2016 ASTRO RADIATION/CANCER BIOLOGY PRACTICE EXAMINATION AND STUDY GUIDE

Produced by the Radiation/Cancer Biology Practice Examination and Study Guide task force

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Preface to the 2016 Edition

In recognition of the critical need to develop new ways to promote education in the biologic basis of radiotherapy, the Radiation and Cancer Biology Committee of ASTRO appointed a subcommittee to develop a dynamic web-based educational tool for radiation oncologists to further their studies of radiation and cancer biology. The *ASTRO Radiation/Cancer Biology Practice Examination and Study Guide* is the product of these efforts. This exam/study guide was created specifically with a goal to stimulate active learning.

It is suggested that users of this exam/study guide attempt to answer the questions in each section and then review the correct answers and explanations. It is anticipated that this approach will lead to a more complete understanding of each topic. References are included whenever possible, with a hypertext link to the abstract and article, for topics that are not addressed fully in the major radiation biology textbooks cited in the exam/study guide. It should be noted that for the selection of references, an emphasis was placed on recent review articles that provide current and comprehensive information on a particular subject.

Radiation and cancer biology are dynamic fields with new results published daily in the scientific literature. The goal for radiation oncologists is to acquire a solid base of knowledge in radiation and cancer biology during their training and to build upon that foundation during their careers through regular reading of the scientific literature as well as attendance at seminars and scientific conferences. The ASTRO Radiation/Cancer Biology Practice Examination and Study Guide is designed to help radiation oncologists achieve this goal. It is hoped that by helping to provide radiation oncologists with a firm foundation in the biologic principles underlying the treatment of cancer with radiation, they will be able to offer more effective radiotherapy and achieve improved clinical outcomes for their patients.

Finally, we would like to thank all of the Associate Editors and Contributors who wrote and carefully reviewed the questions, explanations and references. Most importantly, we thank Dr. Barry Rosenstein for his leadership of and commitment to the subcommittee over the last several years. Without your assistance, creation of the 2016 ASTRO Radiation and Cancer Biology Practice Examination and Study Guide would have not been possible.

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Note on Protein and Gene Nomenclature

The 2016 ASTRO Radiation/Cancer Biology Practice Examination and Study Guide uses the notation system for the name of each gene and protein encoded by that gene that was developed by the HUGO Gene Nomenclature Committee. The details for that system can be found at http://www.gene.ucl.ac.uk/nomenclature/. The guidelines for this sytem stipulate that gene symbols are italicized and designated by upper-case Latin letters or by a combination of upper-case letters and Arabic numerals. The protein encoded by the gene is given the same symbol as the gene, except that the letters are not italicized. Thus, the symbol for the gene mutated in people with the disease ataxia telangiectasia is *ATM* and the protein encoded by that gene is written as ATM.

It should be noted that although the HUGO is widely used in scientific journals and textbooks, this system is rarely used for some proteins and genes. For these genes/proteins, the common symbol has been used in the exam/study guide, but the HUGO symbol is provided in parentheses the first time that the gene/protein is written in the question. For example, *p53* is used in the exam/study guide rather than the official HUGO symbol for this gene, which is *TP53. T*his is noted by indicating *p53 (TP53)* in the question or explanation.

QUESTIONS

I. Interaction of Radiation with Matter

I-1) Which one of the following statements concerning the interaction of photons with matter is CORRECT?

A. The probability of the photoelectric effect decreases with the atomic number of the absorber
B. The predominant interaction of 10 keV photons with soft tissue is the Compton process
C. In the Compton process, the energy of the scattered photon is less than that of the incident photon
D. Pair production occurs for photons with energies less than 1.02 MeV
E. There is only partial absorption of the energy of the incident photon in the photoelectric effect

I-2) Which one of the following is a radiolysis product of water responsible for 2/3-3/4 of the molecular damage caused by the indirect action of ionizing radiation?

A. e_{aq}
B. $^1O_2$
C. OH^-
D. $OH^\bullet$
E. O_2^-

I-3) The approximate minimum photon energy required to cause ionization is:

A. 10-25 eV
B. 100-250 eV
C. 1-2.5 keV
D. 10-25 keV
E. 100-250 keV

I-4) Which of the following X-ray interactions with matter is most important for - producing high-contrast diagnostic radiographs?

A. Compton process
B. pair production
C. photoelectric effect
D. nuclear disintegration
E. coherent scattering

I-5) Which of the following pairs of photon energy and predominant atomic interaction at that energy is correct?

A. 1 keV – pair production
B. 50 keV – triplet production

C. 100 keV – Compton process
D. 2 MeV – photoelectric effect

I-6) Which of the following statements is correct? High LET radiations:

A. include 250 kVp X-rays, 200 MeV protons and 1.1 MV □-rays
B. produce much higher yields of OH radicals than do either X-rays or □-rays
C. are components of solar flares but not of cosmic rays
D. produce less dense ionization tracks than □-rays
E. produce higher levels of clustered lesions in DNA than X-rays

I-7) The lifetime of an $OH^{\bullet}$ radical is approximately:
A. 10^{-15} second
B. 10^{-9} second
C. 10^{-1} second
D. 1 second
E. 1 minute

I-8) Approximately 10,000-20,000 cases of lung cancer each year in the United States are attributed to alpha-particles produced by:
A. Nuclear weapon testing
B. Decrease in the ozone layer
C. Radon gas
D. Chemical contamination

1-9) In pair production, which of the following is true?
A. The incident photon is scattered with reduced energy.
B. Annihilation photons always have an energy of 0.51 MeV each.
C. A pair of orbital electrons are ejected from the atom.
D. Two positrons are emitted at 180 degrees.
E. It cannot occur if the photon energy is above 1.02 MeV.

I-10) Directly ionizing radiation includes all of the following EXCEPT:
A. Electrons
B. Positrons
C. Alpha particles
D. Neutrons
E. Betas.

I-11) Concerning fast neutron interactiobs with matter, which of the following is FALSE?
A. Do not interact with atomic electrons of biological medium.
B. Interact primarily with oxygen in water
C. May cause the ejection of an alpha particle.
D. May activate the target nucleus.
E. May transfer a large fraction of its energy in the process of eleastic scattering.

I-12) Which of the following result from the recombination of the initial water radiolysis products:
A. Solvated electron

B. Solvated proton
C. Hydrogen ion
D. Water
E. Only A and B

1-13) When a live human cell is irradiated by gamma-rays, which one of the following events may eventually cause the most of the damage to DNA.

A. Absorption of radiation energies by the chemical bonds in the DNA molecules.
B. Ionization and excitation on atoms within the DNA structure.
C. Ionization and excitation on atoms within the histones that bound to DNA.
D. Ionization and excitation of the water molecules that surround DNA.
E. Direct damage to the lipids that may later oxidize DNA.

II. Molecular Mechanisms of DNA Damage

II-1) The SF_2 (surviving fraction at 2 Gy) for an irradiated population of cells is most closely correlated with the:

A. Level of γ-H2AX 30 minutes after irradiation
B. Level of γ-H2AX present 24 hours after irradiation
C. Acetylation of H2AZ on lysine 4
D. Rate of DNA single-strand break repair
E. Rate of thymine glycol repair

II-2) Production of which of the following types of DNA damage is not caused by X-rays?

A. Double-strand breaks
B. Thymine glycols
C. Clustered base lesions
D. Oxidized bases
E. Pyrimidine dimers

II-3) A clustered lesion:

A. Results from the creation of multiple double-strand breaks within a particular exon of a gene following exposure to a high LET radiation
B. Involves the formation of several DNA lesions within a highly localized region of DNA
C. Occurs more frequently as the LET of the radiation decreases
D. Represents the repair of multiple lesions within a gene
E. Results from transcription-coupled DNA repair

II-4) Which one of the following assays would be the most appropriate to use for quantitative measurement of DNA double-strand breaks in cells immediately after exposure to ionizing radiation?

A. Alkaline elution
B. Western blotting
C. Neutral comet assay
D. PCR
E. BrdU incoporation assay

II-5) Which statement regarding radiation-induced nuclear foci is correct?

A. ATR is the main apical kinase that responds to radiation-induced double-strand breaks.
B. ERCC1-containing foci indicate the presence of radiation-induced single-strand breaks.
C. Gamma-H2AX foci can be detected within 15 minutes of radiation exposure.

D. p53 forms ATR-dependent foci within minutes of radiation exposure.

II-6) Which gene mutation below would be expected to cause the greatest increase in sensitivity after exposure to a DNA damaging agent which induces double-strand breaks (DSBs):

A. DNA-PKcs null mutation
B. P53 null mutation
C. Activiating K-Ras mutation
D. MLH1 nonsense mutation
E. XRCC1 null mutation

III. Molecular Mechanisms of DNA Repair

III-1) Double-strand DNA breaks caused by ionizing radiation trigger the transcription of DNA damage response genes. Which of the following proteins is a transcriptional transactivator?

A. p21 (CDKN1A)
B. p53 (TP53)
C. ATM
D. CHK1 (CHEK1)
E. TRAIL (TNFSF10)

III-2) Which of the following molecular events occurs earlier than others following the creation of a double-strand DNA break?

A. Destabilization of the mitochondrial outer membrane
B. Inactivation of the CDC25 phosphatases
C. Phosphorylation of CHK1 (CHEK1)
D. Activation of p21 (CDKN1A) transcription
E. Phosphorylation of histone H2AX

III-3) Which of the following statements is FALSE?

A. DNA repair by homologous recombination occurs preferentially in the G_1 phase of the cell cycle
B. Non-homologous end joining is an error-prone repair pathway that involves DNA-PKcs (PRKDC)-associated repair of DNA double-strand breaks
C. The DNA repair proteins MRE11, NBS1 (NBN) and RAD50, localize at nuclear foci corresponding to presumed sites of DNA damage following exposure to DNA-damaging agents
D. A defect in nucleotide excision repair is the basis for the hereditary disorder xeroderma pigmentosum and can lead to increased rates of skin cancer
E. Following the production of DNA double-strand breaks, ATM is converted from an inactive dimer to an active monomer form

III-4) Which of the following proteins is most involved in homologous recombinational repair of radiation-induced DNA double-strand breaks?

A. RAD51
B. XPG (ERCC5)
C. DNA-PKcs (PRKDC)
D. CHK1 (CHEK1)
E. TFIIH

III-5) An agent that inhibits non-homologous end joining (NHEJ) repair of radiation-induced DNA double-strand breaks might be expected to do all of the following, EXCEPT:

A. Affect the immune response
B. Sensitize cells to low dose rate irradiation
C. Decrease normal tissue tolerance during fractionated radiotherapy
D. Increase cellular radioresistance
E. Inhibit sublethal damage recovery

III-6) All of the following proteins are involved in non-homologous end-joining of DNA double-strand breaks, EXCEPT:

A. XRCC4
B. RAD52
C. Artemis (DCLRE1C)
D. KU70 (XRCC6)/KU80 (XRCC5)
E. DNA ligase IV (LIG4)

III-7) A mutation in which of the following genes is LEAST likely to cause an increase in sensitivity to ionizing radiation:

A. *NBS1(NBN)*
B. *BRCA1*
C. *ATM*
D. *MRE11*
E. *XPC*

III-8) Which of the following statements concerning DNA repair is CORRECT?

A. Cells deficient in nucleotide excision repair tend to display hypersensitivity to ionizing radiation
B. A person with LIG4 syndrome is radiation sensitive
C. Mismatch repair involves the action of a DNA glycosylase and an AP endonuclease
D. People with Fanconi anemia exhibit normal sensitivity to DNA cross-linking agents
E. A mutation in *p53 (TP53)* produces an immune deficient phenotype in SCID mice

III-9) Two of the main proteins involved in mismatch repair are:

A. MSH2/MLH1
B. DNA ligase IV (LIG4)/XRCC4
C. KU70 (XRCC6)/KU80 (XRCC5)
D. XPA/XPG (ERCC5)
E. DNA-PKcs (PRKDC)/Artemis

III-10) Which of the following best describes the action of an exonuclease enzyme?

A. Seals breaks in a DNA strand
B. Required for DNA replication
C. Produces nicks within intact DNA strands
D. Controls mRNA synthesis
E. Removes nucleotides from the ends of DNA strands

III-11) Which of the following statements is correct? Base excision repair (BER):

A. When defective, may increase mutation rate, but usually does not dramatically alter cellular radiosensitivity
B. Is the principal pathway responsible for the repair of UV-induced DNA damages
C. Involves the *XP* and *CS* genes
D. Acts primarily on bulky DNA lesions induced by polycyclic aromatic hydrocarbons
E. Is defective in patients with Li-Fraumeni Syndrome

III-12) Which statement regarding the roles of non-homologous end-joining (NHEJ) and homologous recombination (HR) in the repair of ionizing radiation-induced DNA double-strand breaks (DSBs) is TRUE?

A. HR removes DSB from the genome at a faster rate than NHEJ.
B. Defects in HR compromise DSB repair, but do not affect the repair of damage at DNA replication forks.
C. NHEJ requires homologies of 200-600 nucleotides between broken ends of DNA.
D. Defects in NHEJ increase radiosensitivity more than defects in HR in mammalian cells.

III-13) Chemotherapeutic agents frequently produce DNA double-strand breaks by causing stalling and collapse of DNA replication forks. Which of the following pathways is required for the repair of replication-associated double-strand breaks?

A. Non-homologous end-joining (NHEJ)
B. Homologous recombination (HR)
C. Single-strand annealing (SSA)
D. Translesional DNA synthesis (TLS)
E. Nucleotide excision repair (NER)

III-14) A human disorder thought to be due to a DNA repair deficiency is which of the following:

A. Lesch-Nyhan syndrome
B. Xeroderma pigmentosum

C. Tay-Sachs disease
D. Phenylketonuria
E. Down syndrome

III-15) The following statement is true regarding BRCA1 and BRCA2:

A. BRCA1 and BRCA2 mutations account for only a few cases of familial hereditary breast and ovarian cancer
B. BRCA1-deficient cells are resistant to the DNA crosslinking agent mitomycin C
C. The prevalence of BRCA1 mutation is higher than that of BRCA2 mutations
D. BRCA1 and BRCA2 predominantly regulate homologous recombination as opposed to non-homologous end joining
E. The breast cancer risks for carriers of BRCA1 and BRCA2 mutations are similar, but with later age of disease onset for the BRCA1 mutation

IV. Chromosome and Chromatid Damage

IV-1) Which of the following statements concerning chromosome aberrations produced in cells after whole body X-irradiation is TRUE?

A. The formation of terminal deletions follows an exponential dose response
B. Translocations are an unstable type of chromosome aberration
C. The number of dicentric chromosomes detected in peripheral blood lymphocytes remains relatively constant with time
D. SKY (spectral karyotyping) is a useful method for detection of stable aberrations decades following irradiation
E. The minimum dose that can be estimated by scoring dicentric chromosomes is 2 Gy

IV-2) Which of the following types of chromosome aberrations is most responsible for the formation of micronuclei observed after irradiation?

A. Sister chromatid exchanges
B. Chromatid gaps
C. Inversions
D. Quadriradials
E. Acentric fragments

IV-3) Which of the following is the best measure for the presence of radiation-induced chromosome aberrations in interphase cells?

A. Reciprocal translocations
B. Ring chromosomes
C. Dicentric chromosomes
D. Micronuclei
E. Chromatid breaks

IV-4) Which one of the following statements concerning the induction of chromosome aberrations is INCORRECT?

A. Primary radiation-induced breaks can reconstitute without apparent morphological change to the chromosome, rejoin illegitimately with another break site to produce an intra- or inter-chromosomal aberration, or remain "open," leading to a simple break
B. The induction and interaction of DNA double-strand breaks is the principal mechanism for the production of chromosome aberrations
C. Dicentrics, centric rings, and translocations are formed following X-irradiation of cells in the G_0/G_1 phase of the cell cycle, and their formation follows a linear-quadratic dose response
D. Fluorescence *in situ* hybridization (FISH) using multi-colored probes has allowed chromosome aberration complexity to be studied in detail

E. Chromatid type aberrations are observed when cells are irradiated during the G_1 phase of the cell cycle

IV-5) The formation of dicentric chromosome aberrations follows a linear-quadratic dose response curve. This has been interpreted to mean that the production of dicentric chromosomes results from:

A. Two chromosome breaks, produced either by one or by two separate radiation tracks
B. Two chromosome breaks produced by two separate radiation tracks
C. Two chromosome breaks produced by a single radiation track
D. One chromosome break produced by two separate radiation tracks
E. One chromosome break produced by a single track of radiation

IV-6) Which of the following statements concerning chromosome aberrations is TRUE ?

A. A ring chromosome is an example of a chromatid-type aberration
B. A dicentric is a stable chromosome aberration
C. Breakage of a single chromatid in G_2 often leads to the formation of an anaphase bridge
D. Terminal deletions are induced as a linear function of dose
E. For low LET radiation, the yield of dicentric chromosomes is inversely proportional to the dose-rate

IV-7) Increased numbers of chromosome aberrations, especially quadriradials, are frequently found even in the absence of radiation, in which of the following human syndromes?

A. Xeroderma pigmentosum
B. Fanconi anemia
C. Cockayne's syndrome
D. Niemann-Pick disease
E. Li-Fraumeni syndrome

V. Mechanisms of Cell Death

V-1) Pathways that trigger apoptosis culminate in widespread intracellular proteolysis. Which of the following proteases is a downstream executioner that directly participates in the breakdown of numerous cellular proteins?

A. caspase-8 (CASP8)
B. caspase-9 (CASP9)
C. caspase-3 (CASP3)
D. caspase-10 (CASP10)
E. XIAP (BIRC4)

V-2) Which of the following statements regarding cell death following radiotherapy is TRUE?

A. The majority of solid epithelial tumors regress during treatment because of radiation-induced apoptosis.
B. The intrinsic apoptotic pathway can be triggered either by radiation-induced DNA damage or by sphingomyelin-mediated damage to the outer plasma membrane.
C. A novel drug that abolishes the G_1 checkpoint would be expected to reduce the incidence of mitotic catastrophe in irradiated cells.
D. Cells that undergo replicative senescence following radiotherapy are characterized by increased membrane blebbing and DNA fragmentation.
E. The presence of □-H2AX histone foci in irradiated cells is indicative of sphingomyelin activation.

V-3) Radiation-induced cellular senescence is often the result of:
A. Cellular nutrient deprivation
B. Oxidative stress secondary to mitochondrial dysfunction
C. p16-mediated cell cycle arrest
D. Telomere shortening
E. Mitotic catastrophe

V-4) The extrinsic pathway of apoptotic cell death requires:

A. Signals derived from changes in chromatin conformation.
B. Activation of death receptors that translocate from the plasma membrane to the nucleus and degrade DNA.
C. Engagement of death receptors located on the plasma membrane that lead to activation of the initiator caspase-8 (CASP8).
D. p53 (TP53) activation.
E. The triggering of changes in mitochondrial membrane potential.

V-5) One hallmark of the apoptotic process is the display of phosphatidylserine residues on the outer surface of the plasma membrane. This is an important event in terms of the tissue response to ionizing radiation because it:

A. Helps recruit death ligands expressed by neighboring cells to receptors on the cell surface.
B. Stimulates an inflammatory response to remove dying cells from the tissue.
C. Signals the recruitment of phagocytes that engulf the dying cells without causing an inflammatory response.
D. Is required for DNA condensation and fragmentation.
E. Leads to increased ceramide levels.

V-6) Regarding the regulation of apoptosis, which of the following pairs of mammalian proteins and their apoptosis-related functions is FALSE?

A. p53 (TP53) --- upregulation of PUMA
B. DIABLO --- caspase activation
C. XIAP (BIRC4) --- caspase inhibition
D. BAX --- cytochrome c release
E. caspase-3 (CASP3) --- initiator caspase

V-7) Which ONE of the following is a morphological or biochemical feature of apoptosis?

A. Random cleavage of DNA
B. Cellular swelling
C. Lack of dependence on ATP as an energy source
D. Chromatin condensation
E. Rupture of the plasma membrane

V-8) The TUNEL assay used to identify apoptotic cells detects:

A. The action of BAX on the mitochondria
B. Membrane integrity
C. Mitochondrial release of cytochrome c
D. Binding of TNFα (TNF) to its receptor
E. DNA fragmentation

V-9) Which of the following best describes radiation-induced bystander effects?

A. Damage to unirradiated normal tissue noted after irradiation of a tumor.
B. Cell killing that results from irradiation of the cell's cytoplasm in the absence of direct irradiation of the nucleus.
C. Radiation-induced increase in cell membrane permeability that causes increased sensitivity to cytotoxic drugs.
D. DNA and/or chromosomal damage that occurs in unirradiated cells that are proximate to irradiated cells.
E. Intercellular communication that modifies the shoulder region of the radiation survival curve.

V-10) Mitotic death in irradiated cells results primarily from:

A. The mis-rejoining of DNA single strand breaks.
B. DNA ladder formation.
C. Stimulation of the extrinsic death pathway.
D. Mis-assortment of genetic material into daughter cells.
E. An alteration in cell membrane permeability.

V-11) Which of the following concerning autophagy is INCORRECT?

A. Autophagy is a reversible process that can contribute both to tumor cell death and survival.
B. Anti-malaria drugs, chloroquine and hydroxychloroquine, are the only U.S. Food and Drug Administration –approved inhibitors of autophagy for cancer therapy.
C. Autophagy contributes to cellular metabolism by degradation of damaged protein aggregates and organelles.
D. Mitophagy referes to autophagy in mitotic cells.
E. Autophagy is controlled by the Atg family of proteins.

VI. Cell and Tissue Survival Assays

VI-1) Which of the following *in vivo* assays of radiation response does NOT depend on a functional endpoint?

A. LD_{50}
B. Skin nodule formation
C. Myelopathy
D. Breathing rate
E. Cognitive impairment

VI-2) Using the linear-quadratic survival curve model, what would the cell surviving fraction be following a dose of 2 Gy delivered acutely (use α=0.3 Gy^{-1} and β=0.1 Gy^{-2})?

A. 0.01
B. 0.10
C. 0.37
D. 0.50
E. 0.90

VI-3) For the same alpha, beta used in the previous problem, what would be the approximate surviving fraction if the 2 Gy dose were delivered at a low dose rate over a 6 hour period instead of acutely (assume no repopulation takes place during the irradiation)?

A. 0.10
B. 0.20
C. 0.37
D. 0.55
E. 0.90

VI-4) Which clonogenic assay has been used to measure the radiation sensitivity of bone marrow stem cells *in vivo*?

A. Dicentric assay
B. BrdU (BrdUrd) assay
C. Endpoint dilution assay
D. *In vivo*/*in vitro* excision assay
E. Spleen colony assay

VI-5) The components typically required for the analysis of a standard, adherent cell clonogenic survival assay require all of the following, EXCEPT:

A. Calculation of a plating efficiency

B. Colony formation rates at a range of cell densities, for several IR doses
C. Cell line capable of multiple cell divisions
D. Intact apoptosis pathways
E. Nonirradiated control

VII. Models of Cell Survival

VII-1) If a cell line exhibiting a strictly exponential radiation survival curve is exposed to a dose that produces an average of one lethal "hit" per cell, the surviving fraction after this dose would be approximately:

A. 0.01
B. 0.10
C. 0.37
D. 0.50
E. 0.90

VII-2) The α/β ratio is equal to the:

A. Surviving fraction at which the amount of cell killing caused by the induction of irreparable damage equals the amount of cell killing caused by the accumulation of sublethal damage
B. Optimal fraction size to use in a fractionated radiotherapy regimen
C. dose below which a further decrease in fraction size will not affect the surviving fraction for a particular total dose
D. Dq
E. Dose at which the αD component of cell kill is equal to the βD^2 contribution to cell killing

VII-3) Cells from individuals diagnosed with which of the following diseases/syndromes would be expected to have an X-ray survival curve with a relatively large D_0 among the syndromes listed?

A. Nijmegen breakage syndrome
B. LIG4 syndrome
C. ATR-Seckel syndrome
D. Xeroderma pigmentosum
E. Ataxia telangiectasia

VII-4) Which of the following statements concerning cell survival curve analysis is TRUE?

A. The □ □ parameter generally increases as the radiation dose rate decreases
B. The inverse of the D_q corresponds to the final slope of the survival curve
C. The extrapolation number, n, of a survival curve increases with increasing LET of the radiation
D. D_0 is a measure of the incremental increase in cell survival when a given dose is fractionated
E. If n = 1, then $D_{37} = D_0$

VII-5) Reducing the dose rate at which a continuous γ-irradiation is delivered may affect its cell killing efficacy due to several different biological processes. For a total dose of 6 Gy, which pair of dose rate ranges and biological processes resulting in altered cell killing is FALSE?

A. 10 - 1 Gy/min : reoxygenation
B. 1 - 0.1 Gy/min : repair
C. 0.1 - 0.01 Gy/min : redistribution
D. 0.01 - 0.001 Gy/min : repopulation

VII-6) The survival curve for a cell population irradiated with a form of high LET radiation is characterized by a D_{10} of 3 Gy. For a starting population of 10^8 cells, approximately how many cells will survive when a single dose of 18 Gy is given?

A. 10^0
B. 10^1
C. 10^2
D. 10^3
E. 10^4

VII-7) A total dose of 12 Gy of X-rays delivered in 3 Gy fractions reduces cell survival to 10^{-4}. Assuming that cell killing can be modeled using an exponential survival curve, what dose would be required to reduce the surviving fraction to 10^{-6}?

A. 9 Gy
B. 18 Gy
C. 24 Gy
D. 36 Gy
E. 72 Gy

VII-8) In an attempt to generate a radiation survival curve for a new cell line, four cell culture dishes were seeded with 10^2, 10^3, 10^4 and 10^5 cells, and X-irradiated with 0, 3, 6 and 9 Gy, respectively. At the end of a two-week incubation period, a total of 40 colonies was counted on each dish. Which one of the following statements is TRUE?

A. The D_0 for this cell line is 3 Gy
B. The survival curve for this cell line is exponential
C. The n and D_q values for this survival curve are large
D. The cell surviving fraction after a dose of 3 Gy is 0.04
E. The □/ß ratio for this cell line is small

VII-9) What is the approximate $_eD_{10}$ (effective D_{10}) for a particular cell line if the $_eD_0$ is 4 Gy?

A. 2 Gy
B. 4 Gy
C. 6 Gy

D. 9 Gy
E. 12 Gy

VII – 10) When irradiating a cell population with a dose that causes an average of one lethal event per cell, this will likely result in a survival fraction of:

A. 0% of cell survival.
B. 10% of cell survival.
C. 37% of cell survival
D. 63% of cell survival
E. 100% of cell survival

VIII. Linear Energy Transfer

VIII-1) Concerning RBE, OER and LET, which of the following statements is TRUE?

A. Maximum cell killing per dose delivered occurs at an LET corresponding to approximately 1000 keV/μm
B. RBE changes the most over the LET range of 0.1 to 10 keV/μm
C. The relationship between OER and LET is bell-shaped
D. RBE decreases with increasing LET above about 100 keV/μm
E. OER increases with LET

VIII-2) Which of the following statements is correct? Compared with damage from low LET radiation, damage from high LET radiation:

A. Is reduced to a greater extent in the presence of sulfhydryl compounds
B. Shows more potentially lethal damage recovery
C. Exhibits a greater OER
D. Is less subject to split-dose recovery
E. Shows greater sparing when the irradiation is given at a low dose rate

VIII-3) Which of the following statements concerning RBE is TRUE? The RBE:

A. Is lower for neutrons than for protons over the therapeutic energy range
B. For high LET particles is greater for hypoxic cells than for oxygenated cells of the same type
C. For carbon ions is diminished when delivered in several fractions rather than as a single dose
D. For heavy charged particles is greatest at the beginning of the particle tracks

VIII-4) Which of the following pairs of radiation type and approximate LET value is CORRECT?

A. 150 MeV protons – 0.5 keV/μm
B. 1 GeV Fe ions – 20 keV/μm
C. ^{60}Co γ-rays – 15 keV/μm
D. 2.5 MeV α-particles – 5 keV/μm
E. 250 kV X-rays – 10 keV/μm

VIII-5) Which of the following statements concerning LET is INCORRECT?

A. LET is proportional to charge density of a medium.
B. LET is proportional to charge (squared) on the particle moving through a medium.
C. LET is inversely proportional to speed (squared) of the particle.
D. LET is inversely proportional to mass of the particle moving through a medium.
E. LET is related to density of ionizations along the particle's track.

VIII-6) Which statement concerning the linear energy transfer (LET) is CORRECT?

A. LET is equal to the energy transferred by ionizing radiation to soft tissue per unit mass of soft tissue.
B. LET is equal to the number of ion pairs formed per unit track length
C. Once a photon transfers all its energy to an electron, the LET is that of the electron.
D. LET is the quotient of the average energy that a particle lost in causing ionization to the average distance it travels between two consecutive ionizations.
E. The track average method and the energy average method for calculating LET give different numerical values for therapy protons in soft tissue.

VIII-7) How many ion clusters are formed by 55 keV/μm silicon ion along a 1 μm- segment of the ion trajectory through the cell nucleus? Assume silicon ion irradiation with the beam parallel to a cellular monolayer and that ion clusters are uniformely spaced along the silicon ion track.

A. 0.5 cluster every 1 μm or 1 cluster every 2 μm.
B. 5.5 clusters every 1 μm
C. 500 clusters every 1 μm
D. 5,500 clusters every 1 μm.
E. 55,000 clusters every 1 μm

IX. Modifiers of Cell Survival: Oxygen Effect

IX-1) What is the approximate maximum diffusion distance of oxygen from a normally-oxygenated capillary through a typical respiring tissue?

A. 5 nm
B. 15 μm
C. 70 μm
D. 900 μm
E. 2.6 mm

IX-2) A dose of 10 Gy of X-rays reduces the tumor cell surviving fraction to 0.004 in an animal irradiated while breathing air, and to 0.1 in an animal irradiated under nitrogen. An estimate of the hypoxic fraction for this tumor in the air breathing mice would be:

A. 0.0004
B. 0.04
C. 0.25
D. 10
E. 25

IX-3) The K_m for radiosensitization by oxygen (the oxygen concentration at which cellular radiosensitivity is halfway between the fully aerobic and fully hypoxic response) corresponds to an oxygen concentration of approximately:

A. 0.02%
B. 0.5%
C. 3%
D. 15%
E. 30%

IX-4) The most dramatic change in radiation sensitivity occurs over which of the following ranges of oxygen tension (in units of mm Hg or Torr)?

A. 0-30
B. 30-60
C. 60-100
D. 100-250
E. 250-760

IX-5) Which of the following statements concerning the oxygen effect is TRUE?

A. OER values obtained for high energy protons used in radiotherapy are similar to those measured for X-rays
B. During irradiation, an oxygen concentration of about 30% is required to produce full radiosensitization
C. The OER is defined as the ratio of the surviving fraction of cells irradiated with a particular X-ray dose under hypoxic conditions compared to the surviving fraction of cells irradiated with the same dose under aerated conditions
D. Tumors are thought to contain regions of both acute and chronic hypoxia; however, only chronically hypoxic cells can reoxygenate
E. The oxygen effect is principally a manifestation of the increased number of ionizations produced in fully aerated versus hypoxic cells

IX-6) For single, large radiation doses delivered at a high dose rate, the ratio of the OER for X-rays divided by the OER for 15 MeV neutrons is approximately:

A. 0.3
B. 1
C. 2
D. 4
E. 10

IX-7) Which of the following statements concerning the effect of oxygen is TRUE?

A. Oxygen acts as a radiosensitizer because it inhibits DNA repair
B. The OER and RBE both increase with increasing LET
C. Based on pO_2 microelectrode measurements, few human tumors contain regions of hypoxia
D. At an oxygen concentration of about 3%, the radiosensitivity of cells is halfway between a fully aerobic and fully hypoxic response
E. Exposure of cells to hypoxia may stimulate gene transcription

IX-8) Which of the following statements is false when describing tumor hypoxia?

A. In rodent tumors, the hypoxic cell fraction is within the range of 5-50%.
B. Hypoxia is rarely observed in common human tumors.
C. Oxygen diffusion and delivery are limited in some parts of tumors.
D. Hypoxia can enhance tumor progression by means of hypoxia-related gene expression.
E. Hypoxia induces gene amplification and mutation.

IX-9) Which chemical or compound cannot be used to minimize hypoxia-related radioresistance?

A. Nicotinamide and carbogen
B. Perfluorocarbon
C. Vasodilator
D. Misonidazole
E. Nimorazole

IX-10) Oxygen enhancement ratio (OER) changes depending on the type of radiation. Which of the following combination is FALSE?

A. OER 3.0 for x-rays
B. OER 1.6 for neutron
C. OER 3.0 for proton
D. OER 0.5 for energized ions
E. OER 1.0 for alpha-particle

IX-11) Which of the following compounds CANNOT be used to protect mammalian cells from radiation damage?

A. WR-638
B. WR-2721
C. Taxanes
D. Cysteine
E. Amifostine

IX-12) Tyrapazamine (a hypoxic cytotoxin) has recently been developed. Which of the following statements is FALSE when describing the mechanisms and effects of tyrapazamine?

A. If it loses one electron in hypoxic conditions, it becomes cytotoxic.
B. When two electrons are extracted in aerobic conditions, it becomes nontoxic.
C. In aerobic conditions, it can also sensitize cells to radiation.
D. Its uptake is greater for cells in hypoxic conditions than cells in aerobic conditions.
E. The potency of some chemo-agents can be enhanced by the presence of this cytotoxin.

X. Modifiers of Cell Survival: Repair

X-1) An exponentially-growing, asynchronous population of cells is maintained under normal physiological conditions. Which of the following manipulations would NOT reduce killing of these cells by X-rays, as assayed by a clonogenic assay?

A. Cell synchronization in S-phase at the time of irradiation.
B. Irradiation under hypoxic conditions.
C. Irradiation with the dose split into two fractions with a 24 hour interval between fractions rather than given as an acute exposure to the same total dose.
D. Incorporation of bromodeoxyuridine into their DNA prior to irradiation.
E. Addition of cysteine to the cellular growth medium before irradiation.

X-2) For irradiation with X-rays, the increased cell survival observed when a given total dose is delivered at a low dose-rate (~1 Gy/hr) versus high dose-rate (~1 Gy/min) is due primarily to:

A. Repair of DNA double-strand breaks
B. Decreased production of DNA double-strand breaks
C. Induction of free radical scavengers
D. Activation of cell cycle checkpoints
E. Down-regulation of apoptosis

X-3) Relative to the surviving fraction of cells maintained in a non-cycling state for several hours after irradiation, the decreased cell survival observed in cells forced to re-enter the cell cycle immediately after irradiation is evidence for:

A. Rejoining of chromosome breaks
B. Sublethal damage recovery
C. Cell cycle reassortment
D. Translesion of DNA synthesis
E. Expression of potentially lethal damage

X-4) 5 Gy of X-rays is delivered at a high dose rate (1 Gy/min) rather than a low dose rate (1 Gy/hr). Which of the following statements about the effects of this change on cell survival is TRUE?

A. The surviving fraction would change the least for a cell line with a radiation survival curve characterized by a low α/β ratio
B. Treatment of cells during irradiation with an agent that inhibits DNA repair would have a greater impact on the surviving fraction of cells irradiated at the high dose rate
C. More cell killing would occur for irradiation at the high dose rate
D. The difference in the surviving fractions with the two protocols results primarily from repopulation
E. The total number of ionizations produced decreases at the high dose rate

X-5) Exponentially growing cells were maintained at 37°C in 95% air/5% CO_2 and irradiated with either a single dose of 8 Gy of X-rays or two 4 Gy fractions separated by either 2 hours or 8 hours. The surviving fractions for the three treatments were 0.02, 0.15 and 0.08, respectively. The two processes that best account for these differences in survival are:

A. Reassortment and repopulation
B. Repair and reassortment
C. Reoxygenation and repair
D. Repopulation and reassortment
E. Repair and reoxygenation

X-6) Which of the following pairs of radiobiological process and corresponding assay method is CORRECT?

A. Reoxygenation – HIF-1□□(HIF1A) phosphorylation by ATM
B. Potentially lethal damage recovery – tritiated thymidine uptake
C. Cell cycle "age response" – paired survival curve method
D. Sublethal damage recovery – split dose experiment
E. Repopulation – mitotic shakeoff procedure

X-7) Which of the following is a phosphoinositol 3-kinase like kinase (PIKK) that serves as the central orchestrator of the signal transduction response to DSBs?

A. Ku70/80
B. ATM
C. Rad50
D. MSH2
E. p53 (TP53)

X-8) Which of the following is TRUE for potentially lethal radiation damage (PLD)?

A. It is irreversible and irreparable.
B. It is the damage that can be repaired efficiently if cells are allowed to progress through the cell cycle immediately following IR.
C. It is thought to be primarily complex or "dirty" double strand breaks.
D. It can be observed in a "split dose" experiment.
E. It cannot be detected in tumors in vivo.

X-9) Which of the following is false for the split dose experiment and sublethal damage (SLD)?

A. The survivors of the first dose are mainly S phase.
B. The fraction of cells surviving a split dose decreases as the time interval increases due to the repair of SLD.
C. When cells are cycling during the split dose experiments, there is a dip (decrease) in cell survival caused by reassortment.
D. SLD can be repaired before they can interact to form lethal chromosomal damage.
E. SLD is demonstrated by low-LET radiation.

XI. Solid Tumor Assay Systems

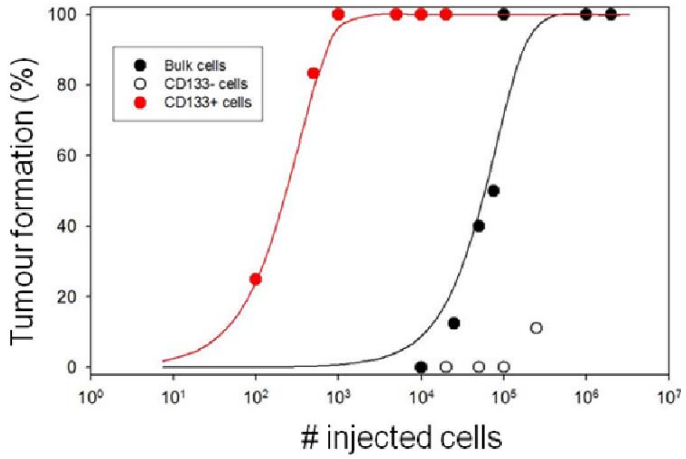
XI-1) Which of the following endpoints would NOT be useful to quantify the response of a tumor to irradiation?

A. Lung colony assay
B. Increase in number of tumors per animal
C. Time to reach a certain size
D. Growth delay
E. Colony forming ability of cells explanted from the tumor

XI-2) The TCD_{50} assay:

A. Measures radiation-induced tumor growth delay
B. Can be conducted using mouse tumors but not human tumor xenografts
C. Gives a measure of the number of cells required to produce a tumor in a mouse
D. Yields results independent of the immune competence of the host animal
E. Measures tumor cure, making it a relevant endpoint for extrapolation to the clinic

XI-3) The number of cells required to produce a tumor "take" in mice is indicated in the graph below for a glioblastoma cell line (bulk) and two sub-lines derived from it ($CD133^+$ and $CD133^-$). Which of the following statements would best explain the experimental findings?



A. $CD133^-$ cells comprise only a small fraction of the total tumor
B. CD133 is a putative marker for cancer stem cells
C. Unsorted bulk cells contain a large fraction of cancer stem cells
D. $CD133^-$ cells are more radiosensitive than $CD133^+$ cells
E. $CD133^+$ cells are more radiosensitive than $CD133^-$ cells

XI-4) A local tumor recurrence after radiotherapy can be caused by:

A. Any surviving cancer cell
B. Any proliferating cancer cell
C. Only cancer cells with the ability to form colonies *in vitro*
D. Only cancer cells with unlimited proliferative potential
E. Only cancer cells that were well-oxygenated during irradiation

XI-5) Which assay or endpoint would provide the best estimate of the radiation response of putative cancer stem cells?

A. Time to first evidence of tumor shrinkage after irradiation
B. Tumor regrowth delay
C. Determining the fraction of proliferating tumor cells 2 weeks after irradiation
D. 50% tumor control dose
E. Quantifying the number of apoptotic tumor cells 6 hours after irradiation

XI-6) In some experiments, tumors treated with radiation and concurrent molecularly-targeted drugs against EGFR and VEGFR displayed longer regrowth delays, but not higher tumor control probabilities, compared to tumors that were treated with radiation only. Which of the following statements provides the most likely explanation for this?

A. The treatment is effective for the bulk of tumor cells, but not for cancer stem cells.
B. The drug did not reach most of the cells due to poor vascular perfusion in the tumor.
C. Experimental error accounts for this, because growth delay and tumor control assays usually yield similar results.
D. Tumor cells generally do not express receptors that are targeted by these drugs.
E. The radiosensitivity of tumor cells does not depend on vascular supply or physiology.

XI-7) Which feature of apoptosis is FALSE?

A. Chromatin condensation
B. Cell shrinkage
C. Preservation of cell and organelles
D. Rapid engulfment by neihboring cells
E. Inflamatory response

XI-8) Which of the following statements is TRUE for the appearance of giant multinucleated cells after radiation?

A. Exhibit an exponential survival curve
B. Are characterized by mitotic catastrophe
C. Dose response relationship describes
D. Mitotic cells are radioresistant
E. Demonstrate increased apoptosis

XI-9) Which of the statements is TRUE for activation of one type of apoptotic pathway?

A. Apoptosis is initiated by PARP.
B. Fas ligand binding its receptor initiates apoptosis.
C. Caspases involved in apoptosis are also involved in necrosis.
D. BCL2 is a pro-apoptotic protein.
E. Anti-apoptotic BAX dimerizes and translocated to the mitochondria.

XII. Tumor Microenvironment

XII-1) Which of the following statements concerning tumor hypoxia is TRUE?

A. Hypoxic regions in tumors may be detected using labeled bortezomib
B. Generally, as tumors increase in size, the hypoxic fraction decreases
C. Regions of chronic hypoxia may develop in tumors due to the intermittent opening and closing of blood vessels
D. In the absence of reoxygenation, it is unlikely that all hypoxic cells would be eliminated from a tumor following a typical course of radiotherapy
E. Acutely hypoxic tumor cells usually exhibit slow reoxygenation while chronically hypoxic tumor cells reoxygenate rapidly

XII-2) Avastin is a monoclonal antibody that targets:

A. Basic fibroblast growth factor (bFGF; FGF2)
B. Hypoxia-inducible factor-1α (HIF-1α; HIF1A)
C. Von Hippel-Lindau (VHL) protein
D. RAS
E. Vascular endothelial growth factor (VEGF; VEGFA)

XII-3) Which of the following responses is least likely to be observed?

A. Exposure to hypoxia increases the expression of angiogenesis-promoting genes.
B. Anti-angiogenic therapy improves tumor oxygenation.
C. A chronic hypoxic environment increases the metastatic potential of tumor cells.
D. Hypoxia inhibits apoptosis in tumor cells.
E. Exposure to hypoxia inhibits cell proliferation

XII-4) Which of the following statements concerning chronically hypoxic cells in tumors is TRUE? Chronically hypoxic cells:

A. Can be selectively targeted for killing by administering certain bioreductive drugs
B. Are resistant to hyperthermia
C. Are located within 10 μm of capillaries
D. Exist in a high pH microenvironment
E. Are a consequence of intermittent blood flow

XII-5) Which of the following statements concerning tumor angiogenesis is TRUE?

A. In the absence of angiogenesis, tumors could not grow beyond a diameter of about 2 cm
B. For most tumor types, a high microvessel density has been negatively correlated with metastatic spread
C. Vascular endothelial growth factor is induced under hypoxic conditions
D. Angiostatin and endostatin are stimulators of angiogenesis

E. Basic fibroblast growth factor is a negative regulator of angiogenesis

XII-6) The regulation of hypoxia-inducible factor-1α (HIF-1α; HIF1A) by oxygen concentration is best described by which of the following statements?

A. Under hypoxic conditions, HIF-1α transcription and translation are up-regulated, causing the protein to translocate from the cytosol to the nucleus
B. Under aerobic conditions, the HIF-1α heme moiety becomes oxygenated. This drives a conformational change in the protein limiting DNA binding and thereby preventing up-regulation of target genes
C. Under hypoxic conditions, HIF-1α is activated by bioreduction, thereby promoting the up-regulation of target genes
D. Under hypoxic conditions, the HIF-1α heme moiety becomes deoxygenated. This causes a conformational change in the protein, enhancing DNA binding and thereby promoting up-regulation of target genes
E. Under aerobic conditions, HIF-1α is hydroxylated by HIF prolyl hydroxylases. This targets the protein for ubiquitination and subsequent proteosomal degradation, thereby preventing the up-regulation of target genes

XII-7) Which of the following statements best describes the "normalization hypothesis" proposed to explain the survival benefit associated with combining anti-angiogenics with traditional chemotherapy agents?

A. Anti-angiogenic therapy stimulates the formation of leaky blood vessels thereby enhancing access of chemotherapy agents to the tumor parenchyma
B. Anti-angiogenic therapy transiently reduces pericyte coverage of tumor blood vessels, which would otherwise form a significant mechanical and biochemical barrier to the delivery of chemotherapy to the tumor
C. Tumor cell-derived, pro-angiogenic factors, render endothelial cells resistant to chemotherapy-induced apoptosis. Anti-angiogenic therapy removes this protection and restores endothelial cell sensitivity to chemotherapy
D. Anti-angiogenic therapy reduces the secretion of anti-apoptotic factors by vascular endothelial cells that would otherwise render nearby cancer cells relatively resistant to chemotherapeutic agents
E. Anti-angiogenic therapy transiently restores the normal balance of pro- and anti-angiogenic factors in tumor tissue, causing reductions in tumor vessel leakiness, dilation and tortuosity, and increasing pericyte coverage

XII-8) At a distance of 150 μm from the nearest tumor blood vessel, one might expect all of the following microenvironmental conditions, EXCEPT:

A. Increased hypoxia
B. Decreased pH
C. Decreased interstitial fluid pressure
D. Decreased glucose

XII –9) Paclitaxel appears to be effective in radiosensitizing tumors *in vivo* for all the following reasons, EXCEPT:

A. Inducing apoptosis
B. Upregulating HIF-1
C. Oxygenating radioresistant hypoxic cells
D. Arresting cells in the radiosensitive G_2/M phase
E. Decreasing interstitial fluid pressure

XII-10) All of the following statements as to why larger tumors are more difficult to control with radiotherapy than smaller tumors are true, EXCEPT:

A. Larger tumors generally contain more radioresistant hypoxic cells than smaller tumors.
B. In order to deliver a curative total dose to a large tumor, the volume of irradiated adjacent normal tissue may become so large as to exceed normal tissue tolerance.
C. A larger primary tumor volume is associated with a higher risk of regional and distant metastatic spread
D. The fraction of rapidly proliferating cells tends to increase with size of the tumor.

XII-11) Which is FALSE about the tumor microenvironment?

A. Blood vessel supply is heterogeneous and irregular.
B. Flow through micro-vessels may be sluggish.
C. There tends to be an increase in vessel density.
D. It has an increased in hypoxic regions.
E. The nutritional support is adequate and homogeneous.

XII-12) Which is FALSE about angiogenesis?

A. For multi-cellular organisms to grow, they must recruit new blood vessels by angiogenesis.
B. This process is normally regulated by pro-angiogenic but not by anti-angiogenic molecules.
C. Angiogenesis is disregulated in cancer.
D. Without blood vessels, tumors cannot grow beyond a critical size or metastasize to other organs.
E. Viable cells are located within 100 micrometers of blood vessels due to the diffusion limits of oxygen.

XII-13) Which is FALSE about anti-angiogenic therapy strategies?

A. The strategies interfere with activators of angiogenesis.
B. The strategies target receptor tyrosine kinases and related signal transductions.
C. The strategies seek to amplify endogenous suppressors of angio-genesis.
D. The strategies use colchicine as an anti-angiogenic agent.
E. The strategies use VEGFR-1 to inhibit angiogenesis.

XII-14) Which of the following statements best describes the “abscopal effect”

A. Localized irradiation of a tumor causes, not only a shrinking of the irradiated tumor, but growth of tumors far from the irradiated area
B. Localized irradiation of a tumor causes, not only a shrinking of the irradiated tumor, but also a shrinking of tumors far from the irradiated area
C. When cells are irradiated, and the medium is transferred to unirradiated cells, these unirradiated cells show increased clonogenic survival
D. The avid response of irradiated low-grade lymphomas to low-dose radiotherapy such as 2 Gy x 2.

XII-15) Which of the following statements concerning vasculogenesis is TRUE?

A. This process is only relevant to the developing embryo and not pathologic state such as cancer.
B. Is similar to angiogenesis but involves the formation of only venous vessels as tumors grow beyond 1-2 mm^3.
C. Tumors use vasculogenesis or angiogenesis in a mutually exclusive fashion.
D. Vasculogenesis is critical for local tumor recurrence following radiotherapy.
E. Vasculogenesis utilizes pre-existing blood vessels during the early stages of tumor development to facilitate tumor growth.

XIII. Cell and Tissue Kinetics

XIII-1) Which of the following CDK or cyclin is paired with the correct phase transition?

A. CDK1 (CDC2) – G_2 into M
B. CDK4 – S into G_2
C. cyclin A – G_2 into M
D. cyclin B – S into G_2
E. cyclin D – M into G_1

XIII-2) Irradiation of an exponentially-growing population of cells in culture with a dose that kills 90% of cells tends to select surviving cells that are initially in which phase of the cell cycle?

A. G_0
B. G_1
C. S
D. G_2
E. M

XIII-3) The typical cell cycle time (T_C) for proliferating cells in human tumors is in the range of:

A. <1 day
B. 1-5 days
C. 6-25 days
D. 26-100 days
E. >100 days

XIII-4) Which of the following statements concerning the cell cycle kinetics of tumors is TRUE?

A. Often, the cell loss factor (□) decreases several weeks after the start of radiotherapy
B. The growth fraction (GF) is the ratio of the number of viable cells to the sum of viable and non-viable cells
C. If the volume doubling time (T_D) is 60 days and the potential doubling time (T_{pot}) is 3 days, then the cell loss factor is 5%
D. T_{pot} has proven useful in predicting tumor response to accelerated radiotherapy
E. Typically, the cell loss factor, □□ is of minor importance in determining a tumor's volume doubling time

XIII-5) Exponentially growing cells are pulse-labeled with tritiated thymidine and sampled as a function of time thereafter. The time required for the percent of labeled mitoses to reach 50% of its maximum value corresponds approximately to:

A. T_S
B. T_C
C. T_{G2}
D. $T_{G1} + T_S/2$
E. $T_{G2} + T_M/2$

XIII-6) If the mitotic index of a cell line is 5%, the growth fraction is 100%, the cell cycle time is 14 hours and the correction factor, λ, is 0.7, how long is mitosis?

A. 0.2 hours
B. 1 hour
C. 2 hours
D. 4 hours
E. 8 hours

XIII-7) Which of the following is the *main* reason why the volume doubling time of a tumor rarely equals its potential doubling time?

A. High cell loss factor
B. High metastatic propensity
C. Long cell cycle time
D. Low hypoxic fraction
E. Low growth fraction

XIII-8) Which of the following statements concerning tumor kinetics is TRUE?

A. Cell-cycle times (T_c) are longer than potential doubling times (T_{pot}) because of the presence of non-proliferating cells
B. The T_{pot} is usually shorter than the volume doubling time because the growth fraction (GF) is usually less than 100%
C. T_{pot} can be determined if the mitotic index (MI) and the duration of S phase (T_s) are known
D. Tumors with long values for T_{pot} are good candidates for accelerated radiotherapy
E. In the absence of cell loss, T_{pot} would equal the volume doubling time (T_D) of the tumor

XIII-9) Which of the following substrates and target sites of the ATM kinase are implicated in the control of the G_2-checkpoint in X-irradiated cells?

A. CHK2 (CHEK2) and MDM2
B. NBS1 (NBN) and CHK2
C. CHK2 and CDC25C
D. CHK2 and p53 (TP53)
E. PUMA and p53 (TP53)

XIII-10) Which of the following pairs of chemicals could be used with flow cytometry to determine the S phase fraction of a cell population and for an estimate of relative DNA content?

A. Bromodeoxyuridine and propidium iodide
B. Tritiated thymidine and hydroxyurea
C. Dichlorohydrofluorescein and cytochrome c
D. H2AX and ethidium bromide
E. Sphingomyelin and ceramide

XIII-11) If a tumor is comprised of cells characterized by a high growth fraction and a short cell cycle time, which of the following would most likely describe its behavior prior to and after treatment with a curative dose of radiation?

A. Slow growth, slow regression
B. Slow growth, rapid regression
C. Rapid growth, rapid regression
D. Rapid growth, slow regression

XIII-12) What is the most probable range of cell cycle time (Tc) and tumor doubling time (Td) for human tumors?

A. Tc, 1 to 5 days and Td, 20 to 30 days
B. Tc, 1 to 5 days and Td, 40 to 100 days
C. Tc, 0.5 to 1 days and Td, 20 to 30 days
D. Tc, 0.5 to 1 days and Td, 40 to 100 days
E. Tc, 1 to 2 days and Td, 120 to 300 days

XIII-13) What is the main reason for the great disparity between the cell cycle time of individual dividing cells and the overall doubling time of the tumor?

A. Intratumor oxygen partial pressure (pO2)
B. Growth fraction
C. Cell loss factor
D. Body temperature where the tumor grow
E. Extra- and intra-cellular acidity (pH)

XIII-14) The cell loss factor represent the ratio of the rate of cell loss to the rate of new cell production. Which one may NOT be the cause of cell loss in tumors?

A. Death from inadequate nutrition
B. Apoptosis (programs cell death)
C. Death from immunologic attack
D. Metastasis

Cell migration

XIII-15) In an untreated tumor with a potential doubling time of 3 days and a cell loss factor of 80%, the volume doubling time is:

A. 2.4 days
B. 3.5 days
C. 3.75 days
D. 15 days
E. 20 days

XIV. Molecular Signaling

XIV-1) Following exposure of cells to 3 Gy from a 6 MV X-ray beam, the ATM protein is activated and phosphorylates multiple intracellular targets. Which of the following is NOT a target for ATM phosphorylation?

A. histone H2AX
B. p53 (TP53)
C. VEGF (VEGFA)
D. BRCA1
E. Artemis

XIV-2) Which of the following pairs of molecular events and their functional consequences is INCORRECT?

A. *VHL* inactivation ---- angiogenesis
B. cyclin D1 repression --- inhibition of proliferation
C. cytochrome c release --- apoptosis
D. ATM phosphorylation ---- epistasis
E. miRNAs mis-expression --- carcinogenesis

XIV-3) Which of the following pairs of transcription factors and genes they directly regulate is INCORRECT?

A. HIF-1 and *VEGF (VEGFA)*
B. p53 (TP53) and *p21(CDKN1A)*
C. FOS and *BRCA2*
D. E2F and *CDC25A*
E. p53 and PUMA

XIV-4) Which of the following statements concerning cytokines is TRUE?

A. NF-κB is the critical cytokine responsible for the development of lung fibrosis following irradiation
B. A paracrine response is the result of a cytokine targeting the same cell that produced the cytokine
C. Most cytokines are tyrosine kinases
D. Cytokines are proteins released by irradiated cells that stimulate tissues to produce a biological response
E. An autocrine response is the result of a cytokine targeting cells adjacent to the cell that produced the cytokine

XIV-5) Which of the following statements concerning the response of NF-κB to ionizing radiation exposure is FALSE?

A. NF-κB is a transcription factor
B. The inhibitor of NF-κB (I□B) is phosphorylated by ATM and degraded, allowing NF-κB to move from the cytoplasm into the nucleus
C. NF-κB generally acts to stimulate apoptosis and enhance the radiosensitivity of cells
D. Both DNA double-strand breaks and reactive oxygen species generated by radiation exposure can activate NF-κB
E. NF-κB is sequestered as an inactive form in the cytoplasm by interaction with an inhibitory subunit of the IκB

XIV-6) Based on functional genomic studies using microarray profiling, which one of the following statements best describes the transcriptional response of irradiated cells and tissues?

A. Many genes are up-regulated by radiation exposure, but down-regulation of genes is rarely observed
B. The transcriptional response to radiation is complex, but for a given cell line, similar responses will be seen between 2 and 24 hours post-irradiation
C. The transcriptional response is dynamic, varying with time after irradiation, but overall is similar for most cell lines examined to date
D. Transcriptional responses depend on the time elapsed after irradiation, and on the cell's tissue of origin, but do not vary significantly between cell types derived from the same tissue, or between different individuals
E. Variability observed in transcriptional profiles between individuals may provide a basis for prediction of individual therapeutic responses in the future as a basis for individualized medicine.

XIV-7) Concerning the p21 (CDKN1A) protein, which of the following statements is TRUE?

A. Its transcription is transactivated by p53 (TP53) in response to ionizing radiation exposure.
B. It is required for entry into S phase of the cell cycle.
C. It is up-regulated only in cells exposed to radiation doses greater than 1 Gy.
D. Overexpression of p21 causes arrest in the G_2 phase of the cell cycle.
E. It binds to BCL-xL (BCL2L1) to promote apoptosis.

XIV-8) The two most frequently activated signaling pathways in prostate cancer are driven by androgen receptor (AR) and PI3K-AKT pathway. Inhibitors of the PI3K pathway are in early clinical trials and AR inhibitors Androgen-deprivation therapy (ADT) confer clinical responses in most patients. Which statement most correctly describes the relationship between these two pathways and explains mechanistically why single inhibition of AR and PI3K-AKT pathways rarely induce tumor regression in preclinical models?

A. ADT represses an androgen receptor gene expression program governing DNA repair and inhibits repair of ionizing radiation–induced DNA damage.
B. AR and PI3K pathways regulate each other by reciprocal negative feedback, such that inhibition of one activates the other.
C. ADT represses the PI3K-AKT-mTOR pathway.
D. ADT activates the unfolded protein response.
E. All of the above.

XIV-9) The phenomenon of "oncogene addiction" most correctly refers to which of the following clinical scenarios.

A. A Chronic Myeloid Luekemia (CML) patient treated with imatinib.
B. An *EGFR*-mutant lung adenocarcinoma patient treated with bevacizumab.
C. A *BRAF*-mutant melanoma patient treated with ipilimumab.
D. An *EML4-ALK* positive lung adenocarcinoma patient treated with olaparib.
E. A Chronic Myeloid Luekemia (CML) patient treated with interferon.

XV. Cancer

XV-1) Which of the following statements is TRUE concerning the retinoblastoma protein (RB1)? RB1:

A. Is the most important downstream effector controlling the G_2 checkpoint
B. Once phosphorylated, releases E2F
C. Is encoded by an oncogene
D. Is phosphorylated by ATM
E. Activity is altered in approximately 10% of cancers

XV-2) Which statement is TRUE concerning the role of p53 (TP53) and p21 (CDKN1A) in the response of the cells to radiation?

A. p21 phosphorylates NBS1 (NBN), thereby stimulating homologous recombinational repair of DNA double-strand breaks
B. p53-mediated G_1 phase arrest results from the inactivation of p21
C. A decrease in the amount of p53 can trigger apoptosis or G_1 arrest
D. p21 inhibits CDK-cyclin activity thereby decreasing phosphorylation of RB1
E. DNA damage initiates a signal transduction pathway that results in a marked increase in transcription of the *p53* gene

XV-3) Which statement is CORRECT concerning the ataxia telangiectasia-mutated (*ATM*) gene and protein?

A. Irradiated cells derived from a person diagnosed with ataxia telangiectasia (AT) exhibit normal S and G_2 checkpoints, but an abnormal G_1 checkpoint
B. Only individuals possessing two defective copy of the *ATM* gene display symptoms of AT
C. *ATM* protein directs the NF-kB transcription factor
D. Cells derived from patients with AT typically display increased levels of p53 (TP53) phosphorylation
E. Irradiation causes autophosphorylation of ATM which converts it from an active monomer to an inactive dimer

XV-4) Which of the following pairs of cancer type and corresponding common genetic alteration in that cancer is FALSE?

A. Pancreatic — *K-RAS*
B. Kidney — *VHL*
C. Colon — *PTCH*
D. Thyroid — *RET*
E. Melanoma - *BRAF*

XV-5) Which of the following pairs of tumor suppressor proteins and their corresponding functions is INCORRECT?

A. APC — signal transduction
B. RB1 — cell cycle regulation
C. p53 (TP53) — cell cycle and apoptosis regulation
D. WT1 — translational regulation
E. BRCA1 — DNA damage repair

XV-6) Which of the following statements is TRUE concerning p53 (TP53)? p53:

A. Is encoded by an oncogene that is activated in the majority of human cancers
B. Is inactivated in the presence of drug-induced DNA damage
C. Inhibits expression of the *GADD45A*, *p21* (*CDKN1A*) and *PCNA* genes
D. Can be inactivated by Epstein-Barr virus (EBV)
E. Is modified by phosphorylation in response to DNA damage

XV-7) Oncogenes were first discovered from the study of:

A. Retroviruses
B. Bacteria
C. Yeast
D. Mice
E. Human cells in culture

XV-8) Which of the following pairs of genes or portions of genes and corresponding descriptors is CORRECT?

A. Tumor suppressor genes – activated in many human tumors
B. Exon – the non-coding region of a gene
C. Promoter – involved in regulating gene transcription
D. DNA repair gene – *EGFR*
E. Oncogene – activated through loss of heterozygosity

XV-9) Which one of the following is NOT a tumor suppressor gene?

A. *PTEN*
B. *BRCA2*
C. *WT1*
D. *NF1*
E. ABL

XV-10) Which of the following statements is TRUE concerning p53 (TP53)?

A. MDM2 binding to p53 inhibits its degradation

B. Irradiation of cells stimulates ATM to act as a phosphatase and remove phosphate groups from p53
C. Following irradiation, p53 activates CDC25C to stimulate the G_2 to M phase transition
D. p53 stimulates the activity of BAX and BID in irradiated cells, resulting in apoptosis

XV-11) Which of the following statements is TRUE concerning the products of the *INK4A/ARF* locus?

A. $p16^{INK4A}$ (CDKN2A) stimulates the hyper-phosphorylation of the RB (RB1) protein resulting in release of the E2F transcription factor
B. $p14^{ARF}$ is induced by the RAS/MEK/MAPK pathway and stimulates cell growth
C. $p16^{INK4A}$ is encoded by a proto-oncogene
D. $p16^{INK4A}$ is activated by the PI3K/AKT pathway and increases synthesis of cyclin D
E. $p14^{ARF}$ inhibits the MDM2-mediated degradation of p53

XV-12) Which of the following represents a potential/actual therapeutic target in the oncogene-addicted tumor?

A. Mutated KIT and/or PDGFR in gastrointestinal stromal tumors (GIST).
B. Translocated ABL1 (previous symbol ABL) in T-cell acute lymphoblastic leukemia.
C. Amplified MYC in non-small cell lung carcinoma.
D. Translocated ALK in small cell lung carcinoma.
E. Mutated Notch1 in chronic myeloid leukemia.

XVI. Total Body Irradiation

XVI-1) An employee working in a nuclear power plant is accidentally exposed to a total body γ-ray dose of 2 Gy. Ten days after the accident, you draw blood and submit it for hematologic analysis. Which of the following would you expect to see?

A. A decrease in hemoglobin concentration and platelet counts
B. A decrease in platelet count and an increase in lymphocyte count
C. A decrease in lymphocyte count, but no effect on hemoglobin concentration
D. An increase in neutrophil count, but no effect on hemoglobin concentration
E. No effect on lymphocytes, hemoglobin, neutrophils or platelets

XVI-2) A terrorist preparing a "dirty bomb" containing ^{210}Po received a total body dose equivalent of approximately 8 Sv resulting from an accidental ingestion of this radioisotope. He did not seek medical attention and died 7 days later from acute radiation toxicity. Which of the following would you expect to see at autopsy?

A. Complete bone marrow aplasia
B. Mitotic arrest of intestinal crypt cells
C. Cerebral edema
D. Microvasculitis
E. Brain necrosis

XVI-3) Which of the following pairs of total body radiation effects and approximate threshold dose is CORRECT?

A. Gastrointestinal syndrome – 2 Gy
B. LD_{50} (no medical intervention) – 3.5 Gy
C. LD_{50} (best current medical treatment) – 15 Gy
D. Cerebrovascular syndrome – 5 Gy
E. Hematopoietic syndrome – 0.2 Gy

XVI-4) The death of a person 30-60 days following a total body radiation dose close to the LD_{50} would likely be due to damage to the:

A. Heart
B. Bone marrow
C. Central nervous system
D. Brain
E. Gastrointestinal system

XVI – 5) Which of the following statements is correct? After total body irradiation, the prodrome of the radiation syndrome:

A. Is not seen unless doses exceed 10 Gy

B. Occurs after the exposed person has recovered from the GI syndrome
C. Can be ameliorated through treatment with amifostine approximately 3-5 hours after the exposure
D. Includes GI symptoms such as anorexia, nausea and vomiting that occur within minutes to hours following exposure and lasting hours to days, depending on the radiation dose
E. Is characterized by hematopoietic system damage, but no effects related to the gastrointestinal system

XVI-6) Following a total body dose of 12 Gy, an exposed individual will not show the bone marrow syndrome because:

A. Higher doses than 12 Gy are needed to cause the bone marrow syndrome
B. The individual will likely die within 5-16 days from the GI syndrome, before overt symptoms of the bone marrow syndrome occur
C. This dose is not sufficiently high to cause any radiation syndrome
D. A bone marrow transplant will likely have been given and would mask the symptoms of the bone marrow syndrome
E. At this dose the radiation syndrome prodrome will be so severe it will overshadow the bone marrow syndrome

XVI-7) For individuals accidentally exposed to radiation, a bone marrow transplant is potentially useful when the radiation dose is within a narrow range. That dose window is approximately:

A. 1-2 Gy
B. 3-4 Gy
C. 8-10 Gy
D. 15-20 Gy
E. Bone marrow transplants have no potential usefulness at any dose

XVI-8) Which of the following statements is FALSE regarding the symptoms that make up the prodrome after total body irradiation:

A. After receiving 2 Gy, almost 80% of those affected experience nausea and vomiting within 1 hour
B. Serotonin-receptor antagonists are recommended in the management of nausea and vomiting after total body irradiation
C. Time to onset of prodromal symptoms is inversely related to radiation dose
D. Diarrhea is a prodromal symptom
E. The severity of prodromal symptoms is directly related to radiation dose

XVI-9) Which of the following is NOT recommended as part of routine management of the gastrointestinal radiation syndrome after accidental total body irradiation:

A. Antiemetics
B. Antibiotics

C. Antidiarrheals
D. Corticosteroids
E. Oral nutritional support

XVII. Clinically Relevant Normal Tissue Responses to Radiation

XVII-1) Which of the following statements concerning the effects of radiation on the heart is TRUE?

A. Pericarditis is the main manifestation of radiation-induced heart disease among radiotherapy patients
B. In the absence of concurrent chemotherapy, cardiomyopathy is observed during or shortly after the completion of radiotherapy
C. An increased incidence of cardiovascular disease among Hodgkin's disease survivors who received mediastinal radiotherapy has not been observed
D. The critical structure associated with the pathogenesis of radiation-induced heart disease appears to be the endothelial lining of blood vessels
E. An excess relative risk for myocardial infarction has been detected in the Japanese atomic bomb survivors, but only among those who received doses greater than 10 Gy

XVII-2) All of the following complications have been observed after high-dose irradiation of short segments of bone, EXCEPT:

A. Osteoradionecrosis
B. Stress fractures
C. Growth retardation after irradiation of epiphyseal plates in children
D. Radiation-induced bone sarcomas
E. Bone marrow failure

XVII-3) Common manifestations of delayed anorectal radiation toxicity include all of the following, EXCEPT:

A. Intestinal obstruction
B. Bleeding
C. Intermittent incontinence
D. Pain
E. Urgency

XVII-4) Acute radiation esophagitis presents as dysphagia or a substernal burning sensation with as early as 2 weeks after the start of conventionally fractionated radiation therapy. Medical management most aoften involves:

A. Angiotensin converting enzyme inhibitors
B. Gene therapy with manganese superoxide dismutase
C. Non-steroidal anti-inflammatory drugs
D. Pentoxifylline
E. Vitamin C

XVII-5) One type of radiation-induced bone injury is mandibular radionecrosis (MORN). Which of the following is NOT a risk factor for MORN?

A. Presence of teeth
B. Pre-existing dental disease
C. Use of fluorinated water
D. Tooth extraction after radiotherapy
E. Use of large doses per fraction during treatment

XVII-6) Which of the following types of blood cells is most radioresistant?

A. Granuocyte/monocyte colony forming cells (GM-CFC)
B. Spleen-colony forming units (CFU-S)
C. Macrophages
D. Unprimed T-helper cells
E. B-cells

XVII-7) What portion of the gastrointestinal tract generally exhibits the greatest acute radiation-induced injury for a given dose?

A. Stomach
B. Oropharynx
C. Small intestine
D. Large intestine
E. Esophagus

XVII-8) Which of the following statements concerning radiation-induced damage to the eye is TRUE?

A. The threshold X-ray dose for a radiation-induced cataract is approximately 10 Gy
B. It is often possible to distinguish a radiation-induced cataract from an age-induced one
C. The neutron RBE for cataract formation is about 5 for low total doses
D. The tolerance dose for the production of blindness is lower than for cataract formation
E. The length of the latency period for cataract formation is independent of radiation dose

XVII-9) Which of the following statements is TRUE concerning radiation effects on the bone marrow?

A. In general, B cells are more radiosensitive than T cells
B. Following total body irradiation, thrombocytopenia is typically observed before neutropenia
C. Lymphocyte counts do not decrease until several weeks after total body irradiation
D. Individuals suffering from the bone marrow syndrome usually die of severe anemia
E. There is no late effect pathology associated with bone marrow irradiation

XVII-10) Which of the following statements is TRUE concerning the effects of radiation on the gonads?

A. Older women are more sensitive to radiation-induced sterility than younger women
B. An acute dose of 3 Gy can both destroy the gametogenic epithelium and eliminate the production of sex hormones in adult men
C. Spermatids and spermatozoa are quite radiosensitive whereas spermatogonia are relatively radioresistant
D. A minimum waiting period of 5 years is recommended for both men and women before attempting procreation following radiotherapy, in order to reduce the risk of radiation-induced genetic effects
E. If sterility in the male is not produced within the first month after the start of radiotherapy, it is unlikely to ever occur

XVII-11) With respect to radiation-induced toxicity in the lung, which of the following statements is FALSE?

A. The likelihood of the injury is dependent on the volume irradiated
B. A characteristic pulmonary late effect, radiation pneumonitis, typically arises about 6-12 months following the completion of radiotherapy
C. The dose response curve for lung injury following whole lung irradiation is steep regardless of the dose per fraction used
D. Lung toxicity is enhanced when radiation is combined with many different types of chemotherapy
E. Several cell types are involved in the development of pulmonary late effects, including the type II pneumocyte, the alveolar macrophage and vascular endothelial cells

XVII-12) The oral mucosa and skin present with many similar pathological features during their progression toward radiation toxicity. Which of the following statements regarding the overlapping pathologies observed in these tissues is FALSE?

A. Oral mucositis is a result of the death and consequent desquamation of the epithelial layers, and is therefore an analogous event to the radiodermatitis (dry/moist desquamation) seen as an early response in irradiated skin
B. Erythema secondary to vasodilation is observed in skin following doses greater than about 2 Gy, similar to the case for mucositis
C. Radiation effects in both oral mucosa and skin are dependent on total dose, fraction size and volume irradiated
D. Possible late effects in both skin and oral mucosa include ulceration and fibrosis
E. The development of dental caries following oral radiotherapy is similar mechanistically to the infections that accompany radiation-induced dermal ulcers; both result from ischemic necrosis due to the loss of small blood vessels

XVII-13) With respect to radiation-induced heart disease (RIHD), which one of the following statements is FALSE?

A. Individuals 20-65 years of age have a lower risk for the development of radiation-induced coronary artery disease compared with other age groups
B. The parietal pericardium may be damaged by radiation therapy, with the injury typically presenting as an increased thickness of the fibrous layer
C. The risk of pericarditis increases with increasing dose per fraction
D. The majority of cardiac complications observed are consistent with the hypothesis that the most radiosensitive cells are the cardiomyocytes
E. Cardiac effects are described as “delayed”, and typically appear months to years after radiotherapy

XVII-14) With respect to the morphologic changes associated with radiation-induced liver disease, notably veno-occlusive disease (VOD), all of the following may be observed, EXCEPT:

A. Heavy congestion in the sinusoids
B. Atrophy of the liver plates
C. Fiber-filled lumen of the sublobular veins
D. Apoptotic Kupffer cells filled with hematoxylin
E. Subacute morphological changes

XVII-15) Which of the following statements regarding radiation-related inflammatory effects is FALSE?

A. Following radiation injury, the extent of neutrophil infiltration into the irradiated volume is positively correlated with the severity of the late complication
B. A distinct inflammatory phase is a major component of many acute tissue reactions
C. In both experimental animals and humans, late infiltrations of activated macrophages have been noted in irradiated tissues such as lung and oral mucosa
D. Total body irradiation to doses of 1 Gy or more can lead to abnormalities in T cell immunity

XVII-16) Which of the following statements concerning irradiation of the CNS is FALSE?

A. Selective damage to gray matter would preclude radiation as the cause of injury
B. Demyelination and white matter necrosis are common manifestations of radiation-induced injury to the CNS
C. Oligodendrocytes and vascular endothelial cells are considered to be the principal target cells for radiation-induced damage to the CNS
D. Most forms of radiation injury to the CNS are characterized by distinct pathognomonic characteristics specific to radiation-induced damage
E. Cognitive deficits are a late effect seen in both children and adults

XVII-17) Which of the following statements is correct? Following acute irradiation of the skin:

A. Epilation and the loss of sebaceous gland secretions follow similar time courses
B. The first visible reaction is moist desquamation, typically observed within 24 hours of irradiation
C. Epilation is only observed at doses much greater than those that cause the main wave of erythema observed at about one week
D. Pigment changes are typically seen within days due to the high proliferation rate of melanoblasts
E. It is usually possible to predict the extent of late reactions based on the severity of early reactions

XVII-18) Which of the following conditions is NOT an expected manifestation of radiation-induced heart disease?

A. Accelerated coronary atherosclerosis
B. Hypertrophic cardiomyopathy
C. Cardiac fibrosis
D. Pericarditis
E. Cardiac myocyte degeneration

XVIII. Mechanisms of Normal Tissue Radiation Responses

XVIII-1) Which of the following cytokines is generally considered both anti-inflammatory and immunosuppressive?

A. Interleukin 1
B. Interleukin 6
C. Interleukin 8
D. Interleukin 10
E. Tumor necrosis factor alpha (TNF□)

XVIII-2) Studies with laboratory animals have shown that all of the following interventions can reduce lethality after total body irradiation, EXCEPT:

A. Fluid and electrolyte therapy
B. Inhibitors of poly(ADP-ribose) polymerase (PARP)
C. Antibiotics
D. Probiotics
E. Blood product administration

XVIII-3) With regard to the retreatment tolerance of previously-irradiated normal tissues, which of the following statements is FALSE?

A. The lung is capable of long-term recovery after doses that are below the tolerance dose for radiation pneumonitis
B. Re-irradiation tolerance for acute damage in rapidly-dividing mucosal tissues is generally observed
C. The spinal cord is capable of moderate long-term recovery after irradiation
D. Re-irradiation tolerance of the kidney increases with increasing time interval between treatments, indicating continuous repair of sub-threshold damage
E. The onset of late bladder damage occurs much earlier in animals that were re-irradiated following a low sub-tolerance intital radiation dose, as opposed to being treated to tolerance in a single course of therapy

XVIII-4) Which of the following statements concerning radiation-induced late effects is TRUE?

A. Most late effects develop primarily as a direct result of endothelial cell killing
B. Most late effects are due to the loss of parenchymal cell clonogens
C. Radiation-induced late effects produce unique pathological responses
D. The development of late effects shares many elements in common with both acute and chronic wound-healing responses in normal tissues
E. Once present, late effects are irreversible

XVIII-5) With regard to the latency period for the expression of radiation-induced normal tissue injury, which of the following statements is CORRECT?

A. The latency period for early-responding tissues decreases markedly with increasing radiation dose
B. Shortening the overall treatment time by accelerating radiotherapy substantially reduces the latency period for early-responding tissues
C. Shortening the overall treatment by accelerating radiotherapy tends to increase the latency period for late-responding tissues
D. The higher the total radiation dose, the shorter the latency period for many late-responding tissues
E. The latency period for early-responding tissues depends on the rate of vascular endothelial cell turnover

XVIII-6) For normal tissues such as spinal cord, a small dosimetric hotspot could be disastrous in terms of increasing the likelihood for a serious late complication. However, a small volume receiving a high dose during lung irradiation may not lead to any late sequelae. The best explanation for this observation is that:

A. The spinal cord has a large functional reserve, but the lung does not
B. Target cells in the lung are better able to repair radiation damage than their counterparts in the spinal cord
C. Surviving clonogens in the lung can repopulate rapidly, whereas those in the spinal cord cannot
D. Migration of cells from outside the irradiated volume helps to augment lung function, but this process does not occur in the spinal cord
E. The putative functional subunits in the lung are arranged in parallel, whereas those in the spinal cord are arranged in series

XVIII-7) Radiation effects in the nervous system typically arise as a consequence of damage to:

A. Axons
B. Neurons
C. Oligodendrocytes and glial cells
D. The perikaryon
E. Dendrites

XVIII-8) Which of the following statements is TRUE concerning irradiation of the salivary glands?

A. Serous acinar cells die only by mitotic catastrophe after irradiation
B. The serous acinar cells of the parotid and submaxillary glands are considered the target cells for radiation-induced salivary gland damage
C. Salivary dysfunction is a late radiation effect rarely observed earlier than six months following treatment
D. Mucous cells are more radiosensitive than serous cells
E. Dose fractionation results in significant sparing of serous cells

XVIII-9) Which statement concerning TGF-□1 (TGFB1) and bFGF (FGF2) is TRUE?

A. The pro-fibrotic activities and role in radiation-induced fibrosis of TGF-□□ are mediated by SMAD3
B. Stimulation of TGF-□□ synthesis should improve the therapeutic ratio
C. bFGF has been shown to sensitize endothelial cells to radiation-induced apoptosis
D The serum concentration of TGF-□□ generally decreases following lung irradiation

XVIII-10) Regarding radiation fibrosis, which of the following statements is TRUE?

A. Fibrosis occurs in only a select few tissues and organs
B. The severity of late fibrosis can be predicted based on radiotherapy treatment parameters and is not tissue-dependent
C. Radiation fibrosis is typically inhomogenous; some affected areas could be densely collagenous whereas others may have only a few fibrous bands, despite both areas having received the same dose
D. Irradiated bone marrow commonly develops regions of fibrosis
E. Increases in collagen deposition are associated with the down-regulation of fibrogenic cytokines.

XVIII-11) The cells thought to be responsible for radiation-induced cognitive dysfunction reside in:

A. Medulla oblongata
B. Cerebral cortex
C. Substantia nigra
D. Hippocampus
E. Hypothalamus

XIX. Therapeutic Ratio

XIX-1) For tumors greater than 5 cm in size, what strategy is likely to improve response to radiation therapy?

A. Stimulation of repair in hypoxic cells
B. Inhibition of reoxygenation
C. Radioprotection of aerobic cells
D. Inhibition of repair in aerobic cells
E. Stimulation of repopulation in hypoxic cells

XIX-2) A 1 cm-diameter tumor that contains 10^7 clonogenic cells is irradiated with daily dose fractions of 1.8 Gy. The effective dose response curve has been determined and is exponential with a D_{10} of 8 Gy. What total dose will correspond to the TCD_{90} (90% probability of tumor control), assuming no cell proliferation between dose fractions?

A. 32 Gy
B. 40 Gy
C. 48 Gy
D. 56 Gy
E. 64 Gy

XIX-3) Based on the information presented in the previous question, what would be the TCD_{90} if a surgical excision removed 99% of the tumor clonogens prior to radiotherapy (assume that the surgery did not otherwise affect the growth fraction of the tumor).

A. 24 Gy
B. 32 Gy
C. 40 Gy
D. 48 Gy
E. 56 Gy

XIX-4) For a tumor that requires 18 days to double its diameter, what is the approximate cell cycle time of its constituent cells (assume no cell loss and that all cells are actively dividing)?

A. 6 days
B. 9 days
C. 12 days
D. 15 days
E. 18 days

XIX-5) For a standard course of radiotherapy, which of the following properties of a tumor would NOT be expected to adversely affect tumor control?

A. Low SF_2
B. Short T_{pot}
C. Slow reoxygenation
D. Large number of tumor clonogens
E. Early onset of repopulation

XIX-6) Which of the following represents a possible mechanism by which a novel compound could increase tumor response to fractionated radiotherapy if applied prior to each dose fraction?

A. Prevents cell cycle redistribution
B. Causes G_2 phase arrest
C. Increases reoxygenation
D. Radioprotects normal tissues
E. Stimulates DNA repair

XIX-7) All of the following could affect the slope of a tumor control probability (TCP) curve, EXCEPT:

A. Tumor size
B. Tumor oxygenation
C. Intrinsic tumor cell radiosensitivity
D. Volume of normal tissue in the radiation field
E. Histopathological tumor type and grade

XIX-8) A new agent that can alter blood flow is being assessed for its potential clinical usefulness in combination with radiation therapy. Which of the following effects on blood flow would be expected to result in therapeutic gain and thus lead to a potentially useful agent in the clinic?

A. Increased in both tumors and normal tissues
B. Increased in normal tissues but decreased in tumors
C. Decreased in normal tissues and in tumors
D. Not altered in normal tissue but decreased in tumors
E. Increased in normal tissues and not altered in tumors

XIX-9) Which of the following statements is correct? Normal tissue regeneration/repopulation:

A. Occurs in acutely responding normal tissues during the course of a standard radiotherapy protocol
B. Interferes with reoxygenation
C. Is the reason that prolonging overall treatment time spares late responding normal tissues
D. Occurs at the same rate after irradiation in acutely and late responding tissues
E. Is the reason why accelerated fractionation protocols increase reactions in late responding normal tissues

XX. Time, Dose, Fractionation

XX-1) Which of the following total doses, given as daily 1.5 Gy fractions, is approximately equivalent to a conventional schedule of 30 fractions of 2 Gy for late normal tissue reactions? Assume the α/β ratio is equal to 3 Gy.

A. 53 Gy
B. 60 Gy
C. 67 Gy
D. 75 Gy
E. 81 Gy

XX-2) Assuming no difference in overall treatment time, which of the following statements is CORRECT concerning isoeffect curves?

A. Tissues with a greater repair capacity have steeper isoeffect curves.
B. Increased proliferation of the critical cell population during the course of radiotherapy will decrease the slope of the isoeffect curve.
C. Tissues with steep isoeffect curves have high α/β ratios.
D. Isoeffect curves for tumor control will be steeper if significant reoxygenation occurs between dose fractions.

XX-3) A total dose of 70 Gy delivered in 2 Gy fractions is used to treat a particular tumor. Assume that the tumor is characterized by an α/β ratio of 2 Gy and a T_{pot} of 30 days. For the dose-limiting normal tissue, the α/β ratio is 4 Gy. Which one of the following treatment schedules would most likely yield the highest therapeutic ratio?

A. Standard fractionation
B. Accelerated treatment
C. Split-course treatment
D. Hyperfractionation
E. Hypofractionation

XX-4) Which of the following fractionation schedules would likely produce the highest incidence of late normal tissue toxicity? (Assume α/β = 2 Gy for the critical normal tissue injury)

A. 20 Gy in 4 fractions over 1 week
B. 24 Gy in 6 fractions over 2 weeks
C. 45 Gy in 15 fractions over 3 weeks
D. 50 Gy in 25 fractions over 5 weeks
E. 60 Gy in 60 fractions over 6 weeks

XX-5) A standard treatment protocol for a particular type of cancer is 60 Gy delivered in once-daily 2 Gy fractions. If the fraction size is decreased to 1.3 Gy in an attempt to reduce the incidence of late effects, approximately what total dose should be delivered to maintain the same level of tumor control? (Assume an equal effect per fraction, no repopulation, and an α/β ratio for the tumor of 10 Gy.)

A. 64 Gy
B. 68 Gy
C. 72 Gy
D. 76 Gy
E. 80 Gy

XX-6) Which of the following statements is correct? One goal of hyperfractionation is to:

A. Decrease toxicity to early-responding tissues
B. Deliver the total radiation dose in a shorter overall time
C. Reduce the number of fractions used
D. Prevent tumor cell repopulation
E. Decrease the incidence of late effects while maintaining or improving tumor control

XX-7) Which of the following statements is TRUE concerning experimental support for the hypothesis that late-responding tissues have lower α/β ratios than early-responding tissues?

A. High LET radiations exhibit RBEs that are greater for early effects than for late effects
B. The use of hyperfractionation results in an increased severity of late effects if the dose is titrated to produce equal early effects
C. Isoeffect curves are steeper for late effects than for early effects
D. When a treatment plan is changed from many small doses to a few large fractions and the total dose is titrated to produce equal early effects, late effects tend to be less severe

XX-8) A clinician changes from the usual fractionation schedule of 1.8 Gy given once per day to an accelerated treatment using 1.6 Gy fractions delivered twice per day. In order to avoid the possibility of reduced normal tissue tolerance due to incomplete repair, what should be the minimum inter-fraction interval for the accelerated schedule?

A. 0.5-1 hour
B. 1-2 hours
C. 2-3 hours
D. 3-6 hours
E. 6-8hours

XX-9) A conventional treatment for a particular type of tumor is 25 fractions of 2 Gy delivered once per day. A hyperfractionated regimen is proposed that would consist of 1.2 Gy fractions delivered twice per day. What would be the approximate therapeutic gain in changing from the standard to hyperfractionated schedule if both were designed to produce the same probability of late complications? (Assume that there is no tumor cell repopulation during treatment, full repair of sublethal damage occurs, the tumor has an α/β ratio of 10 Gy and the normal tissue has an α/β ratio of 2 Gy.)

A. 0.8
B. 1.0
C. 1.2
D. 1.4
E. 1.6

XX-10) All of the following processes could be involved in the increased efficacy of radiation dose fractionation in the clinic, EXCEPT:

A. Sublethal damage repair in normal tissues between fractions
B. Reoxygenation in tumors
C. Redistribution/reassortment of cells in tumors
D. Repopulation of critical cell populations in normal tissues
E. Potentially lethal damage repair in tumors

XX-11) Data suggests that treatment breaks are detrimental to tumor control in head and neck cancer. The radiobiological basis of this phenomenon is:

A. Redistribution
B. Reoxygenation
C. Repair
D. Repopulation
E. Radiosensitization

XX-12) Continuous hyperfractionated accelerated radiation therapy (CHART) involved all of the above EXCEPT:

A. Short overall treatment time of 12 consecutive days
B. Three fractions of radiation per day
C. Total dose of 50 – 54 Gy
D. Low dose per fraction (1.4 – 1.5 Gy)
E. Concurrent chemotherapy

XX-13) Data has suggested that overall treatment time is crucial for which of the following tumors:

A. Head and neck cancer
B. Endometrial cancer
C. Melanoma
D. Breast cancer
E. Basal cell carcinoma

XXI. Brachytherapy

XXI-1) Which of the following equations would be most appropriate to use when calculating the BED for treatment involving a permanent radioactive implant?

Use:
n – number of fractions
d – dose per fraction
α and β – tissue specific dose response curve parameters
T – duration of irradiation
T_K – time at which accelerated proliferation begins
T_{pot} – potential doubling time
h_M – incomplete repair factor
μ – repair rate constant = $0.693/t_{½}$
$t_{½}$ – tissue repair half-time
R_0 – initial dose-rate
R – dose-rate
λ – radioactive decay constant = $0.693/T_{1/2}$
$T_{1/2}$ – radioactive half life of isotope

A. $BED = nd\left[1 + \frac{d}{\alpha/\beta}\right]$

B. $BED = nd\left[1 + \frac{d}{\alpha/\beta}\right] - \left[\frac{0.693(T - T_K)}{\alpha T_{pot}}\right]$

C. $BED = nd\left[1 + \frac{d(1 + h_M)}{\alpha/\beta}\right]$

D. $BED = RT\left\{1 + \left[\frac{2R}{\mu(\alpha/\beta)}\right]\left[1 - \frac{1 - e^{-\mu T}}{\mu T}\right]\right\}$

E. $BED = \frac{R_0}{\lambda}\left\{1 + \left[\frac{R_0}{(\mu + \lambda)(\alpha/\beta)}\right]\right\}$

XXI-2) Which of the following radiobiological processes contributes to the inverse dose rate effect?

A. Repair of sublethal damage
B. Accumulation of cells in S phase
C. Proliferation
D. Repair of potentially lethal damage
E. Redistribution

XXI-3) All of the following are used for brachytherapy implants EXCEPT:

A. Cesium-137
B. Iridium-192
C. Iodine-125
D. Iodine-131
E. Gold-198

XXI-4) Which of the following has a half-life of 30 years?

A. Cesium-137
B. Iridium-192
C. Iodine-125
D. Iodine-131
E. Gold-198

XXI-5) Iridium-192 is characterized by the following EXCEPT:

A. It is the most widely used radionuclide for brachytherapy procedures in the US.
B. It has a small source size.
C. It is used for permanent implants.
D. The lower photon energy makes radiation protection easier than radium or cesium.
E. It is available for use with computer-controlled remote afterloaders.

XXII. Radiobiological aspects of alternative dose delivery systems

XXII-1) Which of the following statements about carbon ion therapy is FALSE?

A. For a given dose to the tumor in the Bragg peak, carbon ions produce better sparing of normal tissues in the entrance region of the beam than either protons or photons
B. Carbon ions have a high RBE in the Bragg peak region
C. There is reduced scattering in both the lateral and longitudinal directions for carbon ions compared to protons
D. There is a greater variation in radiosensitivity between oxygenated and hypoxic tumor cells using carbon ions compared with photons
E. PET verification can be used for carbon ion treatment plans

XXII-2) Which of the following statements concerning intensity-modulated radiation therapy (IMRT) is CORRECT? IMRT:

A. Employs significantly higher energy photon beams than unmodulated radiation dose-delivery techniques.
B. Results in a fewer radiation therapy-induced second cancers in children than in adults.
C. Is most conformal if used in the conventional 1.8-2.0 Gy/fraction radiotherapy protocol.
D. Allows for higher doses to acutely responding normal tissues while decreasing dose to late responding normal tissues.
E. The whole body patient dose is increased with IMRT, compared to treatment plans involving unmodulated beams due to leakage from head, there is scatter from collimator.

XXII-3) Which one of the following statements concerning radiolabeled immunoglobin therapy is FALSE?

A. One disadvantage associated with the use of ^{90}Y-labeled antibodies is that the relatively low energy (<100keV) and short range β-particles emitted, limit the so-called "crossfire effect"
B. Radiation safety is an important issue with the use of ^{131}I-labeled compounds because this isotope emits γ-rays that may pass through the patient
C. The dose-limiting organ associated with the use of Baxxar/tositumomab is the bone marrow
D. Both Zevalin and Bexxar target CD20

XXII-4) Which of the following 5Rs of radiobiology likely has a negative impact on severely hypofractionated schedules (1-5 fractions) used in stereotactic body radiotherapy?

A. Radiosensitivity
B. Repair/recovery
C. Redistribution/reassortment

D. Repopulation
E. Reoxygenation

XXIII. Chemotherapeutic agents and radiation therapy

XXIII-1) Which of the following is a small molecule tyrosine kinase inhibitor?

A. Trastuzumab
B. Erlotinib
C. Bevacizumab
D. Sirolimus
E. Cetuximab

XXIII-2) Which of the following statements is TRUE concerning bortezomib? Bortezomib is:

A. An agent that stimulates ubiquitin-mediated degradation of I□B
B. FDA-approved for use in the treatment of pancreatic cancer
C. A drug that specifically targets EGFR signaling pathways
D. A proteasome inhibitor
E. A monoclonal antibody

XXIII-3) Which of the following best describes the mechanism of action of the chemotherapeutic agent, irinotecan?

A. Inhibits ribonucleotide reductase
B. Stimulates thymidylate synthase
C. Interferes with the action of topoisomerase I
D. Inhibits DNA helicases
E. Generates DNA crosslinks

XXIII-4) Sorafenib targets which of the following proteins?

A. p53 (TP53)
B. Cyclooxygenase 2
C. RAF1 kinase
D. Histone deacetylase
E. DNA-PKcs (PRKDC)

XXIII-5) Which of the following best describes the mechanism of action of tipifarnib?

A. Inhibitor of BCR/ABL
B. Interferes with PTEN
C. Inhibitor of RAD9
D. Activator of p53 (TP53)
E. Inhibitor of farnesyl transferase

XXIII-6) Which of the following molecularly-targeted agents is an epidermal growth factor receptor inhibitor?

A. Bevacizumab
B. Combretastatin
C. Imatinib
D. Cetuximab
E. Rituximab

XXIII-7) Which of the following pairs of drug and description is CORRECT?

A. Glutathione – hypoxic cell cytotoxin
B. Nimorazole – most abundant cell sulfhydryl
C. Tirapazamine – radioprotector
D. Amifostine – bioreductive drug
E. Gefitinib – small molecule tyrosine kinase inhibitor

XXIII-8) Which of the following pairs of a chemotherapeutic agent and its potential target is CORRECT?

A. Etoposide – topoisomerase II
B. Topotecan – microtubules
C. Bevacizumab – EGFR
D. Sunitinib – histone deacytlase
E. 5-fluorouracil – glutathione

XXIII-9) Which of the following statements is correct? Multi-drug resistance:

A. Generally leads to cross-resistance to radiation
B. Is often induced by pre-exposure to ionizing radiation
C. Can be caused by either an increase in p-glycoprotein or other proteins that increase drug efflux
D. Generally results in relatively small changes in sensitivity of cells or tumors to chemotherapy agents
E. Is a transient response to intensive treatment and usually resolves within 4-6 weeks

XXIII-10) Which of the following pairs of chemotherapy drugs and the dependence of their toxicity on oxygenation status is INCORRECT?

A. Bleomycin – more toxic under aerated conditions
B. Tirapazamine – more toxic under hypoxic conditions
C. 5-Fluorouracil – no difference in toxicity between aerated and hypoxic conditions
D. Mitomycin C – more toxic under aerated conditions
E. Misonidazole – more toxic under hypoxic conditions

XXIII-11) Which of the following statements concerning photodynamic therapy is INCORRECT? Photodynamic therapy:

A. Reduces tumor burden through direct tumor cell killing rather than indirectly through damage to tumor vasculature
B. Is generally used to treat either superficial tumors or those that can be accessed with fiberoptic probes
C. Involves the use of a drug activated by visible light
D. Is toxic through the formation of singlet oxygen
E. Is maximally effective in aerobic tissues

XXIII-12) Cisplatinum has all of the following properties EXCEPT:

A. It inhibits DNA synthesis more than RNA or protein synthesis.
B. It is cell-cycle non-specific.
C. It is similar in efficacy to its isometer, trans-platinum.
D. It causes both interstrand and intrastrand crosslinking.
E. It is used as a radiosensitizer with concurrent radiation therapy.

XXIII-13) Which of the following is associated with cardiac toxicity?

A. Adriamycin
B. Cisplatinum
C. Bleomycin
D. Methotrexate
E. Docetaxol

XXIII-14) Which of the following effects is associated with improved survival in patients treated with radiation therapy and cetuximab for head and neck cancer:

A. Hair loss
B. Erythema
C. Acneiform rash
D. Desquamation
E. Pruritis

XXIII-15) Which of the following targeted agents is an immune checkpoint inhibitor?

A. Bevacizumab
B. Ipilimumab
C. Imatinib
D. Cetuximab
E. Crizotinib

XXIII-16) Which of the following pairs of a targeted agents and its potential target is CORRECT?

A. Crizotinib – anaplastic lymphoma kinase

B. Imantinib – programmed cell death 1 (PD-1)
C. Cetuximab – ABL kinase
D. Sunitinib – Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)
E. Sorafenib – CD20

XXIII-17) Synthetic lethality refers to:

A. Indirectly targeting non-druggable cancer promoting lesions by inhibition of druggable lethal interactors.
B. Overexpression of a gene that is synthetic lethal selectively kills cancer cells but is tolerated by normal cells.
C. Loss of function mutations in the DNA damage response may provide selectivity in targeting tumor cells.
D. PARP inhibition in BRCA1/2 deficient cells acts through the induction of large scale genomic damage when the repair of DSBs by error prone mechanisms occurs rather than through homologous recombination.
E. Chemoresistance by either ATM or p53 loss can be abrogated via synthetic lethal strategies by targeting p53 in ATM deficient cells or targeting ATM in p53 deficient cells.

XXIII-18) The use of which of the following targeted agents administered with high dose radiotherapy for metastatic melanoma, has been recently shown to induce the abscopal effect resulting in distant immune modulation and tumor response?

A. Ipilimumab
B. Bevacizumab
C. Cetuximab
D. Trastuzumab
E. Rituximab

XXIII-19) Panitumumab (Vectibix™) is now indicated in the management of *k-ras wildtype* metastatic colorectal cancer that has progressed through primary chemotherapy. In mechanism of action, panitumumab is most similar to which of the following agents?

A. Rituximab
B. Cetuximab
C. Bevacizumab
D. Infliximab
E. Sunitinib

XXIII-20) Yttrium-90, a beta-emitting radioisotope is used in the management of several malignant conditions. It can be medically used in all the following forms except:

A. Bound to an anti-CD20 antibody in the treatment of certain Non-Hodgkins Lymphomas

B. Bound to resin microspheres in the treatment of hepatic metastases from colorectal cancer
C. Bound to glass microspheres in the treatment of hepatocellular carcinoma
D. Bound to metal needles in the local treatment of primary breast adenocarcinoma

XXIV. Radiosensitizers, Radioprotectors and Bioreductive Drugs

XXIV-1) Which of the following statements concerning hypoxic cell sensitizers and bioreductive drugs is TRUE?

A. One possible reason that clinical trials of hypoxic cell radiosensitizers yielded disappointing results is the dose limitation imposed by severe hypotension that often developed in patients receiving higher doses of the drugs
B. Bioreductive drugs are synthesized in a pro-drug form that, upon administration, are oxidized and thereby activated to a cytotoxic intermediate
C. Bioreductive drugs are more toxic to aerobic cells than to hypoxic ones
D. Clinical trials of nimorazole have yielded results indicating a significant improvement in both local control and overall survival in patients with head and neck cancer treated with this drug and radiotherapy
E. Hypoxic cell radiosensitizers are most effective in combination with hyperfractionated radiotherapy

XXIV-2) The enzyme inhibited by 5-fluorouracil that is most closely associated with both its cytotoxic and radiosensitizing effects is:

A. Dihydrofolate reductase
B. Thymidylate synthase
C. RAD50
D. Tyrosine kinase
E. Ligase IV

XXIV-3) Radiosensitization produced by gemcitabine is associated with the inhibition of which of the following enzymes?

A. Topoisomerase I
B. DNA-PKcs (PRKDC)
C. DNA polymerase
D. Ribonucleotide reductase
E. Sphingomyelinase

XXIV-4) Sulfhydryl radioprotectors reduce radiation-induced toxicity by:

A. Preventing the formation of free radicals
B. Scavenging free radicals
C. Stimulating host immune responses
D. Inhibiting ion pair formation
E. Increasing intracellular oxygen

XXIV-5) Which of the following statements concerning amifostine is TRUE?

A. Amifostine is most effective when administered orally
B. Amifostine's dose-limiting toxicity is peripheral neuropathy
C. Amifostine does not readily cross the blood-brain barrier
D. Maximum radioprotection against acute toxicities is achieved when amifostine is administered after irradiation
E. Amifostine does not require metabolic activation for its activity as a radioprotector

XXIV-6) One proposed mechanism through which cisplatin acts as a radiosensitizer is by:

A. Inhibiting the production of dTMP
B. Interfering with DNA repair
C. Inhibiting the proteasome
D. Blocking growth factor receptors
E. Deacetylating histones

XXIV-7) Treatment with temozolomide improves survival in glioblastomas that receive radiation therapy, particularly if there is:

A. Epigenetic silencing of O6-methylguanine-DNA methyltransferase
B. Epigenetic silencing of microRNA expression
C. Epigenetic silencing of PTEN
D. Expression of the mutant receptor EGFRvIII
E. Expression or amplification of Her2/neu

XXIV-8) Rapamycin, everolimus and temsirolimus may act as a radiosensitizers by inhibiting:

A. K-ras
B. mTOR
C. MAPK
D. p38
E. EGFR

XXIV-9) Wee1 inhibitors have been tested as radiosensitizers because they:

A. Interfere with the G2/M checkpoint
B. Block phosphorylation of MAPK-related proteins
C. Suppress NHEJ repair
D. Are selectively toxic in hypoxic tumor cells

XXV. Hyperthermia

XXV-1) Which of the following statements concerning the Arrhenius analysis of mammalian cell killing by heat is FALSE?

A. This analysis suggests proteins as the likely targets for heat-induced cell killing
B. The break point in the Arrhenius plot reflects the development of thermotolerance
C. The Arrhenius relationship has been used to define the temperature dependence of the rate of heat-induced cell killing
D. An Arrhenius curve plots the log of the slopes ($1/D_0$) of heat survival curves as a function of temperature
E. The break point in the Arrhenius plot occurs at approximately 39°C

XXV-2) Hyperthermia combined with radiation may be effective in cancer therapy because:

A. Tumor cells are intrinsically more sensitive to heat than normal cells
B. Hypoxic tumor cells, which may be at a low pH and nutritionally-deprived, exhibit enhanced sensitivity to heat
C. Heat increases the number of ionizations produced by a given dose of radiation
D. Normal tissues tend to retain more heat than tumors
E. Heat can produce maximum radiosensitization even if delivered several days after irradiation

XXV-3) Which of the following statements concerning hyperthermia is TRUE?

A. Heat-induced radiosensitization occurs because heat produces additional DNA damage
B. Hyperthermia transiently down-regulates genes that encode heat shock proteins
C. Upon heating, the heat shock transcription factor, HSF1, stimulates production of heat shock proteins
D. Heat shock proteins facilitate the aggregation of nuclear proteins
E. Hyperthermia does not induce apoptotic cell death

XXV-4) Which of the following statements concerning thermotolerance is TRUE?

A. Thermotolerance is a heritable resistance to heat-induced cell killing
B. A brief exposure to a temperature above 43°C results in resistance to a subsequent additional heat treatment delivered immediately after the 43°C treatment, but at a lower temperature
C. Thermotolerance develops during the heating of tissues at temperatures higher than 43°C
D. The onset and decay of thermotolerance correlate with the appearance and disappearance of proteins associated with the repair of heat-induced DNA strand breaks
E. Heat shock proteins are molecular chaperones

XXV -5) Concerning the effects of heat on cells, which of the following statements is FALSE?

A. Cells of low pHe (extracellular pH) or low Phi (intracellular pH) are sensitive to heat.
B. Cycling cells are more sensitive to heat than non-cycling cells.
C. Both aerated and acutely hypoxic cells have similar sensitivity to heat.
D. Initial shoulder of hyperthermic survival curve suggests the repair of sublethal damage.
E. Heated cells die by apoptosis and the damage is expressed early.

XXV-6) Blood perfusion through normal and tumor tissues can be modified by heating. Which of the following statements is FALSE?

A. For both tumors and normal tissues, all functional capillaries are open and used to capacity.
B. Normal tissues have a relatively high ambient blood flow, which increases in response to thermal stress.
C. Tumor tissues have unresponsive neo-vasculature to heat and are incapable of augmenting blood flow.
D. Hyperthermia can induces compression and occulusion of tumor blood vessels.
E. Tumors get hotter than surrounding normal tissues because of ineffective dissipation of heat.

XXV-7) When hyperthermia (HT) is combined with radiotherapy (RT), there are complementary actions in inactivating tumor cells. Which of the following statements are true, EXCEPT?

A. HT causes protein inactivations while RT causes DNA double strand breaks in cells.
B. HT preferentially kills cells in S phase and RT cells in G_2/M phases of the cell cycle.
C. HT causes damage preferentially in hypoxic (acidic) regions and RT in aerobic (neutral) regions of tumors.
D. HT above 43^{o}C causes inhibition of repair of RT-induced damage.
E. Long duration mild hyperthermia (42^{o}C) cannot inhibit the repair of sublethal radiation damage (SRD), because of the low temperature.

XXVI. Radiation Carcinogenesis

XXVI-1) Which of the following statements concerning possible long-term consequences of radiotherapy is FALSE?

A. Compared to the general population, individuals who survive an initial cancer are at a decreased risk for developing a second cancer
B. There is an increased incidence of second tumors among patients initially treated for soft tissue sarcomas
C. Radiotherapy to the breast or chest wall of young women is associated with long-term cardiotoxicity and an increased risk of second breast cancers
D. Breast cancer patients with a BRCA2 defect are at increased risk of developing ovarian cancers as well as second breast cancers in either the treated or untreated breast

XXVI-2) In children, which of the following organs is the most sensitive to the induction of both benign and malignant tumors by X-rays?

A. Bone marrow
B. Intestine
C. Breast
D. Thyroid
E. Lung

XXVI-3) Among those who develop fatal cancers after total body irradiation, approximately what percentage are leukemias?

A. 0.1%
B. 2%
C. 15%
D. 40%
E. 80%

XXVI-4) For children epilated by X-rays for the treatment of tinea capitis, which of the following organs did NOT demonstrate an excess relative risk for a radiation-induced malignancy?

A. Brain
B. Thyroid
C. Pharynx
D. Bone marrow
E. Breast Cancer

XXVI-5) Which of the following statements is correct? Cancers induced in humans by whole-body irradiation with low total doses:

A. Include excess breast cancers in female radium dial painters
B. Can be distinguished from those occurring naturally
C. Clearly follow an exponential dose response
D. Exhibit similar latency periods for both leukemias and solid tumors
E. Are more likely to appear in individuals who were young at the time of irradiation

XXVI-6) Which of the following statements concerning radiation-induced effects among survivors of the atomic bombings of Hiroshima and Nagasaki is TRUE?

A. There is no change in the incidence of heart disease among survivors who received less than 5 Gy
B. Susceptibility to radiation-induced breast cancer increases with increasing age at the time of exposure
C. The latency period between irradiation and the appearance of most solid tumors is 1-3 years
D. Statistically significant increases in mortality from non-cancer causes with increasing dose have been observed
E. For a population of 1,000 people, each exposed to an acute, whole body dose of 1 Sv, roughly 8 would die from a radiation-induced cancer according to current radiation risk estimates

XXVI-7) Which of the following is NOT a general conclusion of epidemiological studies of irradiated human populations?

A. Most regulatory and advisory committees recommend that risk estimates derived from acute exposures be reduced by a Dose and Dose-Rate Effectiveness Factor (DDREF) of approximately 1.5 - 2.0 in order to apply them to chronic, low dose and low dose-rate exposures
B. Analyses of the Japanese A-bomb survivor data indicate that radiation risk is dependent on age at exposure, time since exposure, and gender
C. For solid tumors in A-bomb survivors, a linear-quadratic fit to the data is significantly better than a linear fit
D. Studies of populations living near nuclear power plants, or of populations exposed to elevated background radiation, usually do not provide a direct quantitative estimate of risk.
E. Based on the BEIR VII estimates, human exposure to ionizing radiation accounts for a lifetime excess cancer risk (both fatal and non-fatal) of roughly 1% per 100 mSv

XXVI-8) Which of the following is an example of a stochastic effect of radiation:

A. Mental retardation from exposure of the fetus in utero
B. Acute radiation toxicity from high dose exposure
C. Mental retardation resulting from mutations in the sperm passed on to the offspring

D. Development of cardiac toxicity following high dose exposure
E. Bystander effect

XXVI-9) Which of the following is true about thyroid carcinomas that occurred as a result of radiation exposure from the Chernobyl nuclear power plant accident?
A. They were induced by 137Cs radiation that settled on the ground.
B. Most of the tumors involved rearrangements of Bcl2 and Myc
C. The tumors could have been reduced in number by administering KI to the population.
D. Initial cancers were induced predominantly in adults that had been exposed.
E. The peak in incidence was approximately 20y after exposure.

XXVI-10) Which of the following tumors are considered to be highly radiogenic?
A. breast, thyroid, leukemia
B. prostate, pancreas, gallbladder
C. leukemia, brain, melanoma
D. Hodgkin's disease, cervix, ovary
E. Muscle, brain, liver

XXVII. Heritable Effects of Radiation

XXVII-1) Which of the following statements is correct? The genetically significant dose (GSD) is:

A. of particular concern with respect to radon inhalation
B. approximately 1 Sv and corresponds to the average annual dose received from all medical procedures involving ionizing radiation performed in the United States
C. the annual average gonadal dose to a population adjusted for the relative child expectancy of that population
D. an estimate of the number of children born each year with a radiation-induced mutation
E. the extrapolated lifetime gonadal dose for an individual

XXVII-2) Which one of the following statements is TRUE concerning radiation mutagenesis?

A. Radiation produces unique mutations not otherwise seen spontaneously
B. It has been reported that children of radiotherapy patients have an increased incidence of genetic abnormalities compared to children whose parents had not been irradiated prior to conception
C. Roughly 25% of the spontaneous mutations in humans can be attributed to exposure to background radiation
D. The genetic doubling dose for humans has been estimated at 1-2 Sv
E. The absolute mutation rate in humans is approximately 8% per Sv

XXVII-3) Which of the following statements is FALSE regarding studies of the Japanese A-bomb survivors by the Radiation Effects Research Foundation (RERF)?

A. Significantly more mutations are noted in children born to parents where one or both had been irradiated, and these data form the basis for the genetic doubling dose estimate for humans
B. More than 60% of the survivor cohort received acute exposures less than 100 mSv
C. One strength of the RERF Life Span Study is that robust dose estimates are available for the survivors
D. A cohort of survivors, called the Adult Health Study, receive thorough clinical examinations every two years
E. Risk estimates for radiation-induced late effects and genetic effects continue to evolve as the survivor cohort ages and their children and grandchildren are followed

XXVII-4) Which of the following statements concerning the landmark “mega-mouse” study of radiation mutagenesis, is INCORRECT?

A. The dose response curve for radiation-induced mutations was linear with no threshold
B. Radiation dose-rate was not found to affect the induction of mutations
C. Males were more susceptible to radiation-induced mutations than females
D. Mutation rates at the different loci studied varied widely
E. The estimated doubling dose for mutations was approximately 1 Gy

XXVII-5) Which of the following statements is true about radiation exposure of the male and female reproductive systems?

A. The dose to induce temporary sterility in the female is 2Gy
B. The latent period for temporary sterility in the female is 1y
C. Radiation sterility in the male affects hormone balance, libido, and physical capability.
D. The dose for low sperm count in the male is 0.15Gy.
E. The dose for permanent sterility in the female is 1.0Gy.

XXVII-6) Which of the following statements is true about ionizing radiation induced mutations?

A. Radiation-induced mutations can be identified by their T to A transitions.
B. High LET radiations tend to cause small deletions and low LET radiations tend to cause large deletions.
C. The spectrum of mutations observed with ionizing radiation is similar to that observed with UV exposure.
D. Exposure of sperm low-dose-rate radiation generally gives fewer mutations than exposure to the same dose at a high dose rate.
E. The relative dose to double mutation rates is 5Gy.

XXVIII. Radiation Effects in the Developing Embryo and Fetus

XXVIII-1) Based on animal studies, the most radiosensitive gestational age in terms of embryonic mortality in humans is approximately:

A. 0-1 weeks
B. 1-4 weeks
C. 4-8 weeks
D. 8-15 weeks
E. 15-40 weeks

XXVIII-2) Which of the following pairs of gestational stage and linked radiation-induced developmental defect is CORRECT?

A. preimplantation – congenital malformations
B. organogenesis – prenatal death
C. early fetal period – mental retardation
D. late fetal period – neonatal death
E. entire gestation period – malformations of the kidney

XXVIII-3) Mental retardation as a result of radiation exposure in utero is most likely to occur when the radiation is given at what weeks of gestation?

A. 0-4 weeks
B. 4-8 weeks
C. 8-15 weeks
D. 16-25 weeks
E. 25-40 weeks

XXVIII-4) Once a pregnancy is declared, the maximum permissible dose to the fetus is:

A. 0.005 mSv per month
B. 0.05 mSv per month
C. 0.5 mSv per month
D. 5 mSv per month
E. 50 mSv per month

XXVIII-5) Prenatal death as a result of radiation exposure in utero is most likely to occur during:

A. Pre-implantation
B. Implantation
C. Early organogenesis
D. Late organogenesis
E. The fetal period

XXVIII-6) The following conditions have been reported after high-dose human embryonic/fetal irradiation, EXCEPT:

A. Microcephaly
B. Spina bifida
C. Mental deficiency
D. Cardiac abnormalities
E. Ear abnormalities

XXVIII-7) Which of the following is true about risks associated with exposure of the embryo and fetus in utero?

A. Exposure in utero results in a period of high risk for mental retardation at 8-15 weeks of gestation and lower risk at 15-25 weeks of gestation.
B. Exposure in utero has not been considered to provide a risk for carcinogenesis to the fetus.
C. Exposure in the preimplantation phase usually results in permanent growth retardation.
D. The LD50 for oocyte killing in humans is around 5Gy.
E. Exposure of the fetus in utero has not been associated with changes in school performance or IQ.

XXIX. Radiation Protection

XXIX-1) A woman begins working at a nuclear power plant on her 18th birthday. According to NCRP guidelines, when she reaches her 20th birthday, she would have been permitted a total work-related lifetime effective dose equivalent of:

A. 5 mSv
B. 50 mSv
C. 100 mSv
D. 200 mSv
E. 300 mSv

XXIX-2) Suppose that on her 21st birthday, the same radiation worker as described in the previous question declared that she was 3 months pregnant. What additional dose limit to the fetus has the NCRP recommended for the duration of her pregnancy?

A. She would not be allowed any additional radiation exposure once the pregnancy was declared
B. 1 mSv
C. 10 mSv
D. 50 mSv, assuming that she had no measurable exposure yet that year
E. 0.5 mSv per month

XXIX-3) What are the NCRP maximum permissible annual dose limits for the eye and to localized skin areas for radiation workers?

A. 50 mSv to the eye and skin
B. 150 mSv to the eye and skin
C. 150 mSv to the eye and 50 mSv to the skin
D. 150 mSv to the eye and 500 mSv to the skin
E. 500 mSv to the eye and150 mSv to the skin

XXIX-4) In the United States, the average annual effective dose equivalent from all sources of radiation is closest to:

A. 0.2 mSv
B. 1 mSv
C. 3 mSv
D. 6 mSv
E. 15 mSv

XXIX-5) Which of the following pairs of person's occupation or status and maximum annual effective dose equivalent permitted is CORRECT? (These values exclude doses from exposure to background radiation, both natural and man-made)

A. radiation oncologist – 200 mSv per year
B. member of the general public – 1 mSv per year
C. sixteen-year old high school student who works part time in a laboratory – 0 mSv
D. nuclear power plant worker – 10 mSv
E. relative who transports a radiotherapy patient to and from treatment – 20 mSv

XXIX-6) The Maximum Permissible Dose (MPD) recommended annually for radiation workers:

A. is the dose workers would receive if the workplace adhered strictly to the principles of ALARA
B. is 100 times higher than that for members of the general public
C. does not include dose received from medical procedures
D. includes dose contributions from man-made sources only
E. is the same under both NCRP and ICRP guidelines

XXIX-7) In estimating the doses to individuals and their critical organs, and also in assessing potential risks to both individuals and populations, various correction factors are required. Which one of the following statements regarding these terms is FALSE?

A. For a particular tissue or organ, the proportion of the risk for stochastic effects resulting from uniform, whole-body irradiation is called the "tissue weighting factor"
B. The dose equivalent to the most sensitive tissue or organ following uniform, whole-body irradiation is called the "committed dose equivalent"
C. The sum of the individual dose equivalents received over a defined time period by an irradiated population is called the "collective dose"
D. The average absorbed dose in a particular tissue or organ that is weighted for radiation quality is called the "equivalent dose"
E. The sum of weighted equivalent doses for all tissues and organs of the body is called the "effective dose"

XXIX-8) The largest contributor to radiation exposure of the US population each year is:

A. Radon
B. Cosmic radiation
C. Medical x-rays/procedures
D. Nuclear fallout
E. Consumer products

XXIX-9) Fluoroscopy for medical purposes causes the most significant reactions in what organ?

A. Skin

B. Brain
C. Lungs
D. Heart
E. Bone

XXX. Molecular Techniques used in Radiation and Cancer Biology

XXX-1) siRNAs and miRNAs:

A. bind to and inhibit the replication of specific genes
B. stimulate RNA synthesis
C. are typically 1 kb in size
D. stimulate protein synthesis
E. inhibit the translation of specific genes

XXX-2)

XXX-2) Which of the following statements concerning gel electrophoresis is TRUE?

A. DNA molecules are negatively charged so will migrate toward the positive electrode of the electrophoresis apparatus
B. SDS is a detergent used for the separation of DNA molecules of different size
C. The higher the concentration of agarose in a gel, the faster DNA molecules will migrate
D. Polyacrylamide gels are used to separate large DNA molecules whereas agarose gels are used for smaller-sized DNAs
E. The higher the molecular weight of the molecule, the faster it will migrate through a gel

XXX-3) Which of the following statements is TRUE concerning the use of PET imaging?

A. ^{18}F-2-deoxy-2-fluoro-D-glucose (^{18}F-FDG) has a radioactive half-life of approximately 10 days
B. A PET imaging camera detects positrons generated from the decay of radiopharmaceuticals
C. The uptake of ^{18}F-FDG is typically lower in areas of inflammation
D. An important advantage to using ^{18}F-FDG-PET/CT fusion images for radiotherapy treatment planning is that they provide both functional and anatomical information
E. Tumors tend to show a reduced uptake of ^{18}F-FDG

XXX-4) Which of the following assays would best determine whether a particular radiation sensitivity syndrome is characterized by defective repair of DNA double-strand breaks?

A. quantitation of γ-H2AX foci
B. western blot
C. alkaline comet assay
D. southern hybridization
E. northern hybridization

XXX-5) Which statement regarding next generation sequencing is false: Next generation sequencing:

A. Unlike capillary sequencing requires the cloning and amplification of DNA sequence containing phage libraries
B. Is a massively parallel process with a million or more simultaneous DNA sequence reads.
C. Is not hampered by homopolymer repeat sequences.
D. Is generally short DNA reads of less than 100 bases.
E. Bases are read by sequential computer-mediated image analysis.

XXX-6) Pulsed-field gel electrophoresis can be used to:

A. determine a cell's karyotype
B. detect DNA interstrand crosslinks
C. separate protein molecules on the basis of both molecular weight and charge
D. monitor the repair/rejoining of large pieces of DNA after the production of double-strand breaks
E. determine the rate of base versus nucleotide excision repair

XXX-7) Which one of the following is NOT a method for studying gene expression at the protein level?

A. immunohistochemistry
B. ELISA
C. northern blots
D. western blots
E. two-hybrid screening

XXX-8) Which of the following statements is INCORRECT concerning techniques to detect wild-type p53 in exponential cultures of human HCT116 colorectal cancer cells:

A. Western blotting with the p53 protein-specific antibody.
B. Flow cytometry to analyze the cycling characteristics of HCT116 cells 12 h after exposure to 6 Gy Xrays.
C. Northern blotting to measure WAF1/CIP1 mRNA expression in HCT116 cells 3 h after exposure to 6 Gy Xrays.
D. Immunoblotting with a cyclin E-specific antibody to detect cyclin E activity 12 h after 6 Gy X rays.
E. Immunoblotting with an antibody against p53 phosphorylated at the serine 15 residue 1 h after 6 Gy X rays.

XXXI. Molecular Imaging

XXXI -1) The most commonly used biologically active molecule for PET scanning is a fluoridinated analog of which of the following:

A. Phosphate
B. Glucose
C. Calcium
D. Albumin
E. Sphingomyelin

XXXI-2) The following nucleoside has been radiolabeled in an effort to image DNA synthesis with PET:

A. Adenosine
B. Guanosine
C. Thymidine
D. Uridine
E. Cytidine

XXXI-3) Which statement regarding the Hounsfield unit scale is CORRECT? The Hounsfield unit scale is:

A. Specific to ultrasound imaging (US)
B. Specific to positron emission tomography (PET)
C. Specific to single photon emission computed tomography (SPECT)
D. Specific to magnetic resonance imaging (MRI)
E. Specific to computed tomography (CT)

XXXI -4) Which of the following statements concerning computed tomography (CT) is CORRECT?

A. Tissues that strongly absorb X-rays appear black while others that absorb poorly appear white on CT images.
B. Iodine-based contrast agents are mainly used in the imaging of the digestive system by CT scanning.
C. Water has an X-ray attenuation of 0 Hounsfield units or 0 HUs.
D. Organ-specific radiation doses from CT scans are negligibly low compared to those associated with conventional radiography.
E. CT devices and image reconstruction software are regulated by the U.S. Nuclear Regulatory Commission (NRC).

XXXI -5) Which of the following statements concerning the prognostic significance of pre-therapy [18-F] fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging in patients is CORRECT?

A. SUV score values are directly correlated with local tumor control.

B. SUV score values are inversely proportional to patient's body weight.
C. SUV score values can be used to distinguish between quiescent and proliferating tumors.
D. SUV score values are insensitive to extending time between radioisotope injection and PET scan.
E. Typical doses of FDG for the clinically useful PET imaging are in a range of 15 Ci.

ANSWERS, EXPLANATIONS AND REFERENCES

GENERAL REFERENCES

Please note: Unless specific references are indicated along with the answer and explanation to a question, the material addressed in each question can be found in one or more of the following textbooks:

The major textbook used in radiation biology is:

Hall EJ and Giaccia AJ, Eds. *Radiobiology for the Radiologist*, 6th Ed. Lippincott Williams & Wilkins, Philadelphia, 2006

Additional useful textbooks include:

Joiner M and van der Kogel A, Ed. *Basic Clinical Radiobiology*, 4th Ed. Arnold, London, 2009

Lehnert S, *Biomolecular Action of Ionizing Radiation*. Taylor and Francis, London, 2007.

Dale R and Jones B, Ed. *Radiobiological Modelling in Radiation Oncology*. The British Institute of Radiology, 2007

Tannock IF, Hill RP, Bristow RG, *et al.*, Eds. *The Basic Science of Oncology*, 4th Ed. McGraw-Hill, Medical Pub. Division, New York, 2005

Mettler FA and Upton AC, Eds., *Medical Effects of Ionizing Radiation*, 3rd Ed. W.B. Saunders, Philadelphia, 2008

Chapters on radiation and cancer biology in the major radiation oncology textbooks:

Haffty B and Wilson L, Eds., *Handbook of Radiation Oncology: Basic Principles and Clinical Protocols.* Jones and Bartlett Publishers, Sudbury MA, 2009

Halperin EC, Perez CA, Brady LW, *et al.*, Eds., *Principles and Practice of Radiation Oncology*, 5th Ed. Lippincott Williams & Wilkins, Philadelphia, 2007

Gunderson LL and Tepper JE, Eds., *Clinical Radiation Oncology*, *Second Edition* Churchill Livingstone, New York, 2007

Leibel SA and Phillips TL, Eds., *Textbook of Radiation Oncology*, 2nd Ed. W.B. Saunders, Philadelphia, 2004

The following represent overviews of radiation and cancer biology that are recommended:

Rosenstein BS, Chapter 2. The Biologic Basis of Radiotherapy: in *Handbook of Radiation Oncology: Basic Principles and Clinical Protocols.* Haffty B and Wilson L, Eds. Jones and Bartlett Publishers, Sudbury MA, 2009

Rosenstein BS, Chapter 3. Molecular Radiobiology: in *Handbook of Radiation Oncology: Basic Principles and Clinical Protocols.* Haffty B and Wilson L, Eds. Jones and Bartlett Publishers, Sudbury MA, 2009

McBride WH and Withers R, Chapter 2. Biological Basis of Radiation Therapy. pp. 76 108, in *Principles and Practice of Radiation Oncology*, 5th Ed. Halperin EC, Perez CA, Brady LW, *et al.*, Eds., Lippincott Williams & Wilkins, Philadelphia, 2007

Baumann M, Cordes N, Hasse M and Zips D, Chapter 3. Molecular Cancer and Radiation Biology. pp. 109-125, in *Principles and Practice of Radiation Oncology*, 5th Ed. Halperin EC, Perez CA, Brady LW, *et al.*, Eds., Lippincott Williams & Wilkins, Philadelphia, 2007

Jain RK and Kozak KR, Chapter 4. Molecular Pathophysiology of Tumors, pp.126-141, in *Principles and Practice of Radiation Oncology*, 5th Ed. Halperin EC, Perez CA, Brady LW, *et al.*, Eds., Lippincott Williams & Wilkins, Philadelphia, 2007

Zeman EM, Chapter 1. Biologic Basis of Radiation Oncology, pp. 1-46, in *Clinical Radiation Oncology, Second Edition*. Gunderson LL and Tepper JE, Eds. Churchill Livingstone, New York, 2007 *(Third Edition due in 2010)*

Willers H, Held KD. Introduction to clinical radiation biology. *Hematol Oncol Clin North Am* 20:1-24, 2006. PubMed

Bernier J, Hall EJ and Giaccia A, Radiation Oncology: A Century of Achievements. Nat Rev Cancer 4: 737-747, 2004. PubMed

Constine LS, Milano MT, Friedman D et al. Late Effects of Cancer Treatment on Normal Tissues pp. 320-355, in *Principles and Practice of Radiation Oncology*, 5th Ed. Halperin EC, Perez CA, Brady LW, *et al.*, Eds., Lippincott Williams & Wilkins, Philadelphia, 2007

Dewey W and Bedford J, Chapter 1. Radiobiologic Principles., pp. 3-30 in *Textbook of Radiation Oncology*, 2nd Ed. Leibel SA and Phillips TL, Eds. W.B. Saunders, Philadelphia, 2004

Section 1. Cancer Biology in *Cancer Medicine, 6th ed.* Kufe DW, Holland JF and Frei E, Eds., BC Decker, Hamilton, Ont., 2003

Note: Those whose primary language is Spanish may find a new Spanish language Radiation Oncology textbook to be of value:

N Urdaneta, A Vera, R E Peschel, and L H Wilson, *Radiotheripa Oncologia*, *Enfoque Multidisciplinario.* This textbook includes chapters reviewing different aspects of radiobiology, radiologic physics, radiation protection, and radiation epidemiology, as well as different areas of clinical radiation oncology. Editorial Disinlimed, Columbia, 2009.

I. Interaction of Radiation with Matter

I-1) C In the Compton process, a photon interacts with an atom causing ejection of an orbital electron. The incident photon, now with reduced energy, continues along a deflected path. The probability of the photoelectric effect increases with atomic number of the absorber. The predominant interaction of 10 keV photons in soft tissue is the photoelectric effect. Pair production occurs for photons with energies greater than 1.02 MeV, and results in the complete conversion of the photon's energy into the production of a positron and electron. For the photoelectric effect, there is complete absorption of the photon's energy, resulting in ejection of an electron that possesses energy equal to the difference between the incident photon's energy and the electron's binding energy.

I-2) D 65-75% of the damage caused by indirect action is mediated by the hydroxyl radical, $OH^{\bullet}$. Little biological damage is caused by the hydrated electron e_{aq}. $^1O_2$ is produced primarily by photosensitizers and, rarely by ionizing radiation. Neither OH^- nor O_2^- are primary radiolysis products, although O_2^- can be produced secondarily by reaction of e_{aq} with O_2.

Mitchell JB, Russo A, Kuppusamy P*, et al.*, Radiation, Radicals, and Images. Ann N Y Acad Sci 899:28-43, 2000. PubMed

I-3) A On average, about 25 eV is required to create an ion pair in water, although the minimum energy needed to eject an electron is only 12.6 eV.

I-4) C The photoelectric effect is the predominant interaction responsible for producing high quality diagnostic radiographs. At relatively low photon energies, the photoelectric effect is the most likely photon interaction and is the desirable type of photon/tissue interaction since there is complete photon absorption with no production of secondary photons. The other possible tissue interactions at the photon energies used in diagnostic radiology are the Compton effect and coherent scattering. For these interactions, a deflected photon traveling in an altered direction is produced at the site of interaction. If these secondary photons are permitted to reach the film, there would be a reduction in image sharpness and loss of spatial resolution.

I-5) C The predominant atomic interaction for 100 keV photons is the Compton process. The minimum energy required for pair and triplet production is 1.02 MeV. The photoelectric effect is predominant for photon energies in the range of 10 keV.

I-6) E High LET (linear energy transfer), or densely ionizing, radiations include particles such as 290 MeV carbon ions, □-particles and neutrons. 250 kVp X-rays, 200 MeV protons and 1.1 MV □-rays are all low LET, or sparsely ionizing, radiations. Although high LET radiations produce more clustered lesions (multiply damaged sites) in DNA than low LET radiations, they actually produce lower yields of OH radicals because of the extensive ion and radical recombination within spurs and

blobs. High LET radiations, such as iron ions or carbon ions, are components of cosmic rays, while solar flares are composed largely of energetic protons (which are low LET).

Goodhead DT: The initial physical damage produced by ionizing radiations. *Int J Radiat Biol* 56: 623-34, 1989. PubMed

I-7) B. The initial ionization process takes approximately 10^{-15} second. The primary radicals produced by the ejection of an electron typically have a lifetime of 10^{-10} second. The resulting hydroxyl radical has a lifetime of approximately 10^{-9} second. The DNA radicals subsequently produced have a lifetime of approximately 10^{-5} second.

I-8) C. Alpha-particles are emitted during the decay of heaving radionuclides that occur in nature, including radon. Radon gas escapes from the soil and builds up inside homes, where it is breathed and can contribute to lung cancer.

1-9) B. Annihition photons always have an energy of 0.51 MeV each. Annihilation photons have energy equal to the rest energy of the positron and electron.

1-10) D. Neutrons are not charged particles and, therefore, cannot ionize atoms directly. However, they transfer some of their energy to protons or light nuclei, which then cause ionization. Thus they are indirectly ionizing.

1-11) B. Fast neutrons with kinetic energy between a few and several tens of MeV are slowed down in bioliogical media mainly by elastic collisions with hydrogen nuclei (protons) of the cellular water. A fraction of energy lost by fast neutron in elastic collision with oxygen nuclei is less than 10% of that with hydrogen nuclei. For the beams of neutrons used in radiation therapy recoil protons from elastic collistions produce a large density of ionizations along their tracts. Neutrons do not interact with atomic electrons but with atomic nuclei. Alpha particles can be produced by neutron capture reactions with isotopes of both carbon and oxygen, but the probability is strongly dependend on the neutron energy and target material. Example:

$$^{17}O + n \rightarrow {}^{14}C + \alpha$$

Neutron absorption in a target nucleus is called actiovation. This is a process by which neutron radiation induces radiaoactivity in materials and it occurs when atomic nuclei capture free neutrons, becoming heavier and entering excited states. The excited nucleus often decays immediately by emitting gamma rays, or particles such as beta particles, alpha particles, fission products and neutrons (in nuclear fission). Neutron activation is a potential health hazard in therapy with high energy photons, because in radiation therapy with photons with eneegy > 10 MeV neutrons are generated in linacs through interactions of photons with nuclei of high atomic number materials and activate the linac head and the beam collimator systems. These photoneutrons have energy 0.1 to 2 MeV, are highly penetrating, have the quality factor of 20, and significantly add to patient's off-field dose.

1-12) D. The main initial products of resulting from irradiation of pure water

are the short-lived free radicals, hydrogen radical (H•) (10%), hydroxyl radical (•OH) (45%) and the solvated electrons (e^-_{aq}) (45%), These react with DNA or with each other. Thus: •OH + H• → H_2O

The remaining recombination reactions of free radicals are:

$e^-_{aq} + e^-_{aq} + 2\ H_2O \rightarrow H_2 + 2\ OH^-$

•OH + •OH → H_2O_2

H• + H• → H_2

These reactions always compete with reactions that lead to direct damage of the biological molecules. The relative efficiency of the recombinations will depend on the separation of the short-lived free radicals after the passage of the charged particle and so depend on LET. At small LET values the spacing of the ionizations is large so, for example, •OH radicals are widely separated and therefore the probability of combining to form H_2O_2 is low. As LET increases, the spacing between ionizations decreases and the probability of an •OH from one ionization with an •OH from another ionization event along the track increases. The yield of hydrogen peroxide increases rapidly with LET of about 20 - 150 keV/μm, the rnge of LET where direct damage to DNA dominates over indirect damage fro the free radicals.

Gray LH. The initiation and development of cellular damage by ionizing radiation. Brit J Radiol 26:609-618, 1953.

I-13) D. The indirect effect mediated by free-radical reactions involving water are most responsible to cause DNA damage upon low LET irradiation

II. Molecular Mechanisms of DNA Damage

II-1) B The nucleosome contains an octamer of core histones: H3, H4, H2A, and H2B. Histone variants and their post-translational modifications regulate chromosomal functions; the post-translational modifications include acetylation, methylation, and phosphorylation. Histone H2A has nine subtypes, among them the H2AX variant, which is involved in the response to DNA damage. Production of DNA double-strand breaks (DSBs) by ionizing radiation leads to the rapid phosphorylation of histone H2AX on serine 139 (γ-H2AX). The specificity of this reaction provides a reliable yardstick for DSBs and the means to spatially localize DSBs within the nuclei of cells (the γ-H2AX focus assay). The degree of H2AX phosphorylation measured at a specific time after induction of the DSBs represents a balance between the rate of phosphorylation following DNA damage and the dephosphorylation that occurs as DNA repair progresses. SF_2, the cell surviving fraction after 2 Gy, is a model-independent measure of radiation sensitivity. It has been shown that the number of phosphorylated sites remaining 24 hours after irradiation directly correlates with intrinsic radiosensitivity. In contrast, after a 30 minute incubation, H2AX has been phosphorylated, but there has been little time for repair. A correlation between cell survival and the repair of either DNA single-strand breaks or thymine glycols has not been observed.

Klokov D, MacPhail SH, Banáth JP, *et al.*, Phosphorylated histone H2AX in relation to survival in tumor cells and xenografts exposed to single and fractionated doses of X-rays. *Radiother Oncol* 80: 223-229, 2006. PubMed

Banáth JP, MacPhail SH, Olive PL, Radiation sensitivity, H2AX phosphorylation, and kinetics of repair of DNA strand breaks in irradiated cervical cancer cell lines. *Cancer Res* 64: 7144-7149, 2004. PubMed

II-2) E In contrast with the other forms of damage listed, pyrimidine dimers are principally produced following absorption of photons in the ultraviolet wavelength range and are not produced by X-rays.

II-3) B A clustered lesion, which has been hypothesized to play an important role in cell lethality, involves the formation of several DNA damages within a highly localized region of DNA.

Georgakilas AG. Processing of DNA damage clusters in human cells: current status of knowledge. *Mol Biosyst* 4:30-35, 2008. PubMed

Hada M, Georgakilas AG. Formation of Clustered DNA Damage after High-LET Irradiation: A Review. *J Radiat Res* 49:203-210, 2008. PubMed

II-4) C The neutral comet assay is used to measure DNA double-strand breaks. Alkaline elution is used to measure single-strand breaks and some base damages, western

blotting is for detection of proteins, PCR (polymerase chain reaction) is used to amplify DNA sequences, and annexin V-labeling can be used to detect apoptosis.

II-5) C One important characteristic of the cellular response to DNA lesions is the spatiotemporal manner by which repair and other proteins are recruited to the site of damaged DNA. Frequently, these protein accumulations can be visualized as subnuclear "foci" using immunofluorescence microscopy. Ionizing radiation-induced DNA double-strand breaks activate ATM kinase, which phosphorylates multiple damage response and repair proteins. ERCC1 is involved in nucleotide excision repair, in addition to roles in homologous recombination and replication fork repair, but does not form subnuclear foci. Histone H2AX is phosphorylated by ATM within 15 minutes after irradiation, and can be visualized using a phospho-specific antibody. These gamma-H2AX foci are regarded a marker for radiation-induced DNA double-strand breaks in cells. p53 itself does not form foci, though specific ATM-dependent phospho-forms of p53 might be detected as foci. ATM functions in response to double strand breaks. By contrast, ATR is activated during every S-phase to regulate the firing of replication origins, the repair of damaged replication forks and to prevent the premature onset of mitosis. Although ATR is activated in response to many different types of DNA damage including double strand breaks (DSB), a single DNA structure that contains a single-stranded DNA may be responsible for its activation. Furthermore, p53 does not form ATR-dependent foci.

Lisby M, Rothstein R. Choreography of recombination proteins during the DNA damage response. *DNA Repair* (Amst). 8:1068-76, 2009. PubMed

Kinner A, Wu W, Staudt C, Iliakis G. Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res*. 36:5678-94, 2008. PubMed

II-6) A. The DNA-PKcs gene encodes a critical protein involved in canonical NHEJ repair of DSBs, and thus loss of the gene confers significant sensitivity to agents that induce DSBs. P53 mutations would not be expected to induce radiation sensitivity. K-Ras mutations that are activating may induce radioresistance. MLH1 nonsense mutations would induce microsatellite instability and resistance to alkylating agents, but not sensitivity to DSB-inducing agents.

III. Molecular Mechanisms of DNA Repair

III-1) B In response to various forms of DNA damage including double-strand breaks, p53 is stabilized and binds to the promoters of numerous target genes, including p21, activating their transcription. This transcriptional transactivation by p53 is an important component of the cellular DNA damage response. ATM and CHK1 are protein kinases that are activated in response to double-strand breaks. TRAIL is a ligand that induces cell death through the extrinsic apoptosis pathway.

Batchelor E, Loewer A, Lahav G. The ups and downs of p53: understanding protein dynamics in single cells. *Nat Rev Cancer* 9:371-7, 2009. Pub Med

Murray-Zmijewski F, Slee EA, Lu X. A complex barcode underlies the heterogeneous response of p53 to stress. *Nat Rev Mol Cell Biol* 9:702-12, 2008. Pub Med

Szumiel I. Intrinsic radiation sensitivity: Cellular signaling is the key. *Radiat Res* 169:249-258, 2008. PubMed

Sengupta S, Harris CC. p53: traffic cop at the crossroads of DNA repair and recombination. *Nat Rev Mol Cell Biol* 6:44-55, 2005. Pub Med

III-2) E Phosphorylation of histone H2AX to γ-H2AX occurs within several minutes of a cell being irradiated. This modification is triggered by ATM and serves to mark the chromosomal site of the DNA break for the subsequent recruitment of signaling proteins, such as CHK1 kinase. Activated CHK1 phosphorylates and inactivates CDC25 proteins, thereby causing the arrest of the cell cycle. p21 transcription is induced several hours after DNA damage, following the stabilization of p53 (TP53).

Bonner WM, Redon CE, Dickey JS, *et al*. GammaH2AX and cancer. *Nat Rev Cancer* 8:957-967, 2008. PubMed

Kinner A, Wu W, Staudt C, *et al.* Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res* 36:5678-5694, 2008. PubMed

III-3) A Homologous recombination requires a second copy of the relevant DNA duplex. Although homologous recombination can take place in G_1 phase, using the homologous chromosome as the template for repair, it occurs much more frequently after replication when the template strand is the sister chromatid, located in close proximity to the damaged strand. The sister chromatid is created during S-phase and serves as a template from which to copy the intact DNA sequence to the site of the damaged strand of DNA. It has been estimated that homologous recombination occurs 1000-fold more frequently in S and G_2 than in G_1. In G_1, the principal form of DNA double-strand break repair is non-homologous recombination

Powell SN, Kachnic LA. Therapeutic exploitation of tumor cell defects in homologous recombination. *Anticancer Agents Med Chem* 8:448-460, 2008. PubMed

Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res* 18:99-113, 2008. PubMed

III-4) A RAD51 is a recombinase and plays a critical role in homologous recombinational repair of DNA double-strand breaks. XPG is an endonuclease that cleaves the DNA strand on the 3' side of the damage site; it also stabilizes the nucleotide excision repair pre-incision complex that is essential for the 5' incision by the XPF (ERCC4) endonuclease. The catalytic unit of DNA protein kinase (DNA-PKcs) plays a central role in non-homologous end joining of DNA double-strand breaks through its recruitment by the KU70 (XRCC6)/80 (XRCC5) heterodimer to sites of DNA double-strand breaks, forming the DNA-dependent protein kinase holo-enzyme complex (DNA-PK). CHK1 is a serine/threonine protein kinase and a key mediator of the DNA damage-induced checkpoint pathway. TFIIH is associated with nucleotide excision repair.

Iijima K, Ohara M, Seki R, *et al*. Dancing on damaged chromatin: functions of ATM and the RAD50/MRE11/NBS1 complex in cellular responses to DNA damage. *J Radiat Res (Tokyo)* 49:451-464, 2008. PubMed

Zhang J, Powell SN. The role of the BRCA1 tumor suppressor in DNA double-strand break repair. *Mol Cancer Res* 3:531-539, 2005. PubMed

Willers H, Dahm-Daphi J, Powell SN. Repair of radiation damage to DNA. *Br J Cancer* 90:1297-1301, 2004. PubMed

III-5) D Inhibition of non-homologous end joining would be expected to *decrease* cellular radioresistance. An effect on immune response would be anticipated because inhibition of NHEJ would affect V(D)J recombination, thereby affecting antigen recognition. Cells and tissues would be sensitized to low dose-rate irradiation since the recovery that occurs at low dose-rates depends, at least in part, upon repair of double-strand breaks by NHEJ. Normal tissue tolerance doses would likely decrease due to radiosensitization. Sublethal damage recovery would be inhibited since this process depends, at least in part, on the repair of double-strand breaks.

Lieber MR. The mechanism of human nonhomologous DNA end joining. *J Biol Chem* 283:1-5, 2008. PubMed

III-6) B RAD52 plays a central role in homologous recombinational repair (HR) of DNA double-strand breaks through recruitment of RAD51 to single-stranded DNA complexed with RPA. RAD52 does not appear to be involved in NHEJ. XRCC4 is an adaptor protein that tightly complexes with DNA ligase IV, which directly mediates DNA-strand joining by NHEJ. The KU70/KU80 heterodimer recruits DNA-PKcs (PRKDC) to the site of DNA double-strand breaks to form a multiprotein complex that keeps broken DNA ends in close proximity and provides a platform for the enzymes required for end processing and ligation. DNA-PKcs phosphorylates the Artemis protein, thereby activating it for endonucleolytic activity. The Artemis:DNA-PKcs complex cleaves 5´ and 3´ nucleotide overhangs, which prepares double-strand breaks for ligation by XRCC4 and DNA ligase IV.

Weterings E, Chen DJ. The endless tale of non-homologous end-joining. *Cell Res* 18:114-124, 2008. PubMed

van Gent DC, van der Burg M. Non-homologous end-joining, a sticky affair.*Oncogene* 26:7731-7740, 2007. PubMed

III-7) E *XPC* is a gene whose product is involved in nucleotide excision repair. Mutations in *XPC* result in the human genetic disease xeroderma pigmentosum, characterized by extreme sensitivity to ultraviolet light. Mutations in all of the other genes result in human genetic diseases characterized by sensitivity to ionizing radiation. This includes Nijmegen breakage syndrome (*NBS1*), familial breast cancer (*BRCA1*), ataxia telangiectasia (*ATM*), and ataxia telangiectasia-like disorder (*MRE11*).

Pollard JM, Gatti RA. Clinical radiation sensitivity with DNA repair disorders: an overview. *Int J Radiat Oncol Biol Phys* 74:1323-31, 2009. PubMed

Lavin MF. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signaling and cancer. *Nat Rev Mol Cell Biol* 9:759-769, 2008. PubMed

Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol* 9:297-308, 2008. PubMed

Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer* 8:193-204, 2008. PubMed

Digweed M, Sperling K. Nijmegen breakage syndrome:clinical manifestation of defective response to DNA double-strand breaks. *DNA Repair* 3:1207-1217, 2004. PubMed

Taylor AM, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. *DNA Repair* 3:1219-1225, 2004. PubMed

III-8) B People diagnosed with LIG4 syndrome are radiation sensitive because these individuals are deficient in the DNA ligase IV enzyme (LIG4), which plays a central role in non-homologous end joining of double-strand breaks. Cells deficient in nucleotide excision repair exhibit normal sensitivity to ionizing radiation since this repair process plays little or no role in the repair of damages induced by ionizing radiation, but are very sensitive to UV. Base excision repair, not mismatch repair, involves the action of a DNA glycosylase and an AP endonuclease. People with Fanconi anemia are highly sensitive to DNA cross-linking agents due to inhibition of the mono-ubiquitination of FANCD2, a downstream Fanconi anemia protein, following genotoxic stress. The immune deficient phenotype in SCID mice is caused by a defect in XRCC7 (DNA-PK_{cs}); this defect causes a defect in NHEJ and a radiosensitive phenotype, as well as the defect in V(D)J rejoining that leads to the immune deficit. Defects in several genes are now known to cause SCID phenotypes; mutation in the common human disease of the same name differs from that in the well-known mouse strain.

Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat Rev Genet* 8:735-748, 2007. PubMed

O'Driscoll M, Gennery AR, Seidel J, *et al.*, An overview of three new disorders associated with genetic instability: LIG4 syndrome, RS-SCID and ATR-Seckel syndrome. *DNA Repair* 3:1227-35, 2004. PubMed

III-9) A MSH2 and MLH1 play a central role in mismatch repair. Regarding the other proteins, XPA/XPC are involved in nucleotide excision repair and the remainder play roles in NHEJ.

Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* 7:335-46, 2006. PubMed

III-10) E An exonuclease cleaves one nucleotides at a time beginning at the end of a DNA strand.

III-11) A Defects in base excision repair may increase mutation rate, but generally do not alter cell survival after ionizing radiation, with the exception of mutation in the *XRCC1* gene which confers a slight increase in radiation sensitivity. Defects in nucleotide excision repair (NER) increase sensitivity to UV, but not to ionizing radiation. The xeroderma pigmentosum (*XP*) and Cockayne Syndrome (*CS*) genes are involved in NER. BER acts to remove damaged bases from DNA, including those damaged by ionizing radiation, but NER acts on pyrimidine dimers, single-strand breaks and bulky adducts. The gene defective in most patients with Li-Fraumeni Syndrome is *p53*, although some patients with that condition have mutations in *CHK2*.

Hegde ML, Hazra TK, Mitra S. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. *Cell Res* 18:27-47, 2008. PubMed

Caldecott KW. Single-strand break repair and genetic disease. *Nat Rev Genet* 9:619-631, 2008. PubMed

III-12) D Two principal recombinational DNA repair pathways have been identified, homologous recombination (HR) and non-homologous end-joining (NHEJ), each of which employs separate protein complexes. DSB repair by HR requires an undamaged template molecule that contains a homologous DNA sequence, typically derived from the sister chromatid in the S and G2 phase cells. In contrast, NHEJ of double-stranded DNA ends, which can occur in any cell-cycle phase, does not require an undamaged partner and does not rely on extensive homologies between the recombining ends (typically 2-6 bp of microhomology are used). Defective HR can be causally linked to impaired DNA replication, genomic instability, human chromosomal instability syndromes, cancer development, and cellular hypersensitivity to DNA damaging agents. Cells with genetic defects in NHEJ (such as mutation of DNA-PK, XRCC4, or DNA ligase IV) display a more pronounced hypersensitivity to ionizing radiation than cells defective in HR (such as mutation of BRCA1, BRCA2, or RAD51).

Helleday T, Lo J, van Gent DC, Engelward BP. DNA double-strand break repair: from mechanistic understanding to cancer treatment. *DNA Repair* (Amst). 6:923-35, 2007. PubMed

van Gent DC, van der Burg M. Non-homologous end-joining, a sticky affair. *Oncogene*. 26:7731-40, 2007. PubMed

Willers H, Dahm-Daphi J, Powell SN. Repair of radiation damage to DNA. *Br J Cancer*.90:1297-301, 2004. PubMed

III-13) B Several DNA repair pathways, including TLS, NER, and HR, can be mobilized at stalled DNA replication forks, depending on the type of fork-blocking lesion. Chemotherapy-induced DNA lesions, such as interstrand crosslinks, interfere with the progress of the replicative DNA helicase or DNA polymerases, thereby leading to replication fork blockage or demise, producing DNA gaps or one-sided DNA double-strand breaks (DSBs). Uncoupling of the replicative DNA helicase from the polymerases may occur, generating excessive single-stranded DNA, which could in turn be the target of endonucleoytic processing, resulting in a one-sided DSB. In addition, single-stranded breaks induced by endogenous and exogenous sources may lead to the formation of one-sided DSBs due to runoff of the replication fork. In the repair of one-sided DSBs, HR appears to be the only pathway leading to their productive resolution. This entails resection of the DSB to form a 3′-tailed end for Rad51 filament assembly and DNA strand invasion and ultimately reconstruction of the replication fork.

Helleday T, Lo J, van Gent DC, Engelward BP. DNA double-strand break repair: from mechanistic understanding to cancer treatment. *DNA Repair* (Amst) 6:923-35, 2007. PubMed

Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res* 18:99-113, 2007. PubMed

III-14 B Xeroderma pigmentosum

III-15 D BRCA1 and BRCA2 predominantly regulate homologous recombination as opposed to non-homologous end joining

III-16 C Azathioprine

IV. Chromosome and Chromatid Damage

IV-1) D Spectral karyotyping (SKY) uses fluorescence staining of chromosomes employing uniquely-colored probes specific for individual chromosomes, thus allowing them to be distinguished from each other on the basis of color. Stable translocations are revealed using SKY as a single chromosome that appears to be multi-colored. The formation of terminal deletions follows a linear dose response since these are single-hit aberrations. Translocations can be stable aberrations since they do not necessarily lead to cell death. The number of dicentric chromosomes detected in peripheral blood lymphocytes decreases with time after irradiation since these are unstable aberrations that ultimately cause the death of the lymphocyte progenitors and stem cells. The minimum dose that can be detected through scoring dicentric chromosomes is roughly 0.25 Gy.

Braselmann H, Kulka U, Baumgartner A, *et al*. SKY and FISH analysis of radiation-induced chromosome aberrations: a comparison of whole and partial genome analysis. *Mutat Res* 578:124-33, 2005. PubMed

Tucker JD, Cofield J, Matsumoto K*, et al.* Persistence of chromosome aberrations following acute radiation: I. Paint translocations, dicentrics, rings, fragments, and insertions. *Environ Mol Mutagen* 45:229-248, 2005. PubMed

IV-2) E Micronuclei are created due to the presence of acentric fragments, which form in the progeny of irradiated cells that undergo mitosis in the presence of one or more asymmetrical chromosome aberrations. Sister chromatid exchanges are reciprocal exchanges between chromatids of the same chromosome that are not readily induced by ionizing radiation. Inversions result when two breaks are produced in a single chromosome and the resulting excised chromosomal fragment reinserts itself back into the chromosome, but with the opposite polarity. A quadriradial is a chromatid-type aberration that may arise from illegitimate interchromosomal recombination, accompanied by crossing-over. Chromatid gaps appear as loss of genetic material from a single chromatid arm and may be caused by incomplete breaks.

IV-3) D Individual chromosome aberrations can, in general, be detected readily only during mitosis. However, some chromosome aberrations lead to the formation of micronuclei, which develop when a pseudo nuclear membrane forms around acentric chromosome fragments or whole chromosomes that did not segregate properly into daughter cells during the previous mitosis. Micronuclei are observed in peripheral lymphocytes and thus can be seen in interphase cells.

Muller WU, Nusse M, Miller BM, *et al*. Micronuclei: a biological indicator of radiation damage. *Mutat Res* 366:163-169, 1996. PubMed

IV-4) E Chromatid type aberrations are produced in cells only when irradiation follows DNA synthesis in S phase.

IV-5) A The formation of dicentric chromosomes is linear at low radiation doses, but follows a quadratic function at higher doses. Two distinct mechanisms are thought to be responsible for these two components of the linear-quadratic dose response curve. The linear portion of the dose response relationship is assumed to result from the simultaneous induction of two chromosome breaks by a single track. The quadratic portion is assumed to result from the two chromosome breaks being produced by two separate radiation tracks.

IV-6) D Terminal deletions are induced as a linear function of dose since they result from a single chromosomal break. A ring chromosome is an example of a chromosome-type aberration, not a chromatid-type aberration. A dicentric is an unstable aberration since it results in the formation of an acentric fragment and ultimately causes cell death. Breaks in two chromatids, followed by illegitimate rejoining, produce an anaphase bridge. The yield of dicentric chromosomes increases with increasing dose-rate for low LET radiation.

Leonard A, Rueff J, Gerber GB*, et al.* Usefulness and limits of biological dosimetry based on cytogenetic methods. *Radiat Prot Dosimetry* 115:448-454, 2005. PubMed

Rodrigues AS, Oliveira NG, Gil OM*, et al.*, Use of cytogenetic indicators in radiobiology. *Radiat Prot Dosimetry* 115:455-460, 2005. PubMed

IV-7) B Blood cells from individuals with Fanconi anemia are often found to have high numbers of chromosome aberrations, especially quadriradials. These complex aberrations increase dramatically with exposure to DNA cross-linking agents such as mitomycin c.

V. Mechanisms of Cell Death

V-1) C Apoptotic signals trigger a series of proteolytic events known as the caspase cascade. There are at least 14 human caspases, which fall into two categories: the initiator caspases (caspases-2, -8, -9 and -10), which activate the downstream caspases, and the executioner caspases (caspases-3, -6 and -7), which cleave cellular substrates. The actions of the executioner caspases produce the cellular effects that distinguish apoptosis from other forms of cell death. XIAP is a protein that binds to and inhibits the action of caspases.

Li J, Yuan J. Caspases in apoptosis and beyond. *Oncogene* 27:6194-206, 2008. PubMed

V-2) B The intrinsic apoptotic pathway can be triggered either by damage to DNA or by damage to the plasma membrane. Radiation acts directly on the plasma membrane, activating acid sphingomyelinase, which generates ceramide by enzymatic hydrolysis of sphingomyelin. Ceramide then acts as a second messenger in initiating an apoptotic response via the mitochondrial system. Mitotic catastrophe, and not apoptosis, is the major mechanism of cell death in epithelial tumors. Inhibition of the G_1 checkpoint in irradiated cells may *increase* the probability of mitotic catastrophe since cells are more likely to enter mitosis with damaged chromosomes. Radiation-induced senescent cells cease dividing and can remain metabolically active for extended periods before dying, but do not show membrane blebbing and DNA fragmentation, which are characteristic of apoptosis. □-H2AX foci noted in the nuclei of irradiated cells are indicative of the presence of DNA double-strand breaks.

Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol.* 9:231-241, 2008. PubMed

Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol.* 9:1004-1010 2008. PubMed

Rupinder SK, Gurpreet AK, Manjeet S. Cell suicide and caspases. *Vascul Pharmacol* 46:383-393, 2007. PubMed

Ch'ang HJ, Maj JG, Paris F, *et al.* ATM regulates target switching to escalating doses of radiation in the intestines. *Nat Med* 11:484-490, 2005. PubMed

Chu K, Teele N, Dewey MW, *et al.*, Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. *Radiat Res* 162:270-286, 2004. PubMed

Kolesnick R, Fuks Z. Radiation and ceramide-induced apoptosis. *Oncogene* 22:5897-5906, 2003. PubMed

V-3) B The term “senescence” refers to the loss of cellular replicative potential leading to a reduced capability to repopulate the tissue after exposure to genotoxic agents,

including ionizing radiation. Senescence is most often the result of a permanent arrest in G_1, associated with elevated expression of the cell cycle inhibitors $p16^{INK4A}$ (CDKN2A) and p21 (CDKN1A, WAF1/CIP1). Importantly, senescence is not a type of cell death *per se* because cells remain morphologically intact and metabolically active when senescent. Mitochondrial dysfunction is a hallmark of apoptotic cell death, not senescence. Telomere shortening is not associated with radiation-induced senescence. Nutrient deprivation can lead to autophagy, a type of programmed cell death distinct from apoptosis.

Ohtani N, Mann DJ, Hara E. Cellular senescence: its role in tumor suppression and aging. *Cancer Sci* 100:792-7, 2009. PubMed

V-4) C There are two principal pathways that can lead to apoptotic death. One of these, the extrinsic pathway, involves extracellular signaling through death receptors located on the plasma membrane such as TRAILR-1 (TNFRSF10A), TRAILR-2 (TNFRSF10B) or FAS (CD95/APO-1). These death receptors are activated in response to ligand binding of TRAIL (TNFSF10) or FAS ligand (FASLG/CD95-L) and signal through a series of adapter molecules leading to the recruitment of the major initiator pro-caspase, procaspase-8. The activation of procaspase-8 is thought to occur via an induced proximity model leading to its conversion to the active enzyme, caspase-8. Ionizing radiation can elicit activation of the extrinsic pathway leading to apoptosis. The other pathway by which ionizing radiation can elicit an apoptotic response is the intrinsic pathway. This can be stimulated by DNA damage leading to signaling to mitochondria, changes in mitochondrial membrane potential, release of cytochrome c, and activation of procaspase-9.

Cotter TG. Apoptosis and cancer: the genesis of a research field. *Nat Rev Cancer* 9:501-7, 2009. PubMed

V-5) C Apoptosis helps maintain tissue homeostasis because cells that are undergoing an apoptotic response recruit phagocytes that clear the dying cells, also known as "apoptotic corpses", from the tissue without stimulating an inflammatory response. The exposure of phosphatidylserines (phospholipids) on the exterior of the plasma membrane is the signal that initially recruits phagocytes. Ordinarily, phosphatidylserine is sequestered within the phospholipid bilayer and is not displayed on the cell's surface. The process of necrosis, which involves rupture of the cell membrane and the leakage of cellular contents into the surrounding tissue, does elicit an inflammatory response. While DNA condensation and fragmentation are important steps in the apoptotic process, they are not coordinated directly through the exposure of phosphatidylserine on the plasma membrane. A number of stimuli lead to increased ceramide levels, including TNF, FasL and ionizing radiation, but not phosphatidylserine.

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. *Nature* 450:435-9, 2007. PubMed

V-6) E The characteristic changes associated with apoptosis are due to activation of a family of intracellular cysteine proteases, known as caspases. Initiator caspases are the first to be activated; these include caspases-2, -8, -9 and -10. Initiator caspases cleave and activate the effector/executioner caspases, including caspases-3, -6, and -7, which then cleave, degrade or activate other cellular proteins. Activation of caspases is regulated by members of the BCL2 family and by the inhibitors of apoptotic protein (IAP) family. BAX is one of a series of pro-apoptotic members of the BCL2 family. These pro-apoptotic BCL2 family members regulate the release of cytochrome c from mitochondria and elicit the subsequent activation of caspases. Another important function of p53 is that it causes upregulation of pro-apoptotic PUMA. X-linked IAP (XIAP) inhibits the activity of caspases directly. DIABLO is a pro-apoptotic protein that prevents IAPs from inhibiting caspases. BAX and p53 are required for some forms of DNA damage-induced apoptosis.

Letai AG. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. *Nat Rev Cancer* 8:121-132, 2008. PubMed

Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 9:47-59, 2008. PubMed

Ow YP, Green DR, Hao Z, *et al.*, Cytochrome c: functions beyond respiration. *Nat Rev Mol Cell Biol* 9:532-542, 2008. PubMed

Riedl SJ, Salvesen GS. The apoptosome: signalling platform of cell death. *Nat Rev Mol Cell Biol* 8:405-413, 2007. PubMed

V-7) D During the apoptotic process, endonucleases cut the DNA at precise sites corresponding to the linker region between nucleosomes. This leads to the formation of fragments that are multiples of 80 bp units. There is no cell swelling, such as occurs in necrosis, but rather cell shrinkage after the apoptotic process begins, followed by condensation of chromatin at the periphery of the nucleus. Apoptosis is an energy-

dependent process requiring ATP. During the apoptotic process, the plasma membrane initially remains intact, but later fragments and surrounds the apoptotic bodies.

Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. *Nat Rev Cancer* 5:231-237, 2005. PubMed

Okada H, Mak TW. Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat Rev Cancer* 4:592-603, 2004. PubMed

Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science* 306:990-995, 2004. PubMed

V-8) E The terminal deoxynucleotidyl transferase (TdT) mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) technique has been used to identify apoptotic cells. It is based upon the binding of TdT to the exposed 3'-OH terminal ends of DNA fragments generated during apoptosis and catalyzes the addition of modified deoxynucleotides, conjugated with biotin or fluorescein, to the DNA termini.

V-9) D While damage to cellular DNA was long considered the major initiator of cellular responses to ionizing radiation, more recent evidence suggests the involvement of non-targeted pathways, including radiation-induced bystander effects. Bystander effects are defined as radiation-like effects observed in cells that are not themselves irradiated, but that are in communication with irradiated cells through their location near these cells or by stimuli transferred from the irradiated cells through the intracellular medium. Various endpoints have been measured as bystander effects, including enhanced cell killing, induction of apoptosis, presence of chromosome aberrations and micronuclei, presence of DNA double-strand breaks, increased oxidative stress, genetic effects (including induction of mutations, and neoplastic transformation) and altered gene expression

Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy. *Nat Rev Cancer* 9:351-60, 2009. PubMed

V-10) D Mitotic death in most irradiated cells results primarily from mis-assortment of genetic material into daughter cells as a result of the formation of asymmetrical chromosome aberrations. This aberrant mitosis triggers mitotic catastrophe, which is characterized by cells exhibiting multiple tubulin spindles and centrosomes as well as the formation of multinucleate, giant cells that contain uncondensed chromosomes. Single strand breaks are repaired rapidly and do not appear to play an important role in cell lethality. DNA ladder formation is characteristic of apoptosis. An alteration in cell permeability occurs in cells undergoing necrosis.

Chu K, Teele N, Dewey MW*, et al.* Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. *Radiat Res* 162:270-286, 2004. PubMed

Ianzini F, Mackey MA. Delayed DNA damage associated with mitotic catastrophe following X-irradiation of Hela S3 cells. *Mutagenesis* 13:337-344, 1998. PubMed

V-11) D.Autophagy can be nonselective or selective. Nonselective, bulk degradation of cytoplasm and organelles by autophagy provides material to support metabolism during periods of cellular stress. For example, autophagy provides internal nutrients, when external ones are unavailable. Whether mechanisms exist to prevent bulk autophagy from consuming essential components, such as a cell's final mitochondrion, remains unclear, and in some cases such consumption may lead to cell death. Selective autophagy of proteins and of organelles such as mitochondria (mitophagy), ribosomes (ribophagy), endoplasmic reticulum (reticulophagy), peroxisomes (pexophagy), and lipids (lipophagy) occurs in specific situations.

VI. Cell and Tissue Survival Assays

VI-1) B Skin nodule formation is not a functional endpoint; it is a clonogenic assay measuring survival of individual epidermal cells regrowing *in situ*. All of the other assays cited represent non-clonogenic, functional endpoints for assaying radiation damage.

VI-2) C Using the equation $S = e^{-(\alpha D + \beta D^2)}$, the surviving fraction would be $e^{-[(0.3)(2)+(0.1)(2)^2]} = e^{-[(0.6)+(0.4)]} = e^{-1} = 0.37$

VI-3) D If the dose was delivered at a low dose rate, the surviving fraction would increase due to repair of sublethal damage during the course of irradiation. If one assumes that there is full repair of sublethal damage during the 6 hr irradiation (which is probably an oversimplification), sublethal damage would not contribute to cell killing. The □ component of the LQ equation would therefore approach zero. Therefore, the surviving fraction can be estimated as $e^{-(0.3)(2)} = e^{-0.6} = 0.55$

VI-4) E The spleen colony assay involves the ability of donated bone marrow stem cells, injected intravenously into lethally-irradiated recipient mice, to form discrete splenic colonies. The higher the radiation dose received by the donated marrow, the fewer colonies (relative to the number of cells injected) will form in the recipients' spleens. This technique allows a cell survival curve to be generated *in vivo*.

Till J, McCulloch EA. Direct measurement of radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14:213-222, 1961. PubMed

VI-5) D The clonongenic survival assay measures the ability of single cells to divide continuously after a given exposure, which typically measures colony formation 7-14 days after exposure to the agent. It requires a normalization in which the number of colonies formed is divided by the number of cells seeded (in the absence of any DNA damaging agent), which yields the plating efficiency. Surviving fraction is then calculated for each dose of a given agent by dividing the number of colonies formed by the number of cells seeded, and normalizing to the "0 Gy" plating efficiency. Mutliple doses and cell densities typically are needed for the adequate analysis of cell survival. This is not a short-term growth delay assay, and thus a cell capable of multiple cell divisions is needed. DNA damaging-agents induce cell death via a number of pathways, including apoptosis. However, apoptosis is not the sole pathway.

VII. Models of Cell Survival

VII-1) C Assuming that all cells in the cell population are identical and that cell killing is a random, probabilistic process that follows a Poisson distribution, one model that can calculate the radiation dose that produces an average of one lethal hit is the single-target single-hit model. From the equation that describes this model, $S = e^{-D/D_0}$, the dose, D, at which there would be an average of one hit per cell would be equal to D_0, the constant of proportionality. Therefore, $S = e^{-1} \sim 0.37$.

VII-2) E The α/β ratio represents the dose at which the αD component of cell killing, assumed to result from the induction of irreparable damage, is equal to the lethality produced by the βD^2 component of cell killing that results from the accumulation of sublethal damage.

VII-3) D Cells derived from an individual diagnosed with xeroderma pigmentosum are defective in nucleotide excision repair. These cells are sensitive to UV radiation since this form of radiation produces damages such as pyrimidine dimers that are removed through the nucleotide excision repair pathway. Because DNA double-strand breaks are important lesions responsible for lethality in cells exposed to X-rays and DNA double-strand break repair is generally normal in cells derived from a person diagnosed with xeroderma pigmentosum, the D_0 determined from a radiation survival curve for these cells would not be particularly small. People with Nijmegen breakage syndrome, LIG4 syndrome, ATR-Seckel syndrome and ataxia telangiectasia, who possess mutations in either *NBS1*, *LIG4*, *ATR* or *ATM*, respectively, are all characterized by defects in strand break repair or repair-related signaling. Therefore, at least a small increase in radiosensitivity (a decrease in the D_0) *would* be expected in cells derived from people with these syndromes.

VII-4) E If n =1, the survival curve has no shoulder and D_{37} (dose resulting in a survival fraction of 0.37) equals the D_0. The □ parameter would decrease with decreasing dose rate as sublethal damage became less important. The inverse of the D_0, not the D_q, is equal to the final slope of the survival curve. The extrapolation number, n, of a survival curve decreases with increasing LET of the radiation until reaching a value of 1.0 for very high LET radiations. The D_0 would not necessarily be a good predictor for the effect of fractionation on survival; D_q or n would be better.

VII-5) A Reoxygenation generally occurs over a period of hours to days. Therefore, little to no reoxygenation of hypoxic cells is likely during irradiations performed at dose rates in the 1-10 Gy/min range since, for a total dose of 6 Gy, the irradiation times would only vary from 0.6-6 minutes. If the total treatment time is long enough that significant repair of sublethal damage (half-time on the order of 0.5-1.0 hour) can occur during irradiation, repair does influence cell survival. The irradiation time would vary from 6-60 minutes for dose-rates in the range of 1-0.1 Gy/min and significant repair would occur. Movement of the surviving cells through the cell cycle (causing redistribution of viable cells from resistant phases into sensitive phases) can influence the radiosensitivity of the cell population when irradiation times are

increased to several hours (for the dose-rate range of 0.1-0.01 Gy/min, times of 1-10 hours would be needed to produce 6 Gy). Repopulation can lead to an increase in the number of cells during irradiation and, hence, to an increase in the total number of surviving cells when a radiation dose is delivered over days (10-100 hours are required to produce a total dose of 6 Gy over a range of 0.01-0.001 Gy/min).

VII-6) C For a high LET radiation, it can be assumed that the survival curve is exponential, or near exponential and cell survival can be modeled using the single-target, single-hit equation ($S = e$), or the simplified form of the linear quadratic equation in which β is zero ($S = e^{-\alpha D}$). Using either of these equations, 3 Gy reduces the surviving fraction to 10^{-1}, and a dose of 18 Gy therefore would reduce survival to 10^{-6}. Therefore, irradiating 10^8 cells with 18 Gy would result in thesurvival of: (10^8 cells) x (10^{-6} surviving fraction) = 10^2 cells.

VII-7) B An exponential survival curve can be modeled using the single-target, single-hit equation ($S = e$), or the simplified form of the linear quadratic equation in which β is zero ($S = e^{-\alpha D}$). Since four 3 Gy fractions reduce the surviving fraction to 10^{-4}, and assuming an equal effect per fraction, each 3 Gy fraction reduces the surviving fraction by 10^{-1}. Accordingly, two additional 3 Gy fractions (producing a total dose of 18 Gy) would yield a surviving fraction of 10^{-6}.

VII-8) B The survival curve for this cell line is exponential because each incremental dose of 3 Gy decreased the surviving fraction by an additional factor of 0.1. Thus, this survival curve can be modeled using an exponential equation which can be expressed as either $S= e^{-\alpha D}$ (linear-quadratic model) or $S = e^{-D/D0}$ (target theory model). The D_0 is equal to the $D_{10}/2.3$, or 1.3 Gy, not 3 Gy. The n and D_q values for this survival curve are equal to 1 and 0 Gy, respectively, and are therefore small, not large. The surviving fraction after a single dose of 3 Gy can be calculated from the colony forming efficiency of the irradiated cells (40/1000), divided by the plating efficiency (PE) of the unirradiated cells (40/100), which is equal to 0.04/0.4 or 0.1. Since this survival curve can be represented by $S = e^{-\alpha D}$, the β term of the linear-quadratic equation must approach zero, so the α/β would be very high, and in fact will be undefined if the β term is actually zero.

VII-9) D The ${}_eD_{10}$ is equal to the ${}_eD_0$ multiplied by 2.3, or 4 Gy x 2.3 = 9.2 Gy

VIII-10) C When the irradiated cell populaiton recived an average of 1 lethal hit, it results in 37% of survival based on Poison statstics.

VIII. Linear Energy Transfer

VIII-1) D RBE decreases with increasing LET above about 100 keV/μm. This is thought to be due to the "overkill" effect in which many more ionizations (and damage) are produced in a cell traversed by a very high LET particle than are minimally necessary to kill it, thereby "wasting" some of the energy. Maximum cell killing occurs at an LET of approximately 100 keV/μm, not 1000 keV/μm. RBE shows the greatest changes for LET values between roughly 20 and 100 keV/μm. OER decreases slowly with increasing LET for low LET values, but falls rapidly after LET exceeds about 60 keV/μm and, therefore, does not follow a bell-shaped curve.

VIII-2) D There is little or no split-dose recovery following high LET radiation exposure because the single dose survival curves for high LET radiations have little or no shoulder. There is also little or no potentially lethal damage recovery, oxygen effect or radioprotection afforded by the presence of sulphydryl compounds. Delivery of a radiation dose at a low dose rate leads to less sparing for a high LET radiation compared with a low LET radiation.

VIII-3) B The RBE for high LET particles is greater for hypoxic cells than for well-oxygenated cells of the same type because there is little or no oxygen effect for high LET radiation. The RBE is greater for neutrons than it is for protons in the therapeutic energy range because the high energy protons used in radiotherapy are of a relatively low LET and therefore possess an RBE of approximately 1.1. The RBE for carbon ions, or any other type of high LET radiation, is greater for a fractionated irradiation compared with an acute exposure because of the substantial sparing exhibited with the reference X-rays with fractionation. The RBE for charged particles is low at the beginning of the particle track and greatest near the end of the track, in the Bragg peak region. RBE does show a fractionation dependence; it decreases with increasing fraction size.

VIII-4) A 150 MeV protons have an LET of approximately 0.5 keV/μm. 1 GeV Fe ions, ^{60}Co γ-rays, 2.5 MeV α-particles and 250 kV X-rays have LET values of approximately 143, 0.2, 166 and 2 keV/μm, respectively.

Miller RC, Martin SG, Hanson WR, et al. Effect of track structure and radioprotectors on the induction of oncogenic transformation in murine fibroblasts by heavy ions. Adv Space Res 22: 1719-1723, 1998.

Balcer-Kubiczek EK, Zhang XF, Harrison GH, et al. Delayed expression of hpS2 and prolonged expression of CIP1/WAF1/SDI1 in human tumor cells irradiated with X-rays, fission neutrons and 1 GeV/nucleon Fe ions. Int J Radiat Biol 75: 529-541, 1999.

VIII-5) D LET does not directly dependent on mass of the particle, but rather indirectly through kinetic energy relationships.

VIII -6) C Photons, such as 250 KV X-rays, in passing through tissue produce

no ionizations directly but only by setting in motion atomic electrons of tissue molecules. Electrons set in motion by incident photons have a broad energy distribution which is dissipated in tracts with LET ranging from about 0.4 to 40 keV/μm. Radiation therapy high energy photons can generate neutrons with energy between 0.1 to 2 MeV through photon interactions with nuclei of high atomic number materials that constitute the linac head and collimator systems. These neutrons in passing through tissue also produce no ionization directly but by setting protons in motion by knock on collitions with hydrogen nuclei of the cellular water molecules. Protons set in motion by photoneutrons dissipate energy over a range of LET up to about 90 keV/μm.

Naseri A, Mesbahi A. A review of photoneutrons characteristics in radiation therapy with high-energy photon beams. Reports of Practical Oncology and Radiotherapy 15 (2010) 138-144.

VIII-7) C On the average, the formation of a three-ion cluster requires dissipation of 110 eV. Thus, $\frac{55\ keV}{\mu} \times \frac{1000\ eV}{1\ keV} \times \frac{1\ ion\ cluster}{110\ eV} = \frac{500\ ion\ clusters}{\mu}$ or 1 cluster every 20 Å (1 μm = 10,000 Å). This spacing of ion clusters along the silicon ion track corresponds to a 20 Å diameter of of the DNA helix.

IX. Modifiers of Cell Survival: Oxygen Effect

IX-1) C In a typical respiring tissue, the approximate distance that oxygen can diffuse from a normally oxygenated capillary before cellular hypoxia is detectable is approximately 70 µm. Howevr, the oxygen diffusion distance will depend on the partial pressure of oxygen in the capillary and on the rate of oxygen consumption by the tissue, and therefore shows some variability.

IX-2) B The fraction of cells in a tumor that are hypoxic can be estimated using the paired survival curve method. This corresponds to the surviving fraction of cells irradiated in normally oxygenated tumors divided by the surviving fraction of cells from a tumor made fully hypoxic by asphyxiating the host with nitrogen immediately prior to irradiation; this is assumed to render all of the tumor cells radiobiologically hypoxic. Thus the estimate for the fraction of hypoxic cells would be 0.004/0.1 = 0.04.

IX-3) B The K_m value occurs at an oxygen concentration of roughly 0.5-1% or 3-8 mm Hg.

IX-4) A The most dramatic change in radiation sensitivity occurs over an oxygen tension range of 0-30 mm Hg (Torr). Cells irradiated under an oxygen partial pressure at the low end of this range are maximally radioresistant, whereas irradiation at 30 mm Hg oxygen results in near maximum radiosensitization.

IX-5) A Since the high energy protons used in radiotherapy have an LET similar to that of X-rays, their OER values are also similar. An oxygen partial pressure greater than about 2-3% during irradiation will result in essentially full radiosensitization. The OER is defined as the ratio of the radiation dose needed to cause a certain biological effect in hypoxic cells divided by the dose needed to produce the same effect in aerated cells. Both acutely and chronically hypoxia cells can reoxygenate. The increased cell killing resulting from irradiation in the presence of oxygen is thought to be the result of increased radical damage and damage fixation by oxygen. The initial number of ionizations produced by radiation in the aerated and hypoxic cells would be the same.

Dewhirst MW. Relationships between cycling hypoxia, HIF-1, angiogenesis and oxidative stress. *Radiat Res* 172:653-665, 2009. PubMed

Ljungkvist AS, Bussink J, Kaanders JH, van der Kogel AJ. Dynamics of tumor hypoxia measured with bioreductive hypoxic cell markers. *Radiat Res* 167:127-145, 2007.

Ljungkvist AS, Bussink J, Kaanders JH, van der Kogel AJ. Dynamics of tumor hypoxia measured with bioreductive hypoxic cell markers. *Radiat Res* 167:127-145, 2007. PubMed

IX-6) C Since the X-ray OER is typically about 3 and the OER for 15 MeV neutrons is about 1.6, then the ratio of the OERs is about 2.

IX-7) E Exposure of cells to hypoxia, as in other stress situations, leads to changes in expression of a number of stress genes, many of which are responsive to the transcription factor, hypoxia-inducible factor-1α (HIF-1α) (HIF1A). Under normoxic conditions, HIF-1α is hydroxylated on proline residues by oxygen-dependent prolyl hydroxylases. The hydroxylated prolines bind to the von Hippel-Lindau (VHL) protein, which is a component of the E3 ubiquitin-protein ligase complex that ubiquitinates HIF-1α and targets it for degradation. Oxygen acts as a radiosensitizer principally through its ability to "fix" radiation-induced DNA damage and does not inhibit DNA repair. The OER decreases with increasing LET, whereas the RBE increases with LET until reaching a maximum at approximately 100 keV/μm, and then decreases. Measurements with pO_2 microelectrodes and bioreductive probes have demonstrated that hypoxic cells are often present in human tumors.

Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R. Reactive oxygen species and HIF-1 signalling in cancer. *Cancer Letters* 266:12-20, 2008. PubMed

Rockwell S. Oxygen delivery: implications for the biology and therapy of solid tumors. *Oncol Res* 9:383-390, 1997. PubMed

IX-8) B Hypoxia has been observed and documented in human mammary tumors by using Eppendorf oxygen microelectrode.

IX-9) C Vasodialator works mostly to the blood vessels of normal tissues, which will result in pulling more blood from tumors making tumors more hypoxic.

IX-10) D OER for energized ions should be 1.0. By definition, OER cannot be smaller than 1.0.

IX-11) C Taxanes bind to microtubules and adversely affect their function by enhancing and preventing disassembly. Taxanes act as mitotic inhibitors, blocking cells in the G_2/M phase of the cell cycle and, if the concentration is sufficient, killing them in this phase.

IX-12) D There is no difference in the uptake of the chemical by aerobic and hypoxic cells but obious difference in the action of cell kill because of the amount of oxygen available in the cells.

X. Modifiers of Cell Survival: Repair

X-1) D Bromodeoxyuridine incorporated into cellular DNA in place of thymidine acts as a radiation sensitizer, so cell killing would be enhanced, not reduced. S-phase is the most radioresistant phase of the cell cycle, so cell killing would be decreased relative to that for an asynchronous population. Oxygen is a radiation sensitizer, so cell killing would decrease in cells made hypoxic before irradiation. Splitting the dose into two fractions separated by 24 hours would allow sublethal damage recovery and possibly cellular proliferation to take place between fractions. . Therefore, cell killing would be less than if the total dose had been delivered acutely. Cysteine is a sulfhydryl-containing compound that scavenges radiation-induced free radicals; it therefore acts as a radioprotector and reduces cell killing.

X-2) A For irradiations at a low dose-rate of ~1 Gy/hr, cell survival increases primarily due to cellular repair, generally assumed to be repair of DNA double-strand breaks during irradiation.

X-3) E Potentially lethal damage recovery is operationally defined as an increase in cell survival after delivery of a large, single radiation dose under environmental conditions not conducive to progression of cells through the cell cycle for several hours after irradiation. If non-cycling cells are forced to re-enter the cell cycle immediately after irradiation, rather than remaining quiescent, potentially lethal damage will be "expressed" and therefore the surviving fraction will be lower. Sublethal damage recovery is operationally defined as an increase in cell survival noted when a total radiation dose is delivered as two fractions with a time interval between the irradiations, as opposed to a single exposure. Repair of DNA damage and rejoining of chromosome breaks presumably underlie both the sublethal and potentially lethal damage recovery. Cell cycle reassortment has a sensitizing effect on a population of cells receiving multi-fraction or protracted irradiation regimens, since, under these conditions, cells in a resistant phase of the cell cycle that survive the initial irradiation may progress through the cell cycle between fractions, leading to their reassortment into more sensitive phases of the cell cycle by the time the next dose is delivered. However, this process is irrelevant under the conditions described here, in which only a single radiation dose was administered. Translesion DNA synthesis is an error-prone process in which certain DNA polymerases synthesize DNA using a DNA strand that contains damage as a template, resulting in error-prone DNA synthesis.

X-4) C When a dose of 5 Gy is delivered at a dose rate of 1 Gy/min, irradiation requires 5 minutes. When 5 Gy is delivered at 1 Gy/hr, irradiation requires 5 hours. Extensive repair of sublethal damage will occur during the low dose rate, but not the high dose rate irradiation. As a result, the β component of cell killing will decrease, which, in the limit, would result in a strictly exponential, shallow survival curve. Therefore, more cell killing would occur when a dose of 5 Gy is delivered at a high dose rate rather than a low dose rate. The surviving fraction would change the least for a cell line with a radiation survival curve characterized by a high, not low, α/β ratio. Treatment with an agent that inhibited DNA repair would have little impact during the 5 minute irradiation that would occur at the high dose rate. In contrast, such a treatment would markedly reduce cell survival for the 5 hour irradiation required at the low dose rate since, in the absence of the agent, substantial repair would take place during the course of the irradiation. The increase in the surviving fraction for this low dose rate irradiation is primarily a consequence of sublethal damage recovery and not repopulation, as the repopulation would only occur for overall treatment times on the order of days.. The number of ionizations produced is a reflection of the total dose delivered and does not vary with the dose rate.

X-5) B Compared to the cell surviving fraction after the single 8 Gy dose, the increase in cell survival noted for the two 4 Gy doses delivered with a 2 hour interfraction interval was due to repair of sublethal damage (SLDR). Although SLDR also occurred when the interfraction interval was 8 hours, cells surviving the first dose also reassorted from the radioresistant phases they were in at the time of the initial irradiation (e.g. late S) into more radiosensitive phases (e.g. G_2 and M) resulting in an overall surviving fraction for the 8 hour interval that was lower than that for the split dose protocol with a 2 hour interval between fractions. It is unlikely that much repopulation would take place during the total time of 8 hours needed to complete the irradiations. Reoxygenation would not be an issue for cells maintained in a 95% air environment, which are well aerated.

X-6) D Sublethal damage recovery is operationally defined and demonstrated using a split dose protocol. Potentially lethal damage repair is detected by changing the post-irradiation environment and observing the effect on survival. Incorporation of tritiated thymidine into DNA would not specifically measure PLDR, but would reflect DNA synthesis and other forms of DNA repair. Reoxygenation would best be assayed by performing repeat measurements during the course of radiotherapy using an oxygen electrode or an hypoxia maker, such as pimonidazole, that is metabolized and incorporated exclusively into hypoxic cells. Cell cycle age response is best demonstrated using a cell synchronization technique, followed by irradiation of cohorts of cells in particular cell cycle phases and a clonogenic survival assay. Repopulation can be assayed *in vitro* by counting the number of cells present as a function of time after irradiation. The mitotic shake off technique is used to collect synchronous populations of cells for use in experiments examining age response functions.

X-7) B ATM (Ataxia Telangictasis Mutated). Cells deficient in ATM activity display cell cycle checkpoint defects and IR sensitivity.

X-8) C PLD is believed to be complex DSBs that are repaired slowly as compared to simple DSBs. Therefore, cells that are left in stationary phase after irradiation display enhanced survival as they have time to repair complex DSBs before resuming progression through the cell cycle.

X-9) B The fraction of cells surviving a split dose increases with increasing time between the two doses because of the repair of SLD.

XI. Solid Tumor Assay Systems

XI-1) B An increase in the number of tumors per animal would be a reflection of metastatic spread of the tumor, and would not necessarily reflect the radiation response of the primary tumor *per se*. All of the other assays can be used to quantify the response of tumors to irradiation.

XI-2) E TCD_{50} quantifies the dose required to cure 50% of a group of matched tumors and, hence, is a highly relevant endpoint for extrapolation to the clinic where the goal is tumor cure. The assay can be conducted using mouse tumors or human tumor xenografts, although suppression of the host immune system when using xenografts is crucial so as to minimize misleading results due to rejection of implanted cells. The TD_{50} assay is used to measure the number of cells required to cause a tumor in mice and has historically been used to determine tumor cell survival curves, to assess the number of clonogens in a tumor, and to study host factors that influence tumor development.

XI-3 B CD133 has been described as a putative marker for cancer stem cells in glioblastoma. Injection of 100 CD133+ cells is sufficient to initiate tumor formation in >30% of nude mice thus confirming CD133 as a potential cancer stem cell marker. The unsorted bulk cells contain cancer stem cells, but based upon the requirement to use about 100-1,000 more cells to form the same level of tumors as purified CD133+ cells, it appears that <1% of the cells in the tumor are stem cells. CD133- cells were derived from a glioblastoma and are therefore not normal, although they possess a very limited ability to form tumors. In this experiment, no data are provided regarding radiation sensitivity or radiation resistance of the cells.

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. *Nature* 444:756-60, 2006. PubMed

XI-4) D Results from tumor transplantation experiments indicate that only a small proportion of all cancer cells have an unlimited proliferative capacity and are able to maintain their population within the tumor (self-renewal). In analogy to in vitro colony formation, tumor cells with the ability to cause a local recurrence after radiotherapy have been termed "clonogenic cells"; which may correspond to putative "cancer stem cells". The existence of cancer stem cells, which are defined by the ability for self-renewal and generation of the heterogeneous lineages in the tumor, has been hypothesized.

Baumann M, Zips D, Appold S. Radiotherapy of lung cancer: technology meets biology meets multidisciplinarity. *Radiother Oncol* 91:279-81, 2009. PubMed

Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat Rev Cancer* 8:545-54, 2008. PubMed

XI-5) D It has been suggested that a small proportion (less than 1%) of all cells in a tumor are cancer stem cells. If correct, this hypothesis suggests that all cancer stem cells must be inactivated to achieve permanent local tumor control. In theory, one surviving cancer stem cell is sufficient to cause a local recurrence after irradiation. Thus, the rate of permanent local tumor control is a direct measure of radiation response of cancer stem cells. In contrast, tumor shrinkage and growth delay are dominated by the response of the bulk of cancer cells and not specific for the radiation response of cancer stem cells. Cancer cells with a limited proliferative capacity and doomed cancer stem cells might undergo a number of cell divisions before they permanently stop to proliferate and ultimately die. Thus, determination of proliferating cells will not provide information about the radiation response of cancer stem cells. Cancer cells can die upon radiation in different ways: mitotic catastrophe, apoptosis, autophagy, senescence and necrosis. None of these modes of cell death is likely to be specific for cancer stem cells. Given that many solid tumors exhibit resistance to undergoing apoptosis and the controversial data from studies comparing the rate of apoptosis with radiation response of tumors, it is unlikely that the rate of apoptosis after irradiation will be a proper parameter to determine the response of irradiated cancer stem cells.

XI-6) A There are indeed some examples in the literature showing a discrepancy between growth delay and tumor control probability. In these experiments various molecular targeting approaches in combination with radiation were investigated. Though difficult to prove, the assumption of a differential effect on cancer stem cells and non-cancer stem cells is the mostly likely explanation for these results. It is likely that the drug reached the tumor since there was an effect on tumor growth. Cancer cells generally express EGFR and cell survival following irradiation is affected by vascular supply. The observed discrepancy between growth delay and local tumor control in some experimental settings suggests that the latter assay is the preferable endpoint to evaluate new therapeutic approaches with curative intent.

Baumann M, Krause M, Hill R. Exploring the role of cancer stem cells in radioresistance. *Nat Rev Cancer* 8:545-54, 2008. PubMed

Baumann M, Krause M, Zips D, et al. Selective inhibition of the epidermal growth factor receptor tyrosine kinase by BIBX1382BS and the improvement of growth delay, but not local control, after fractionated irradiation in human FaDu squamous cell carcinoma in the nude mouse. *Int J Radiat Biol* 79:547-59, 2003. PubMed

Zips D, Krause M, Yaromina A, et al. Epidermal growth factor receptor inhibitors for radiotherapy: biological rationale and preclinical results. *Journal of Pharmacy and Pharmacology* 60:1019-28, 2008. PubMed

XI-7) B There is no inflammatory response in apoptosis. Inflammatory response is a feature of necrosis.

XI-8) B Radio resistant cells display mitotic catastrophe caused by aberrant mitosis which is associated with formation of multinucleate, giant cells that contain uncondensed chromosomes.

XI-9) B Fas ligand binds its receptor and triggers the external death receptor pathway.

XII. Tumor Microenvironment

XII-1) D In the absence of reoxygenation, it is unlikely that all hypoxic cells would be eliminated from a tumor possessing even a small percentage of hypoxic cells due to their ~3-fold greater radioresistance compared with aerated cells. Hypoxic regions in tumors can be detected using a labeled nitroimidazole compound rather than bortezomib (Velcade), which is a proteasome inhibitor. Generally, although not without exceptions, as tumors increase in size, the hypoxic fraction also increases since the typically abnormal tumor vasculature is insufficient to maintain oxygen demand. Regions of *acute* or transient hypoxia may develop in tumors due to intermittent blood flow and it is the acutely hypoxic cells that tend to exhibit rapid reoxygenation, whereas chronically hypoxic cells generally reoxygenate more slowly.

Dewhirst MW. Relationships between cycling hypoxia, HIF-1, angiogenesis and oxidative stress. *Radiat Res* 172:653-665, 2009. PubMed

XII-2) E Avastin is a monoclonal antibody that targets vascular endothelial growth factor (VEGF).

Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 8:579-591, 2008. PubMed

Shih T, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Clin Ther* 28):1779-802, 2006. Review. PubMed

XII-3) D An increased apoptotic index is often observed in hypoxic regions of tumors. The gene for vascular endothelial growth factor (*VEGF/VEGFA*) is one of the major genes under the control of the hypoxia responsive promoter, HRE, which binds the transcription factor, HIF-1. Studies in animal models have indicated that treatment with anti-angiogenics can cause "normalization" of tumor blood vessels and result in a transient improvement in tumor oxygenation before vessels start to deteriorate. Studies with animal models and in the clinic have linked increased hypoxia in tumors to increased tumor aggressiveness and metastatic potential. Exposure to severe hypoxia inhibits progression of cells through the cell cycle and therefore inhibits proliferation.

Rockwell S, Dobrucki IT, Kim EY, Marrison ST, Vu VT. Hypoxia and radiation therapy: Past history, ongoing research, and future promise. *Curr Mol Med* 9:442-458, 2009. PubMed

XII-4) A Chronically hypoxic regions in a tumor can be targeted for elimination by administering certain bioreductive drugs that preferentially kill hypoxic, but not aerobic, cells. *Acutely* hypoxic cells are a consequence of intermittent blood flow in tumors. Chronically hypoxic cells tend to be sensitive to hyperthermia because they exist in an acidic (*low* pH) microenvironment. Based on model calculations of oxygen consumption rates in respiring tissues, and through the use of hypoxia markers, it has been shown that chronically hypoxic cells rarely appear closer than about 70 μm from capillaries.

Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer* 4: 437-47, 2004. PubMed

XII-5) C Vascular endothelial growth factor (VEGF) is induced under hypoxic conditions through the action of hypoxia-inducible transcription factors that bind to the *VEGF* promoter to stimulate its transcription and, as a consequence, to stimulate angiogenesis. In the absence of angiogenesis, tumors would only be expected to reach a diameter of about 2 mm, not 2 cm. Microvessel density, a measure of angiogenesis, has been correlated *positively* with metastatic spread for most tumor types. Angiostatin and endostatin are inhibitors of angiogenesis, whereas basic fibroblast growth factor is a positive regulator of angiogenesis.

Kerbel RS. Tumor angiogenesis. *N Engl J Med* 358:2039-49, 2008. PubMed

XII-6) E Hypoxia-inducible factor-1 (HIF-1) is a heterodimer that acts as a key regulator of several oxygen-responsive proteins, including erythropoietin and vascular endothelial growth factor. HIF-1 was first identified as a DNA-binding protein that mediated the up-regulation of the erythropoietin gene under hypoxic stress. Subsequent studies have implicated HIF-1 in the regulation of a broad range of oxygen responsive genes including *VEGF*, VEGF receptors, angiopoietins, nitric oxide synthase, fibroblast growth factors and platelet-derived growth factor. Under aerobic conditions, HIF-1α is hydroxylated by HIF prolyl hydroxylases. Hydroxylation at two prolyl residues targets HIF-1α to the von Hippel-Lindau E3 ubiquitin ligase resulting in HIF-1α ubiquitination and subsequent proteosomal degradation, thereby limiting upregulation of target genes. Because the hydroxylation catalyzed by prolyl hydroxylases requires molecular oxygen, HIF escapes inactivation under hypoxic conditions.

Kaelin WG Jr. The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. *Nat Rev Cancer* 8:865-873, 2008.PubMed

Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. *Nat Rev Cancer* 8:967-975, 2008. PubMed

Bristow RG, Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer* 8:180-192, 2008. PubMed

Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat Rev Cancer* 8:425-437, 2008. Erratum in: *Nat Rev Cancer* 8:654, 2008. PubMed

XII-7) E When administered as single agents, certain anti-angiogenic drugs have not yielded a long-term survival benefit. In contrast, combining the same anti-angiogenic agents with chemotherapy *has* produced a significant survival benefit in colon cancer and previously untreated lung and breast cancers. If the anti-angiogenic agent, in combined regimens, were destroying tumor vasculature, one would expect decreased tumor blood flow and compromised delivery of chemotherapy to the tumor. Thus, the survival benefits produced by the addition of an anti-angiogenic drug to traditional chemotherapeutic regimens appears paradoxical. One possible explanation for this has been termed the "normalization hypothesis". Under the pressure of pro-angiogenic factors, tumor vasculature is structurally and functionally abnormal. Anti-angiogenic therapy (transiently) restores the balance of pro- and anti-angiogenic factors. Consequently, immature and leaky blood vessels are pruned, pericyte coverage increases, and the basement membrane becomes more homogenous and normal. The resultant vascular bed is less leaky, dilated and tortuous and is better organized. These morphological changes also result in functional changes, including decreased interstitial fluid pressure, increased tumor oxygenation and improved penetration of drugs into the tumor parenchyma. Due to improved drug delivery, chemotherapy is more efficacious. However, sustained or high-dose anti-angiogenic therapy may drive an imbalance favoring anti-angiogenic factors producing an inadequate tumor blood supply and compromising chemotherapy efficacy.

Jain RK. Lessons from multidisciplinary translational trials on anti-angiogenic therapy of cancer. *Nat Rev Cancer* 8:309-316, 2008. PubMed

Jain RK. Antiangiogenic therapy for cancer: current and emerging concepts. *Oncology* 19(4 Suppl 3):7-16, 2005. PubMed

Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307: 58-62, 2005. PubMed

XII-8) C Solid tumors develop regions of increased hypoxia (decreased pO_2), decreased pH, decreased glucose and *increased* (not decreased) interstitial fluid pressure.

XII-9) B Paclitaxel has been shown to increase the radiation sensitivity of tumors by inducing apoptosis, increasing oxygenation of hypoxic cells in tumors, arresting cells in the radiosensitive G_2/M phase of the cell cycle and decreasing interstitial fluid pressure. Some of these studies have been conducted in animal models and some in human breast cancer patients. No studies have indicated upregulation of HIF-1, and one might expect HIF-1 to be degraded more rapidly after reoxygenation occurs.

Taghian AG, Abi-Raad R, Assaad SI, *et al.* Paclitaxel decreases the interstitial fluid pressure and improves oxygenation in breast cancers in patients treated with neoadjuvant chemotherapy: clinical implications. *J Clin Oncol* 23:1951-1961, 2005. PubMed

Griffon-Etienne G, Boucher Y, Brekken C, *et al.* Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical implications. *Cancer Res* 59:3776-3782, 1999. PubMed

Milas L, Milas MM, Mason KA. Combination of taxanes with radiation: preclinical studies. *Semin Radiat Oncol* 9:12-26, 1999. PubMed

XII-10) D The fraction of proliferating cells tends to decrease with increasing tumor volume.

Dubben HH, Thames HD, Beck-Bornholdt HP. Tumor volume: a basic and specific response predictor in radiotherapy. *Radiother Oncol* 1998; 47:167-74. PubMed

Bentzen SM, Thames HD. Tumor volume and local control probability: clinical data and radiobiological interpretations. *Int J Radiat Oncol Biol Phys* 1996; 36:247-51. PubMed

XII-11) E Tumor masses exhibit abnormal blood vessel networks which fail to provide adequate and homogeneous nutritional support.

XII-12) B This process is normally regulated by a balance of pro-and anti-angiogenic molecules.

XII-13) D Colchicine is being used for an anti-vascular therapy aimed at damaging tumor blood vessels not for anti-angiogenic therapy.

XII-14) B Localized irradiation of a tumor causes, not only a shrinking of the irradiated tumor, but also a shrinking of tumors far from the irradiated area. This once obscure radiotherapy anecdotal phenomenon is having a revival in the form of radiation in combination with immune checkpoint

inhibitors such as anti-CTLA-4 and anti-PD-1 with sometimes startingly responses

Ehlers G, Fridman M. Abscopal effect of radiation in papillary adenocarcinoma. Br J Radiol.1973;46:220–2.

Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012;366:925-931

XII-15) **D** Vasculogenesis is the term used for the formation of new blood vessels, arterial and venous, when there are no pre-existing ones and can occur in the post-natal state from circulating endothelial progenitor cells. During early tumor development both vasculogenesis and angiogenesis are likely employed. Because tumor irradiation abrogates local angiogenesis, the tumor must rely on the vasculogenesis pathway for re-growth after irradiation. Tumor irradiation produces a marked influx of CD11b+ myeloid cells into the tumors, and these are crucial to the formation of blood vessels in the tumors after irradiation and for the recurrence of the tumors.

PMID 24338942 & 26788072

XIII. Cell and Tissue Kinetics

XIII-1) A CDK1 (and cyclin B/A) is associated with the G_2 to M phase transition. CDK4 and cyclin D are associated with G_1 into S, cyclin A with S into G_2, cyclin B with G_2 into M, and cyclin D with G_1 into S phase.

Schwartz GK, Shah MA. Targeting the cell cycle: A new approach to cancer therapy. *J Clin Oncol.* 23:9408-9421, 2005. PubMed

Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 432:316-323, 2004. PubMed

XIII-2) C A dose that kills 90% of the cells in the population would leave a surviving cell population heavily enriched in the radioresistant cells in late S phase.

XIII-3) B The typical T_C for human tumor cells *in vivo* is in the range of 1-5 days.

XIII-4) A The cell loss factor (□) often appears to decrease several weeks after the start of radiotherapy, which has the net effect of slowing tumor regression. The growth fraction is the ratio of the number of proliferating cells to the sum of proliferating and quiescent cells. If the T_D, the observed tumor volume doubling time, is 60 days, and the T_{pot}, the potential doubling time calculated from the cell cycle time and the growth fraction is 3 days, then the cell loss factor is 95%. Although T_{pot} (as measured from a tumor biopsy derived from patients previously given bromodeoxyurdine) has not proven to be a robust predictor of long term outcome after accelerated radiotherapy, it might still be useful for the pre-selection of patients most likely to benefit from accelerated treatment. For carcinomas at least, the cell loss factor is usually the *major* determinant of the discrepancy between a tumor's potential doubling time and overall volume doubling time.

XIII-5) E The length of time required for the first radioactively-labeled S phase cells to first enter mitosis, as measured using the percent-labeled mitosis technique, would correspond to the duration of G_2 phase (T_{G_2}). The additional time required for the cells to completely fill the mitotic compartment (i.e., 100% labeled mitoses) would be equal to the length of M (T_M). Therefore, the time to reach 50% of the maximum point corresponds to T_{G_2} plus $T_M/2$.

XIII-6) B Using the equation $MI = \lambda T_M/T_C$, where MI is the mitotic index, T_M is the length of mitosis and T_C is the total cell cycle time, then $T_M = (MI)(T_C/\lambda) = (0.05)(14 \text{ hours})/0.7 = 1$ hour. (Even without performing this calculation, it should be noted that the duration of mitosis for most mammalian cells is typically ~1 hour.)

XIII-7) A A tumor's volume doubling time rarely equals its potential doubling time because most tumors have high cell loss factors. Formation of metastases represents only one of many reasons for cell loss, and usually is only a minor contributor. Human tumor cells typically have cell cycle times of a few days whereas tumor volume doubling times are generally on the order of months. The presence of a *high* hypoxic fraction would probably contribute to a low growth fraction, which would affect both T_{pot} and volume doubling time. If hypoxia were a significant cause of cell death, it would affect the cell loss factor and therefore affect the volume doubling time. The presence of non-proliferating cells affects both the tumor volume doubling time and the potential doubling time, and does not cause a difference between them. Similarly, non-viable cells (whether hypoxic or aerobic) have similar effects on the tumor volume doubling time and the potential doubling time.

XIII-8) E The cell loss factor is equal to $1-(T_{pot}/T_D)$. Therefore, if the cell loss factor were zero, then the T_{pot} would equal the T_D. The mean T_C is *shorter* than the T_{pot} becauseT_{pot} also considers the presence of quiescent cells, and the growth fraction in tumors is generally less than 100%. For solid tumors, the T_{pot} is generally much shorter than the T_D because the cell loss factor is typically quite high. The GF is taken into account in the determination of T_{pot}, so it does not affect the relationship between the T_{pot} and the T_D. T_{pot} can be calculated, from the labeling index (LI) and the duration of S phase (T_S), using the equation $T_{pot} = \lambda T_S/LI$. (where λ is a constant ranging from about 0.6 to 1.0) It has been suggested, that tumors with short pretreatment values for T_{pot}, (suggesting the presence of rapidly proliferating cells and a high growth faction), would be most likely to benefit from accelerated

radiotherapy, but this has not been confirmed in clinical trials performed to date.

XIII-9) C Regulation of the G_2 checkpoint by ATM is thought to occur through activation of CHK2, which phosphorylates CDC25C phosphatase, thereby preventing it from dephosphorylating CDK1 (CDC2), a step necessary for the progression from G_2 into M phase. The remaining proteins listed in answer choices A-D are all targets for phosphorylation by the ATM kinase, and, consequently, are implicated in various cell cycle control pathways, although not the G_2 checkpoint. CHK2 and MDM2 are involved in control of the G_1–S phase transition. Upon phosphorylation by CHK2, p53 is stabilized, causing cell cycle arrest in G_1. ATM also phosphorylates MDM2, which reduces the ability of MDM2 to negatively regulate p53. NBS1 and CHK2 are implicated in S phase progression. PUMA ("p53-upregulated modulator of apoptosis") is a pro-apoptotic gene that can induce cell death via a p53-dependent pathway.

Hurley PJ, Bunz F. ATM and ATR: components of an integrated circuit. *Cell Cycle* 6:414-7, 2007. PubMed

Lukas J, Lukas C, Bartek J. Mammalian cell cycle checkpoints: signalling pathways and their organization in space and time. *DNA Repair (Amst)* 3: 997-1007, 2004. PubMed

XIII-10) A Bromodeoxyuridine is incorporated into DNA in place of thymidine, so it can be used to label cells in S-phase. The incorporated bromodeoxyuridine is assayed using a fluorescently-labeled anti-BrdUrd antibody. Propidium iodide fluoresces when incorporated into DNA. The amount of fluorescence is directly proportional to the DNA content, which in turn is a reflection of the cell cycle phase in which the cell is located.

XIII-11) C Tumor types with a high growth fraction and short cell cycle time would be expected to grow more rapidly. Such a tumor would also be expected to regress rapidly after irradiation since irradiated cells generally die as they attempt to progress through mitosis.

XIII-12) B The doubling time of human tumors (Tb) is characteristically 40 to 100 days and the cell cycle time is relatively short, 1 to 5 days. This has important implications, which often are overlooked, in the use of cyclic-specific chemotherapeutic agents or radiosensitizing drugs for which it is the cell cycle time that is relevant.

XIII-13) C The high rate of cell loss in human tumors largely accounts for the great disparitybetween Tc and Tb.Values for the cell-loss factor vary from 0% to more than 90% for the tumors in laboratory animals.

XIII-14) E Cell migration within a tumor has recently been described from the study of microbeam radiation therapy (MRT). Since cell migration occurs within 200 mirometers (interspace between microbeams), the cells still stay in the tumor mass without affecting cell loss from the tumor.

Crosbie JC, Anderson RL, Rothkamm K, Restall CM, Cann L, Ruwanpura S, Meachem S, Yagi N, Svalbe I, Lewis RA, Williams BR, Rogers PA.: Tumor cell response to synchrotron microbeam radiation therapy differs markedly from cells in normal tissues. Int J Radiat Oncol Biol Phys. 1;77(3):886-94, 2011 (doi: 10.1016/j.ijrobp.2010.01.035).

XIII-15) D Volume doubling time = potential doubling time / (1 – cell loss factor).

XIV. Molecular Signaling

XIV-1) C ATM is a kinase that is activated in response to the presence of DNA double-strand breaks, such as would be generated by exposure to ionizing radiation. Activated ATM phosphorylates multiple, distinct target proteins, including histone H2AX, p53, BRCA1 and Artemis. Phosphorylation of H2AX (to γ-H2AX) results in chromatin modification that facilitates the recruitment of factors needed for DNA repair. The tumor suppressors, p53 and BRCA1, activate cell cycle checkpoint and/or DNA repair processes in response to genotoxic stress. VEGF is a secreted factor that promotes angiogenesis and is not a direct target of ATM phosphorylation.

Kitagawa R, Kastan MB. The ATM-dependent DNA damage signaling pathway. *Cold Spring Harb Symp Quant Biol* 70: 99-109, 2005. PubMed

XIV-2) D Epistasis is a form of gene interaction in which an allele for one trait (at one locus) influences the expression of alleles (at a different locus) for another, independent trait; this process is unrelated to ATM phosphorylation. Inactivation of the *VHL* (von Hippel-Lindau tumor suppressor) gene results in overexpression of many environmental stress-inducible mRNAs including those involved in energy metabolism, apoptosis and angiogenesis through activation of vascular endothelial growth factor (VEGF). Release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria into the cytoplasm is a primary mitochondrial apoptogenic activity. Cyclin D1 repression is associated with anti-proliferation effects and its overexpression has been observed in some human cancers (including pancreatic, lung and esophageal). MicroRNAs (miRNAs) are small non-protein-coding RNAs that function as negative regulators of gene expression under normal physiological conditions. Mis-expression of, or mutations in, miRNAs are associated with the development of various human cancers, including B-cell chronic lymphocytic leukemia, colorectal and breast cancers.

Esquela-Kerscher A and Slack FJ. Oncomirs --- microRNAs with a role in cancer. *Nature Rev* 6: 259-269, 2006. PubMed

Kaelin WG Jr. The von Hippel-Landau tumor suppressor gene: roles in cancer and oxygen sensing. *Cold Spring Harb Quant Biol* 70:159-166, 2005. PubMed

XIV-3) C FOS is a transcription factor that has been shown to modulate a variety of genes involved in stress responses, although not *BRCA2*. HIF-1 is a hypoxia inducible factor known to regulate the expression of the *VEGF* gene and thus the regulation of angiogenesis. p53 is a transcription factor that induces expression of p21. E2F is known to regulate a large

number of proteins involved in cell cycle progression, including *CDC25A*. PUMA is the major mediator of p53-dependent apoptosis following ionizing radiation in most cell types. PUMA, a pro-apoptotic BH3-only member of BCL2 family protein promotes BAX/BAK and mitochondria-dependent apoptosis in various cell types.

Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nature med 10; 789-799, 2004.

XIV-4) D Cytokines are proteins released by cells, including irradiated cells, that stimulate tissues to mount a biological response. NF-κB is a transcription factor (not a cytokine), whereas TGF-β1 (TGFB1) is an important example of one of the cytokines that has been associated with the development of lung fibrosis following irradiation. A paracrine response is the result of a cytokine targeting cells within a tissue or organ, other than those that produced the cytokine. In contrast, an autocrine response is the result of a cytokine targeting the cell from which it was produced. Cytokines generally do not have tyrosine kinase activity. BCLX is regulated through a number of transcription factors, including AP-1, STATS and NF□B.

XIV-5) C Nuclear factor (NF)-κB generally exerts a pro-survival influence through interference with apoptotic signals. It accomplishes this through the TNF receptor signaling pathway which, upon activation by an apoptotic signal, is coupled via the FADD adaptor to a caspase cascade involving the initiator caspases-8 or -10. However in some cell types, this may not occur since it may be opposed through parallel triggering by TNF of a signaling pathway that activates NF□B via the TRADD and TRAF adaptors. Active NF-κB induces transcription of a set of genes that encode the anti-apoptotic IAP's ("inhibitors of apoptosis"). NF-κB can also exert an anti-apoptotic effect by inducing transcription of anti-apoptotic proteins such as BCL-xL (BCL2L1), which act to prevent cytochrome c release and the subsequent caspase-9 activation. IκB binds to NF-κB to prevent its translocation to the nucleus. Following formation of DNA double-strand breaks and reactive oxygen species in irradiated cells, kinases (including ATM) phosphorylate IκB, targeting it for ubiquitination and degradation, which allows NF-κB to translocate to the nucleus from the cytoplasm where it can act as a transcription factor. NF-κB can exist as hetero- or homodimers of five different subunits. Different heterodimers activate different sets of genes, while p50 and p52 homodimers, lacking transactivation domains, can selectively repress expression of their target genes. Post-transcriptional modifications and cofactor binding also help shape the specificity of the NF-κB response. Competition between p53 (TP53) and NF-κB for CBP/p300 may play an important role in determining the balance between apoptosis and cell cycle arrest following irradiation.

Habraken Y, Piette J. NF-kappaB activation by double-strand breaks. *Biochem Pharmacol* 72:1132-41, 2006. PubMed

Magne N, Toillon RA, Bottero V *et al.* NF-kappaB modulation and ionizing radiation: mechanisms and future directions for cancer treatment. *Cancer Letters* 231:158-168, 2006. PubMed

XIV-6) E Many genes are both up- and down-regulated following irradiation in both a time and tissue-dependent manner. In addition, variation is also seen between cells derived from the same tissue and between tissue samples taken from different individuals. This inter-individual variation is seen both in the response to stressors such as ionizing radiation and in the normal basal gene expression patterns. One of the major driving factors in the science of microarray profiling is the hope that a better understanding of this variability in gene expression may lead to a more "personalized" diagnosis of disease, prognosis and prediction of the best therapeutic approach for cancer and other diseases.

Snyder AR, Morgan WF. Gene expression profiling after irradiation: clues to understanding acute and persistent responses? *Cancer Metastasis Rev* 23:259-68, 2004. PubMed

Amundson SA, Bittner M, Fornace AJ. Functional genomics as a window on radiation stress signaling. *Oncogene* 22:5828-33, 2003. PubMed

XIV-7) A *p21* is one of the most strongly p53-transactivated genes, and codes for the p21 protein. It responds robustly at both the mRNA and protein levels to ionizing and UV radiation, as well as to most other stress-inducing agents. p21 is a CDK inhibitor, and also binds to PCNA to prevent entry of cells into S phase. The predominant role of p21 appears to be in mediating G_1 phase arrest, although it also plays roles in differentiation, senescence, and regulation of apoptosis.

Child ES, Mann DJ. The intricacies of p21 phosphorylation: protein/protein interactions, subcellular localization and stability. *Cell Cycle* 5:1313-9, 2006. PubMed

XiV-8 B Prostate cancer is characterized by its dependence on androgen receptor and frequent activation of PI3K signaling. AR transcriptional output is decreased in human and murine tumors with *PTEN* deletion and that PI3K pathway inhibition activates AR signaling by relieving feedback inhibition of HER kinases. Similarly, AR inhibition activates AKT signaling by reducing levels of the AKT phosphatase PHLPP. Thus, these two oncogenic pathways cross-regulate each other by reciprocal feedback. Inhibition of one activates

the other, thereby maintaining tumor cell survival. However, combined pharmacologic inhibition of PI3K and AR signaling causes near complete prostate cancer regressions in a *Pten*-deficient murine prostate cancer model and in human prostate cancer xenografts, indicating that both pathways coordinately support survival.

Refs.
PMID 21575859

XIV-9) **A** Oncogene addiction was first coined by Bernard Weinstein. Oncogene addiction is the phenomenon that despite the diverse array of genetic lesions typical of cancer – some tumors rely on one single dominant oncogene for growth and survival, so that inhibition of this specific oncogene product is sufficient to halt the neoplastic phenotype. Answer "A" is correct as imatinib targets BCR-ABL. The other answers are all examples of oncogene addicted cancers that are treated with agents that do not target the dominant oncogene product.

Refs.
PMID 21953712

XV. Cancer

XV-1) B RB1 is the product of the *RB1* tumor suppressor gene (not an oncogene) and functions upon phosphorylation by CDK4/6 to release E2F. E2F then activates genes associated with the G_1 checkpoint. RB1 is functionally inactivated in virtually all human cancers, either directly or indirectly through p53 (TP53) as the p53-dependent induction of p21 (CDKN1A) regulates cyclin E/CDK2 and cyclin A/CDK2 complexes, both of which phosphorylate RB1. The RB1 and p53 signaling pathways are dysregulated in nearly all human cancers.

Mittnacht S. The retinoblastoma protein -- from bench to bedside. *Eur J Cell Biol* 84:97-107, 2005. PubMed

Massague J. G1 Cell-cycle control and cancer. *Nature* 432:298-306, 2004. PubMed

Classon M, Harlow E. The retinoblastoma tumour suppressor in development and cancer. *Nat Rev Cancer* 2:910-917, 2002. PubMed

XV-2) D p21 inhibits CDK-cyclin activity, which has the effect of decreasing the phosphorylation of RB1. ATM, and not p21, phosphorylates NBS1 thereby stimulating homologous recombinational repair. p53-mediated G_1 arrest results from transactivation of p21 by p53. An *increase* in the amount of p53 can result in apoptosis or G_1 arrest. DNA damage does initiate a signal transduction pathway that results in increased amounts of p53, however this occurs by stabilization of the existing protein, rather than by increased transcription of the gene that encodes it.

XV-3) B The ATM protein contains a highly conserved C-terminal kinase domain resembling a phosphatidylinositol-3-kinase (PI3K); this kinase is an important component of a number of DNA damage repair pathways. Irradiated AT cells exhibit abnormal G_1, S and G_2 cell cycle checkpoints. Only individuals who possess two mutated copies of the *ATM* gene (which are usually protein truncation-type mutations that cause complete loss of the ATM protein) display symptoms of AT. Cells derived from patients with AT typically display *decreased* levels of p53 phosphorylation. Irradiation causes autophosphorylation of ATM which converts it from an inactive dimer into the active monomeric form, not vice versa.

Dupre A, Boyer-Chatenet L, Gautier J. Two-step activation of ATM by DNA and the Mre11-Rad50-Nbs1 complex. *Nat Struct Mol Biol* 13:451-457, 2006. PubMed

Lavin MF, Birrell G, Chen P, *et al.* ATM signaling and genomic stability in response to DNA damage. *Mutat Res* 569:123-132, 2005. PubMed

Lobrich M, Jeggo PA. The two edges of the ATM sword: co-operation between repair and checkpoint functions. *Radiother Oncol* 76:112-118, 2005. PubMed

Lobrich M, Jeggo PA. Harmonising the response to dsbs: a new string in the ATM bow. *DNA Repair* 4:749-759, 2005. PubMed

XV-4) C Carcinogenesis is a multistep process with multiple genetic alterations occurring at particular stages of cancer progression. Alterations in *PTCH* are associated primarily with basal cell skin carcinoma and medulloblastoma. EGFR and VEGF are frequently overexpressed in colon cancer, but their lack of a relationship with progression and survival has led to their prognostic value being questioned. The other genetic changes listed have been observed at moderate-to-high frequency in the associated tumors, suggesting their importance in the etiology of these diseases. For example, over 90% of human pancreatic cancers harbor an activating point mutation in the *K-RAS* gene at codon 12. Hereditary medullary thyroid carcinoma (MTC) is caused by autosomal dominant gain-of-function mutations in the *RET* proto-oncogene. BRAF mutation is present in 50% of cutaneous melanomas and provide the therapeutic target for vemurafenib.

Doger FK, Meteoglu I, Tuncyurek P, *et al.* Does the EGFR and VEGF expression predict the prognosis in colon cancer? *Eur Surg Res* 38:540-544, 2006. PubMed

Kouvaraki MA, Shapiro SE, Perrier ND, *et al.* RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. *Thyroid* 15:531-44, 2005. PubMed

Marchese R, Muleti A, Pasqualetti P, *et al.* Low correspondence between K-ras mutations in pancreatic cancer tissue and detection of K-ras mutations in circulating DNA. *Pancreas* 32:171-177, 2006. PubMed

Sudarshan S, Linehan WM. Genetic basis of cancer of the kidney. *Semin Oncol* 33:544-551, 2006. PubMed

XV-5) D WT1 is a transcription factor which, when mutated or absent, is associated with the development of Wilms tumor. Loss of APC plays a role in gastrointestinal carcinogenesis due to its normal involvement in cell signal transduction. RB1 and p53 are both tumor suppressors that regulate cell cycle progression; p53 also regulates apoptosis. BRCA1 protein is part of

the DNA repair complex, but likely has several other functions as well, including regulation of the cell cycle and maintenance of genomic stability.

XV-6) E p53 is modified post-translationally by phosphorylation or by acetylation in response to DNA damage. p53 is encoded by a tumor suppressor gene (not an oncogene) that is inactivated in more than half of all human cancers. The DNA repair pathways that regulate p53 include not only NHEJ and HRR, but also MMR, BER and NER so that p53 plays a universal role in DNA damage surveillance and repair. DNA damage causes p53 to become stabilized and active, not inactive. p53 *increases* expression of *GADD45A*, *p21* and *PCNA*. Viruses that contain proteins that inactivate p53 include human papilloma virus, SV40 and adenovirus, but not EBV.

Sengupta S, Harris CC. p53: Traffic cop at the crossroads of DNA repair and recombination. *Nat Rev Mol Cell Biol* 6:44-55, 2005. PubMed

Viktorsson K, De Petris L, Lewensohn R. The role of p53 in treatment responses in lung cancer. Biochem Biophys Res Comm 331:868-880, 2005. PubMed

Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 432:316-323, 2004. PubMed

Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. *Nature* 432:307-315, 2004. PubMed

Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. *Nat Rev Cancer* 3:117-129, 2003. PubMed

XV-7) A Retroviruses, viruses with genomes composed of RNA instead of DNA, can cause cancers in animals (example: Rous sarcoma virus in chickens). Usually, this occurs because the retroviruses contain modified (often mutated) proto-oncogenes captured from the genomes of their vertebrate hosts.

XV-8) C The promoter region is the regulatory portion of a gene that plays a critical role in directing whether the gene is transcribed or not. Tumor suppressor genes are generally *inactivated* in many cancers, typically resulting in a loss of control over cell proliferation. Exons are the expressed, or coding, regions of genes, whereas introns are the non-coding sequences. The protein encoded by the *EGFR* (epidermal growth factor receptor) gene is found on the surface of some cell types to which epidermal growth factor binds,

stimulating cells to divide. Loss of heterozygosity is a common mechanism by which tumor suppressor genes are inactivated.

XV-9) E *ABL* is an oncogene whereas *PTEN*, *BRCA2*, *WT1* and *NF1* are all tumor suppressor genes.

Park BH, Vogelstein B. Tumor suppressor genes. In *Cancer Medicine 7,* Kufe DW, Bast RC, Hait W, *et al*., Eds. B.C. Decker, Hamilton, pp 85-103, 2006.

Pierotti MA, Frattini M, Sozzi G, *et al*., Oncogenes. In *Cancer Medicine 7,* Kufe DW, Bast RC, Hait W, *et al*., Eds. B.C. Decker, Hamilton, pp 68-84, 2006.

Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 10:789-799, 2004. PubMed

XV-10) D p53 stimulates the activity of BAX and BID in irradiated cells, resulting in apoptosis. MDM2 binding to p53 stimulates degradation of p53. Irradiation of cells activates ATM to add phosphate groups to p53. Following irradiation, p53 inhibits CDC25C which inhibits the G_2 to M phase transition. Lymphocytes and thymocytes with a mutant p53 tend to be more radioresistant than their normal counterparts.

XV-11) E $p14^{ARF}$ inhibits the MDM2-mediated degradation of p53. $p16^{INK4A}$ is a cell cycle inhibitor that prevents phosphorylation of RB by CDK4. $p14^{ARF}$ is an MDM2 inhibitor thereby causing p53 levels to increase, resulting in greater cell cycle inhibition. $p16^{INK4A}$ is encoded by a tumor suppressor gene.

Sharpless NE. INK4a/ARF: a multifunctional tumor suppressor locus. *Mutat Res* 576:22-38, 2005. PubMed

XV-12) A. The oncogene addiction model postulates that some tumors rely on the continued activity of single dominant oncogene for growth and survival. Thus, according to the oncogene addition model, inactivation of this key single oncogene will halt malignant proliferation by inducing cell-cycle arrest, differentiation, senescence or other forms of cell death, depending on tissue contexts. Products of the oncogenes listed in this question are all the addictive oncoproteins in human cancers but cancers and oncogenes to which cancers are addicted are incorrectly paired in choices B - E. The receptor kinases KIT and/or PDGFR display activating mutations in more than 90% of gastrointestinal stromal tumors (GIST) (choice A). This observation supported the use of the multi-target small-molecule tyrosine kinase inhibitor, imatinib mesylate (Gleevac) in GISTs. The correct matches in other choices are: Translocated ABL1 in chronic

myeloid leukemia; Amplified MYC in small-cell lung carcinoma; Translocated ALK in non-small cell lung carcinoma; Mutated Notch1 in T-cell acute lymphoblastic leukemia.

Torti D, Trusolino L. Oncogene addition as a fundamental rationale for targeted anti-cancer therapy: promises and perils. EMBO Molecular Medicine 3; 623-636, 2011.

Dietel M, Jöhrens K, Laffert MV, Hummel M, Bläker H, Pfitzner BM, Lehmann A, Denkert C, Darb-Esfahani S, Lenze D, Hepper FL, Koch A, Sers C, Klauschen F, Anagnostopoulos I. A 2015 update on predictive molocular pathology and its role in targeted cancer therapy: a review focusing on clinical relevance. Cancer Gene Ther 22; 417-430, 2015.

XVI. Total Body Irradiation

XVI-1) C Ten days after a total body dose of 2 Gy, one would expect lymphocyte and neutrophil counts to decrease, but hemoglobin concentration and platelet counts to remain normal. Platelets will not decrease until ~20 days after a 2 Gy exposure. Hemoglobin will not decrease unless much higher doses are received and a longer time period has elapsed.

ACR Disaster Preparedness for Radiology Professionals, A Primer for Radiologists, Radiation Oncologists and Medical Physicists, Government Version 3.0 available through the ASTRO website at: http://www.astro.org/GovernmentRelations/RadiationDisasterManagement/documents/prepbroch_001.pdf

Planning Guidance for Nuclear Detonation, first edition Jan 2009, Homeland Security Council Interagency Policy Coordination Subcommittee for Preparedness and Response to Radioological and Nuclear Threats. Available on the ASTRO website at: http://www.astro.org/GovernmentRelations/WhatsHappeningInWashington/documents/NucDetPlanGuide.pdf

Turai I, Veress K, Gunalp B, *et al*. Medical response to radiation incidents and radionuclear threats. *BMJ* 328:568-72, 2004. PubMed

Waselenko JK, MacVittie TJ, Blakely WF, *et al.* Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. *Ann Intern Med* 140:1037-1051, 2004. PubMed

XVI-2) B This individual will experience the GI syndrome and die before bone marrow would become completely aplastic, although there probably would be some hypoplasia in the marrow, spleen and lymph nodes. A characteristic feature observed in people who die from the GI syndrome is mitotic arrest in the intestinal crypt cells. The other changes (cerebral edema, microvasculitis, brain necrosis) would be expected with the cerebrovascular syndrome, which would not occur unless the total dose received was at least 3-4 fold higher than 8 Sv.

Pellmar TC, Rockwell S. Priority list of research areas for radiological nuclear threat countermeasures. *Radiat Res* 163:115-123, 2005. PubMed

Leikin JB, McFee RB, Walter FG, *et al.* A primer for nuclear terrorism. Disease-a-Month 49:485-516, 2003. PubMed

Cassatt DR, Kaminski JM, Hatchett RJ, *et al*. Medical countermeasures against nuclear threats: radionuclide decorporation agents. *Radiat Res* 170:540–548, 2008. PubMed

XVI-3) B The human LD_{50} (dose to result in lethality in 50% of an irradiated population) in the absence of medical intervention is estimated at 3.5 Gy. The dose thresholds for the hematopoietic, gastrointestinal and cerebrovascular syndromes are roughly 2 Gy, 8 Gy and 20 Gy, respectively. The LD_{50} with the best current medical treatment is about 7 Gy.

XVI-4) B The death of a person 30-60 days following a total body radiation dose close to the LD_{50} would be due to damage to the bone marrow, resulting in the gradual reduction in the level of peripheral blood elements. Infection due to loss of white blood cells and/or hemorrhage due to the loss of platelets are typically the cause(s) of death. Usually, death from ablation of the bone marrow would not be manifest until about a month or two after irradiation; this is a reflection of the normal turnover rates of the mature blood components, which would not be replaced in the absence of functioning bone marrow stem cells. Death from radiation damage to the heart, liver or kidney would not occur within two months following irradiation. Death due to damage to the gastrointestinal system usually takes place within 5-16 days following irradiation and would not be likely with a dose near the LD_{50} since it requires higher doses to be manifest.

XVI-5) D The prodrome of the radiation syndrome is a spectrum of early symptoms that occur shortly after whole body irradiation, lasts for a limited amount of time and varies in time of appearance, duration and severity depending on the dose. GI symptoms such as anorexia, nausea and vomiting occur when

an individual is exposed to doses near the LD_{50}; at higher doses symptoms such as fever and hypotension are also seen. The radioprotector amifostine would not be expected to ameliorate these symptoms if given after irradiation.

XVI-6) B Following a total body dose of 12 Gy, an irradiated individual will likely die within 5-16 days from the GI syndrome. Thus, death will occur before the symptoms of the bone marrow syndrome are manifest, usually starting at about 20 days and resulting in death at 30-60 days. The bone marrow syndrome, resulting from damage to bone marrow stem cells, occurs after doses in the 2-8 Gy region.

DuBois AT, King GL, Livengood DR (eds). *Radiation and the Gastrointestinal Tract*, CRC Press, Boca Raton, 1995.

XVI-7) C Bone marrow transplants are only useful when the radiation dose to the exposed person is within about 8-10 Gy. At lower doses, an exposed person will likely survive with appropriate medical care. For doses above 10 Gy, death from effects on the GI tract will occur, despite use of all effective currently available treatments.

XVI-8) A After exposure to 2 Gy, 50% or less will experience nausea and vomiting. Typically this occurs within 2-6 hours of exposure.

XVI-9) D Systemic corticosteroids are not recommended, without a specific indication for use.

Dainiak N, Gent RN, Carr Z, Schneider R, Bader J, Buglova E, Chao N, Coleman CN, Ganser A, Gorin C, Hauer-Jensen M, Huff LA, Lillis-Hearne P, Maekawa K, Nemhauser J, Powles R, Schünemann H, Shapiro A, Stenke L, Valverde N, Weinstock D, White D, Albanese J, Meineke V. Disaster Med Public Health Prep. 2011 Oct;5(3):183-201. PubMed link

XVII. Clinically Relevant Normal Tissue Responses to Radiation

XVII-1) D The critical target structure associated with the development of radiation-induced heart disease appears to be the endothelial lining of blood vessels, particularly arteries. Irradiation of endothelial cells is thought to induce an early stimulation of a pro-inflammatory signaling cascade that enhances arteriosclerosis and microvascular dysfunction. Historically, radiation pericarditis represented a significant complication of large-volume radiation therapy to the breast or mediastinum to doses greater than 40 Gy. However, with current treatment methods, a much smaller heart volume is irradiated, so radiation pericarditis is now infrequently observed. Cardiomyopathy during or shortly after radiotherapy is only observed in patients who received combined anthracycline chemotherapy. Following mediastinal radiotherapy of Hodgkin's disease, a statistically-significant increase in the risk of fatal cardiovascular disease, primarily attributable to myocardial infarction, has been reported among patients surviving 10 years or more. Similarly, an increased risk of myocardial infarctions was also reported after post-operative radiotherapy for breast cancer. One of the most important recent findings among the survivors of the Japanese atomic bombings is that mortality from myocardial infarction is significantly increased more than 40 years after receiving acute doses as low as 1-2 Gy.

Schultz-Hector S, Trott KR. Radiation-induced cardiovascular diseases: Is the epidemiologic evidence compatible with the radiobiologic data? *Int J Radiat Oncol Biol Phys* 67:10-18, 2007. PubMed

XVII-2) E Bone marrow failure is not a concern after localized irradiation because of the limited volume of bone marrow irradiated and compensation from the unirradiated marrow volume. Osteoradionecrosis and stress fractures, on the other hand, can be major problems. In children, growth retardation is a concern after irradiation of growth zones. Bone sarcoma is the most common secondary neoplasm following irradiation of bony structures.

Tai P, Hammond A, Dyk JV, *et al.* Pelvic fractures following irradiation of endometrial and vaginal cancers-a case series and review of literature. *Radiother Oncol* 56:23-28, 2000. PubMed

Meadows AT, Baum E, Fossati-Bellani F, *et al.* Second malignant neoplasms in children: an update from the Late Effects Study Group. *J Clin Oncol* 3:532-538, 1985. PubMed

XVII-3) A Intestinal obstruction is not a common manifestation of delayed anorectal injury after pelvic radiation therapy. In contrast, bleeding, rectal pain, and

urgency *are* common manifestations of radiation proctitis. Post-treatment anal sphincter dysfunction is also common and frequently associated with intermittent incontinence.

Fajardo LF, Berthrong M, Anderson RE. *Radiation Pathology*. Oxford University Press, Oxford, 2001.

Yeoh E, Botten R, Russo A, *et al.* Chronic effects of therapeutic irradiation for localized prostatic carcinoma on anorectal function. *Int J Radiat Oncol Biol Phys* 47:915-924, 2000. PubMed

Zimmermann FB, Feldmann HJ. Radiation proctitis. Clinical and pathological manifestations, therapy and prophylaxis of acute and late injurious effects of radiation on the rectal mucosa. *Strahlenther Onkol* 174:85-89, 1998. PubMed

XVII-4) C NSAIDs can help prevent esophagitis by decreasing inflammation. Although ACEIs have proven effective in the treatment of radiation nephropathy and pneumopathy, there are no data supporting their use in treating radiation-induced esophagitis. Intra-esophageal administration of MnSOD-plasmid liposomes has been shown to protect the mouse esophagus from both single dose and fractionated irradiation. These studies have been recently been translated to a phase clinical I trial, but benefit of this approach has not been proven in humans. Both pentoxifylline and vitamin E have been shown, in combination, to prevent and cause significant regression of radiation-induced fibrosis in breast cancer patients treated with radiotherapy.

Delanian S, Porcher R, Rudant J, *et al.* Kinetics of response to long-term treatment combining pentoxifylline and tocopherol in patients with superficial radiation-induced fibrosis. *J Clin Oncol* 23: 8570-8579, 2005. PubMed

Epperly MW, Kagan VE, Sikora CA, *et al.* Manganese superoxide dismutase-plasmid/liposome (MnSOD-PL) administration protects mice from esophagitis associated with fractionated radiation. *Int J Cancer* 96: 221-231, 2001. PubMed

Tarhini AA, Belani CP, Luketich JD, Argiris A, Ramalingam SS, Gooding W, Pennathur A, Petro D, Kane K, Liggitt D, Championsmith T, Zhang X, Epperly MW, Greenberger JS. Hum Gene Ther. 2011 Mar;22(3):336-42. PubMed link

Jacobson G, Bhatia S, Smith BJ, Button AM, Bodeker K, Buatti J. Int J Radiat Oncol Biol Phys. 2013 Mar 1;85(3):604-8. PubMed link

Delanian S, Porcher R, Balla-Mekias S, Lefaix JL. J Clin Oncol. 2003 Jul 1;21(13):2545-50. PubMed link

XVII-5) C Use of fluorinated water as a part of normal dental hygiene would, if anything, help prevent dental caries and reduce the risk of MORN. MORN is most commonly precipitated by post-radiotherapy tooth extraction secondary to poor dentition. Early studies from the 1960's and 1970's at MD Anderson Cancer Center showed that patients with teeth were at a significantly greater risk of MORN than patients without teeth. However, current treatment practices do not require the removal of all teeth prior to radiotherapy, but rather, recommend careful dental care. Radiation tolerance of the mandible is also affected by pre-irradiation dental disease, fraction size and gender (males more susceptible).

Murray CG, Herson J, Daly TE *et al.* Radiation necrosis of the mandible: a 10 year study. Part I. Factors influencing the onset of necrosis. *Int J Radiat Oncol Biol Phys* 6: 543-548, 1980. PubMed

Reuther T, Schuster T, Mende U, *et al.* Osteonecrosis of the jaws as a side effect of radiotherapy of head and neck tumour patients-a report of a thirty year retrospective review. *Int J Oral Maxillofac Surg* 32:289-295, 2003. PubMed

Grant B, Fletcher G. Analysis of complications following megavoltage therapy for squamous cell carcinomas of the tonsillar area. *Am J Roentgenol* 96:28-36, 1966. PubMed

XVII-6) C Macrophages are among the most radioresistant cells in the body and are capable of surviving large doses of radiation. GM-CFC and CFU-S, which are progenitor cells, are radiosensitive, as are unprimed T-cells and B-cells.

XVII-7) C In documented cases of humans dying from gastrointestinal syndrome after whole-body irradiation, the small intestine typically showed the most denudation relative to the other sites, likely due to the greater presence of radiosenstivie crypt cells.

XVII-8) B It is often possible to distinguish a radiation-induced cataract from an age-related cataract as a radiation-induced cataract usually begins at the posterior portion of the lens and an age-related cataract more commonly appears in the anterior portion of the lens. The threshold dose for cataract formation is now known to be well below 10 Gy. Several recent studies, which included early lens opacities as well as cataracts that interfere with vision, have longer followup times than previous research with greater statistical power. This work suggests a low threshold (under 1 Gy) and are even statistically consistent with no threshold for cataract induction. The low-dose neutron RBE for cataract formation is greater than 20. The tolerance dose for the production of blindness is greater than that for cataract formation. The latency period for the induction of a radiation-induced cataract decreases with increasing dose.

Ainsbury A, Bouffler SD, Dörr W, *et al*. Radiation cataractogenesis: A review of recent studies. *Radiat Res* 172:1-9, 2009. PubMed

Neriishi K, Nakashima E, Minamoto A, *et al*. Postoperative cataract cases among atomic bomb survivors: radiation dose response and threshold. *Radiat Res* 168:404-408, 2007. PubMed

Worgul BV, Kundiyev YI, Sergiyenko NM, *et al*. Cataracts among Chernobyl clean-up workers: implications regarding permissible eye exposures. . *Radiat Res* 167:233-243, 2007. PubMed

XVII-9) A In general, B cells are more radiosensitive than T cells. Following total body irradiation, neutropenia is observed prior to thrombocytopenia. For even modest doses, a decrease in lymphocyte count can be detected within 1-2 days following total body irradiation. Serial blood counts over this period can be useful in assessing dose and guiding treatment after an accidental exposure. Individuals suffering from the bone marrow syndrome usually die of infection and/or hemorrhage. Survivors of total bone marrow irradiation demonstrate a late loss of bone marrow architecture characterized by tissue replacement with lipid cells.

XVII-10) A Older women are more sensitive to the induction of radiation-induced sterility than younger women, presumably due to their diminished number of oocytes compared with younger women. A dose of 3 Gy can destroy the gametogenic epithelium, but would not eliminate the production of sex hormones in adult men. Spermatids and spermatozoa are more radioresistant than spermatogonia. Based on animal data, a minimum waiting period of 3-6 months is recommended for both men and women before attempting procreation following radiotherapy, in order to reduce the risk of radiation-induced genetic effects. A modest radiation dose is unlikely to kill many of the more mature members of the spermatogenic series, although it could be lethal to most of the spermatogonial stem cells. Thus, even if there is no significant drop in sperm count within the first 30 days after the start of irradiation, this does not preclude the possibility that sterility could occur about a month or two later. This is a reflection of the turnover time (approximately 70 days) required for a spermatogonia stem cell to develop into a mature spermatozoa.

XVII-11) B There are two well-defined pulmonary toxicities: an acute radiation pneumonitis (seen 8-16 weeks post-radiation), and a late effect, radiation fibrosis (appearing months to years after therapy). Volume irradiated has been shown to be a particularly critical factor with respect to the degree of pulmonary toxicity observed. Many radiation oncologists are using the V20 or V30, the dose received by 20-30% of the lung, as a defining limiting factor. Regarding lung tolerance dose, as expected, large single doses to the entire lung induce steep dose responses, with incidences of radiation pneumonitis being reported at ~5% following 8.2 Gy, but rising to 50% following 9.3 Gy. With increasing fractionation, higher total doses can be tolerated, yet the dose response curves remain steep, with a reported 5% incidence following a dose of 26.5 Gy, rising to a 50% probability when the total dose is increased to 30 Gy, the latter frequently being observed in the pediatric population. Tolerance doses are affected significantly by a broad range of chemotherapeutic agents, which have been shown to act synergistically or independently to enhance toxicity. Laboratory animal models have identified multiple cell types that appear to play critical roles in the development of radiation-induced late effects in the lung.

Roberts KB, Rockwell S. Radiation pneumonitis. In: *Fishman's Pulmonary Diseases & Disorder*, *4th Ed*, (A.P. Fishman, Ed.) McGraw-Hill, New York, 2009.

Werner-Wasik M, Yu X, Marks LB, *et al.* Normal-tissue toxicities of thoracic radiation therapy: esophagus, lung, and spinal cord as organs at risk. *Hematol Oncol Clin N Am* 18:131-160, 2004. PubMed

McDonald S, Rubin P, Phillips TL, *et al.* Injury to the lung from cancer therapy: Clinical syndromes, measurable endpoints, and potential scoring systems. *Int J Radiat Oncol Biol Phys* 31:1187-1203, 1995. PubMed

XVII-12) E The oral mucosal response to irradiation is indeed similar to that seen in skin. However, the formation of dental caries is a direct consequence of the killing of saliva-secreting acini cells in the salivary glands, ultimately leading to xerostomia. This results in the loss of saliva's normal antibacterial action and acidification of the mouth. This is in contrast to the infections observed in irradiated skin which are a downstream consequence of damage to small blood vessels.

Prott FJ, Handschel J, Micke O, *et al.* Long-term alterations of oral mucosa in radiotherapy patients. *Int J Radiat Oncol Biol Phys* 54:203-210, 2002. PubMed

Hopewell JW. The skin: its structure and response to ionizing radiation. *Int J Radiat Biol* 57:751-773, 1990. PubMed

XVII-13) D Vascular endothelial cells are the most radiosensitive cells in the heart, with direct radiation damage to this population leading to protein leakage, fibrin deposition and the up-regulation of such cytokines as TGF-β1. Many other cell types within the heart contribute to the development of RIHD, but of them all, the cardiac myocyte, a fixed post-mitotic cell, is the most radioresistant. A number of large clinical trials, particularly those performed in Hodgkin's disease patients, have indicated that the populations most at risk for RIHD are young females and the elderly, and that the important factors governing tissue tolerance are total dose, fraction size and volume irradiated. Typically, late effects in the heart occur months to years after therapy. One structure that may be affected by radiation therapy is the parietal pericardium, with an associated fibrous thickening due to collagen replacing the external adipose layer.

McGale P, Darby SC. Low doses of ionizing radiation and circulatory diseases: A systematic review of the published epidemiological evidence. *Radiat Res* 163:247-257, 2005. PubMed

Little MP, Tawn EJ, Tzoulaki I, *et al*. A systematic review of epidemiological associations between low and moderate doses of ionizing radiation and late cardiovascular effects, and their possible mechanisms. *Radiat Res* 169:99-109, 2007. PubMed

Prosnitz RG, Chen YH, Marks LB. Cardiac toxicity following thoracic radiation. *Semin Oncol* 32:S71-80, 2005. PubMed

Fajardo LF, Berthrong M, Anderson RE. *Radiation Pathology*. University Press, Oxford, 2001.

King V, Constine LS, Clark D, *et al.* Symptomatic coronary artery disease after mantle irradiation for Hodgkin's disease. *Int J Radiat Oncol Biol Phys* 36:881-889, 1996. PubMed

Boivin JF, Hutchison GB, Lubin JH, *et al.* Coronary artery disease mortality in patients treated for Hodgkin's disease. *Cancer* 69:1241-1247, 1992. PubMed

XVII-14) D The Kupffer cells, hepatic-specific phagocytes, often increase in size during the progression of VOD and can contain large amounts of *hemosiderin*, a pigment that is a breakdown product of hemoglobin derived from phagocytized erythrocytes that have leaked from damaged vasculature. Hematoxylin is a nuclear stain widely used in histology that would not be expected to be found in the liver. Although the VOD lesion presents at about 90 days post-irradiation and is technically a late effect, nonetheless it is typically defined clinically and morphologically as a "subacute" effect. The morphologic hallmark of VOD is the presence of lesions with severely congested sinusoids in the central zones of the lobules, and an accompanying atrophy of the central portion of the liver plates. The lumen of the central and sublobular veins are filled with a dense network of reticulin fibers that frequently contain trapped red cells.

Lawrence TS, Robertson JM, Anscher MS, *et al.* Hepatic toxicity resulting from cancer treatment. *Int J Radiat Oncol Biol Phys* 31:1237-1248, 1995. PubMed

XVII-15) A Although transient neutrophil infiltration is a recognized early step in the normal wound healing process, it appears to play little or no part in the development of radiation-induced late effects. Radiation has both direct and indirect effects on various components of the inflammatory system. Indirectly, radiation exposure can be considered pro-inflammatory, with an "-itis" being a commonly observed early radiation response in many tissues and organs, e.g. lung (pneumonitis), skin (radiodermatitis) and the alimentary tract (mucositis). In many of these tissues, the inflammation is

mediated by activated macrophages that recognize the chronic dysregulation characteristic of irradiated tissues during the development of late effects. However, radiation's direct effects on inflammatory cells are more anti-inflammatory in nature. For example, it has been recognized both in the Japanese A-bomb survivors and in the Chernobyl cleanup workers that total body irradiation (TBI) doses of 1 Gy and above can lead to abnormal T cell immunity, possibly due to altered T cell differentiation and increased cell killing.

Kuzmenok O, Potapnev M, Potapova S, *et al.* Late effects of the Chernobyl radiation accident on T cell-mediated immunity in cleanup workers. *Radiat Res* 159:109-116, 2003. PubMed

Akiyama M. Late effects of radiation on the human immune system: an overview of immune response among the atomic-bomb survivors. *Int J Radiat Biol* 68:497-508, 1995. PubMed

XVII-16) D There are typically no distinct pathognomonic characteristics of CNS injury that would unambiguously identify radiation as the causative agent.

XVII-17) A Following irradiation of the skin, the dose and time course for epilation and loss of sebaceous gland secretion are similar. Following skin irradiation, the first visible evidence of damage is a transient erythema that is observed within 24 hours following irradiation, whereas moist desquamation would only be observed after a few weeks. Epilation is observed at doses similar to those that cause the main wave of erythema that is typically manifested about one week following irradiation. Pigment changes typically appear long after irradiation due to the low proliferation rate of melanoblasts. It is usually not possible to predict the extent of late reactions based upon the severity of early reactions because early reactions result from killing of epidermal stem cells, whereas late reactions likely occur due to vascular damage in the dermis.

Geleijns J, Wondergem J. X-ray imaging and the skin: radiation biology, patient dosimetry and observed effects. *Radiat Prot Dosimetry* 114:121-125, 2005. PubMed

XVII-18) B Hypertrophic cardiomyopathy is not considered a common feature of radiation-induced heart disease. Accelerated coronary atherosclerosis, on the other hand, is an important source of morbidity and mortality after irradiation of intra- or peri-thoracic tumors. Cardiac myocyte degeneration and cardiac fibrosis (adverse cardiac remodeling) may contribute to post-radiation congestive heart failure. Fibrotic thickening of the pericardium

and pericardial exudate may occur and could lead to constrictive pericarditis.

Schultz-Hector S: Radiation-induced cardiotoxicity: experimental data: In Dunst J, Sauer R (eds): *Late Sequelae in Oncology*. Springer-Verlag, Berlin 1995:181-189.

Levitt SH. Cardiac morbidity and mortality following radiotherapy. In Dunst J, Sauer R (eds): *Late Sequelae in Oncology*. Springer-Verlag, Berlin 1995:197-203.

XVIII. Mechanisms of Normal Tissue Radiation Responses

XVIII-1) D IL-10 is produced by a variety of different cell types, particularly monocytes/macrophages and lymphocytes. It is a major anti-inflammatory cytokine that inhibits the initiation and effector phases of cellular immune responses as well as a variety of inflammatory responses. The other cytokines (IL-1, IL-6, IL-8, and TNF□) are all considered pro-inflammatory. There is considerable overlap between the activities of TNF□ and IL-1. TNF□ is secreted mainly by activated monocytes/macrophages and has profound pro-inflammatory effects. It also stimulates the secretion of many other cytokines, including IL-1, IL-6, and IL-8. IL-1 is also a key mediator of host response to infection and inflammation. The main cellular sources of IL-1 are cells of the monocyte and macrophage lineage. Similar to TNF□, IL-1 induces several secondary cytokines, including IL-6 and IL-8. Upon stimulation by IL-1 and/or TNF□, IL-6 and IL-8 are produced by a large number of different cell types, including monocytes, fibroblasts, endothelial cells and epithelial cells.

Taylor A, Verhagen J, Blaser K, *et al.* Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. *Immunology* 117:433-442, 2006. PubMed

The Cytokine Factsbook. Edited by Fitzgerald KA, O'Neill LAJ, Gearing AJH, Callard RE. Academic Press, London, 2001.

XVIII-2) B The radiation dose-dependent lethality and reduction in gut crypt cell survival is significantly *potentiated*, not reduced, in PARP-deficient mice and in mice treated with a PARP inhibitor. Treatment with fluids, electrolytes, antibiotics, and blood products is part of the standard supportive care after exposure to total body irradiation.

Delanian S, Lefaix JL. Current management for late normal tissue injury: radiation-induced fibrosis and necrosis. *Semin Radiat Oncol* 17:99-107, 2007. PubMed

Moulder JE, Cohen EP. Future strategies for mitigation and treatment of chronic radiation-induced normal tissue injury. *Semin Radiat Oncol* 17:141-148, 2007. PubMed

Brook I, Elliott TB, Ledney GD, *et al.* Management of postirradiation infection: lessons learned from animal models. *Military Med* 169:194-197, 2004. PubMed

XVIII-3) D In the kidney, the tolerance to retreatment *decreases* with time, indicating a continuous progression of renal injury in the interval between treatments.

Experimental studies in mice given initial radiation doses approximately 30-50% of the BED_t (tolerance dose) showed that the lungs could be re-irradiated with doses equivalent to the BED_t provided a sufficient time interval between the first and second treatments had elapsed. Re-irradiation tolerance for acute damage in rapidly dividing mucosal tissues is commonly observed. Rodent and monkey data indicate that, contrary to popular belief, the spinal cord is capable of considerable recovery from the injury caused by an initial radiation treatment and can subsequently be retreated with at least a partial tolerance dose. In the bladder, the latency period before expression of injury is shorter in animals that were re-irradiated, as opposed to being treated to tolerance in a single course of therapy, even after low, sub-tolerance initial radiation doses.

Cohen EP, Robbins ME. Radiation nephropathy. *Semin Nephrol* 23:486-499, 2003. PubMed

Stewart FA, Oussoren Y, Van Tinteren H, *et al.* Long-term recovery and reirradiation tolerance of mouse bladder. *Int J Radiat Oncol Biol Phys* 18:1399-1406, 1994. PubMed

Ang KK, Price RE, Stephens LC *et al.* The tolerance of primate spinal cord to re-irradiation. *Int J Radiat Oncol Biol Phys* 25:459-464, 1993. PubMed

Stewart FA, Luts A, Lebesque JV. The lack of long-term recovery and reirradiation tolerance in the mouse kidney. *Int J Radiat Biol* 56:449-462, 1989. PubMed

Terry NH, Tucker SL, Travis EL. Residual radiation damage in murine lung assessed by pneumonitis. *Int J Radiat Oncol Biol Phys* 14:929-938, 1988. PubMed

XVIII-4) D Despite the recent surge in interest in radiation-induced late effects, the precise mechanisms responsible for their development and progression remain unclear. Historically, late effects were considered to be a consequence of the radiation-induced killing of either parenchymal or vascular target cell populations, and as such, were thought to be inevitable, progressive and untreatable. More recent findings suggest that this hypothesis is overly simplistic. Radiation-induced late effects are now viewed as the result of dynamic interactions between multiple cell types within the tissue. The parenchymal cells are no longer viewed as passive bystanders, merely dying as they attempt to divide, but rather are thought to be active participants in an orchestrated, yet limited, response to injury. In general, irradiating late-responding normal tissues leads to an acute inflammatory response followed by an aberrant chronic inflammatory/wound healing response in which vascular and parenchymal cell dysfunction and cell loss, associated with chronic overproduction of particular cytokines and growth factors, result in fibrosis and/or necrosis, depending on the particular organ involved. This new paradigm promises novel approaches to the mitigation of radiation-induced normal tissue complications, including the possibility that late effects might be reduced by the application of therapies directed at altering steps in the cascade of events leading to the clinical expression of the injury. There are no pathognomonic features characteristic of irradiated late-responding normal tissues.

Milano MT, Constine LS, Okunieff P. Normal tissue tolerance dose metrics for radiation therapy of major organs. *Semin Radiat Oncol* 17:131-140, 2007. PubMed

Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nature Rev Cancer* 6:702-713, 2006. PubMed

Denham JW, Hauer-Jensen M. The radiotherapeutic injury - a complex "wound". *Radiother Oncol* 63:129-145. 2002. PubMed

Stone HB, McBride WH, Coleman CN. Modifying normal tissue damage postirradiation. *Radiat Res* 157:204-223, 2002. PubMed

XVIII-5) D Early-responding tissues exhibit radiation-induced injury during or shortly after a course of radiotherapy. Late reactions are manifested months or years following the completion of radiotherapy. The classical model of radiation-induced normal tissue injury hypothesizes that normal tissue injury involves the loss of specific target cell clonogens. In early-responding tissues such as the skin and oral mucosa, clonogenic cell turnover is rapid, as is clonogenic cell death. Thus, the latency period, i.e., the period before the expression of radiation-induced injury, is short. This latency period is fixed in early-responding tissues, since it depends on the time it takes for cells to move from the stem cell compartment through the transit compartment, and finally to the terminally-differentiated, non-dividing parenchymal cell that is lost through normal wear and tear. In contrast, target cell turnover is slow or non-existent in late-responding tissues and therefore the latency period is long. Shortening the overall treatment time may cause greater cell depletion and increase the severity of early reactions since the time available for cell repopulation would be limited under these circumstances. This might result in more pronounced "consequential" late effects; however the latent period for these late effects would, if anything, decrease, rather than increase. According to classical theory, the decrease in the latency period with dose for late effects was thought to be due to the enhanced cell killing resulting from the use of higher doses. It is now recognized that this cell killing likely plays only a limited role in the development of most late responses. In contrast, it is thought that when irradiation of a tissue may give rise to a late radiation reaction, there is initially an acute inflammatory response followed by an aberrant chronic inflammatory/wound healing response. Vascular and parenchymal cell dysfunction and cell loss then occur which are accompanied by a chronic overproduction of particular cytokines and growth factors, ultimately resulting in the manifestation of radiation toxicity. Thus, it is now thought that the process ultimately leading to the development of late radiation effects actually begins relatively quickly after irradiation. Presumably, the speed and/or intensity of this process is somewhat dose dependent such that the length of time necessary before a late effect is observed clinically decreases with increasing dose. There is no relationship between the latency period for early-responding tissues and endothelial cell turnover; if anything, the latter has been considered a target cell for injuries in late-responding tissues.

Wheldon TE, Michalowski AS. Alternative models for the proliferative structure of normal tissues and their response to irradiation. *Br J Cancer* Suppl 7: 382-385, 1986. PubMed

XVIII-6) E The reason why a large dose to a small length of the spinal cord may cause severe radiation injury, such as myelopathy, is that the inactivation of even a single functional subunit (FSU) can disrupt the function of the entire organ for tissues whose FSUs are arranged in a serial fashion. In contrast, a high

dose to a small volume of the lung may have little impact because the remainder of the lung will continue to function normally because its FSUs are arranged in parallel.

XVIII-7) C The effects of radiation on the nervous system arise primarily as a consequence of damage to oligodendrocytes and glial cells. Although radiation likely does cause some damage to neurons as well, this alone does not seem to manifest itself as a frank nervous system injury.

Johannesen TB, Lien HH, Hole KH, *et al.* Radiological and clinical assessment of long-term brain tumour survivors after radiotherapy. *Radiother Oncol* 69:169-176, 2003. PubMed

Tofilon PJ, Fike JR. The radioresponse of the central nervous system: a dynamic process. *Radiat Res* 153:357-370, 2000. PubMed

XVIII-8) B The serous acinar cells of the parotid and submaxillary glands are considered to be the targets for radiation-induced salivary gland damage. Serous acinar cells typically die by apoptosis and not mitotic catastrophe following irradiation. Salivary dysfunction is an early radiation response that often begins during radiotherapy. Mucous cells are more radioresistant than serous cells. Fractionation results in relatively little sparing from radiation-induced killing of serous cells, as is typical for cells with a pro-apoptotic tendency.

Eisbruch A, Rhodus N, Rosenthal D, *et al.* The prevention and treatment of radiotherapy-induced xerostomia. *Semin Radiat Oncol* 13:302-308, 2003. PubMed

Chao KS, Majhail N, Huang CJ, *et al.* Intensity-modulated radiation therapy reduces late salivary toxicity without compromising tumor control in patients with oropharyngeal carcinoma: a comparison with conventional techniques. *Radiother Oncol* 61:275-280, 2001. PubMed

XVIII-9) A TGF-□□□ plays a central role in radiation-induced fibrosis as it causes epithelial to mesenchymal cell trans-differentiation and promotes the influx of fibroblasts and production of extracellular matrix. TGF-□□ activates SMAD proteins, including SMAD3, which modulates the transcription of target genes with pro-fibrotic activities. It is thought that stimulation of TGF-β1 synthesis would cause fibrosis, thereby *decreasing* the therapeutic ratio. bFGF has been shown to protect, not sensitize, endothelial cells to radiation-induced apoptosis. In addition, TGF-□□ has anti-inflammatory activity.

Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer* 69:702-13, 2006. PubMed

Bierie B, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer* 6:506-20, 2006. PubMed

Kirshner J, Jobling MF, Pajares MJ, *et al.* Inhibition of transforming growth factor-beta1 signaling attenuates ataxia telangiectasia mutated activity in response to genotoxic stress. *Cancer Res* 66:10861-9, 2006. PubMed

Flanders KC. Smad3 as a mediator of the fibrotic response. *Int J Exp Pathol* 85:47-64, 2004. PubMed

Chen Y, Okunieff P, Ahrendt SA. Translational research in lung cancer. *Semin Surg Oncol* 21:205-219, 2003. PubMed

Paris F, Fuks Z, Kang A, *et al.* Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* 293:293-297, 2001. PubMed

XVIII-10) C Fibrosis is one of the most common late radiation effects, noted in the majority of irradiated tissues and organs. Although the appearance of fibrosis is both time and dose dependent, its extent and severity vary not only within an organ, but also among individuals. Bone marrow is one of the few tissues where fibrosis is rarely seen and, in general, fibrosis appears only if a tumor or inflammatory lesion was present prior to irradiation. Bone marrow is usually replaced by adipose tissue. Much of the regulation of collagen deposition is mediated through the action of fibrogenic cytokine families and is characterized by the upregulation of such cytokines as TGF-β1.

Travis EL. Genetic susceptibility to late normal tissue injury. *Semin Radiat Oncol* 17:149-55, 2007. PubMed

Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer* 6:702-13, 2006. PubMed

Anscher MS, Vujaskovic Z. Mechanisms and potential targets for prevention and treatment of normal tissue injury after radiation therapy. *Semin Oncol* 32:S86-91, 2005. PubMed

Robbins ME, Zhao W. Chronic oxidative stress and radiation-induced late normal tissue injury: a review. *Int J Radiat Biol* 80:251-259, 2004. PubMed

Dent P, Yacoub A, Contessa J, *et al.* Stress and radiation-induced activation of multiple intracellular signaling pathways. *Radiat Res* 159:283-300, 2003. PubMed

Williams J, Chen Y, Rubin P, *et al.* The biological basis of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 13:182-188, 2003. PubMed

Denham JW, Hauer-Jensen M. The radiotherapeutic injury -- a complex 'wound'. *Radiother Oncol* 63:129-145, 2002. PubMed

XVIII-11) D The hippocampus is thought to be a region of the brain that is important for cognitive function. Several studies are investigating the potential benefit of avoiding radiotherapy to this area of the brain.

Gondi V, Tomé WA, Mehta MP. Radiother Oncol. 2010 Dec;97(3):370-6. PubMed link

XIX. Therapeutic Ratio

XIX-1) C Normal tissues are typically well-oxygenated, but tumors may contain a fraction of radioresistant hypoxic cells that are likely to be critical for tumor control. Larger tumors are more likely to harbour hypoxic regions. Thus, any treatment that enhances the survival of aerobic cells or reduces the survival of hypoxic cells, could theoretically result in an improved therapeutic ratio.

XIX-2) E In order to achieve a 90% tumor control rate, the total dose delivered must reduce the number of surviving clonogenic cells to an average of 0.1. This is based on the equation $P = e^{-(N)(SF)}$, where P is the probability of tumor cure (90% or 0.9 in this case), N is the initial number of tumor clonogens (10^7) and SF is the surviving fraction resulting from the irradiation protocol. Thus, for 10^7 clonogenic cells, a total dose that reduces the surviving fraction to 10^{-8} (i.e., an average of 0.1 clonogen surviving in each tumor or 1 cell surviving out of every 10 tumors irradiated) must be used to achieve a 90% control rate. Since the survival curve is exponential with a D_{10} of 8 Gy, it would be necessary to use a dose of 64 Gy.

XIX-3) D A surgical excision of 99% of the tumor would reduce the initial number of clonogens to 10^5. Thus to achieve a 90% control rate, a dose of 48 Gy would be required, corresponding to a final clonogen surviving fraction of 10^{-6}.

XIX-4) A A doubling of a tumor's diameter reflects about an 8-fold increase in cell number, which would require 3 cell divisions to accomplish. Thus, if 18 days were required to complete 3 cell cycles, the cell cycle time must be 6 days.

XIX-5) A A low value for SF_2 indicates that the surviving fraction of tumor cells following irradiation with 2 Gy is low. This should be *advantageous* for radiotherapy, as it suggests that the tumor cells are relatively radiosensitive. That being said, a consistent, positive correlation between low SF_2 and high tumor control probability has yet to be established. A short T_{pot} would be deleterious to tumor control because it suggests a high potential for vigorous repopulation during the course of treatment. Slow reoxygenation may also limit the effectiveness of treatment as hypoxic cells would remain hypoxic and radioresistant for a longer portion of the overall treatment time than if they had reoxygenated rapidly and efficiently. A large number of clonogenic cells would require a higher total dose for their eradication and this might increase the probability of adverse normal tissue effects. Early onset repopulation would also be deleterious as the tumor cells would then be proliferating for a longer time during the course of radiotherapy and the

cell population that must be killed to cure the tumor would therefore be larger.

XIX-6) B An agent that arrested cells in the radiosensitive G_2 phase of the cell cycle before irradiation could increase the response of tumors to a fractionated treatment protocol if applied prior to each dose of radiation. Prevention of cell cycle redistribution would diminish response since the surviving cells could remain in a radioresistant portion of the cell cycle rather than being permitted to cycle and traverse into more radiosensitive phases, where a subsequent radiation dose would have a greater probability of causing lethality. Inhibition of reoxygenation would also reduce tumor response to radiation since this would prevent the conversion of surviving radioresistant hypoxic cells to more sensitive aerated cells. Radioprotection of normal tissues would have no bearing on tumor response *per se*, although it could improve the therapeutic ratio overall, assuming the tumor was not similarly protected. Stimulation of DNA repair would reduce tumor response since a greater proportion of tumor cells may survive if treated with an agent that enhanced their repair capacity.

XIX-7) D The slope of a tumor control probability (TCP) curve is determined by factors that introduce heterogeneity into the population of tumors under study. Tumor heterogeneity can be caused by variations in tumor size, oxygenation, tumor cell radiosensitivity or histological type and grade of the tumor. While it may be important for toxicity and therefore for the therapeutic ratio, the volume of normal tissue in the radiation field does not affect the tumor control probability.

XIX-8) A In order for there to be a therapeutic gain, the differential between the radiation response of tumor and normal tissue must be increased. Since blood flow is usually not compromised in normal tissues, the radiobiological oxygen effect would not be enhanced by increasing blood flow. However, since many tumors contain hypoxic cells, increasing blood flow to the tumor could result in radiation sensitization. In contrast, decreasing blood flow to tumors would not be expected to be advantageous, since it could cause increased hypoxia and thus radiation resistance.

XIX-9) A Regeneration/repopulation can occur in early responding tissues such as skin during the course of a standard course of radiotherapy, increasing the tolerance of these tissues to radiation. The apparent slower kinetics of late responding tissues suggests that no repopulation occurs in these tissues compared to acutely responding tissues. If such were to take place, then this would reduce, not increase, late effects, irrespective of the fractionation schedule. Repopulation/regeneration plays no role in reoxygenation.

XX. Time, Dose, Fractionation

XX-1) C This can be calculated using the linear-quadratic formula that allows comparison of two different fractionation schedules. Biologically effective dose (BED) = (nd) x (1+d/α/β), where n is the number of fractions and d is dose per fraction. If the two schedules are isoeffective $BED_1 = BED_2$, which reduces to $n_1d_1/n_2d_2 = (\alpha/\beta+d_2)/(\alpha/\beta+d_1)$.

XX-2) A An isoeffect curve describes the relationship between total dose for a given level of tissue effect and one of the different fractionation parameters (overall time, dose per fraction, number of fractions, etc). Isoeffect curves are often plotted with the log of the total dose on the y-axis and the log of the fraction size (from high to low) on the x-axis. Tissues with a greater repair capacity will show greater sparing with increasing fractionation (smaller fraction sizes) and therefore will have steeper isoeffect curves. Increased proliferation will cause an increase in the slope of an isoeffect curve because it would take a higher total dose to kill the larger number of cells produced during the course of treatment. Tissues with steep isoeffect curves have low, not high, α/β ratios. Reoxygenation decreases the slope of the isoeffect curve because it decreases the number of radioresistant hypoxic cells and hence reduces the total dose required to control the tumor, everything else being equal.

Withers HR. Biologic basis for altered fractionation schemes. *Cancer* 55:2086-95, 1985. PubMed

XX-3) E In principle, a *hypofractionated* protocol would yield the highest therapeutic ratio because, for either standard or small fraction sizes (i.e., hyperfractionation), there would be greater sparing of this tumor (α/β ratio = 2 Gy) than for the critical dose-limiting normal tissue (α/β ratio = 4 Gy). There would not be much point to using accelerated treatment since this is a relatively slow-growing tumor (T_{pot} = 30 days), nor would split course treatment be indicated since, again, the □/ß ratio suggests greater recovery in the tumor versus the normal tissue.

Withers HR, Taylor JM, Maciejewski B. The hazard of accelerated tumor clonogen repopulation during radiotherapy. *Acta Oncol* 27:131-46, 1988. PubMed

XX-4) C Since the focus of this question concerns late effects, the overall treatment time (a maximum of 6 weeks) should not be an important determinant of outcome. The BEDs calculated for each of the different fractionation schedules are 70, 72, 113, 100 and 90 Gy_2, respectively. The protocol of 45

Gy delivered in 15 fractions results in the greatest value for BED and, therefore, should be the most likely to produce late normal tissue complications. This illustrates the point that both fraction size *and* total dose play important roles in determining the probability of late effects.

XX-5) A Since there is the assumption of an equal effect per fraction and no repopulation, the basic BED equation, BED = nd(1+d/α/β), can be used. Thus, the standard treatment results in a BED of (30)(2 Gy)(1+2 Gy/10 Gy) = 72 Gy_{10}. Therefore, in order to determine the number of fractions to be used if the fraction size is reduced to 1.3 Gy, BED = 72 Gy_{10} = n(1.3 Gy)(1+1.3 Gy/10 Gy) = 1.47n. Thus, 49 fractions of 1.3 Gy should be used, to a total dose of 64 Gy.

XX-6) E One goal of hyperfractionation is to improve the therapeutic ratio by decreasing the incidence of late reactions, while maintaining or improving tumor control. Therapeutic gain can be achieved only if the late-responding normal tissue has a lower α/β ratio than the tumor. Hyperfractionation would be likely to have no effect on early-responding tissues or may slightly increase toxicity; it would not decrease these toxicities. For hyperfractionation, the larger number of smaller-sized dose fractions is typically delivered over about the same overall treatment time as conventional therapy, meaning that there would be no change in the potential of surviving tumor clonogens to repopulate.

XX-7) C Isoeffect curves are steeper for late effects than for early effects, meaning that late-responding tissues are more sensitive to changes in dose per fraction than early-responding tissues (and tumors). RBEs for high LET forms of radiation are greater for late effects compared to early effects when hyperfractionation is used. Hyperfractionation would *reduce* the severity of late effects if the total dose was titrated to maintain the same level of early effects. When a treatment plan is changed from many small doses to a few large fractions and the total dose is titrated to produce equal early effects, late effects would be more severe.

XX-8) E Results from clinical trials of hyperfractionation and accelerated fractionation employing more than one fraction per day have shown worse late complications when the time between fractions was less than 6 hours. This finding has been attributed to incomplete repair, because sublethal damage recovery is generally slower in late-responding tissues. It has since been suggested that even an inter-fraction interval of 6 hours may not be sufficient for those normal tissues with the slowest repair rates and that a longer time between fractions may be necessary to avoid a reduction in tolerance dose.

Nguyen LN, Ang KK. Radiotherapy for cancer of the head and neck: altered fractionation regimens. *Lancet Oncol* 3:693-701, 2002. PubMed

Bentzen SM, Saunders MI, Dische S, *et al*. Radiotherapy-related early morbidity in head and neck cancer: quantitative clinical radiobiology as deduced from the CHART trial. *Radiother Oncol* 60:123-35, 2001. PubMed

Landuyt W, Fowler J, Ruifrok A, *et al*. Kinetics of repair in the spinal cord of the rat. *Radiother Oncol* 45:55-62, 1997. PubMed

XX-9) C The BEDs for the standard protocol are 60 Gy_{10} and 100 Gy_2, respectively, for the tumor and late-responding normal tissue, as determined from the equation $BED = nd(1+d/\alpha/\beta)$. Assuming the BED of 100 Gy_2 for the normal tissue is maintained for the hyperfractionated protocol, this would correspond to a total dose of 1.2 Gy per fraction multiplied by 52 fractions, or 62.4 Gy. Putting these values into the BED equation for the tumor, the BED would increase from 60 Gy_{10} for the standard treatment, to 70 Gy_{10} for the hyperfractionated treatment. Hence, the therapeutic index (TI), BED_{tumor}-hyperfractionated/BED_{tumor}-standard divided by BED_{normal}-hyperfractionated /BED_{normal}-standard, equals 70 Gy_{10}/60 Gy_{10}/100 Gy_2/100 Gy_2 = 1.2.

XX-10) E Sublethal damage repair and repopulation in normal tissues treated with fractionated radiation therapy, as well as reoxygenation in tumors and possible redistribution of proliferating tumor cells into more sensitive phases of the cell cycle, could all contribute to increased efficacy of dose fractionation, at least in theory. Potentially lethal damage repair in tumors would not contribute to the efficacy of dose fractionation as this would enhance the survival of tumor cells.

XX-11) D Accelerated repopulation is triggered several weeks after the initiation of a course of radiation therapy. A dose increase of approximately 0.6 Gy per day is needed to compensate for this repopulation. Hence, any interruptions in treatment, once it has begun, can compromise tumor control due to accelerated repopulation.

XX-12) E The CHART protocol was performed in the 1990s in the UK. It involved 36 fractions over 12 consecutive days with three fractions delivered daily. Each fraction was between 1.4 – 1.5 Gy with a total dose of 50 – 54 Gy. The strategy was based on the thought that low dose/fraction would minimize late effects and a short treatment time would maximize tumor control. There was no concurrent chemotherapy given.

XX-13) E Data has shown that local tumor control is decreased by about 1.4% for each day that treatment is prolonged for head and neck cancer and 0.5% for uterine cervix cancer. There is no data suggesting a similar effect in melanoma, breast or basal cell cancer.

XXI. Brachytherapy

XXI-1) E An equation that takes into account the complete decay of the brachytherapy source is most appropriate for calculation of the BED for a permanent radioactive implant. Choice A is the correct equation to use for standard external beam therapy when the dose is delivered typically over a 1-2 minute period or for high dose-rate brachytherapy. Choice B takes into account repopulation during the course of radiotherapy and should be used to compare fractionated protocols of different durations. Choice C is used for treatment with closely-spaced, multiple fractions per day when incomplete repair may be an issue. Choice D is used to calculate the BED for a brachytherapy treatment at a constant, low dose rate.

XXI-2) E Decreasing the dose rate over the range from 1 Gy/min to 0.01 Gy min generally results in an increase in the surviving fraction following irradiation with a specific dose of radiation due to repair of sublethal damage. The inverse dose-rate effect is the observation that, as the dose rate declines further over a critical range, cellular survival *decreases* as the same constant dose is delivered. This effect relates to reassortment of cells into the radiosensitive phase of the cell cycle through the DNA damage-induced G_2 block. It would not be anticipated that repair of sublethal damage, the accumulation of cells in S phase, proliferation, or repair of potentially lethal damage would cause an inverse dose rate effect, since each of these processes would increase cell survival.

XXI-3) D Cesium-137, Iridium-192, Iodine-125, and Gold-198 are all available for brachytherapy implants. Iodine-131 is an unsealed isotope that is administered systemically for diagnostic or therapeutic purposes.

XXI-4) A Cesium-137 has a half-life of 30 years. Iridium-192 has a half-life of 74.2 days. Iodine-125 has a half-life of 60.2 days. Iodine-131 has a half-life of 8.0 days. Gold-198 has a half-life of 2.7 days.

XXI-65) C Iridium-192 is the most widely used radionuclide in part because of its convenience, due to its small size, its low photon energy simplifying radiation protection and its ability to be used in remote afterloaders. It is used for temporary implants and not used for permanent implants.

XXII. Radiobiological aspects of alternative dose delivery systems

XXII-1) D Carbon ions represent a high LET form of radiation and, as such, display *less* dependence upon oxygen for cell killing (and therefore have a lower OER). Hence, there should be fewer hypoxic tumor cells surviving carbon ion therapy than following treatments using either X-rays or protons. Basic research with light ions established that carbon ions suitable for radiotherapy (~400 MeV/amu) have superior depth-dose profiles from the entrance region of the beam up through the Bragg peak. An additional advantage is the reduction in lateral and longitudinal scatter. Two centers, one at the HIMAC in Chiba, Japan, and the other at the GSI in Darmstadt, Germany, have been treating with carbon ions since the mid to late 1990's and another center at Hyogo, Japan came on line in 2002. Carbon ions show an increased RBE for both cells irradiated *in vitro* and tissues exposed *in vivo.* The exact RBE depends on the energy of the beam and the characteristics of the cells at risk. It is possible to verify the carbon ion treatment plan using PET since a small fraction of the ions undergo nuclear fragmentation when a beam of carbon ions penetrates a thick absorber. Often, one or two neutrons are stripped, converting the stable ^{12}C to the positron emitting isotopes ^{11}C and ^{10}C. These isotopes travel with almost the same velocity as the main beam and stop in nearly the same location. They have short half-lives and as the emitted positron combines with an electron in an annihilation reaction, two 0.51 MeV photons are produced that can be detected by a PET scanner. As a consequence, the high dose treatment volume can be visualized.

Jones B, The Case for Particle Therapy. Br J Radiol 79, 937: 24-31, 2006. PubMed

Amaldi U, Kraft G. Radiotherapy with beams of carbon ions. *Reports on Progress in Physics* 68:1861-1882, 2005.

XXII-2) E The whole body patient dose is higher with IMRT because in addition to leakage from head, there is scatter from collimator. Regarding other choices, intensity-modulated radiation therapy (IMRT) usually employs a linear accelerator at mega-voltage energies, which are similar to or lower than energies used to deliver treatment doses with an unmodulated field (A). The higher risk of IMRT radiotherapy-induced second cancers in pediatric patients than in adult patients is a direct consequence of the smaller size of the body of a child compared with an adult. As originally discussed by Hall (2006), radiogenic organs are closer to the treatment site in a child and thus receive larger radiation doses than when a comparable treatment is delivered to an adult (B). IMRT is

most conformal if all target volumes are treated simultaneously using different fraction sizes (C). This permits graded dose levels to the gross tumor with embedded normal tissues and tissues at risk for tumor spread (normal tissues surrounding the gross tumor and lymph nodes). Such a treatment strategy is called the simultaneous integrated boost (SIB). The SIB strategy uses the same plan for the entire course of treatment to deliver prescribed doses to treated volumes. The effect of the modified fractionation on acute and late toxicity of normal tissue is taken into account in treatment planning (D). The SIB-IMRT fraction sizes are estimated using an isoeffect relationship based on the linear-quadratic (LQ) equation using the values of LQ model parameters (such as α/β ratios and tumor doubling time) for the isodose calculations for various tissues components in treatment volume.

Hall EJ. Intensity-modulated radiation therapy, protons and the risk of second cancers. *Int J Radiat Oncol Biol Phys* 65:1-7, 2006. PubMed

Abo-Madyan Y, Aziz MH, Aly M, Schneider F, Sperk E, Clausen S, Giordano FA, Herskind C, Steil V, Wenz F. Second cancer risk after 3D-CRT, IMRT and VMAT for breast cancer. Radiother Oncol 110:471-476, 2014.Mohan R, Wu Q, Manning M, Schmidt-Ullrich R. Radiobiological considerations in the design of fractionation strategies for intensity-modulated radiation therapy of head and neck cancers. Int J Radiat Oncol Biol Phys 46:619-630, 2000.

XXII-3) A ^{90}Y emits β-particles with a relatively high energy (0.9 MeV) and long range that can penetrate several millimeters into the tissue. Thus, there *is* a significant crossfire effect, i.e., cells adjacent to those that have taken up the radioisotope are also irradiated. Radioimmunotherapy (RIT) involves treatment with a targeted radiopharmaceutical that combines a tumor-selective monoclonal antibody conjugated to a radionuclide, typically a medium-range β-emitter. Two radiopharmaceuticals have been approved by the FDA for the management of relapsed and refractory CD20-positive low-grade B-cell non-Hodgkin's lymphoma (NHL): ^{90}Y-ibritumomab tiuxetan (Zevalin) and ^{131}I-tositumomab (Bexxar). Both drugs are composed of a murine antibody selective for the CD20 surface antigen found on over 95% of NHL B-cells (in addition to all normal, mature B cells). ^{90}Y is a pure β-emitter with a short effective half-life; therefore, very little of the radioactivity produced by Zevalin escapes the patient, minimizing the radiation safety hazard. However, a surrogate imaging isotope, such as ^{111}In, must be incorporated into the Zevalin framework to allow positional localization. Bexxar incorporates ^{131}I, which is a medium-energy, mixed-

spectrum β- and γ-emitter with a γ emission at 364 keV that can be detected using a gamma camera. Because of the penetrating γ-rays of ^{131}I and eight-day half-life, more rigorous radiation safety precautions must be used with Bexxar.

Hernandez MC and Knox SJ. Radiobiology of Radioimmunotherapy with ^{90}Y Ibritumomab Tiuxetan (Zevalin). *Semin Oncol* 30:6-10, 2003. PubMed

Pohlman B, Sweetenham J, Macklis RM. Review of clinical radioimmunotherapy. *Expert Rev Anticancer Ther* 6:445-461, 2006. PubMed

XXII-1) E Most human tumors except for very small ones have radioresistant hypoxic cells. The negative influence of hypoxic cells against local tumor control is greater in hypo-fractionated radiotherapy compared to conventional therapy. SBRT treatments are usually completed within <1 to 2 weeks and re-oxygenation during the course of SBRT therapy is very limited to negligible. Laboratory and clinical data suggest an intra-fraction interval of at least 3 days to increase possibility for re-oxygenation of tumor cells between fractions.

Shibamoto Y, Hashizume C, Baba F, Ayakawa S, Manabe Y, Nagai A, Miyakawa A, Murai T, Iwata H, Mori Y, Mimura M, Ishikura S. Stereotactic body radiotherapy using a radiobiology-based regimen for stage I nonsmall cell lung carcinoma. Cancer 118: 2078-2084, 2012.

XXIII. Chemotherapeutic agents and radiation therapy

XXIII-1) B Erlotinib is an EGFR small molecule tyrosine kinase inhibitor. Trastuzumab is an anti-HER2 antibody. Bevacizumab is an anti-VEGF antibody. Sirolimus binds to the FKBP12 complex and inhibits mTOR (FRAP1), a downstream target of the PI3K/AKT pro-survival signaling pathway that is activated by radiation exposure. Cetuximab is a monoclonal antibody against EGFR.

Petroulakis E, Mamane Y, Le Bacquer O, *et al.* mTOR signaling: implications for cancer and anticancer therapy. *Br J Cancer* 94:195-199, 2006. PubMed

Spalding AC, Lawrence TS. New and emerging radiosensitizers and radioprotectors. *Cancer Invest* 24:444-56, 2006. PubMed

Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* 5:341-354, 2005. PubMed

XXIII-2) D Bortezomib inhibits the chymotrypsin-like activity of the 26S proteasome and inhibits the degradation of many proteins that undergo ubiquitin-mediated degradation. NF-κB is not directly targeted by proteasome inhibitors, however, proteasome inhibitors indirectly promote NF-□B being kept in its inactive form by blocking the ubiquitin-mediated degradation of its repressor, IκB. EGFR signaling pathways are not a target for bortezomib. Bortezomib is FDA-approved for treatment of multiple myeloma, not pancreatic cancer.

Richardson PG, Mitsiades C, Hideshima T, *et al*. Bortezomib: proteasome inhibition as an effective anticancer therapy. *Ann Rev Med* 57:33-47, 2006. PubMed

XXIII-3) C The active metabolite of irinotecan, which is a camptothecin analog, specifically inhibits formation of the cleavable complex between topoisomerase I and DNA, causes the formation of DNA single strand breaks and is an inhibitor of DNA replication. Examples of drugs that target ribonucleotide reductase, thymidylate synthase or create DNA crosslinks are gemcitabine, 5-FU and cisplatin.

Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer* 6:789-802, 2006. PubMed

XXIII-4) C Sorafenib is a targeted agent that has been approved by the FDA for use in patients with renal cell carcinoma. It is a small molecule multi-kinase

inhibitor that targets RAF1, KIT, FLT3, VEGFR (KDR) and PDGFR. RAF1 is a component of the RAS signaling cascade, a pathway that is often overactive in cancer, including renal cell carcinoma. Sorafenib also inhibits other kinases, including ones involved in tumor angiogenesis.

Chinnaiyan P, Allen GW, Harari PM. Radiation and new molecular agents, part II: targeting HDAC, HSP90, IGF-1R, PI3K, and RAS. *Semin Radiat Oncol* 16:59-64, 2006. PubMed

Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* 6:38-51, 2006. PubMed

Wilhelm S, Carter C, Lynch M, *et al.* Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 5:835-844, 2006. PubMed

Sridhar SS, Hedley D, Siu LL. Raf kinase as a target for anticancer therapeutics. *Mol Cancer Ther* 4:677-85, 2005. PubMed

XXIII-5) E Tipifarnib is a compound used in targeted therapy for several hematologic malignancies. Tipifarnib acts by inhibiting farnesyl transferase, an enzyme that is required for the sustained activity of the RAS signaling cascade. The RAS pathway is overactive in many malignancies and inhibition of farnesyl transferase activity can block RAS pathway activity.

Mesa RA. Tipifarnib: Farnesyl transferase inhibition at a crossroads. *Expert Rev Anticancer Ther* 6:313-319, 2006. PubMed

XXIII-6) D A newer class of chemotherapeutic agents includes drugs engineered to interact with defined tumor-associated molecular targets. All the choices are examples of targeted agents, but of those listed, only cetuximab specifically targets the epidermal growth factor receptor (EGFR), a member of an important family of transmembrane signaling proteins. Cetuximab has been shown to be an effective radiosensitizer in a randomized Phase III clinical trial. EGFR signaling is associated with control of normal cell growth and differentiation, as well as tumorigenesis and disease progression in malignant tissues. EGFR is over-expressed in most solid tumors (breast, lung, colorectal cancers), and high levels of expression are positively correlated with aggressive tumor growth, reduced survival and radioresistance. Because tumor cells depend on continued stimulation by growth factors, inhibition of the EGFR pathway might provide an effective means of controlling tumor growth. Of the remaining choices, bevacizumab targets VEGF and limits growth of new tumor blood vessels. Combretastatin is one of several low molecular-weight vascular-disrupting

agents (VDAs) that target the established tumor vasculature. These drugs diminish tumor blood flow by increasing vascular permeability. Imatinib is an inhibitor of a small family of tyrosine kinases, including BCR-ABL, KIT and PDGFR; specifically, imatinib blocks the ATP-binding site of the p210 tyrosine kinase domain of the BCR-ABL fusion protein in chronic myeloid leukemia. Rituximab is a monoclonal antibody against CD20, which has a direct anti-tumor effect in CD20-positive lymphomas by inducing apoptosis and cell lysis.

Bonner JA, Harari PM, Giralt J, *et al.* Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 354:567-578, 2006. PubMed

Chaplin DJ, Horsman MR, Siemann DW. Current developmental status of small-molecule vascular disrupting agents. *Curr Opin Investig Drugs* 7:522-528, 2006.PubMed

Cvetkovic RS, Perry CM. Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Drugs* 66:791-820, 2006. PubMed

XXIII-7) E Gefitinib is a small molecule tyrosine kinase inhibitor. Glutathione is a cellular sulfhydryl compound. Nimorazole is a hypoxic cell radiosensitizer. Tirapazamine is a hypoxic cell cytotoxin. Amifostine is a radioprotector.

XXIII-8) A Etoposide targets topoisomerase II. Topotecan targets topoisomerase I, bevacizumab targets VEGF, sunitinib is a tyrosine kinase inhibitor with multiple targets including EGFR, FLT3, VEGFR and KIT, and 5-fluorouracil targets thymidylate synthase.

Atkins M, Jones CA, Kirkpatrick P: Sunitinib maleate. *Nat Rev Drug Discov* 5: 279-80, 2006. PubMed

XXIII-9) C Multi-drug resistance develops relatively frequently in cells and tumors exposed to chemotherapeutic agents. The primary mechanism by which this occurs is an increase in levels of p-glycoprotein or one of the other proteins that non-specifically efflux xenobiologics from cells. These multidrug resistant resistant cells rapidly and efficiently efflux foreign molecules and thus maintain low, non-toxic intracellular drug levels even in the presence of high extracellular drug concentrations that would normally be lethal. Induction of multi-drug resistance by one drug can lead to resistance to a broad spectrum of related and unrelated drugs, which kill cells by different mechanisms. Cells or tumors that have become multi-drug resistant through

this mechanism do not become radioresistant, as radiation cannot be effluxed. Radiation exposure does not cause multi-drug resistance. The differences in the sensitivity of multi-drug resistant and non-resistant cells can be very large, often producing differences of several orders of magnitude in survival for a given drug dose. Multidrug resistance represents a permanent change in the cell phenotype and is not transient. Other changes in tumor cells can also increase resistance to multiple drugs. For example, increased glutathione levels would increase resistance to a spectrum of drugs with a mechanism of action involving radicals. Similarly, an increase in the activity of DNA a repair pathway could lead to improved repair of drug damage and increased survival. Resistance from these mechanisms is not nearly as dramatic as the drug resistance induced by the efflux proteins described above, but is important to radiotherapy because the changes can also cause small increases radioresistance.

Baguley BC. Multidrug resistance in cancer. *Methods Mol Biol* 596:1-14, 2010. PubMed

Hall MD, Handley MD, Gottesman MM. Is resistance useless? Multidrug resistance and collateral sensitivity. *Trends Pharmacol Sci* 30:546-556, 2009. PubMed

XXIII-10) D The bioreductive properties of mitomycin C make it more toxic to many cells under hypoxic conditions.

XXIII-11) A Photodynamic therapy (PDT) requires a photosensitizer, oxygen and light to produce the active, toxic species, singlet oxygen. PDT has been used to treat both superficial tumors as well as more deep-seated tumors that can be accessed endoscopically and exposed to light using fiberoptic probes. Although direct tumor cell killing may occur, particularly when there is a long drug-light exposure that allows free diffusion of the photosensitizer into tumor tissue, in most instances, the main photosensitizing effect occurs while the drug is confined to the tumor vasculature and result in damage to cells therein, which leads to the indirect killing of tumor cells as a result of the vascular damage. Because oxygen is required for the PDT reaction, PDT is ineffective in hypoxia.

Chen B, Pogue BW, Hoopes PJ, *et al.* Combining vascular and cellular targeting regimens enhances the efficacy of photodynamic therapy. *Int J Radiat Oncol Biol Phys* 61:1216-26, 2005. PubMed

Santiago R, Hahn S, Glatstein E. Chapter 73: Clinical Applications of Photodynamic Therapy pp 1625-1637 In *Textbook of Radiation Oncology,*

2nd ed. Leibel SA and Phillips TL, Eds., W.B. Saunders, Philadelphia, 2004.

Hasan T, Ortel B, Moor A, *et al.* Photodynamic Therapy of Cancer. pp. 605-622, in *Cancer Medicine*, 6th Ed. Kufe DW, Holland JF and Frei E, Eds. BC Decker, Hamilton, Ont., 2003.

XXIII-12) C Cisplatinum is a chemotherapeutic agent that causes DNA synthesis inhibition by causing both interstrand and intrastrand crosslinking. It is cell-cycle non-specific. It is used as a radiosensitizer with concurrent radiation. It is much more effective than its isomer, trans-platinum.

XXIII-13) A Adriamycin has been linked to cardiac toxicity. Cisplatinum has been linked to ototoxicity and renal toxicity. Bleomycin has been linked to pulmonary toxicity. Docetaxel has been linked to peripheral neuropathy.

XXIII-14) F Data suggests that ptaients treated with definitive radiation and cetuximab have improved overall survival if they developed a grade 2 or more acneiform rash as compared to patients with no rash or grade 1 rash (HR 0.49).

Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU, Sur RK, Raben D, Baselga J, Spencer SA, Zhu J, Youssoufian H, Rowinsky EK, Ang KK. Lancet Oncol. 2010 Jan;11(1):21-8. PubMed link

XXIII-15) B Ipilimumab is an antibody against the immune checkpoint molecule CTLA-4. Bevacizumab is the humanized monoclonal antibody that inhibits vascular endothelial growth factor A (VEGF-A). Imantinib is a small molecular inhibitor of receptor tyrosine kinases most selective for Bcr-Abl, but also less so against c-kit and PDGF-R Cetuximab is a monoclonal antibody against EGFR. Crizotinib is a small molecular inhibitor of ALK and ROS1 kinases.

XXIII-16) A Crizotinib is a small molecular inhibitor of ALK and ROS1 kinases. Imantinib is a small molecular inhibitor of receptor tyrosine kinases most selective for Bcr-Abl, but also less so against c-kit and PDGF-R. Cetuximab is a monoclonal antibody against EGFR. Sunitinib and sorafenib are "dirty" multi-targeted receptor tyrosine kinase inhibitors.

XXIII-17) B Abrogation of a gene that is synthetic lethal selectively kills cancer cells.

Reinhardt HC, Jiang H, Hemann MT, Yaffe MB. Cell Cycle. 2009 Oct 1;8(19):3112-9. PubMed link

XXIII-18) A A case of the abscopal effect of radiation therapy in a patient with metastatic melanoma receiving treatment with ipilimumab has been reported. Correlative immunologic analyses suggest that radiation therapy modified the immune system and facilitated the abscopal effect.

Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, Mu Z, Rasalan T, Adamow M, Ritter E, Sedrak C, Jungbluth AA, Chua R, Yang AS, Roman RA, Rosner S, Benson B, Allison JP, Lesokhin AM, Gnjatic S, Wolchok JD. N Engl J Med. 2012 Mar 8;366(10):925-31. PubMed link

XXIII-19) B Panitumumab is a humanized IgG2monoclonal antibody to the epidermal growth factor receptor (EGFR). Cetuximab is a chimeric IgG1 antibody also targeting the EGFR and is used in the treatment of metastatic k-ras wild type colorectal cancers as well as in head and neck cancers. In contrast, Rituximab targets CD20 (primarily in B-cell lymphomas), bevacizumab targets the VEGF receptor and sunitinib is a tyrosine kinase receptor inhibitor. Infliximab is a monoclonal antibody against tumor necrosis factor-α and is used in the treatment of autoimmune disorders such as rheumatoid arthritis and psoriasis.

XXIII-20) D Zevalin is an anti-CD 20 antibody tagged with Yttrium-90 used for the systemic radioisotope based-treatment of widespread B-cell lymphomas. Y90 ccontaining resin microspheres are FDA approved for the treatment of hepatic metastases from mCRC and Y90 containing glass microspheres (Theraspheres™) are used in the treatment of primary hepatocellular carcinomas.

XXIV. Radiosensitizers, Radioprotectors and Bioreductive Drugs

XXIV-1) D Metaanalysis has shown that when combined with radiotherapy, nimorazole significantly improves both local control and overall survival in select subsets of patients with head and neck cancer. One reason that clinical trials of hypoxic cell sensitizers may have yielded disappointing results was because of the dose-limiting peripheral neuropathy; this cumulative toxicity severely limited the total dose of sensitizers that could be given over a course of radiotherapy. Bioreductive drugs are compounds that are metabolically-*reduced* under hypoxic conditions to yield cytotoxic species. Because the bioreduction occurs preferentially under hypoxic conditions, these drugs are selectively toxic to hypoxic cells and not aerobic ones. In laboratory studies, hypoxic cell radiosensitizers are most effective when given in high doses, with large radiation doses; there effectiveness in model tumor systems decreases with increasing fractionation. One would expect from these laboratory studies that they would be more effective in combination with hypofractionated radiotherapy regimens or radiosurgery, rather then with standard radiotherapy regimens or hyperfractionated regimens.

Overgaard J, Hansen HS, Overgaard M, *et al*. A randomized double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85. *Radiother Oncol* 46:135-146, 1998. PubMed

Overgaard J. Hypoxic radiosensitization: Adored and ignored. *J Clin Oncol* 25:4066-4074, 2007. PubMed

Rockwell S, Dobrucki IT, Kim EY, *et al*. Hypoxia and radiation therapy: Past history, ongoing research, and future promise. *Current Mol. Med* 9:441-459, 2009. PubMed

XXIV-2) B Thymidylate synthase is the enzyme inhibited by 5-fluorouracil, leading to the inhibition of DNA synthesis (as well as that of ribosomal and messenger RNA). These account for the drug's cytotoxic and radiosensitizing effects.

XXIV-3) D In its active metabolite form, gemcitabine inhibits ribonucleotide reductase, which likely accounts for its action as a radiosensitizer. The inhibition of this enzyme affects DNA synthesis by preventing the *de novo* biosynthesis of deoxyribonucleoside triphosphate precursors.

XXIV-4) B Sulfhydryl radioprotectors reduce radiation toxicity by scavenging free radicals.

XXIV-5) C Amifostine does not readily cross the blood brain barrier and therefore affords little radioprotection to tissues in the CNS. Amifostine must be administered intravenously for maximal efficacy. Hypotension, nausea/vomiting, fatigue and fever/rash are the main toxicities associated with amifostine. Amifostine should be administered 15-30 minutes before radiotherapy, not after. It is a pro-drug that is metabolized by alkaline phosphatase to the free thiol metabolite that acts as the direct radioprotective agent.

Grdina DJ, Kataoka Y, Murley JS. Amifostine: mechanisms of action underlying cytoprotection and chemoprevention. *Drug Metabol Drug Interact* 16:237-79, 2000. PubMed

Yuhas JM. Active versus passive absorption kinetics as the basis for selective protection of normal tissues by S-2-(3-aminopropylamino)-ethylphosphorothioic acid. *Cancer Res* 40:1519-24, 1980. PubMed

XXIV-6) B One mechanism that has been proposed to account for the radiosensitizing effect of cisplatin is through inhibition of DNA double-strand break repair.

Wilson GD, Bentzen SM, Harari PM. Biologic basis for combining drugs with radiation. *Semin Radiat Oncol* 16:2-9, 2006. PubMed

XXIV-7) A Methylation of the promoter for MGMT (O^6-methylguanine-DNA methyltransferase) through an epigenetic mechanism (no gene mutation) decreases expression of this DNA repair gene. When tumor cells express MGMT, they repair the alkylation of DNA caused by temozolomide. Therefore, patients with MGMT expressing glioblastomas derive little benefit from concurrent temozolomide and radiation therapy. In contrast, when MGMT is silenced in glioblastomas, temozolomide causes significant DNA damage through alkylation, which increases the radiosensitivity.

Stupp R, Mason WP, van den Bent MJ, *et al*., Radiotherapy Plus Concomitant and Adjuvant Temozolomide for Glioblastoma. *NEJM* 352, 10: 987-996, 2005. PubMed

Hegi ME, Diserens A, Gorlia T, *et al*., *MGMT* Gene Silencing and Benefit from Temozolomide in Glioblastoma. *NEJM* 352, 10: 997-1003, 2005. PubMed

XXIV-8) B Rapamycin, everolimus and temsirolimus are all inhibitors of the mTOR (mammalian target of rapamycin). mTOR functions downstream of PI3K to promote cell survival. Inhibition of mTOR blocks these pro-survival pathways.

Murphy JD, Spalding AC, Somnay YR, *et al*., Inhibition of mTOR radiosensitizes soft tissue sarcoma and tumor vasculature. *Clin Cancer Res* 15, 2: 588-596, 2009. PubMed

Sabatini DM, mTOR and cancer: Insights into a complex relationship. *Nature Reviews Cancer* 6: 729-734, 2006. PubMed

XXIV-9) A IR induces a G2/M checkpoint arrest which allows sufficient time for the repair of DSBs before the initiation of mitosis, since cell division in the presence of an unrepaired DSB could lead to mitotic catastrophe. Blockade of this checkpoint via inhibition of Wee1, which enforces it, would lead to significant radiosensitization. The Wee1 inhibitor, MK1775, has been tested in DIPG and GBM as a radiosensitizer

XXV. Hyperthermia

XXV-1) E The break point in the Arrhenius plot occurs at a temperature of approximately 43°C.

Dewey WC, Hopwood LE, Sapareto LA, *et al*. Cellular responses to combinations of hyperthermia and radiation. *Radiology* 123:463-474, 1977. PubMed

XXV-2) B One reason why hyperthermia in combination with radiation may be effective in cancer therapy is because hypoxic tumor cells may be at a low pH and nutritionally-deprived and are therefore particularly sensitive to hyperthermia, whereas they are resistant to radiation. Tumor cells are not intrinsically more sensitive to heat than normal cells. Heat does not affect the number of ionizations produced by a given dose of radiation. Preferential retention of heat by normal tissues would represent a disadvantage to the use of hyperthermia. Maximum radiosensitization is produced when the two agents are given simultaneously, or nearly so. At long times after irradiation, there is less heat-induced radiosensitization because much of the radiation damage has been repaired.

XXV-3) C At normal body temperature, heat shock proteins (HSPs) help maintain the HSF1 transcription factor in an inactive state, but upon exposure to higher temperatures, HSPs bind to unfolded proteins triggering the release of HSF1, thereby initiating additional transcription of the HSP gene. HSPs are molecular chaperones, which are proteins that bind to non-native or (partially) unfolded proteins and assist in their correct assembly by preventing their non-productive aggregation. A major mechanism for heat-induced radiosensitization is inhibition of the re-polymerization step in the repair of radiation-induced base damage; therefore heat does not produce DNA damage directly. Heat-induced cell death may occur by prompt apoptosis or by delayed death secondary to mitotic failure. In addition, apoptosis-resistant cells may die a necrotic death or die due to permanent cell cycle arrest following a heat treatment.

Moyer HR, Delman KA. The role of hyperthermia in optimizing tumor response to regional therapy. *Int J Hyperthermia* 24:251-261, 2008. PubMed

Dewhirst MW, Vujaskovic Z, Jones E, *et al*. Re-setting the biologic rationale for thermal therapy. *Int J Hyperthermia* 21:779-790, 2005. PubMed

XXV-4) E Heat shock proteins (HSPs) belong to the family of proteins called molecular chaperones that help prevent protein aggregation. Thermotolerance is a transient acquired resistance to heat that is *not* heritable by the progeny of the treated cells. Once the protein damage is removed by HSPs following heat treatment, the HSPs rebind HSF1, thereby decreasing the level of HSPs in an autoregulatory loop and restoring normal heat sensitivity. Step-down heating results in greater *sensitivity* to a subsequent heat treatment at a lower temperature due to inhibition by the initial 43ºC treatment of the development of thermotolerance. Thermotolerance develops *during* the heating of tissues at temperatures lower than 43°C. The onset and decay of thermotolerance correlates with the appearance and disappearance of heat shock proteins, and is not related to the repair of DNA damage.

XXV-5) D Due to the difference in the mode of action, it is important not to draw conclusions for heat based on the interpretation of radiation dose-response curves. The amount of energy involved in cell inactivation is a thousand times greater for heat than for x-rays.

XXV-6) A All functional capillaries in tumors are open and used to capacity, even under ordinary conditions. But many capillaries in normal tissues are closed under ambient conditions.

XXV-7) E Long duration mild hyperthermia is as good as 4ºC incubation in eliminating the repair of RT-induced SRD, which is particularly true in a low dose-rate for brachytherapy.

XXVI. Radiation Carcinogenesis

XXVI-1) A Cancer survivors constitute 3.5% of the US population, but second primary malignancies among this high risk group now account for 16% of all cancers diagnosed. A high frequency of second primary tumors among soft tissue sarcoma patients has been reported and includes a particularly high risk of developing a new soft tissue sarcoma. Radiotherapy to the breast or chest wall of young women is associated with long-term cardiotoxicity and an increased risk of second breast cancers. Genetic factors, as well as the potential carcinogenic effects of treatment, can affect the probability of second cancers in survivors. BRCA 2 patients are at increased risk of subsequent ovarian cancer, as well as cancers in the irradiated and unirradiated breast. Patients with Li-Fraumeni syndrome and other familial cancer syndromes would likewise be at increased risk of developing second malignancies unrelated to the carcinogenic effects of their initial treatments.

Armstrong GT, Liu Q, Yasui Y *et al.* Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. *J Clin Oncol* 27:2328-38, 2009. PubMed

Robison LL.Treatment-associated subsequent neoplasms among long-term survivors of childhood cancer: the experience of the Childhood Cancer Survivor Study. *Pediatr Radiol* 39 Suppl 1:S32-7, 2009. PubMed

Sadetzki S, Mandelzweig L. Childhood exposure to external ionising radiation and solid cancer risk. *Br J Cancer* 7;100(7):1021-5, 2009. Review. PubMed

XXVI-2) D Both benign nodules and malignant tumors of the thyroid can be induced by radiation. None of the other sites shows such an increased incidence of benign tumors due to X-irradiation.

Boice JD. Radiation-induced thyroid cancer -- what's new? *J Natl Cancer Inst* 97:703-705. PubMed

Cardis E, Kesminiene A, Ivanov V, *et al*. Risk of thyroid cancer after exposure to 131I in childhood. *J Natl Cancer Inst* 97:724-32, 2005. PubMed

XXVI-3) C For a population exposed to whole body irradiation, approximately 15% of the fatal cancers are leukemias.

Finch SC. Radiation-induced leukemia: lessons from history. *Best Pract Res Clin Haematol* 20(1):109-18, 2007. Review. PubMed

XXVI-4) C An excess incidence was not detected for head and neck cancers in the group of children irradiated for tinea capitis. Brain cancers, thyroid cancers, adenomas and (non-CLL) leukemias were all observed at an excess incidence in children who were X-irradiated compared with children who only received topical medications for treatment of tinea capitis or ringworm. X rays for tinea capitis are also associated with the late development of breast cancer.

Shore RE, Moseson M, Harley N, *et al.* Tumors and other diseases following childhood x-ray treatment for ringworm of the scalp (Tinea capitis). *Health Phys* 85:404-408, 2006. PubMed

Modan et al, Lancet, Vol 333, 1989, Pages 629-631

XXVI-5) E The susceptibility to radiation-induced cancer decreases with increasing age at the time of irradiation. By repeatedly licking their paint brushes, radium dial painters ingested significant quantities of radium-containing paint while painting watch dials and subsequently developed an excess number of bone tumors as a result of the incorporation of radium into their growing bones, and the continuous low-dose-rate irradiation received by these tissues over the next decades. Radiation-induced cancers cannot at this time be distinguished from cancer occurring naturally, although molecular markers diagnostic of radiation exposure may eventually be identified. The current consensus among radiation protection organizations is that the most appropriate dose response curve for radiation carcinogenesis is one that increases linearly with increasing radiation dose, and with no dose threshold (linear no-threshold or LNT model). However, this hypothesis has been challenged by those who believe that low radiation doses are less harmful than predicted by the LNT model, and possibly even beneficial (often referred to as hormesis). The LNT model has also been criticized by those who believe that bystander effects may result in increased risks at low doses over those predicted by the LNT model. Hematological malignancies have shorter latency periods compared to solid tumors.

Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2 (2006) National Research Council, National Academies Press, 2006.

Charles MW. LNT -- an apparent rather than a real controversy? *J Radiol Prot* 26:325-329, 2006. PubMed

Tubiana M, Aurengo A, Averbeck D, *et al.* The debate on the use of linear no threshold for assessing the effects of low doses. *J Radiol Prot* 26:317-324, 2006. PubMed

Tubiana M, Aurengo A, Averbeck D, *et al*. Recent reports on the effect of low doses of ionizing radiation and its dose-effect relationship. *Radiat Environ Biophys* 44:245-251, 2006. PubMed

XXVI-6) D Statistically significant increases in non-cancer disease mortality with increasing radiation dose have been observed, particularly for diseases of the circulatory, digestive, and respiratory systems. There is also an increased risk of heart disease among people who were exposed to doses less than 5 Gy. Among the Japanese A-bomb survivors, susceptibility to radiation-induced breast cancer was found to dramatically *decrease* with increasing age at time of exposure, with women over 50 years of age showing little or no excess. The latency period for the appearance of most radiation-induced solid tumors is far greater than 1-3 years, ranging from 10-60 years post-exposure. It is estimated that 8% of people exposed to 1 Sv would die from a radiation-induced cancer. Thus, in a population of 1,000 people, approximately 80 would develop a fatal cancer.

Nakachi K, Hayashi T, Hamatani K, *et al*. Sixty years of follow-up of Hiroshima and Nagasaki survivors: current progress in molecular epidemiology studies. *Mutat Res* 659:109-17, 2008. PubMed

Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2 (2006) National Research Council, National Academies Press, 2006. PubMed

Taylor CW, McGale P, Darby SC. Cardiac risks of breast-cancer radiotherapy: a contemporary view. *Clin Oncol (R Coll Radiol)* 18:236-46, 2006. PubMed

XXVI-7) C The risk estimates based on the Radiation Effects Research Foundation (RERF) analyses for solid tumors are well fit using a linear model; a linear-quadratic model provides a much better fit to the leukemia dose response data. Although the BEIR VII Committee conducted an analysis of the data related to the DDREF, and uses a value of 1.5 for its own risk estimations, historically the factor of 2.0 has been applied to adjust for lower doses and

dose-rates. RERF data clearly indicate that radiation risk is dependent on age at exposure, time since exposure, and gender. Population studies frequently have more limitations compared to more quantitative case-control studies, including smaller population sizes and uncertainties associated with dose estimations, confounding factors, and lack of relevant control populations. The BEIR VII estimates the lifetime additional cancer risk is about 1% following 100 mSv.

Preston DL, Cullings H, Suyama A, *et al*. Solid cancer incidence in atomic bomb survivors exposed in utero or as young children. *J Natl Cancer Inst* 100:428-36, 2008. PubMed

XXVI-8) C Mental retardation following exposure of the sperm is due to mutations that are passed on to the offspring; mutations are a stochastic effect of radiation. Mental retardation caused by exposure of the fetus has a dose-threshold and is considered to be a deterministic effect. All of effects noted here are deterministic because they do not involve mutations in either germline or somatic cells.

XXVI-9) C Administration of KI to the children that had been exposed to 131I as a result of the accident would have reduced the number of thyroid cancers by decreasing exposure to the radioactive I. While 137Cs was released as part of the accident, 131I was the cause of the thyroid cancers that occurred predominantly in children within 7-10y following exposure.

XXVI-10) A From the atomic bomb survivors leukemia (other than CLL), breast cancer, thyroid cancer, bladder cancer were all considered to be highly affected by radiation exposure.

XXVII. Heritable Effects of Radiation

XXVII-1) C The genetically significant dose (GSD) is the annual average gonadal dose to a population adjusted for the relative child expectancy of that population. Radon exposure does not contribute significantly to the GSD because the decay products of radon are deposited almost entirely in the lung. The GSD resulting from medical procedures performed annually in the U.S. is estimated at 0.3 mSv, not 1 Sv. Although the GSD can be used to estimate the number of children born each year with a radiation-induced mutation, the GSD itself is an estimate of the average gonadal dose to the population (including potential parents), not an estimate of the effects on offspring. The GSD is an annual population dose, not an individual lifetime dose. .

XXVII-2) D The dose to double the incidence of mutations in humans has been estimated at approximately 1-2 Sv. Radiation does not induce characteristic mutations, but only increases the incidence of mutations that occur spontaneously. A higher incidence of genetic abnormalities has *not* been found in the children of radiotherapy patients compared with children whose parents had not been irradiated prior to conception. The best estimates are that no more than 1-6% of spontaneous mutations in humans are due to exposure to background radiation. The absolute mutation rate for humans has been estimated at 0.1-0.6% per Sv.

Boice JD Jr, Tawn EJ, Winther JF, *et al.* Genetic effects of radiotherapy for childhood cancer. *Health Phys* 85:65-80, 2003. PubMed

Neel JV. Reappraisal of studies concerning the genetic effects of the radiation of humans, mice, and Drosophila. *Environ Mol Mutagen* 31:4-10, 1998. PubMed

XXVII-3) A Studies of the Japanese A-bomb survivors by RERF have served as a "gold standard" for radiation epidemiology. One of the key findings has been that there has NOT been a statistically significant increase in mutations in the F1 generation (approximately 70,000 individuals), despite the original expectation that there might be based on animal experiments. Therefore, the doubling-dose estimate for radiation-induced genetic mutations in humans is based on mouse data, coupled with estimates of the human spontaneous mutation rates. A majority of the survivor cohort received a relatively low radiation exposure of less than 100 mSv. A recently revised dosimetry model (DS02) provides improved estimates of individual exposures. The Adult Health Study cohort members receive thorough clinical exams every two years and have provided data and biological samples that remain an important resource for future study.

Fujiwara S, Suyama A, Cologne JB, et al. Prevalence of adult-onset multifactorial disease among offspring of atomic bomb survivors. *Radiat Res* 170:451-457, 2008. PubMed

International Commission on Radiological Protection, ICRP Publication 90: Biological Effects after Prenatal Irradiation (Embryo and Fetus), 1st Ed., Elsevier, New York, 2004.

Schull WJ. The children of atomic bomb survivors: a synopsis. *J Radiol Prot* 23: 369-84, 2003. PubMed

Streffer C, Shore R, Konermann G, et al: Biological effects after prenatal irradiation (embryo and fetus). A report of the International Commission on Radiological Protection. *Ann ICRP* 33:5-206, 2003. PubMed

XXVII-4) B The mutation rate decreased significantly when the dose rate was reduced. This was attributed to repair processes that take place during irradiation at low dose rates.

Russell LB, Russell WL. Frequency and nature of specific-locus mutations induced in female mice by radiations and chemicals: a review. *Mutat Res* 296:107-127, 1992. PubMed

XXVII-5) D The dose for low sperm count and reduced fertility in the male is 0.15Gy. There is no temporary sterility or latent period for the female. Radiation sterility does not affect hormone balance, libido, and physical capability. The dose to produce permanent sterility in the female is 2Gy in a mature woman.

XXVII-6) D Low dose-rate exposure usually results in few mutations than the same dose given at a high dose rate. T to A transitions are usually found following exposure to UV but not to ionizing radiation. The dose to

double mutations is around 1Gy. High LET radiations tend to cause large deletions and low LET radiations tend to cause small deletions.

XXVIII. Radiation Effects in the Developing Embryo and Fetus

XXVIII-1) A Based on animal studies, the most sensitive period during gestation, when radiation exposure may cause embryonic lethality, is during the period immediately following conception but prior to implantation in the uterine wall.

XXVIII-2) C Irradiation during the early fetal period, corresponding to weeks 8-15 of gestation in humans, is associated with the greatest risk for mental retardation. The main risks during preimplantation, organogeneis and the late fetal period are prenatal death, congenital malformations, growth retardation and carcinogenesis, respectively. There is an increased risk of carcinogenesis following irradiation throughout the gestation period.

XXVIII-3) C Radiation during 8-15 weeks of gestation is mostly likely to cause mental retardation, compared to the other periods of gestation.

XXVIII-4) C Once a pregnancy is declared, the maximum permissible dose to the fetus is 0.5 mSv per month. Prior to declaration, there are no special limits except for the general limits for radiation workers.

XXVIII-5) A Radiation exposure is most likely to be lethal in the earliest phase of the prenatal period, after conception, before implantation.

XXVIII-6) B Spina bifida is a neural tube defect typically associated with folate deficiency, not ionizing radiation exposure.

XXVIII-7) A Exposure in utero does result in a high risk for mental retardation which is accompanied by low IQ and poor school performance at 8-15w and a 4x lower risk at 15-25 weeks of gestation. Ultrasound is used for monitoring the fetus in utero because of concerns about cancer induction. The LD50 for oocytes is approximately 0.5Gy.

XXIX. Radiation Protection

XXIX-1) C A radiation worker is permitted either 50 mSv per year for each year that the person was engaged in radiation work or else a lifetime dose equal to his/her age multiplied by 10 mSv, whichever is less. Based on the lifetime dose rule, this woman would have been permitted 200 mSv as of her 20th birthday. However the 50 mSv per year rule dictates that her maximum allowable dose would be only 100 mSv.

NCRP Report 116. Limitation of Exposure to Ionizing Radiation, 1993 PubMed

XXIX-2) E The NCRP recommendations state that a worker who has declared a pregnancy may receive a maximum dose of 0.5 mSv per month to the fetus.

XXIX-3) D A radiation worker is permitted 150 mSv to the eye and 500 mSv to the skin in any given year. These limits are based on risk estimates for the production of radiation-induced deterministic effects.

However, In 2011 the ICRP published a statement that changed their previously recommended threshold doses for some tissue reaction effects, including the lens of the eye, the heart and the cerebrovascular system. The ICRP now recommends an equivalent absorbed dose limit for the lens of the eye of 0.5 Gy in a single exposure. For chronic occupational exposures, the ICRP recommends an equivalent dose limit for the lens of the eye of 20 mSv in a year, averaged over defined periods of 5 years, with no single year exceeding 50 mSv. The NCRP is currently discussing the ICRP recommendation, but has not yet made a change in the previous recommendation.

XXIX-4) D The average annual effective dose for a person residing in the US is approximately 6 mSv. This total includes an average radon contribution of 2 mSv; cosmic, terrestrial and internal radioactivity of 1 mSv. In addition, an average of 3 mSv from man-made sources, primarily from diagnostic and nuclear medicine procedures, is received. This increase from the often quoted 1980 figure of 0.5 mSv associated with medical procedures is the result primarily from the significant increase in the use of CT scans over the past 30 years. CT scans deliver relatively high radiation doses compared with other imagining modalities.

Mettler FA Jr, Bhargavan M, Faulkner K, *et al*. Radiologic and nuclear medicine studies in the United States and worldwide: frequency, radiation dose, and

comparison with other radiation sources -- 1950-2007. Radiology 253:520-531, 2009. PubMed

XXIX-5) B A member of the general public is permitted 1 mSv per year for "chronic" radiation exposure over extended periods, or 5 mSv per year for an infrequent exposure. Radiation workers, including radiation oncologists and nuclear power plant employees, may receive 50 mSv per year. A person under the age of 18 may be exposed to radiation up to 1 mSv per year if the potential exposure occurs as part of an educational or training program. The person transporting the radiotherapy patient to and from treatment, presumably an infrequent event, would be considered to be a member of the general public and therefore would be allowed 5 mSv per year.

XXIX-6) C The MPD defines occupational exposure and does not include the dose received from medical procedures. In nearly all cases, the MPD is greater than the dose that would be obtained with strict adherence to the principles of ALARA, which stipulate that personnel should receive doses "as low as reasonably achievable". The MPD recommendations for radiation workers are typically 10-50 fold higher than for members of the general public. The NCRP and ICRP guidelines treat age differently in establishing the MPD. The MPD for younger workers is therefore greater under NCRP guidelines than under ICRP guidelines, but the MPD for older workers is greater under ICRP guidelines than under the NCRP guidelines.

XXIX-7) B A "committed dose equivalent" is the dose equivalent to a tissue or organ that will be received over a 50-year period from the ingestion of radioactive material(s).

NCRP (1993) *Limitation of Exposure to Ionizing* Radiation*: NCRP Report No. 116*. Bethesda, MD. PubMed

ICRP (1991). *1990 Recommendations of the International Commission on Radiological Protection. ICRP Publication 60*. *Ann of the* ICRP 21, 1-3 Pergamon Press, Oxford.

XXIX-8) A Radon accounts for 55% of the annual exposure of the US population to radiation.

XXIX-9) A The maximum dose with fluoroscopy is to the skin. Early transient erythema may occur with doses of 2 Gy, dry desquamation with single doses of 14 Gy, and moist desquamation with single doses of 18 Gy or more.

XXX. Molecular Techniques used in Radiation and Cancer Biology

XXX-1) E The use of miRNAs (microRNAs) and siRNAs (small interfering RNAs) has become an important tool for so-called "gene silencing" or RNA interference (RNAi). miRNAs and siRNAs bind to and inhibit the transcription of specific genes and/or they can silence cytoplasmic mRNAs either by stimulating their cleavage or by inhibiting translation. miRNAs and siRNAs are 21-26 nucleotide (nt) RNA molecules that can be distinguished based on the mechanisms through which they were created. miRNAs are produced from transcripts that form stem-loop structures, whereas siRNAs are produced from long double-stranded RNA precursors. In the initiation phase of RNAi, the ribonuclease-III enzyme Dicer cleaves double-stranded RNA molecules into 21–23-nt short interfering siRNA duplexes. In the effector phase of RNAi, the siRNA becomes unwound and assembles into RISC (RNA-induced silencing complex). The activated effector complex recognizes the target by siRNA–mRNA base pairing and cleaves the mRNA strand with its endoribonuclease activity.

XXX-2) A In gel electrophoresis, DNA molecules are negatively charged and therefore migrate towards the positive electrode. Sodium dodecyl sulfate (SDS), a detergent, is used to denature *proteins*, not DNA, so that the proteins can be separated by size on a gel. The higher the concentration of agarose in the gel, the slower DNA molecules will migrate. Polyacrylamide gels are generally used to separate small DNA molecules whereas agarose gels are used for large sized DNA. The *lower* the molecular weight of the molecule, the more rapidly it will migrate through a gel.

XXX-3) D An important advantage to the use of FDG-PET/CT fusion imaging for radiotherapy treatment planning is that it provides both functional and anatomical information. The radioactive half-life of ^{18}F is 110 minutes, not 10 days. PET imaging cameras detect the 0.51 MeV photons produced by annihilations resulting from the interaction of a positron and electron. The uptake of ^{18}F-FDG is typically *higher*, not lower, in areas of inflammation and in tumors.

XXX-4) A Quantitation of DNA repair foci using a monoclonal antibody raised against γ-H2AX is currently considered the most sensitive assay for the repair of DNA double-stranded breaks, although there is some controversay about whether □-H2AX foci are also formed in response to other types of DNA changes. The *neutral* comet assay also can be used to measure DNA double-

strand breaks, although it is generally considered less sensitive than □-H2AX.

XXX-5) A Capillary sequencing requires in vivo cloning and amplification where NGS uses adaptor ligation of DNA fragments and binding to a matrix for DNA sequencing.

Metzker ML. Nat Rev Genet. 2010 Jan;11(1):31-46. PubMed link

XXX-6) D Pulsed-field gel electrophoresis is a technique in which a gel is subjected to electrical fields alternating in orientation, which allows very large DNA fragments to migrate and separate. This technique enables the detection of the repair/rejoining of DNA double-strand breaks following irradiation with a biologically-relevant dose.

XXX-7) C Northern blotting is used to study RNA.

XXX-8) A Wild-type p53 protein is not detectable, because p53 mRNA is short-lived ($T_{1/2}$ = 8 min) in unstressed cells. The induction of DNA double strand breaks by X-irradiation initiates a p53-dependent signal transduction cascade, including the induction of WAF1/CIP1 mRNA. The WAF1/CIP1 gene encodes the p21 protein. Activation of WAF1/CIP1 inhibits the cyclin E/cyclin-dependent kinase 2 complex, an event that stops cells from progressing through G_1. Phosphorylation of p53 at serine-15 in response to ionizing radiation correlates with both accumulation of total p53 as well as its transactivation of downstream genes.

Johnson DG, Walker CL. Cyclins and cell cycle checkpoints. Annu Rev Pharmacol Toxicol 39: 295-312, 1999.

El-Deiry WS, Harper JW, O'Connor PM, et al. WAF1/CIP1 is induced in p53-mediated G_1 arrest and apoptosis. Cancer Res 54: 1169-1174, 1994.

Waldman T, Kinzler KW, Vogelstein B. p21 is necessary for the iated G_1 arrest in human cancer cells. Cancer Res 55: 5187-5190, 1995.

Pandita TK, Lieberman HB, Lim DS, et al. Ionizing radiation activates the ATM kinase throught the cell cycle. Oncogene 19: 1386-1391, 2000.

XXXI. Molecular Imaging

XXXI-1) B 18-Fluorodeoxyglucose is the most commonly used metabolic radiotracer for PET scanning at present.

XXXI-2) D 18-Fluorine labeled thymidine has been used to image DNA synthesis in humans in vivo.

Salskov A, Tammisetti VS, Grierson J, Vesselle H. Semin Nucl Med. 2007 Nov;37(6):429-39.PubMed link

XXXI-3) E The Hounsfield unit scale is a standardized approach to interpreting reconstructed images obtained with a computerized tomography (CT) scanner. CT is a technique that relies on differential levels of X-ray attenuation by tissues within the body to produce digital images reflecting anatomy. Hounsfield units (HU) are numerical values that reflect these differences in density and composition, and thus X-ray attenuation, between various tissue types. Radiologists use software that automatically that assigns HUs to every voxel of a CT scan to enable efficient scan interpretation. In oral radiology, approximate HUs can be derived using grayscale levels in cone beam CT (CBCT) images.

XXXI-4) C The Hounsfield unit (HU) scale relates X-ray attenuation in various tissue types (μ) to X-ray attenuation in water μ(water) through the equation:

$$\text{HU} = 1000 \times \frac{\mu - \mu(water)}{\mu(water)}$$

Obviously, the HU of water is 0, based on the above equation. Other choices are incorrect. Barium-based rather than iodine-based contrast agents are used in the imaging of the digestive system by CT. Organ doses from diagnostic CT procedures are typically estimated to be in the range of 10 mGy per scan and as much as 100 mGy from multiple CT scans. For example, cumulative doses from 2-3 head CTs to the brain are 50-60 mGy. For comparison, X-ray doses from chest radiography are 0.1 mGy and X-ray doses from mammography are 0.4 mGy. The Nuclear Regulatory Commission (NRC) has as part of its responsibilities the regulation of radioactive materials used medically, including diagnostic radionuclides used in PET imaging. CT scanners and image reconstruction software as well as other "medical devices" such as linear accelerators and associated treatment planning software are regulated by the U.S. Food and Drug Administration.
References:

Lusic H, Grinstaff MW. X-ray computed tomography contrast agents. Chem Rev 113: 1641-1666, 2013.

Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, Howe NI, Ronckers CM, Rajaraman P, Craft AW, Parker L, Berrington de Gonzalez A. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumors: a retrospective cohort study. Lancet 380:499-505, 2012.

Miglioretti DL, Johnson E, Williams A, Greenlee RT, Weinmann S, Solberg LI, Feigelson HS, Roblin D, Flynn MJ, Vannman N, Smith-Bindman R. The use of computed tomography in pediatrics and the associated radiation exposure and estimated cancer risk. JAMA Pediatr 167:700-707, 2013.

McCollough CH, Bushberg JT, Fletcher JG, Eckel LJ. Answer to common questions about the use and safety of CT scans. Mayo Clin Proc 90: 1380-1392, 2015.

XXXI-5) C SUV, the standard uptake value, is a standard method of quantifying the radioactive uptake observed in a PET scan image. As a cancer detection method, FDG PET is based on the observation that in normoxic conditions tumor cells primarily use glycolysis for energy production instead of mitochondrial oxidative phosphorylation like normal cells (the Warburg effect). SUV scores of > 15 g/ml usually indicate a tumor which is highly dependent on glucose metabolism, that is, aggressive rather than indolent. It follows that SUV scores inversely correlate with local tumor control. Choices B and E can be eliminated by examining the definition of the standard uptake value,

SUV [g/ml] = (Tissue activity (mCi/ml))/(injected dose (mCi))×patient^' s body weight (g)

Tissue activity (mCi) per lesion volume (ml) are derived from pixel intensities of PET (or PET/CT) images. This quanitity is then divided by the amount of radioactive tracer injected into the patient (mCi) per unit of his/her body weight (g) (specific activity). SUV values increase in value if PET scan is delayed after FDG injection.

References:

Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. Science 324:1029-1033, 2009.

Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. Cell Death and Disease 4:e532, 2013. www.nature.com/cddls.