or p16.
Activation of telomerase

Angiogenesis:

Numerous angiogenic factors have been identified, including specific endothelial cell growth factors (e.g., vascular endothelial growth factor, or VEGF), cytokines and inflammatory agents (e.g., tumor necrosis factor α, or TNF-α, and interleukin-8, or IL-8), fragments of circulatory system proteins (e.g., angiostatin and endostatin), and extracellular matrix components (e.g., thrombospondins, or TSPs).TSP-1 is an important anti-angiogenic factor.

Invasion & Metastasis:
Decreased expression/ loss of function of E-Cadherin
Decreased expression of NCAM

DNA Repair genes:

Suppressor	Syndrome	Tumor
ATM	Ataxia-telangiectasia	Leukemia, lymphoma
XP	Xeroderma pigmentosum	Skin
BRCA1	Hereditary breast cancer 1	Breast
BRCA2	Hereditary breast cancer 2	Breast, ovary
FANC	Fanconi's anemia	Leukemia
NBS	Nijmegen breakage syndrome	Lymphoma
hMSH2	Hereditary nonpolyposis colorectal cancer	Colon
hMLH1	Hereditary nonpolyposis colorectal cancer	Colon
hMSH6	Hereditary nonpolyposis colorectal cancer	Colon
hPMS1	Hereditary nonpolyposis colorectal cancer	Colon

Radiation-induced signal transduction:

The PIKK family is comprised of proteins such as **ATM** and **DNA-PK** that sense and respond to DNA strand breaks.
In unstressed cells, ATM exists as a homodimer in which the kinase domain is sterically blocked by its tight binding to a region that includes serine 1981.

In response to DNA strand breaks, ATM changes conformation and autophosphorylates at serine 1981, resulting in dissociation of the inactive dimer into a monomer. The resulting ATM monomer is the active species that in turn phosphorylates target proteins involved in cell-cycle control and DNA repair such as Nijmegen breakage syndrome (**NBS**), breast cancer 1 (**BRCA1**), structural maintenance of chromosome 1 (**SMC1**), **H2AX**, and **p53BP1**. The **p53** protein is also an ATM target that plays a major role in the regulation of the mammalian cellular stress response, in part through the transcriptional activation of genes involved in cell-cycle control, DNA repair, and apoptosis.

Cell Cycle Checkpoints:

(1) G1-S: Key players in the G_1 restriction point include the protein of the Rb gene, D-type cyclins, and Cdk4 and Cdk6, as well as Cdk inhibitors. The tumor suppressor gene p53 is critical for G1 arrest, as also the ATM and Chk genes.
(2) S: Cyclin A is maximally expressed in S phase and enhances transition of the cell through this phase of the cycle. ATM gene is important for Cyclin A inactivation.
(3) G2-M: Complex of cyclins B and A with Cdk1. ATM & Chk genes are important for G2 arrest.

Progression through the cell cycle is governed by protein kinases, activated by cyclins. Each cyclin protein is synthesized at a discrete phase of the cycle: cyclin D and E in G_1, cyclin A in S and G_2, and cyclin B in G_2 and M. Transitions in the cycle occur only if a given kinase activates the proteins required for progression.

Therapeutic Ratio (Therapeutic Index)

The ratio of the tumor response for a fixed level of normal-tissue damage has been variously called the therapeutic ratio or therapeutic index.

Particle Beam Therapy:

<u>Neutrons</u>

- Neutrons are **indirectly ionizing**. In tissue, they give up their energy to produce **recoil protons, α-particles, and heavier nuclear fragments**.
- Biologic properties of neutrons differ from those of x-rays in several ways: reduced OER, little or no repair of sublethal damage or potentially lethal damage, and less variation of sensitivity through the cell cycle.
- The rationale for the use of neutrons in radiotherapy has changed over the years. The earlier rationale was the reduced OER to overcome the problem

of hypoxic cells. The revised rationale is based on a **higher neutron RBE for slowly growing tumors**.

- An advantage has been proved in clinical trials for neutrons in the treatment of salivary gland and prostate tumors and soft-tissue sarcomas, but not for the majority of cancer sites tested.
- A new generation of hospital-based cyclotrons, generating neutrons by the $p^+ \rightarrow$ Be reaction, are now available, but enthusiasm for neutrons has waned.

Boron Neutron-Capture Therapy

- The principle of boron neutron-capture therapy (BNCT) is to deliver a drug containing boron that localizes only in tumors and then to treat with low-energy thermal neutrons that interact with boron to produce α-particles.
- Boron is a suitable substance because it has a large cross section for thermal neutrons and emits short-range densely ionizing α-particles if bombarded by thermal neutrons. Its chemistry is such that it can be incorporated into a wide range of compounds.
- Many attempts have been made to synthesize boron-containing compounds that are selectively localized in tumors relative to normal tissues, with limited success. They fall into two categories:
  - Low-molecular-weight agents that simulate chemical precursors needed for tumor cell proliferation
  - High-molecular-weight agents such as monoclonal antibodies and bispecific antibodies
- **Thermal** neutrons are poorly penetrating in tissue, with a half-value layer of only **1.5 cm**.
- **Epithermal** neutrons are somewhat more penetrating. They are degraded to thermal neutrons by collisions with hydrogen atoms in tissue. The peak dose is at **2 to 3 cm**, and the high surface dose is avoided.
- Results of clinical trials of the efficacy of BNCT are tantalizing but not definitive.

- The concept of BNCT is very attractive, but there are formidable practical difficulties in making it a treatment modality even for relatively shallow tumors.

Protons

- Protons result in excellent physical dose distributions.
- Protons have biologic properties similar to those of x-rays.
- There is an established place for protons in the treatment of **choroidal melanoma** or tumors close to the **spinal cord**, in which a sharp cutoff of dose is important.
- Hospital-based high-energy cyclotrons with isocentric mounts are now being used to treat a broader spectrum of cancer patients with protons. Their efficacy has yet to be proved in clinical trials, but they offer the obvious physical advantage of good dose distributions with reduced dose to normal structures.

Carbon Ion Radiotherapy

- The use of high-energy carbon ions has experienced a renaissance in Europe and Japan.
- For carbon ions, as for protons, the narrow Bragg peak must be spread out to cover a tumor of realistic dimensions by varying the energy of the beam. For a synchrotron, SOBP is possible by varying the energy from pulse to pulse and deflecting with magnets.
- Inevitably, the RBE varies across the spread-out Bragg peak. In theory, this could be an advantage for carbon ions because it further exaggerates tumor dose relative to normal tissue. In practice, it is a complication because one must estimate RBE values from experiments in model systems.
- A unique attraction of carbon ion therapy is that the target volume can be visualized by **PET** as some ^{12}C ions decay to radioactive ^{11}C and ^{10}C. These are positron-emitting isotopes, which undergo annihilation reaction with neighbouring electrons, producing gamma rays which can be detected on PET, thus the location of the Bragg peak and the high-dose region can be visualised.

Model Tumor Systems:

- The five assays in common use are tumor growth delay measurements, tumor cure (TCD_{50}) assay, tumor cell survival determined by the dilution assay

technique, the production of lung colonies, and in vivo treatment followed by in vitro assay.

- Many human tumor cells can be grown as xenografts in immune-deficient animals. Although the histologic characteristics of the human source tumor are maintained, the stroma is of mouse origin.
- Programmed cell death, or apoptosis, occurs after irradiation in many animal tumors, as well as in human xenographs in nude mice.
- Apoptosis is most important in lymphomas, essentially absent in sarcomas, and intermediate and variable in carcinomas.
- Cells may show signs of dying an apoptotic death by 3 hours after irradiation.

Apoptosis is characterized by a stereotyped sequence of morphologic events that take place in two discrete phases. In the first phase, cells condense and bud to produce many membrane-enclosed bodies. In the second phase, these bodies are phagocytized and digested by nearby tissue cells. Apoptosis characteristically affects scattered individual cells.

The skin, intestinal epithelium, and bone-marrow cells, for example, are rapidly dividing self-renewal tissues and respond early to the effects of radiation.

The spinal cord, lung, and kidney, by contrast, are late-responding tissues.

This reflects the current philosophy that the radiation response of all tissues results from the depletion of the critical parenchymal cells and that the difference in time at which early- and late-responding tissues express radiation damage is a function simply of different cell turnover rates.

Many older papers in the literature ascribe the response of late-responding tissues to vascular damage rather than to depletion of parenchymal cells, but this thesis is becoming increasingly difficult to accept.

Radiotherapy & Cataract:

- A cataract is an opacification of the normally transparent lens of the eye.

- Dividing cells are limited to the preequatorial region of the epithelium. Progeny of these mitotic cells differentiate into lens fibers and accrete at the equator. It is the failure of these cells to differentiate correctly that leads to a cataract, whether spontaneous or radiation induced.
- A unique feature of the lens is that there is no mechanism for the removal of dead or damaged cells.
- The minimum dose required to produce a progressive cataract is about 2 Gy (200 rad) in a single exposure; larger doses are necessary in a fractionated or protracted exposure. The minimum dose increases to 4 Gy (400 rad) spread over 3 weeks to 3 months and 5.5 Gy (550 rad) for more than 3 months.
- The latent period between irradiation and the appearance of a lens opacity is dose related. The latency is about 8 years after exposure to a dose in the range of 2.5 to 6.5 Gy (250-650 rad).
- It is never possible to state unequivocally that a given cataract is radiation induced; however, the appearance of a cataract at the posterior pole of the lens in an individual with a radiation history strongly suggests radiation as the causative agent. On the other hand, it is possible to say with some certainty that some cataracts—for example, nuclear cataracts—do not have a radiation etiology.
- The RBE of neutrons or heavy ions is about 10 at high doses but rises to 50 or more for small doses.
- There is evidence of early cataracts in astronauts exposed to high-energy heavy ions.
- A radiation-induced cataract is a deterministic late effect. There is a practical threshold dose below which cataracts are not produced, and above this threshold the severity of the biologic response is dose related.

Effect of Radiation on Developing Embryo:

- The effects depend on the **stage** of gestation, the **dose,** and also the **dose rate**.
- Gestation is divided into **preimplantation**, **organogenesis,** and the **fetal** period. In humans, these periods correspond to about **0 through 9 days**, **10 days through 6 weeks**, and **6 weeks through term**, respectively.
- The principal effects of radiation on the developing embryo and fetus, aside from cancer, are embryonic, fetal, or neonatal death; congenital

malformations; growth retardation; and functional impairment, such as mental retardation.

- Irradiation during **preimplantation** leads to potential **death** of the embryo. Growth retardation or malformations are not seen in animals from irradiation at this time. The human data are consistent with this conclusion.
- In animals, embryos exposed to radiation in early **organogenesis** exhibit the most severe intrauterine growth retardation, from which they can recover later (i.e., **temporary growth retardation**).
- Irradiation in the **fetal** period leads to the greatest degree of **permanent growth retardation**.
- In animals, lethality from irradiation varies with stage of development. The **embryonic 50% lethal dose is lowest during early preimplantation**; at this stage, embryos killed by radiation suffer a prenatal death and are resorbed. In organogenesis, prenatal death is replaced by neonatal death—death at or about the time of birth. During the fetal stage, the 50% lethal dose approaches that of the adult.
- In animals, the peak incidence of teratogenesis, or gross malformations, occurs if the fetus is irradiated in organogenesis.
- In contrast to what is observed in experimental animals, radiation-induced malformations of body structures **other** than the central nervous system are **uncommon** in the Japanese survivors irradiated **in utero**, although they have been reported in patients exposed to therapeutic doses of medical radiation.
- In the Japanese survivors, irradiation in utero resulted in small head size (**microcephaly**) and **mental retardation**.
- Mental retardation from irradiation occurred primarily at **8 to 15 weeks** of gestational age, with a smaller excess at 16 to 25 weeks. It is thought to be caused by radiation effects on cell migration within the brain.
- The incidence of **severe mental retardation** as a function of dose is apparently linear without threshold at 8 to 15 weeks, with a risk coefficient of **0.4 per Gy** (0.4 per 100 rad). The incidence is about four times lower at 16 to **25 weeks**. The data are also consistent with a **dose threshold of 0.12 to 0.2 Gy** (12-20 rad).
- Small head circumference was more common than mental retardation.

- Data on atomic-bomb survivors indicate that **microcephaly** can result from exposure at 0 to 7 and 8 to **15 weeks** postovulation, but not at later times. There is **little** evidence for a threshold in dose.
- A variety of effects have been documented in experimental animals after irradiation during fetal stages, including effects on the hematopoietic system, liver, and kidney, all occurring, however, after quite high radiation doses.
- There is an association between exposure to diagnostic x-rays in utero and the subsequent development of childhood malignancies.
- The original study of diagnostic x-ray exposure in utero and subsequent malignancies, principally leukemia, was done by Stewart and Kneale at Oxford University, but the same association was observed in the United States by MacMahon. If x-rays are the causative agent, these studies imply that radiation at **low doses in utero increases the spontaneous cancer incidence in the first 10 to 15 years of life by 50%**—that is, by a factor of 1.5 to 2.
- It has been argued for years whether radiation is the causative agent or whether there are other factors involved, such as the selection of a particular group of children prone to cancer.
- Doll and Wakeford in 1997 summarized all of the evidence for and against and concluded that **an obstetric x-ray examination, particularly in the third trimester, increased the risk of childhood cancer by 40%. The risk is increased by a dose of only 10 mGy (1 rad). The excess absolute risk is about 6% per gray (100 rad),** which is not very different from the risk estimates from the atomic-bomb survivors for adult exposure.
- **Until** a pregnancy is declared, **no** special limits apply to women other than those applicable to any radiation worker. Once a pregnancy is declared, the maximum permissible dose to the fetus is 0**.5 mSv (0.05 rem) per month**.
- Once a pregnancy is declared, the duties of a radiation worker should be reviewed to ensure that this limit is not exceeded.
- A dose of **0.1 Gy (10 rad)** to the embryo during the sensitive period of gestation (**10 days to 25 weeks**) often is regarded as the cutoff point above which a therapeutic abortion should be considered to avoid the possibility of an anomalous child. The decision to terminate a pregnancy should be flexible and must depend on many factors in addition to dose.

Radiation Carcinogenesis:

- Deterministic effect has a **threshold** in dose, and the **severity** of the effect is dose related. Radiation-induced cataracts are an example of a deterministic effect.
- Radiation carcinogenesis is a **stochastic** effect; that is, the **probability** of an effect increases with dose, with **no** dose threshold, but the **severity of the effect is not dose related**. Hereditary effects are also stochastic.
- Latency refers to the time interval between irradiation and the appearance of the malignancy.
- The shortest latency is for leukemia, with a peak at **5 to 7 years**.
- For solid tumors, the latency may extend for 60 years or more.
- Regardless of the age at exposure, radiation-induced malignancies tend to appear at the **same age** as spontaneous malignancies of the same type. Indeed, for solid cancers, the excess risk is apparently more like a **lifelong elevation of the natural age-specific cancer risk**.
- The reassessment of radiation-induced cancer risks by the BEIR V committee was based on a **time-related relative risk model**. Excess cancer deaths were assumed to depend on **dose**, **square of the dose**, **age at exposure**, **time since exposure**, and, for some cancers, **gender.**
- For **solid tumors**, the excess cancer incidence was found to be a **linear** function of dose up to about 2 Sv (200 rem).
- **Leukemia** data were best fitted by a **linear-quadratic** function of dose.
- Based on reports of the UNSCEAR and BEIR V committees, the ICRP suggests a risk estimate of excess cancer mortality in **a working population** of **8×10^{-2} per sievert for high doses and high dose rates** and **4×10^{-2} per sievert for low doses and low dose rates.**
- For the **general population**, slightly higher risks apply because of the increased susceptibility of the young. The ICRP estimates are **10×10^{-2} per sievert for high doses and dose rates** and **5×10^{-2} per sievert for low doses and dose rates**.
- The ICRP estimates that, on average, 13 to 15 years of life are lost for each radiation-induced cancer and that death occurs at age 68 to 70 years.

- Radiation induced solid tumors include both carcinomas and sarcomas. Carcinomas can occur in regions receiving high and low doses, whereas **sarcomas** occur only in regions receiving **high** doses.

Hereditary Effects of Radiation:

- In the male, doses as low as **0.15 Gy** (15 rad) result in **oligospermia** (diminished sperm count) after a latent period of about 6 weeks.
- Doses above **0.5 Gy** (50 rad) result in **azoospermia** (absence of living spermatozoa) and therefore **temporary sterility**. Recovery time depends on dose.
- **Permanent sterility** in the **male** requires a **single** dose in excess of **6 Gy** (600 rad).
- In the **male**, fractionated doses cause more gonadal damage than a single dose. Permanent sterility can result from a dose of **2.5 to 3 Gy** (250-300 rad) in a **fractionated** regime over 2 to 4 weeks.
- In the female, radiation is highly effective in inducing permanent ovarian failure, with a marked age dependence on the dose required.
- The dose required for **permanent sterility** in the female varies from **12 Gy** (1,200 rad) prepubertal to **2 Gy (**200 rad) premenopausal.
- The induction of sterility in males does not produce significant changes in hormone balance, libido, or physical capability, but in the female leads to pronounced hormonal changes comparable to natural menopause.
- Exposure of a population can cause adverse health effects in the descendants as a consequence of mutations induced in germ cells. These used to be called “genetic” effects but are now more often called “hereditary” effects.
- Hereditary diseases are classified into three principal categories: **Mendelian, chromosomal,** and **multifactorial**.
- Radiation does **not** produce new, unique mutations but increases the incidence of the same mutations that occur spontaneously.
- Information on the hereditary effects of radiation comes almost entirely from animal experiments. The earlier mutation experiments were carried out with the fruit fly Drosophila melanogaster.

- Relative mutation rates have been measured in the megamouse project by observing seven specific locus mutations. This leads to an estimate of the "doubling dose."
- The **doubling dose** is the dose required to double the spontaneous mutation incidence; put another way, it is the dose required to produce an incidence of mutations **equal** to the spontaneous rate. Based on the mouse data, the doubling dose for low dose-rate exposure is estimated to be **1 Gy** (100 rad).
- Not more than 1 to 6% of spontaneous mutations in humans may be ascribed to background radiation.
- To estimate the risk of radiation-induced hereditary diseases in the human, two quantities are required: (1) the baseline mutation rate for humans, which is estimated to be 738,000 per million, and (2) the doubling dose from the mouse data, which is about 1 Gy (100 rad).
- Two correction factors are needed: (1) to allow that not all mutations lead to a disease—this is the mutation component (MC), which varies for different classes of hereditary diseases; (2) to allow for the fact that the seven specific locus mutations used in the mouse experiments are not representative of inducible hereditary diseases in the human because they are all nonessential for the survival of the animal or cell.
- The International Commission on Radiological Protection (ICRP) estimates that the hereditary risk of radiation is about **0.2%/Sv** for the general population and about **0.1%/Sv** for a working population.
- In terms of detriment, expressed in years of life lost or impaired, congenital anomalies (i.e., resulting from effects on the developing embryo and fetus) are much more important than hereditary disorders.
- Children of the atomic-bomb survivors have been studied for a number of indicators, including congenital defects, gender ratio, physical development, survival, cytogenetic abnormalities, malignant disease and electrophoretic variants of blood proteins. A recent paper estimated the doubling dose to be about 2 Sv (200 rem), with a lower limit of 1 Sv (100 rem) and an upper limit that is indeterminate because the increase in mutations is not statistically significant.

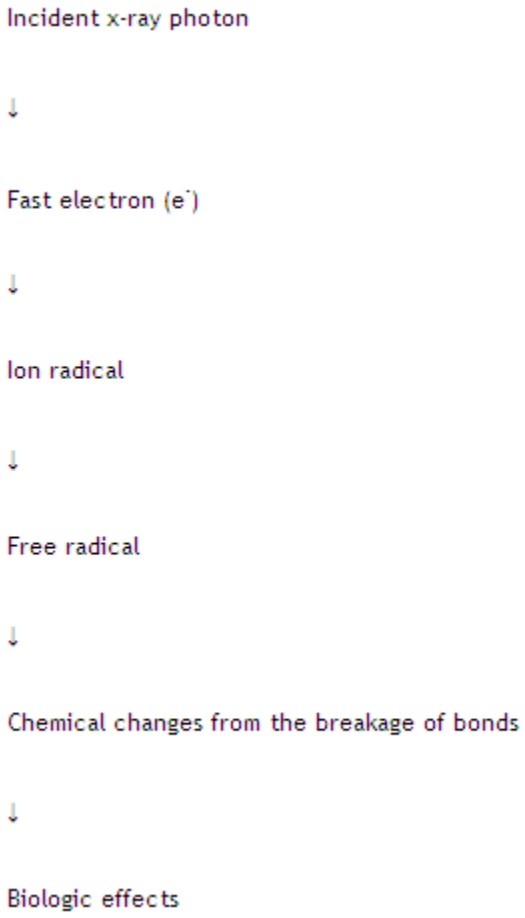
Amifostine:

- Amifostine, (WR-2721)sold under the trade name Ethyol, is the only radioprotective drug approved by the FDA for use in the prevention of xerostomia in patients treated for head and neck cancer.
- Amifostine is a “prodrug” that is unreactive and that penetrates poorly into cells until it is dephosphorylated by the enzyme alkaline phosphatase to the active metabolite WR-1065.
- The rationale for the use of phosphorothioate radioprotectors is that they flood normal tissues rapidly after administration but penetrate tumors much more slowly. The strategy is to begin irradiation soon after administration of the drug to exploit a differential effect.
- The mechanism of action is the scavenging of free radicals and restitution of free-radical damage.
- The dose-reduction factor (DRF) is the ratio of radiation doses required to produce the same biologic effect in the absence and presence of the radioprotector. The best available radioprotectors can attain dose-reduction factor values of 2.5 to 3.0 for bone-marrow death in mice irradiated with x-rays. Dose-reduction factor values close to the oxygen enhancement ratio are possible for γ -rays, but the effectiveness of radioprotectors decreases with increasing linear energy transfer.

Absorption of radiation:

- X- and γ-rays are **indirectly ionizing**; the first step in their absorption is the production of **fast recoil electrons**.
- Biologic effects of x-rays may be caused by **direct action** (the **recoil electron** directly ionizes the target molecule) or **indirect action** (the recoil electron interacts with water to produce a **hydroxyl radical**, which diffuses to the target molecule).
- About **two thirds** of the biologic damage by x-rays is caused by indirect action.
- Neutrons are also **indirectly ionizing**; the first step in their absorption is the production of fast recoil protons, α-particles, and heavier nuclear fragments.
- Most of the biological effects of neutrons is caused by **direct action** (the recoil protons and alpha-particles) directly cause DNA damage).

- Charged particles such as electrons, protons, alpha particles and heavy ions are directly ionising.



Effect of Radiation on DNA & chromosomes:

- A variety of techniques have been used to measure DNA double-strand breaks, including sucrose gradient sedimentation, alkaline and neutral filter elution, nucleoid sedimentation, **pulsed-field gel electrophoresis**, and **single-cell gel electrophoresis** (the comet assay).

- There is good reason to believe that double-strand breaks rather than single-strand breaks lead to important biologic end points, including cell death, carcinogenesis, and mutation.
- Radiation-induced breakage and incorrect rejoining in **prereplication** (G_1) chromosomes may lead to **chromosome** aberrations.
- Radiation-induced breakage and incorrect rejoining in **postreplication** (late S or G_2) chromosomes may lead to **chromatid** aberrations.
- **Lethal** aberrations include **dicentrics, rings,** and **anaphase bridges**.
- **Symmetric translocations** and **small deletions** are **nonlethal**.
- There is a good correlation between cells killed and cells with asymmetric exchange aberrations (i.e., dicentrics or rings).
- The incidence of most radiation-induced aberrations is a linear-quadratic function of dose.
- Scoring aberrations in lymphocytes from peripheral blood may be used to estimate total-body doses in humans accidentally irradiated. The lowest single dose that can be detected readily is **0.25 Gy** (25 rad).
- **Dicentrics** are "**unstable**" aberrations; they are lethal to the cell and are not passed on to progeny. Consequently, the incidence of dicentrics **declines** slowly with time after exposure.
- **Translocations** are "**stable**" aberrations; they persist for many years because they are not lethal to the cell and are passed on to the progeny.

- For **1Gy** of radiation, the number of base damages=**1000,** number of SSBs=**1000,** number of DSBs=**40**
- **Telomeres:** Mammalian telomeres consist of long arrays of **TTAGGG** repeats that range in total length anywhere from **1.5 to 150 kilobases**. Each time a normal somatic cell divides, telomeric DNA is lost from the lagging strand, because DNA polymerase cannot synthesize new DNA in the absence of an RNA primer. Successive divisions lead to progressive shortening, and after **40 to 60 divisions**, the telomeres in human cells are shortened dramatically, so that vital DNA sequences begin to be lost. At this point, the cell cannot divide further and undergoes senescence. Telomere length has been described as the "**molecular clock**" or generational clock, because it shortens with age in somatic tissue cells during adult life. Stem cells in self-renewing tissues, and cancer cells in particular, avoid this problem of aging by activating the enzyme telomerase. **Telomerase** is a **reverse transcriptase** that includes the

complementary sequence to the TTAGGG repeats and so continually rebuilds the chromosome ends to offset the degradation that occurs with each division. In this way, the cell becomes immortal.

Cell Survival Curves:

- A cell is said to have retained its reproductive integrity if it is capable of **sustained** proliferation, that is, if it can grow into a macroscopic colony. A survivor that has retained its reproductive integrity is said to be **clonogenic**.
- The percentage of untreated cells seeded that grow into macroscopic colonies is known as the plating efficiency. The fraction of cells surviving a given dose is determined by counting the number of macroscopic colonies as a fraction of the number of cells seeded. Allowance must be made for the plating efficiency. A cell survival curve is the relationship between the fraction of cells retaining their reproductive integrity and the absorbed dose.
- Conventionally, surviving fraction on a logarithmic scale is plotted on the ordinate against dose on the abscissa. The shape of the survival curve is important.
- The cell survival curve for α-particles and low-energy neutrons (densely ionizing radiations) is a **straight line** on a log-linear plot; that is, survival approximates to an **exponential** function of dose.
- The cell survival curve for x- or γ-rays (sparsely ionizing radiations) has an initial slope, followed by a bending region or shoulder, after which it tends to straighten again at higher doses.
- For the first one or two decades of survival and up to doses used in single fractions in radiotherapy, survival data are adequately represented by the linear-quadratic relationship

  $S = e^{-\alpha D-\beta D2}$

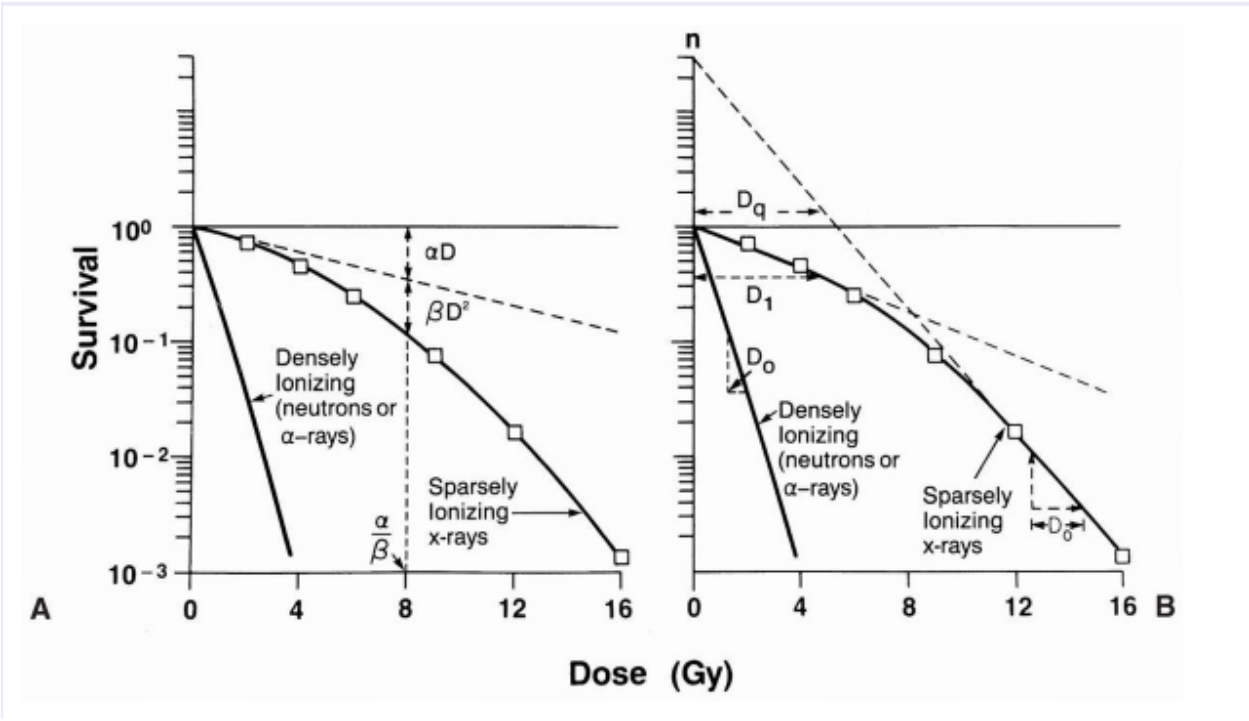
  in which S is the fraction of cells surviving a dose D and α and β are constants representing the linear and quadratic components of cell killing.
- The initial slope of the cell survival curve is determined by α; the quadratic component of cell killing (β) causes the curve to bend at higher doses.
- The ratio α/β is the dose at which linear and quadratic components of cell killing are equal.

- There is good evidence that the nucleus, specifically the DNA, is the principal target for radiation-induced cell lethality. Membrane damage also may be a factor.
- Following exposure to radiation, cells may die attempting the next or a subsequent mitosis (**mitotic death**), or they may die programmed cell deaths (**apoptotic death**).
- In cells that die a **mitotic death**, there is a one-to-one correlation between cell survival and the average number of putative "**lethal" chromosomal aberrations** per cell, that is, asymmetric exchange-type aberrations such as dicentrics and rings.
- In some cell types (such as **lymphoid cells**), **apoptotic death** is dominant following irradiation. Survival is then an **exponential** function of dose; that is, the survival curve is **straight** and shoulderless on the usual log-linear plot. There is also **no** dose-rate effect.
- In some cell types (such as CHO or V79 cells in culture), **mitotic death** is dominant following irradiation. Survival is then a **linear-quadratic** function of dose; that is, the survival curve has a shoulder on the usual log-linear plot. There is usually a **large dose-rate effect**.
- Many cell populations die both mitotic and apoptotic deaths. There is, in general, a correlation between the importance of **apoptosis** and **radiosensitivity**. If apoptosis is dominant, cells are radiosensitive; if apoptosis is absent, cells are radioresistant.
- A number of human syndromes have been found to be associated with radiosensitivity; AT is the best example. Examples are Ataxia Telengiectasia, Fanconi's anemia, Usher's syndrome, Cockayne syndrome, Down's syndrome, Gardner's syndrome, Basal Cell Nevoid Syndrome, Nijmegan Breakage Syndrome.
- There is often a link between sensitivity to killing by radiation and predisposition to cancer.
- If a radiation dose is delivered in a series of equal **fractions**, separated by sufficient time for repair of sublethal damage to occur between doses, the effective dose-survival curve becomes an **exponential** function of dose. The shoulder of the survival curve is repeated many times, so that the effective survival curve is a **straight line** from the origin through a point on the single-dose survival curve corresponding to the daily dose fraction. The average value

of the effective D_0 for the multifraction survival curve for human cells is about **3 Gy** (300 rad).

- The D_{10}, the dose resulting in one decade of cell killing, is related to the D_0 by the expression:

$D_{10} = 2.3 \times D_0$



A=LQ model **B=Multitarget model**

Initial slope, D_1: resulting from single-event killing;

Final slope, D_0: resulting from multiple-event killing

The quantities D_1 and D_0 are the reciprocals of the initial and final slopes. In each case, it is the dose required to reduce the fraction of surviving cells to 37% of its previous value. Because the surviving fraction is on a logarithmic scale and the survival curve becomes straight at higher doses, the dose required to reduce the cell population by a given factor (to 0.37) is the **same** at all survival levels. It is, on average, the dose required to deliver one inactivating event per cell.

The **extrapolation number**, n, is a measure of the **width of the shoulder**. If n is large (e.g., 10 or 12), the survival curve has a broad shoulder. If n is small (e.g., 1.5 to 2), the shoulder of the curve is narrow.

Quasithreshold dose: It is defined as the dose at which the **straight** portion of the survival curve, extrapolated backward, cuts the dose axis drawn through a survival fraction of **unity**.

$\log_e n = D_q/D_0$

Bystander effect:

Defined as the induction of biologic effects in cells that are not directly traversed by a charged particle, but are in close proximity to cells that are.

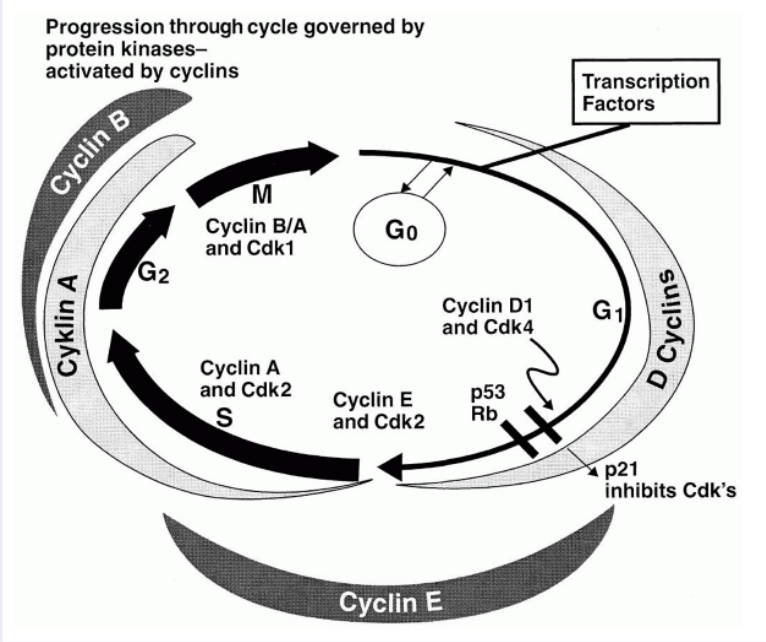
The effect is most pronounced when the bystander cells are in **gap-junction** communication with the irradiated cells. For example, up to **30%** of bystander cells can be killed in this situation.

The bystander effect is much smaller when cell monolayers are **sparsely** seeded so that cells are separated by several hundred micrometers. In this situation, **5 to 10%** of bystander cells are killed, the effect being due, presumably, to cytotoxic molecules released into the medium.

In addition to the experiments described above involving sophisticated single-particle microbeams, there is a body of data involving the transfer of **medium** from irradiated cells, which results in a biologic effect (cell killing) when added to unirradiated cells. These studies, which also evoke the term bystander effect, suggest that irradiated cells secrete a molecule into the medium, that is capable of killing cells when that medium is transferred onto unirradiated cells.

Cell Cycle:

- The whole process of mitosis—in preparation for which the cell rounds up, the chromosome material condenses, and the cell divides into two and then stretches out again and attaches to the surface of the culture vessel—lasts only about **1 hour**. Total cell cycle time is around **24 hours**, whereas the S phase lasts for maximum 15 hours.
- The remainder of the cell cycle, the interphase, occupies all of the intermitotic period.
- Based on tritiated thymidine-labelled DNA using autoradiography/bUDR using fluorescent microscopy.



- Regulation occurs by the periodic activation of different members of the cyclin-dependent kinase (Cdk) family. In its active form, each Cdk is complexed with a particular cyclin.
- The Cdk catalytic subunit by itself is inactive, requiring association with a cyclin subunit and phosphorylation of a key threonine residue to become fully active.
- The Cdk—cyclin complex is reversibly inactivated either by phosphorylation on a tyrosine residue located in the adenosine triphosphate-binding domain, or by association with Cdk inhibitory proteins.
- After the completion of the cell-cycle transition, the complex is inactivated irreversibly by ubiquitin-mediated degradation of the cyclin subunit.
- Entry into S phase is controlled by Cdks that are sequentially regulated by cyclins D, E, and A. Cyclin E expression in proliferating cells is normally periodic and maximal at the G_1/S transition, and throughout this interval it enters into active complexes with its catalytic partner, Cdk2.

- Cells are most sensitive at or close to mitosis.
- Resistance is usually greatest in the latter part of S phase.
- G_2 phase is usually sensitive, perhaps as sensitive as M phase.

- The variation in radiosensitivity as a function of cell age is qualitatively similar for neutrons and x-rays; that is, with both types of radiation, maximum sensitivity is noted at or close to mitosis, and maximum resistance is evident late in S phase. There is, however, a quantitative difference in that the range of radiosensitivity between the most resistant and the most sensitive phases of the cell cycle is much less for fast neutrons than for x-rays. As LET increases, the variation in radiosensitivity through the cell cycle decreases, so that at very high LET, the age-response function is almost flat—that is, radiosensitivity varies **little** with the phase of the cell cycle.
- The oxygen enhancement ratio (OER) varies little with phase of the cell cycle but may be slightly lower for cells in G_1 than for cells in S.

Oxygen effect:

If molecular oxygen is present, DNA reacts with the free radicals (R·). The DNA radical can be chemically restored to its reduced form through reaction with an SH group.

However, the formation of **RO2, an organic peroxide**, represents a non-restorable form of the target material; that is, the reaction results in a change in the chemical composition of the material exposed to the radiation. This reaction cannot take place in the absence of oxygen, since then many of the ionized target molecules are able to repair themselves and recover the ability to function normally. In a sense, then, oxygen may be said to "fix" or make permanent the radiation lesion. This is known as the **oxygen fixation** hypothesis.

By the time a concentration of oxygen corresponding to **2%** has been reached, the survival curve is virtually indistinguishable from that obtained under conditions of normal aeration.

Furthermore, increasing the amount of oxygen present from that characteristic of air to 100% oxygen does **not** further affect the slope of the curve.

- The oxygen enhancement ratio (OER) is the ratio of doses under hypoxic to aerated conditions that produce the same biologic effect.
- The OER for x-rays is about **3** at high doses and is possibly lower (about 2) at doses below about 2 Gy (200 rad).

- The OER decreases as linear energy transfer increases. The OER approaches unity (i.e., **no** oxygen effect) for α-particles. For neutrons, the OER has an intermediate value of about **1.6**.
- To produce its effect, molecular oxygen must be present during the radiation exposure or at least during the lifetime of the free radicals generated by the radiation.
- Most transplantable tumors in animals have been shown to contain hypoxic cells that limit curability by single doses of x-rays. Hypoxic fractions vary from 0 to 50%, with a tendency to average about **15%**.
- The “slow” component is caused by the reoxygenation of chronically hypoxic cells as the tumor shrinks. The “fast” component of reoxygenation is caused by the reoxygenation of acutely hypoxic cells as tumor blood vessels open and close.
- Hypoxia can inhibit the expression of the mismatch repair genes Mlh1, MsH2, and MsH6, leading to increased dinucleotide repeat instability, and the recombinational repair gene Rad51, resulting in decreased levels of homologous recombination.
- Approximately 1% of the genome is transcriptionally regulated by hypoxic stress. A substantial portion of hypoxia-induced genes are regulated by the hypoxia-inducible transcription factor (HIF-1).
- Among chemotherapy agents, Bleomycin, Procarbazine and Dactinomycin aremore effective in presence of oxygen, whereas Mitomycin C & Doxorubicin are more active against hypoxic cells.

Linear Energy Transfer:

> The linear energy transfer (L) of charged particles in medium is the quotient of dE/dl, where dE is the average energy locally imparted to the medium by a charged particle of specified energy in traversing a distance of dl.

Radiation	Linear Energy Transfer, keV/μm	
Cobalt-60 γ-rays	0.2	
250-kV x-rays	2.0	
10-MeV protons	4.7	
150-MeV proton	0.5	
14-MeV neutrons	Track Avg. 12	Energy Avg. 100
2.5-MeV α-particles	166	
2-GeV Fe ions (space radiation)	1,000	

For a given type of charged particle, the higher the energy, the lower the LET and therefore the lower its biologic effectiveness.

Relative Biological Effectiveness:

The RBE of some test radiation (r) compared with x-rays is defined by the ratio D_{250}/D_r, where D_{250} and D_r are, respectively, the doses of x-rays and the test radiation required for equal biological effect.

As the LET increases, the RBE increases slowly at first and then more rapidly as the LET increases beyond 10 keV/μm. Between 10 and 100 keV/μm, the RBE increases rapidly with increasing LET and in fact reaches a maximum at about **100 keV/μm**. Beyond this value for the LET, the RBE again falls to lower values.

LET of about 100 keV/μm is optimal in terms of producing a biologic effect. At this density of ionization, the average separation between ionizing events just about coincides with the diameter of the DNA double helix (20 Å, or 2 nm). Radiation with this density of ionization has the highest probability of causing a **double-strand break** by the

passage of a **single** charged particle. In the case of x-rays, which are more sparsely ionizing, the probability of a single track causing a double-strand break is low, and in general more than one track is required. As a consequence, x-rays have a low biologic effectiveness. At the other extreme, much more densely ionizing radiations (with an LET of 200 keV/μm, for example) readily produce double-strand breaks, but energy is “wasted” because the ionizing events are too close together. Because RBE is the ratio of doses producing equal biologic effect, this more densely ionizing radiation has a lower RBE than the optimal LET radiation. The more densely ionizing radiation is just as effective **per track**, but less effective per unit **dose**.

RBE depends on the following: Radiation quality (LET), Radiation dose, Number of dose fractions, Dose rate, Biologic system or end point

At low LET, corresponding to x- or γ-rays, the OER is between 2.5 and 3; as the LET increases, the OER falls slowly at first, until the LET exceeds about 60 keV/μm, after which the OER falls rapidly and reaches unity by the time the LET has reached about 200 keV/μm.

The radiation weighting factor (W_R) depends on LET.

Equivalent dose is the product of absorbed dose and the radiation weighting factor.

Effective dose equivalent is the sum of the equivalent doses to each tissue and organ exposed multiplied by the appropriate tissue weighting factors.

Repair of DNA damage:

(1) Base excision repair

(2) Nucleotide excision repair

(3) Repair of DSBs: Homologous Recombination Repair (HRR): Error free, occurs in **late S/G2** phase. Uses sister chromatid as template.

Non-homologous End Joining (NHEJ): Error-prone, occurs in **G1** phase. Occurs without sister chromatid.

(4) Single strand annealing: bridge between HRR and NHEJ.

(5) Cross-link repair

(6) Mismatch repair

NHEJ:

NHEJ can be divided into four steps: (1) end recognition, (2) end processing, (3) fill-in synthesis, or end bridging, and (4) ligation.

End recognition occurs when the **Ku heterodimer**, composed of 70-kDa and 83-kDa subunits, and the **DNA-dependent protein kinase catalytic subunit** (DNA-PKcs) bind to the ends of the DNA double-strand break.

DNA polymerase μ has been found to be associated with the Ku/DNA/XRCC4/DNA ligase IV complex and serves as a strong candidate polymerase for the fill-in reaction.

In the final step of NHEJ, ligation of nicked DNA ends that have been processed is mediated by an **XRCC4/DNA ligase IV complex** that is probably recruited by the Ku heterodimer.

HRR:

The immediate response of a cell to a DNA double-strand break is the activation of a group of sensors that serve both to promote DNA repair and to prevent the cell from proceeding in the cell cycle until the break is faithfully repaired. These sensors, **ATM** (ataxia-telangiectasia mutated) and **ATR** (ataxia-telangiectasia and Rad3 related), are protein kinases that belong to the phosphatidylinositol-3-kinase-related kinase (**PIKK**) family and are recruited to the sites of DNA strand breaks induced by ionizing radiation. BRCA 1 & 2 gene proteins are also involved in this process.

Dysregulated homologous recombination can also lead to cancer by loss of heterozygosity (**LOH**).

Radiation damage types:

Radiation damage to mammalian cells can operationally be divided into three categories:

(1) **lethal damage**, which is irreversible and irreparable and, by definition, leads irrevocably to cell death;

(2) **potentially lethal damage (PLD)**, the component of radiation damage that can be modified by postirradiation environmental conditions. PLD is repaired

and the fraction of cells surviving a given dose of x-rays enhanced if postirradiation conditions are **suboptimal** for growth, so that cells do **not** have to attempt the complex process of mitosis while their chromosomes are damaged. If mitosis is delayed by suboptimal growth conditions, DNA damage can be repaired. Resistant human tumors (e.g., melanoma) owe their resistance to large amounts of potentially lethal damage repair.

(3) **sublethal damage (SLD)**, which under normal circumstances can be repaired in hours unless additional sublethal damage is added (e.g., from a second dose of radiation) with which it can interact to form lethal damage. Sublethal damage repair, therefore, is manifest by the increase in survival observed if a dose of radiation is split into two fractions separated by a time interval. The half-life of SLDR for mammalian cells is 1 hour.

The pattern of repair is a combination of three processes occurring simultaneously. First, there is the prompt **repair** of sublethal radiation damage. Second, there is progression of cells through the cell cycle during the interval between the split doses, which has been termed **reassortment.** Third, there is an increase of surviving fraction resulting from cell division, or **repopulation**, if the interval between the split doses is 10 to 12 hours, because this exceeds the length of the cell cycle of these rapidly growing cells.

In general, there is a good correlation between the extent of repair of sublethal damage and the size of the **shoulder** of the survival curve.It is the quadratic component (β) that causes the curve to bend and that results in the sparing effect of a split dose. A **large** shoulder corresponds to a **small** α/β ratio, ie tumors with greater repair of SLD have larger shoulder and lower α/β ratio.

The repair of sublethal damage is simply the repair of double-strand breaks.

The breaks in two chromosomes that must interact to form a lethal lesion such as a dicentric may be formed by (1) a single track breaking both chromosomes (i.e., single-track damage) or (2) separate tracks breaking the two chromosomes (i.e., multiple-track damage).

The component of cell killing that results from single-track damage is the same whether the dose is given in a single exposure or fractionated. The same is not true of multiple-track damage.

For x-rays, dividing the total dose into two equal fractions, separated by 1 to 4 hours, results in a marked increase in cell survival because of the prompt repair

of sublethal damage. By contrast, dividing the dose into two fractions has little effect on cell survival if neutrons are used, indicating **little** repair of sublethal damage.

Effect of dose rate on repair:

As the dose rate is reduced, the survival curve becomes shallower and the shoulder tends to disappear (i.e., the survival curve becomes an exponential function of dose).

Inverse Dose Rate Effect:

Decreasing the dose rate for this HeLa cell line from 1.54 to 0.37 Gy/h (from 154 to 37 rad/h) increases the efficiency of cell killing such that this low dose rate is almost as damaging as an acute exposure. At about 0.37 Gy/h (37 rad/h), cells tend to progress through the cycle and become arrested in G_2, a radiosensitive phase of the cycle. At higher dose rates, they are "frozen" in the phase of the cycle they are in at the start of the irradiation; at lower dose rates, they continue to **cycle** during irradiation.

Acute Effects of Total Body Irradiation:

- The prodromal syndrome varies in time of onset, severity, and duration.
- At doses **close** to the dose that would be lethal to 50% of the population (LD50), the principal symptoms of the **prodromal syndrome** are anorexia, nausea, vomiting, and easy fatigability.
- Immediate diarrhea, fever, or hypotension indicate a **supralethal** exposure.
- **The cerebrovascular syndrome** results from a total-body exposure to about **100 Gy** (10,000 rad) of γ-rays and in humans results in death in **24 to 48 hours**. The cause of death may be **changes in permeability of small blood vessels in the brain**.
- The **gastrointestinal syndrome** results from a total-body exposure to about **10 Gy** (1,000 rad). (range 5-12 Gy). Death occurs in about **5 to 10 days** in humans because of depopulation of the epithelial lining of the gastrointestinal tract, after a latent period of 3 days following the prodrome.
- The **hematopoietic syndrome** results from total-body exposure **to 2.5 to 5 Gy** (250-500 rad). The radiation sterilizes some or all of the mitotically active precursor cells. Symptoms result from lack of circulating blood elements **3 or more weeks later,** after a latent period following the prodrome .
- In all cases, fatalities occur due to depletion of the stem-cell component.
- The **LD50 for humans is 3 to 4 Gy** (300-400 rad) for young adults without medical intervention. It may be less for the very young or the old. Deaths can occur upto 60 days.

- Below LD50,no treatment is ncessary. Between 4-8Gy exposure, barrier nursing, antibiotics, transfusions. Above 10Gy, only symptomatic care.
- Since for exposures >10Gy, peripheral lymphocytes completely disappear from the blood within 24 hours, so assaying their chromosomal abnormalities to gauge dose is not possible in these cases.
- Some people who would otherwise die from the hematopoietic syndrome may be saved by antibiotics, platelet infusions, or bone-marrow transplants.
- The dose window over which **bone-marrow transplants** may be useful is narrow, namely, **8 to 10 Gy** (800-1,000 rad).
- Heavily irradiated survivors of accidents in the nuclear industry have been followed for many years; their medical history mirrors that of any aging population. An expected higher incidence of shortened lifespan, early malignancies after a short latency, and rapidly progressing cataracts has **not** been observed. That is not to say that heavily irradiated individuals are not at increased risk, but an excess cancer incidence can be observed only by a careful study of a large population.

Time Dose Fractionation:

- The Strandquist plot is the relation between total dose and overall treatment time. In this context, “time” includes the number of fractions. On a double log plot, the slope of the line for skin is often close to **0.33**.
- Ellis NSD model: Total dose = $(NSD)T^{0.11}\ N^{0.24}$
- Prolonging overall time within the normal radiotherapy range has little sparing effect on late reactions but a large sparing effect on early reactions.
- Fraction size is the dominant factor in determining late effects; overall treatment time has little influence. By contrast, fraction size and overall treatment time both determine the response of acutely responding tissues.
- Accelerated repopulation starts in head and neck cancer in humans about **4 weeks** after initiation of fractionated radiotherapy. About **0.6 Gy** (60 rad) per day is needed to compensate for this repopulation.
- **Accelerated** treatment: the EORTC trial **22851** of 72 Gy in 45 fractions (three fractions per day) over 5 weeks, showed a significant **increase in local tumor control** (13% at 5 years), but no increase in survival. There was an unexpected increase in late effects, some of which were lethal. The late effects were probably “consequential” late effects, developing out of the severe acute effects. Incomplete repair between fractions also may have been a problem because the time interval between fractions was too short.

- **Hyperfractionation** has been shown in randomized clinical trials of head and neck cancer (EORTC **22971**) to improve local tumor control (from 40% to 59%) and survival with no increase in acute or late effects.
- **Conventional vs Hyperfractionated vs Accelerated split course vs Accelerated concomitant boost:** Evaluated in the RTOG **90-03** trial, which showed significant improvement in local control, trend towards improved DFS but not OS for HF & AF concomitant boost (similar in both arms) vs conventional. Acute toxcities were increased in all altered fractionation arms but late toxicities were similar.
- **CHART:** Similar local control & survival, increased acute toxicities, reduced late toxicities, except myelopathy.
- Overall treatment time is a very important factor for fast-growing tumors. In head and neck cancer, local tumor control is decreased by about **1.4%** (range 0.4-2.5%) for each day that the overall treatment time is prolonged. The corresponding figure for carcinoma of the cervix is about **0.5%** (range 0.3-1.1%) per day.
- Time factor modifications for LQ model: An approximate allowance can be made for **tumor cell proliferation** when comparing protocols involving different overall treatment times. There are two approaches.
- Fowler has suggested corrections based on the T_{pot} value for different tumors.

$$\frac{E}{\alpha} = (nd)\left(1 + \frac{d}{\alpha/\beta}\right) - \frac{0.693}{\alpha}\frac{t}{T_{pot}}$$

For typical **6-week** (39-day) schedules referred to earlier, proliferation may reduce the biologically effective dose **by 8.3 Gy_{10}**, while for typical **7-week** (46 day) schedules, reduction would be **11.6 Gy_{10}**.

- Peters and colleagues have suggested a pragmatic approach in the case of fast-growing squamous cell carcinomas of the head and neck, where corrections for overall time may be more important than number of fractions. They assume that between 5 and 7 weeks after the start of a fractionated regimen, the dose equivalent of regeneration with protraction of treatment is

about **0.5 Gy per day,** rounded down to **3 Gy per week**. The correction will be different for other tumors and probably negligible for prostate cancer.

- Two quite different cell populations may be **radioresistant**:
- A population proliferating so fast that S phase occupies a major portion of the cycle.
- A population proliferating so slowly that many cells are in early G_1 or not proliferating at all, so that many resting cells are in G_0.
- For a given total dose delivered in a given number of fractions, the incidence of late effects is worse for interfraction intervals less than 4 hours compared with interfraction intervals longer than 6 hours. These data imply that the repair of sublethal damage in late-responding tissues is slow, and so current wisdom dictates an interfraction interval **of 6 hours** or more if multiple fractions per day are used.