

# 2023 ASTRO RADIATION AND CANCER BIOLOGY STUDY GUIDE

Produced by the Radiation and Cancer Biology  
Study Guide Task Force

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## Preface to the 2023 Edition

In recognition of the critical need to develop new ways to promote education regarding the biologic basis of radiotherapy, the Education Committee of ASTRO appointed a subcommittee to develop a dynamic web-based educational resource for radiation oncologists studying radiation and cancer biology. The *ASTRO Radiation and Cancer Biology Study Guide* is the product of these efforts. This study guide was created specifically to stimulate active learning.

It is our suggestion that users of this study guide answer all of 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 have been included whenever possible along with a hyperlink to the article abstract for topics that may not be comprehensively addressed in the major radiation biology textbooks cited as a primary reference in the 2023 Study Guide released by the American Board of Radiology (ABR). 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on recent review articles that provide current and comprehensive information on a particular subject; however, key historical references are also cited. Finally, although this is not an exhaustive resource for preparation for the ABR Radiation and Cancer Biology written examination, questions and answers were cross referenced with the 2022 ABR study guide to align with this content.

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 training and to subsequently build upon that foundation throughout their career by regular reading of the scientific literature as well as by attendance at seminars and scientific conferences. The ASTRO Radiation and Cancer Biology Study Guide is designed to help radiation oncologists achieve this goal. It is our hope that providing radiation oncologists in-training with a firm foundation underlying the biologic principles of therapeutic radiation will ultimately yield more effective radiotherapeutic treatment techniques as well as improved clinical outcomes for patients.

Finally, we would like to thank each 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 commitment to this subcommittee via the initiation of this project. Without the assistance and volunteerism of these individuals, production of the 2023 ASTRO Radiation and Cancer Biology Study Guide would have not been possible.

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## Note on Protein and Gene Nomenclature

The 2023 ASTRO Radiation and Cancer Biology 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 system 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 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 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 study guide rather than the official HUGO symbol for this gene, which is *TP53*. This is noted by indicating *p53 (TP53)* in the question or explanation.



# QUESTIONS

## I. Interaction of Radiation with Matter

- I-1) Which 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 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 of a water molecule 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 the specified photon 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 X-rays
  - B. Produce much higher yields of OH radicals than do either X-rays or  $\gamma$ -rays
  - C. Are components of solar flares but not of cosmic rays
  - D. Produce less dense ionization tracks than X-rays
  - E. Produce increased numbers of clustered lesions in DNA than X-rays
- I-7) The lifetime of an OH $\cdot$  radical is approximately:
- A.  $10^{-15}$  second
  - B.  $10^{-9}$  second
  - C.  $10^{-1}$  second
  - D. 1 second
  - E. 1 minute
- I-8) Regarding pair production and annihilation, which of the following is true?
- A. The incident photon is scattered with reduced energy
  - B. Annihilation photons always have an energy of 0.511 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-9) Directly ionizing radiation includes all of the following EXCEPT:
- A. Electrons
  - B. Positrons
  - C. Alpha particles
  - D. Neutrons
  - E. Betas

- I-10) Concerning fast neutron interactions with matter, which of the following is FALSE?
- A. They do not interact with atomic electrons of biological media
  - B. They interact primarily with oxygen in water
  - C. They may cause the ejection of an alpha particle
  - D. They may activate the target nucleus.
  - E. They may transfer a large fraction of its energy in the process of elastic scattering.
- I-11) Which of the following results 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
- 1-12) When a live human cell is irradiated by gamma-rays, which one of the following events may eventually cause 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 are 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

## I. Interaction of Radiation with Matter Answers & Explanations

- I-1) C In the Compton process, a photon interacts with an atom causing the 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 the atomic number of the absorber (Answer Choice A).

The predominant interaction of 10 keV photons in soft tissue is the photoelectric effect (Answer Choice B).

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 (Answer Choice D).

For the photoelectric effect, there is complete absorption of the photon's energy, resulting in ejection of an electron that possesses kinetic energy equal to the difference between the incident photon's energy and the electron's binding energy (Answer Choice E).

- I-2) D 65-75% of the damage caused by indirect action is mediated by the hydroxyl radical, OH<sup>•</sup>.

Little biological damage is caused by the hydrated electron (e<sub>aq</sub>; Answer Choice A).

<sup>1</sup>O<sub>2</sub> is produced primarily by photosensitizers and, rarely, by ionizing radiation (Answer Choice B).

Neither OH<sup>•</sup> nor O<sub>2</sub><sup>•-</sup> are primary radiolysis products, although O<sub>2</sub><sup>•-</sup> can be produced secondarily by reaction of e<sub>aq</sub> with O<sub>2</sub> (Answer Choices C and E).

Mitchell JB, 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. Furthermore, with the photoelectric effect, absorption of photons is dependent on the cube of the atomic number of the material. The resultant differential of absorption in tissue allows for the ability to differentiate between bone, soft tissue, and air.

I-5) C The predominant atomic interaction for 100 keV photons is the Compton process. Sources provide different answers on minimum energy for triplet production with some stating  $2mC^2$  (1.02 MeV) and some stating  $4mC^2$  (2.04 MeV) The photoelectric effect is predominant for photon energies in the range of 10 keV.

I-6) E High linear energy transfer (LET), or densely ionizing, radiations include particles such as 290 MeV carbon ions, GeV cosmic particles and neutrons. 250 kVp X-rays, 200 MeV protons and 1.1 MV X-rays are all low LET, or sparsely ionizing radiations (Answer Choice A).

Although high LET radiations produce more clustered lesions (multiply damaged sites) in DNA than low LET radiations (Answer Choice E), they actually produce lower yields of OH radicals because of the extensive ion and radical recombination within spurs and blobs (Answer Choice B).

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

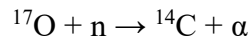
Goodhead DT. The initial physical damage produced by ionizing radiations. *Int J Radiat Biol* 56: 623-34, 1989. [Pubmed](#)

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.

1-8) B Annihilation photons always have an energy of 0.511 MeV each, which is equal to the rest energy of the positron and electron.

1-9) D Neutrons are not charged particles and, therefore, cannot ionize atoms directly. They do, however, transfer some of their energy to protons or light nuclei, which then cause ionization. They are, therefore, indirectly ionizing.

- 1-10) B Fast neutrons with kinetic energy between a few and several tens of MeV are slowed down in biological media mainly by elastic collisions with hydrogen nuclei (protons) of the cellular water. A fraction of energy lost by fast neutrons in elastic collision with oxygen nuclei is less than 10% of that which occurs with hydrogen nuclei. Neutron beams used in radiation therapy can be made using a cyclotron by accelerating protons into a beryllium target. The fast neutrons then recoil protons in target tissue from elastic collisions and produce a large density of ionizations along their tracks. Neutrons do not interact with atomic electrons but, instead, interact with atomic nuclei. Alpha particles can be produced by neutron capture reactions with isotopes of both carbon and oxygen, but the probability is strongly dependent on the neutron energy and target material. Example:

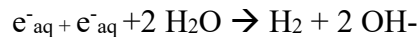


Neutron absorption in a target nucleus is called activation. This is a process by which neutron radiation induces radioactivity in materials. It occurs when atomic nuclei capture free neutrons, becoming heavier and entering excited states. The excited nucleus often decays immediately by emitting gamma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

- 1-11) 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<sup>-</sup><sub>aq</sub>) (45%). These react with DNA or with each other. Therefore,



The remaining recombination reactions of free radicals are:



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 therefore depend on LET. At low LET values, the spacing of the ionizations is large. As a result, •OH radicals are widely separated thereby decreasing the probability of recombination

to form  $\text{H}_2\text{O}_2$ . As LET increases, the spacing between ionizations decreases and the probability of production of an  $\bullet\text{OH}$  from one ionization event as well as an  $\bullet\text{OH}$  from another ionization event along a single track increases. The yield of hydrogen peroxide increases rapidly with LET of about 20 - 150 keV/ $\mu\text{m}$ , the range of LET where direct damage to DNA dominates over indirect damage from the free radicals. Note that LET for photons is in the  $\sim 1$  keV/ $\mu\text{m}$  range, while protons are approximately 10 keV/ $\mu\text{m}$ , carbon atoms are 10-100 keV/ $\mu\text{m}$ , and alpha particles or heavy charged particles are  $>100$  keV/ $\mu\text{m}$ .

Gray LH. The initiation and development of cellular damage by ionizing radiation. *Brit J Radiol.* 26:609-618, 1953. [Pubmed](#)

- I-12) 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) The SF<sub>2</sub> (surviving fraction at 2 Gy) for an irradiated population of cells is most closely correlated with the:
- A. Level of  $\gamma$ -H2AX 30 minutes after irradiation
  - B. Level of  $\gamma$ -H2AX present 24 hours after irradiation
  - C. Acetylation of H2AX on lysine 4
  - D. Rate of DNA single-strand break repair
  - E. Rate of thymine glycol repair
- II-2) Which of the following statements about ionizing radiation (IR) induced DNA damage is correct?
- A. IR causes only DNA double-strand breaks
  - B. IR may produce thymine glycols, but much less frequently than DNA double strand breaks
  - C. IR can cause more clustered lesions at low dose rates than at high dose rates
  - D. IR cannot cause oxidization of nucleotide bases
  - E. IR is unlikely to produce pyrimidine dimers
- II-3) A clustered lesion:
- A. Results from the creation of multiple DNA double-strand breaks (DSBs) within a particular exon of a gene following exposure to 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 (DSBs) in cells immediately following exposure to ionizing radiation?
- A. Alkaline elution
  - B. Western blotting
  - C. Neutral comet assay
  - D. PCR
  - E. BrdU incorporation 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 ( $\gamma$ H2AX) foci can be detected within 15 minutes of radiation exposure
  - D. p53 forms ATR-dependent foci within minutes of radiation exposure
  - E. The remaining  $\gamma$ H2AX foci within the nucleus at 24 hours after irradiation is not related to the cellular sensitivity radiation.
- II-6) Which of the following has been shown to be a reliable surrogate marker for DNA double strand breaks (DSBs) in the cells?
- A. Phosphorylated histone variant H2AX (or gammaH2AX)
  - B. Degraded histone H2AX
  - C. Dephosphorylated H2AX
  - D. Cleavage of Caspase 3
  - E. DNA methylation

## II. Molecular Mechanisms of DNA Damage Answers & Explanations

- 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 ( $\gamma$ -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  $\gamma$ -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<sub>2</sub>, the cell surviving fraction after 2 Gy, is a model-independent measure of radiation sensitivity. The numbers of phosphorylated  $\gamma$ -H2AX foci shortly after the irradiation represent the initial level of DNA damage, but the number of phosphorylated  $\gamma$ -H2AX foci at 24 hours after irradiation represent the residual level of unrepaired DNA double strand break at this time. 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 (UV) wavelength range and are not produced by X-rays. Pyrimidine dimers are cytotoxic, but more of these DNA lesions are required in order to achieve cell death compared to the DNA lesions produced by X-rays. It is estimated that the number of DNA lesions per cell from X-rays necessary to kill 63% of the cell population (thereby allowing 37% to survive) is 40 double-stranded DNA breaks (DSBs). In comparison, 1,000,000 pyrimidine dimers from ultraviolet radiation are needed to kill

63% of the cell population. IR can produce not only DSBs, but also other forms of damage including single strand breaks, thymine glycols, and base damage. These other forms of DNA damage, however, are more readily repaired and are less likely to result in cell death.

- 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 (DSBs). The comet assay is the electrophoresis of single-cells in order to detect DNA damage and its repair. Cells are exposed to ionizing radiation, embedded in agarose, and then subjected to an electrical gradient to move the DNA into the gel. The negatively charged DNA in the cell moves through the agarose toward the positive electric pole. If there are no breaks, the cell's DNA moves all together in a small ball. Double-strand DNA breaks create DNA fragments that are smaller than the unbroken DNA and migrate further into the agarose making what appears like a comet's tail. Alkaline conditions cause the separation of the two strands of the DNA helix and allows the visualization of DNA fragments created by both double-strand and single-strand DNA breaks. In neutral pH conditions, the DNA helix is intact so single-strand breaks do not result in separate fragments and you can only see the fragments created by double-strand DNA breaks.

Alkaline elution is used to measure single-strand breaks and some base damages (Answer Choice A)

Western blotting is for detection of proteins (Answer Choice B). Polymerase chain reaction (PCR) is used to amplify DNA sequences (Answer Choice D). The BrdU incorporation assay measures the amount of new DNA synthesis (Answer Choice E).

- 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. An elevated level of remaining  $\gamma$ -H2AX foci at an extended time (such as 24 hours) after the initial DNA damage is indicative of an impaired DNA repair and cell sensitivity to radiation.

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

Kinner A, et al. 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 level of phosphorylated H2AX has been shown to correlate with the level of DNA double strand breaks.

### 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 the other events 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<sub>1</sub> 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. Impact 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. Adds a new nucleotide to the end of DNA during DNA synthesis.
  - C. Produces nicks within intact DNA strands
  - D. Generates new species of mRNA
  - E. Removes nucleotides from the ends of DNA strands
- III-11) Which of the following statements is CORRECT? Base excision repair (BER):
- A. May increase mutation rate when defective, but usually does not dramatically alter cellular radiosensitivity
  - B. Is the principal pathway responsible for the repair of UV-induced DNA damage
  - 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 DSBs 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.
  - E. The cell cycle status does not affect the choice between HR and NHEJ to repair a DSB.



- III-13) Chemotherapeutic agents frequently produce DNA double-strand breaks (DSBs) by causing stalling and collapse of DNA replication forks. Which of the following pathways has a dominant role in 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) Which of the following statements 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
- III-16) Which of the following gene mutations would be expected to cause the greatest increase in sensitivity after exposure to a DNA damaging agent that induces double-strand breaks (DSBs)?
- A. DNA-PKcs null mutation
  - B. P53 null mutation
  - C. Activating K-Ras mutation
  - D. MLH1 nonsense mutation
  - E. XRCC1 null mutation

- III-17) 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<sub>1</sub> phase arrest results from the inactivation of p21
  - C. A decrease in the amount of p53 can trigger apoptosis or G<sub>1</sub> 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
- III-18) Which statement is CORRECT concerning the ataxia telangiectasia-mutated (*ATM*) gene and Rad3-related (*ATR*) genes and proteins?
- A. Ionizing radiation induced phosphorylation of Chk1 requires either ATM or ATR.
  - B. ATM is recruited to double strand breaks by the Mre11-Rad50-Mbs1 complex
  - C. ATR activation and Chk1 phosphorylation occurs prior to ATM activation
  - 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

### III. Molecular Mechanisms of DNA Repair Answers & Explanations

- III-1) B Transcriptional transactivators increase the expression of a protein by binding to the promoter region of the target gene and turning on transcription. 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 (Answer Choices C and D).

TRAIL is a ligand that induces cell death through the extrinsic apoptosis pathway (Answer Choice E).

Batchelor E, et al. The ups and downs of p53: understanding protein dynamics in single cells. *Nat Rev Cancer*. 9:371-7, 2009. [Pubmed](#)

Murray-Zmijewski F, et al. A complex barcode underlies the heterogeneous response of p53 to stress. *Nat Rev Mol Cell Biol*. 9:702-12, 2008. [Pubmed](#)

Szumiel I. Intrinsic radiation sensitivity: Cellular signaling is the key. *Radiat Res*. 169:249-258, 2008. [Pubmed](#)

Sengupta S and Harris CC. p53: traffic cop at the crossroads of DNA repair and recombination. *Nat Rev Mol Cell Biol*. 6:44-55, 2005. [Pubmed](#)

- III-2) E Phosphorylation of histone H2AX to  $\gamma$ -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<sub>1</sub> 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<sub>2</sub> than in G<sub>1</sub>. In G<sub>1</sub>, 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 (Answer Choice B).

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; Answer Choice C).

CHK1 is a serine/threonine protein kinase and a key mediator of the DNA damage-induced checkpoint pathway (Answer Choice D).

TFIIH is associated with nucleotide excision repair (Answer Choice E).

Iijima K, *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 and 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, et al. Repair of radiation damage to DNA. *Br J Cancer*. 90:1297-1301, 2004. [Pubmed](#)

- III-5) D Inhibition of non-homologous end joining (NHEJ) 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 (Answer Choice A).

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 (Answer Choice B).

Normal tissue tolerance doses would likely decrease due to radiosensitization (Answer Choice C).

Sublethal damage recovery would be inhibited since this process depends at least in part on the repair of double-strand breaks (Answer Choice E).

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 (Answer Choice A).

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 (Answer Choice D).

DNA-PKcs phosphorylate 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 (Answer Choice C and E).

Weterings E and Chen DJ. The endless tale of non-homologous end-joining. *Cell Res*. 18:114-124, 2008. [Pubmed](#)

van Gent DC and 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 (NER). Mutations in *XPC* result in the human genetic disease xeroderma pigmentosum, which is 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, including Nijmegen breakage syndrome (*NBS1*), familial breast cancer (*BRCA1*), ataxia telangiectasia (*ATM*), and ataxia telangiectasia-like disorder (*MRE11*).

Pollard JM and 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 and Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol*. 9:297-308, 2008. [Pubmed](#)

Helleday T, et al. DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer*. 8:193-204, 2008. [Pubmed](#)

Digweed M and Sperling K. Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks. *DNA Repair*. 3:1207-1217, 2004. [Pubmed](#)

Taylor AM, et al. 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 (NHEJ) 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 radiation (Answer Choice A).

Base excision repair (BER), not mismatch repair, involves the action of a DNA glycosylase and an AP endonuclease (Answer Choice C).

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 (Answer Choice D).

The immune deficient phenotype in SCID mice is caused by a defect in XRCC7 (DNA-PK<sub>cs</sub>), which is critical for NHEJ as well as V(D)J rejoining. As a result, a defect in XRCC7 leads to a radiosensitive phenotype as well as the immune deficits seen in the SCID mouse. Defects in several genes are now known to cause SCID phenotypes; the mutation in the common human disease of the same name (severe combined immunodeficiency) 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, 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. XPA/XPG are involved in nucleotide excision repair (Answer Choice D). DNA Ligase IV, Ku70, and DNA-PKcs all play roles in NHEJ (Answer Choices B, C, and E).

Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol.* 7:335-46, 2006. [Pubmed](#)

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

Ligases seal breaks in the DNA strand (Answer choice A). DNA polymerases add a new nucleotide to the end of DNA during DNA synthesis (Answer choice B). Endonucleases produce nicks within intact DNA strands (Answer choice C). RNA polymerase generates new species of mRNA (Answer choice D).

- III-11) A Defects in base excision repair (BER) may increase mutation rate but generally do not alter cell survival after ionizing radiation with the exception of mutation of the *XRCC1* gene, which would confer a slight increase in radiation sensitivity, as it is also involved in single-stranded DNA break repair.

Defects in nucleotide excision repair (NER) increase sensitivity to UV radiation but not to ionizing radiation (Answer Choice B).

The xeroderma pigmentosum (*XP*) and Cockayne Syndrome (*CS*) genes are involved in NER (Answer Choice C).

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 (Answer Choice D).

The gene defective in most patients with Li-Fraumeni Syndrome is *p53*, although some patients with that condition have mutations in *CHK2* (Answer Choice E).

Hegde ML, et al. 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, et al. 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 translesional DNA synthesis (TLS), nucleotide excision repair (NER), and homologous recombination (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 and 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 endonucleolytic 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 is a genetic disorder due to mutation in genes involved in nucleotide excision repair (NER). This phenotype is characterized by extreme sensitivity to ultraviolet light.

- III-15) D BRCA1 and BRCA2 predominantly regulate homologous recombination (HR) as opposed to non-homologous end joining (NHEJ). Fewer than 10% of patients with breast cancer are found to have mutations in these breast cancer susceptibility genes. Due to the negative effect that BRCA1 mutation has on HR, BRCA1-deficient cells are more SENSITIVE, not resistant, to mitomycin C and other DNA crosslinking agents (Answer choice A). The prevalence of BRCA1 mutation is slightly lower than that of BRCA2 mutations in the general US population (1:500 for BRCA1 vs 1:222 for BRCA2). The breast cancer risks for carriers of BRCA1 and BRCA2 mutations are similar at about 70% by the age of 80, but with earlier age of disease onset for the BRCA1 mutation (peaks in 4<sup>th</sup> decade) compared to BRCA2 carriers (peaks in 5<sup>th</sup> decade).

Kara N. Maxwell, Susan M. Domchek, Katherine L. Nathanson, and Mark E. Robson. Population Frequency of Germline BRCA1/2

Mutations. *Journal of Clinical Oncology* 2016 34:34, 4183-4185

Kuchenbaecker KB, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017 Jun 20;317(23):2402-2416. PMID: 28632866.

III-16) A Among the 5 genes listed, all may affect radiation or DSB sensitivity at different levels. However, DNA-PKcs is directly involved in DSB repair and DNA-PKcs mutation is known to cause hypersensitivity to DSB damage and radiation.

III-17) 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<sub>1</sub> arrest results from transactivation of p21 by p53. An *increase* in the amount of p53 can result in apoptosis or G<sub>1</sub> 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.

III-17) B The ATM protein contains a highly conserved C-terminal kinase domain resembling a phosphatidylinositol-3-kinase (PI(3)K); this kinase is an important component of a number of DNA damage repair pathways. Both ATM and ATR are required for IR-induced Chk1 phosphorylation. ATM is recruited to double strand breaks by the Mre11-Rad50-Nbs1 complex. ATR is recruited to single stranded DNA at sites of stalled replication forks by ATR-interacting protein (ATRIP).. 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. ATM activation and Nbs1 recruitment to damaged DNA occurs prior to ATR recruitment and Chk1 phosphorylation.

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

Jazayeri A, Falck J, Lukas C. *et al.* ATM-and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. PMID: 16327781

#### 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 are 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 most reliable 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 terminal deletion
  - 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<sub>0</sub>/G<sub>1</sub> 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<sub>1</sub> 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<sub>2</sub> 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

#### IV. Chromosome and Chromatid Damage Answers & Explanations

- 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 (Answer Choice A).

Translocations can be stable aberrations since they do not necessarily lead to cell death (Answer Choice B).

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 (Answer Choice C).

The minimum dose that can be detected through scoring dicentric chromosomes is roughly 0.25 Gy (Answer Choice E).

Brasemann 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 (Answer Choice A).

Chromatid gaps appear as loss of genetic material from a single chromatid arm and may be caused by incomplete breaks (Answer Choice B).

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 (Answer Choice C).

A quadriradial is a chromatid-type aberration that may arise from illegitimate interchromosomal recombination, accompanied by crossing-over (Answer Choice D).

- 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, et al. Micronuclei: a biological indicator of radiation damage. *Mutat Res.* 366(2):163-169, 1996. [Pubmed](#)

- IV-4) E Chromatid type aberrations are produced in cells only when irradiation follows DNA synthesis in S phase. In this case, only one of the two chromatid has the anomaly. Anaphase bridges are caused by chromatid type aberrations. Chromosomal type aberrations are produced when irradiation occurs prior to DNA synthesis and the anomaly is duplicated on both chromatids of the chromosome. Examples included dicentric or ring chromosomes.

- 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 (Answer Choice A).

A dicentric is an unstable aberration since it results in the formation of an acentric fragment and ultimately causes cell death (Answer Choice B).

Breaks in two chromatids, followed by illegitimate rejoining, produce an anaphase bridge (Answer Choice C).



The yield of dicentric chromosomes increases with increasing dose-rate for low LET radiation (Answer Choice E)

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, an autosomal recessive disorder, are harboring a mutation in one of 22 FANC genes, which are part of the FA/BRCA pathway. Defects in this pathway result in chromosomal instability due to decreased capacity to repair interstrand DNA crosslinks. This results in the development of high numbers of chromosome aberrations, especially quadriradials. These complex aberrations increase dramatically with exposure to DNA cross-linking agents such as mitomycin c.

García-de-Teresa B, Rodríguez A, Frias S. Chromosome Instability in Fanconi Anemia: From Breaks to Phenotypic Consequences. *Genes (Basel)*. 2020 Dec 21;11(12):1528. PMID: 33371494

## 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<sub>1</sub> 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  $\gamma$ -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 TNF $\alpha$  (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 resulting 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 nearby 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. The U.S. Food and Drug Administration–approved anti-malarial drugs chloroquine and hydroxychloroquine are inhibitors of autophagy
  - C. Autophagy contributes to cellular metabolism by degradation of damaged protein aggregates and organelles
  - D. Mitophagy refers to autophagy in mitotic cells
  - E. Autophagy is controlled by the Atg family of proteins
- V-12) Which of the following is NOT a feature of apoptosis?
- A. Chromatin condensation
  - B. Cell shrinkage
  - C. DNA fragmentation
  - D. Rapid engulfment by neighboring cells
  - E. Pro-inflammatory response

V-13)

Which of the statements is TRUE regarding the 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 the execution of apoptosis are also involved in the execution of necrosis
- D. Bcl2 is a pro-apoptotic protein.
- E. Anti-apoptotic Bax dimerizes and translocates to the mitochondria.

## V. Mechanisms of Cell Death Answers & Explanations

- 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](#)

McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. *Cold Spring Harb Perspect Biol*. 7(4). Pii: a026716, 2013. [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<sub>1</sub> 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.  $\gamma$ -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) C The term “senescence” refers to the loss of cellular replicative potential leading to a reduced capability to repopulate a tissue after exposure to genotoxic agents, including ionizing radiation. Senescence is most often the result of a permanent arrest in G<sub>1</sub>, associated with elevated expression of the cell cycle inhibitors p16<sup>INK4A</sup> (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. Depending on the level of tumor suppressor proteins and the oncogenic signal, senescence can be reversible in a small subset of cells though in most cells this process is irreversible.

A clinically relevant scenario for radiation-induced senescence is the loss of salivary gland function and xerostomia commonly seen in head and neck cancer patients undergoing radiotherapy. Another one is radiation-induced premature senescence in fibroblasts that triggers proinflammatory and profibrotic senescence associated secretory phenotype (SASP) and ultimately drives fibrosis in the lung.

Mitochondrial dysfunction is a hallmark of apoptotic cell death, not senescence (Answer Choice B).

Telomere shortening occurs in most normal somatic cells as part of each cell cycle (“end replication problem”) and triggers senescence once a critical low threshold is reached, but telomere shortening tends not to be the cause for radiation-induced senescence which is driven by DNA-damage and cell cycle arrest (Answer Choice D).

Nutrient deprivation can lead to autophagy, and ultimately autophagic death cell distinct from apoptosis (Answer Choice A).

Ohtani N, Mann DJ, Hara E. Cellular senescence: its role in tumor suppression and aging. *Cancer Sci.* 100:792-7, 2009. [Pubmed](#)

Munoz-Espin D and Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Biol.* 15(7):482-96, 2014. [Pubmed](#)

Kuilman T, *et al.* The essence of senescence. *Genes Dev.* 24:2463–2479, 2010. [Pubmed](#)

Nguyena HQ, et al. Ionizing radiation-induced cellular senescence promotes tissue fibrosis after radiotherapy. A review. *Crit Rev Oncol Hematol*. 129:13-26, 2018. [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 such as the adapter molecule Fas-associated death domain (FADD) within the death-inducing signalling complex (DISC). Upon recruitment and oligomerization FADD then binds procaspases-8 and -10, causing their homodimerization and activation.

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.

In most cases, activated caspase-8 induces apoptosis through activation of pro-caspase-3 at the DISC independently of mitochondria. However, in some cells, especially when only a low amount of active caspase-8 is generated (and hence not sufficient amounts of pro-caspase-3), caspase-8 cleaves the 'Bcl-2 homology (BH) 3-only protein' Bid, generating an active fragment (tBid) that activates the (intrinsic) mitochondrial death pathway. In this manner, the extrinsic death signal may be amplified through formation and activation of the apoptosome which contributes to effector caspase activation. In other words, the extrinsic pathway can feed into the intrinsic one and additionally change mitochondrial membrane potential.

Cotter TG. Apoptosis and cancer: the genesis of a research field. *Nat Rev Cancer* 9:501-7, 2009. [Pubmed](#)

Maier P, et al. Cellular Pathways in Response to Ionizing Radiation and Their Targetability for Tumor Radiosensitization. *Int J Mol Sci*. 17(1):. Pii: E102, 2016. [Pubmed](#)



Gupta S, et al. The mitochondrial death pathway: a promising therapeutic target in diseases. *J Cell Mol Med.* 13(6):1004-1033, 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. In fact, uptake of apoptotic cells by macrophages can actually lead to the release of anti-inflammatory mediators such as TGF- $\beta$  and IL-10, and the attenuation of the RIG-I/IRF-3 pathway and the cGAS/STING pathway through proteolytically inactivating RIP kinase 1 or the degradation of cytoplasmic DNA. As a result, apoptosis can decrease the expression of interferons and other inflammatory factors.

Of note, the concept that apoptosis is entirely non-inflammatory isn't always strictly true. An example is the induction of apoptosis in hepatocytes following FAS activation that causes a strong inflammatory response probably because they can't get cleared fast enough by phagocytes.

The exposure of phosphatidylserines (phospholipids) on the exterior of the plasma membrane is the signal that initially recruits phagocytes. Ordinarily, phosphatidylserine is sequestered on the inner leaflet of 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.

Miyaniishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. *Nature* 450:435-9, 2007. [Pubmed](#)

Rock KL and Kono H. The inflammatory response to cell death. *Annu Rev Pathol.* 3:99-126, 2011. [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, and 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. BCL2 family members can be either pro- or anti-apoptotic. 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 multinucleated giant cells that contain uncondensed chromosomes. Mitotic death can be of any molecular mechanism, including apoptosis or necrosis.

Single strand breaks are repaired rapidly and do not appear to play an important role in cell lethality (Answer Choice A).

DNA ladder formation is characteristic of apoptosis (Answer Choice B).

An alteration in cell permeability occurs in cells undergoing necrosis (Answer Choice E).

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](#)

Kroemer G et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. *Cell Death Differ.* 16(1): 3–11, 2009. [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.

Autophagy ('self-eating') tends to refer to macroautophagy: the sequestration process of cytoplasmic material for degradation. (Microautophagy and chaperone-mediated autophagy are other types.) After initiation, an isolation membrane encloses a small portion of cytoplasmic material, including damaged organelles and unused proteins, to form a double-membraned structure called an "autophagosome" that subsequently fuses with lysosomes to become an "autolysosome", in which the cytoplasmic material is degraded by lysosomal enzymes.

The whole process is tightly regulated through at least 30 Atg-autophagy related genes that orchestrate initiation, cargo recognition, packaging, vesicle nucleation expansion and fusion and breakdown. The initial steps center around the Atg1 complex (Atg1–Atg13–Atg17– Atg29–Atg31) that translocates to the ER, (thought to be the major membrane source for autophagy). This leads to recruitment of the autophagy-specific form of the class III PI(3)K complex, which includes Vps34, Vps15, Atg6/Beclin-1 and Atg14, to the ER. To form an autophagosome, elongation and closure of the isolation membrane requires 2 protein conjugation systems, the Atg12–Atg5–Atg16 complex and the Atg8/LC3–phosphatidylethanolamine (PE) complex. Detection of autophagy relies on the redistribution of GFP-LC3 fusion proteins into vesicular structures (which can be autophagosomes or autolysosomes).

'Autophagic cell death' is the excessive version of autophagy, that occurs in the absence of chromatin condensation. In contrast to apoptotic cells, there is little or no association of autophagic cells with cells phagocytes. Although the expression 'autophagic cell death' is a linguistic invitation to believe that cell death is executed by autophagy, the term simply describes cell death with autophagy.

Kroemer G et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. *Cell Death Differ.* 16(1): 3–11, 2009. [Pubmed](#)

Doherty J and Baehrecke EH. Life, death and autophagy. *Nature Cell Biology.* 20(10):1110–1117, 2018. [Pubmed](#)

- V-12) E There is no pro-inflammatory response in pure apoptosis. Apoptosis can in fact be anti-inflammatory through its recruitment of macrophages via “find-and-eat-me” signals; macrophages in turn produce anti-inflammatory TGF- $\beta$ . Pro-inflammatory forms of cell death include necrosis, necroptosis, and pyroptosis.

Bertheloot D, Latz E, Franklin B. Necroptosis, pyroptosis, and apoptosis. *Cellular & Molecular Immunology* 18:1106-1121, 2021. PMID: 33785842

- V-13) B Fas ligand binds its receptor and triggers the external death receptor pathway.

Cleavage of PARP-1 by caspases is considered to be a biochemical hallmark of apoptosis (Answer Choice A).

Different caspases play different roles in the initiation and execution of apoptosis and are not involved in necrosis. Necrosis is the unregulated digestion of cellular components as a result of external factors (Answer Choice C).

Bcl-2 inhibits apoptosis while Bax stimulates apoptosis (Answer Choices D and E).

## 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<sub>50</sub>
  - 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  $\alpha=0.3 \text{ Gy}^{-1}$  and  $\beta=0.1 \text{ Gy}^{-2}$ )?
- A. 0.01
  - B. 0.10
  - C. 0.37
  - D. 0.50
  - E. 0.90
- VI-3) For the same  $\alpha$  and  $\beta$  values 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 radiation doses
  - C. A cell line capable of multiple cell divisions
  - D. Intact apoptosis pathways
  - E. Nonirradiated control

## VI. Cell and Tissue Survival Assays Answers & Explanations

- VI-1) B A functional endpoint for radiation response is a measured endpoint that is downstream of clonogenic survival and may involve measurement of tissue/organ function, the incidence of toxicity, or whole animal survival. Clonogenic endpoints directly measure the replicative capacity of cells (e.g., colony formation). 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.

Hall and Giaccia. *Radiobiology for the Radiologist*, 7<sup>th</sup> Edition. Chapter 19. p306.

Withers, HR. The dose-survival relationship for irradiation of epithelial cells of mouse skin. *Radiation Res.* 40(471): 187-94. 1967. [Pubmed](#) 1967 PMID: 6019041.

- 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  $\beta$  component of the LQ equation would therefore approach zero, leaving the  $\alpha$  component to dominate. The surviving fraction can therefore 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. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 14:213-222, 1961. [Pubmed](#)

- VI-5) D The clonogenic survival assay measures the ability of single cells to divide continuously after a given exposure, and 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. Multiple 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 cell death pathway.

## 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  $\alpha/\beta$  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.  $D_q$
  - E. Dose at which the  $\alpha D$  component of cell kill is equal to the  $\beta D^2$  contribution to cell killing
- VII-3) Cells from individuals diagnosed with which of the following diseases/syndromes would not be expected to have increased sensitivity to X-ray irradiation?
- 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  $\beta$  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  $\gamma$ -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 INCORRECT?
- 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 alpha-beta 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
- VII-11) 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
- VII-12) 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

VII-13) 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

## VII. Models of Cell Survival Answers & Explanations

- 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  $\alpha/\beta$  ratio represents the dose at which the  $\alpha D$  component of cell killing, assumed to result from single hit killing, is equal to the lethality produced by the  $\beta D^2$  component of cell killing that results from multi hit killing.
- VII-3) D Cells derived from an individual diagnosed with xeroderma pigmentosum are defective in nucleotide excision repair (NER). These cells are sensitive to UV radiation because this form of radiation produces damage 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 because DNA double-strand break repair is generally normal in cells derived from a person diagnosed with xeroderma pigmentosum, their cells are not expected to be especially sensitive to X rays. 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, their cells would be expected to be sensitive to X rays.
- VII-4) E Parameters to define a radiation cell survival curve include: the initial slope ( $D_1$ ), a final slope ( $D_0$ ), and some quantity that is a measure of the width of the shoulder. This quantity can be the extrapolation number ( $n$ ) or the quasi-threshold dose ( $D_q$ ). If  $n=1$ , the survival curve has no shoulder and  $D_{37}$  (dose resulting in a survival fraction of 0.37) equals the  $D_0$ .

$D_0$  is a measurement of radiosensitivity based on the exponential part of the survival curve. In a target theory, it can be defined as a dose that gives an average of one lethal hit per cell in a population. Thus, an increase of an exposed dose of  $D_0$  would result in a 37% further reduction of survival. A smaller  $D_0$  value indicates a higher sensitivity to radiation.

For the same radiation dose, radiation delivered at a lower dose rate may produce less cell killing than radiation delivered at a higher dose rate because sublethal damage repair occurs during the protracted exposure.

As the dose rate is reduced, the slope of the survival curve becomes shallower and the shoulder tends to disappear because  $\alpha$  does not change significantly but  $\beta$  trends to zero in the linear quadratic model.

The inverse of the  $D_0$ , not the  $D_q$ , is equal to the final slope of the survival curve.

For densely ionizing radiation (increasing LET), the shoulder of the survival curve tends to disappear.  $N$ , therefore, decreases, until it reaches 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. Little to no reoxygenation of hypoxic cells is therefore likely during irradiation 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 (Answer Choice B).

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; Answer Choice C).

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; Answer Choice D).

- VII-6) C For 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 ( $S = e^{-\alpha D}$ ), or the simplified form of the linear quadratic equation in which  $\beta$  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 the survival of:  $(10^8 \text{ cells}) \times (10^{-6} \text{ surviving fraction}) = 10^2 \text{ cells}$ .

- VII-7) B An exponential survival curve can be modeled using the single-target, single-hit equation ( $S = e^{-\alpha D}$ ), or the simplified form of the linear quadratic equation in which  $\beta$  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  $S = e^{-D/D_0}$  (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  $\beta$  term of the linear-quadratic equation must approach zero, so the  $\alpha/\beta$  would be very high, and in fact will be undefined if the  $\beta$  term is actually zero.
- VII-9) D  $eD_{10}$  is the dose required to kill 90% of population
- $$eD_{10} = 2.3 \times D_0$$
- The  $eD_{10}$  is equal to the  $eD_0$  multiplied by 2.3, or 4 Gy  $\times$  2.3 = 9.2 Gy
- VII-10) C When the irradiated cell population receives an average of 1 lethal hit, it results in 37% cell survival based on Poisson statistics.
- VII-11) 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 effective survival curve is exponential with a  $D_{10}$  of 8 Gy, it would be necessary to use a dose of 64 Gy.
- VII-12) 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  $10^{-6}$ .

- VII-13) 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.

## 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/ $\mu\text{m}$
  - B. RBE changes the most over the LET range of 0.1 to 10 keV/ $\mu\text{m}$
  - C. The relationship between OER and LET is bell-shaped
  - D. RBE decreases with increasing LET above about 100 keV/ $\mu\text{m}$
  - 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. Is greater for high LET particles in hypoxic cells as compared to oxygenated cells of the same type
  - C. Is diminished for carbon ions when delivered over several fractions as compared to a single dose
  - D. Is greatest for heavy charged particles at the beginning of the particle track
  - E. Increases for MeV alpha-particles with increasing dose
- VIII-4) Which of the following pairs of radiation type and approximate LET value is CORRECT?
- A. 150 MeV protons – 0.5 keV/ $\mu\text{m}$
  - B. 1 GeV Fe ions – 20 keV/ $\mu\text{m}$
  - C.  $^{60}\text{Co}$   $\gamma$ -rays – 15 keV/ $\mu\text{m}$
  - D. 2.5 MeV  $\alpha$ -particles – 5 keV/ $\mu\text{m}$
  - E. 250 kV X-rays – 10 keV/ $\mu\text{m}$

- 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) of 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  $55 \text{ keV}/\mu\text{m}$  silicon ion along a  $1 \mu\text{m}$  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 uniformly spaced along the silicon ion track
- A. 0.5 cluster every  $1 \mu\text{m}$  or 1 cluster every  $2 \mu\text{m}$
  - B. 5.5 clusters every  $1 \mu\text{m}$
  - C. 500 clusters every  $1 \mu\text{m}$
  - D. 5,500 clusters every  $1 \mu\text{m}$
  - E. 55,000 clusters every  $1 \mu\text{m}$

## VIII. Linear Energy Transfer Answers & Explanations

- VIII-1) D RBE *decreases* with increasing LET above approximately 100 keV/ $\mu\text{m}$ . 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/ $\mu\text{m}$ , not 1000 keV/ $\mu\text{m}$  (Answer Choice A).

RBE shows the greatest changes for LET values between roughly 20 and 100 keV/ $\mu\text{m}$  (Answer Choice B).

OER decreases slowly with increasing LET for low LET values, but falls rapidly after LET exceeds about 60 keV/ $\mu\text{m}$  and, therefore, does not follow a bell-shaped curve (Answer Choices C and E).

- 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 sulphhydryl 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 Relative Biological Effectiveness (RBE) is defined as:

$$\frac{\text{Dose of Reference Radiation (250 keV X-Rays)}}{\text{Dose of Test Radiation to give the same biological effect}}$$

The reference radiation for calculation of RBE is low LET radiation, such as 250 keV X-rays or Co-60.

The dose of the reference radiation that will achieve the same level of cell killing as high LET particles in hypoxic cells will be *greater* because there is little to no oxygen effect for high LET radiation (Answer Choice B).

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 (Answer Choice A).

The RBE for carbon ions, or any other type of high LET radiation, is greater for fractionated irradiation compared with an acute exposure due

to the substantial sparing exhibited with reference X-rays with fractionation (Answer Choice C).

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 (Answer Choice D).

RBE does show a fractionation dependence; it decreases with increasing fraction size. The RBE for 4 MeV alpha particles will *decrease* with increasing dose because there is more sublethal damage repair with low-LET X-rays at lower doses, and therefore more survival compared with high-LET radiation (Answer Choice E).

- VIII-4) A 150 MeV protons have an LET of approximately 0.5 keV/μm. 1 GeV Fe ions, <sup>60</sup>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 Re.* 22(12): 1719-1723, 1998. [Pubmed](#)

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(5): 529-541, 1999. [Pubmed](#)

- VIII-5) D LET is a measure of local energy deposition along a track of medium. It is inversely proportional to the energy of a given charged particle. The local transfer of energy to medium is more probable at lower energies.

- VIII -6) 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. 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 collisions 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. Answer choice E mainly pertains to neutrons, not protons, where the average method and the energy method for calculating LET give significantly different numbers.

Naseri A and Mesbahi A. A review of photoneutrons characteristics in radiation therapy with high-energy photon beams. Reports of Practical Oncology and Radiotherapy. 15(5):138-144, 2010. [Pubmed](#)

- VIII-7) C On average, the formation of a three-ion cluster requires dissipation of 110 eV. Therefore,

$$\frac{55 \text{ keV}}{\mu} \times \frac{1000 \text{ eV}}{1 \text{ keV}} \times \frac{1 \text{ ion cluster}}{110 \text{ eV}} = \frac{500 \text{ ion clusters}}{\mu}$$

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 the DNA helix.

## 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  $\mu\text{m}$
  - C. 200  $\mu\text{m}$
  - D. 900  $\mu\text{m}$
  - E. 2.6 mm
- IX-2) A dose of 10 Gy of X-rays reduces the tumor cell surviving fraction to 0.001 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. 0.0001
  - B. 0.01
  - 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 partial pressure 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 divided by 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 reaction of O<sub>2</sub> with sulfhydryl compounds to form SOO
- 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 chemical repair of DNA
  - B. The OER and RBE both increase with increasing LET
  - C. Based on pO<sub>2</sub> microelectrode measurements, few human tumors contain regions of hypoxia
  - D. An oxygen partial pressure of about 30 mM Hg results in a radiosensitivity halfway between hypoxic conditions and fully oxygenated conditions.
  - 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 generally within the range of 5-50%
  - B. Hypoxia is rarely observed in common human solid tumors
  - C. Oxygen diffusion and delivery is limited in some parts of tumors
  - D. Hypoxia can enhance tumor progression by means of hypoxia-related changes in gene expression
  - E. Hypoxia induces gene amplification and mutation
- IX-9) Which chemical or compound CANNOT be used to mitigate hypoxia-related radioresistance?
- A. Nicotinamide and carbogen
  - B. Perfluorocarbon
  - C. Amifostine
  - D. Misonidazole
  - E. Nimorazole
- IX-10) Oxygen enhancement ratio (OER) changes depends on the type of radiation. Which of the following combinations is FALSE?
- A. OER 3.0 for x-rays
  - B. OER 1.6 for neutrons
  - C. OER 3.0 for protons
  - D. OER 0.5 for carbon ions
  - E. OER 1.0 for alpha-particles
- IX-11) Tirapazamine and other hypoxic cytotoxins have been in preclinical and clinical development. Which of the following statements is FALSE when describing the mechanisms and effects of tirapazamine?
- A. If it gains one electron in hypoxic conditions, it becomes cytotoxic
  - B. When two electrons are extracted in aerobic conditions, it becomes less toxic
  - C. In normoxic 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 chemotherapy agents can be enhanced by the presence of this cytotoxin

- IX-12) Which of the following statements about tirapazamine is FALSE?
- A. A trial examining the utility of the drug in the definitive treatment of locally advanced cervical cancer was conducted but failed to fully accrue due to lack of drug availability.
  - B. Addition of tirapazamine failed to improve 2-year overall survival in head and neck patients treated with cisplatin-based chemoradiation compared to chemoradiation alone without tirapazamine.
  - C. The use of tirapazamine was not associated with higher rates of esophagitis in limited stage small cell lung cancer patients treated with definitive chemoradiation compared to historical controls.
  - D. In GOG 219, tirapazamine increased gastrointestinal toxicity while having no effect on progression free survival.
  - E. In GOG 219, tirapazamine failed to show benefits in progression free survival but led to significant increase in leukopenia and hepatic/renal dysfunction.
- IX-13) Which of the following is TRUE regarding the use of the hypoxic radiosensitizer nimorazole in treating head and neck cancers with radiotherapy?
- A. The DAHANCA 5-85 trial showed improved locoregional control but not overall survival with the addition of nimorazole.
  - B. Low plasma concentrations of osteopontin were associated with worse outcomes compared to higher concentrations in the DAHANCA 5 trial but was also associated with a higher benefit from the addition of nimorazole.
  - C. The addition of nimorazole improves locoregional control in p16-positive tumors but not p16-negative tumors.
  - D. The addition of nimorazole to radiation is associated with increased acute mucositis and late fibrosis compared to placebo.
  - E. The DAHANCA 5-85 trial showed improved locoregional control but not disease-specific survival with the addition of nimorazole.

## IX. Modifiers of Cell Survival: Oxygen Effect Answers & Explanations

- 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 70-200  $\mu\text{m}$ . 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. Thomlinson and Gray measured 150  $\mu\text{m}$  in their landmark experiments in 1955. Olive et al. (IJROBP 1992) determined that the maximum oxygen diffusion distance using solid tumor cubes incubated with fluorescent probes and found it to range from 107  $\mu\text{m}$  to 192  $\mu\text{m}$ , depending on the cell line. Torres Filho et al. (Proc. Natl. AcadUSA 1994) measured *in vivo* oxygen concentration in a SCID mouse model and found hypoxia to occur at distances  $>200 \mu\text{m}$ .

Thomlinson and Gray. The histological structure of some human lung cancers and the possible implications for radiotherapy. *Br J Cancer*. 9(4): 539-49. 1955. [Pubmed](#) PMID: 13304213

Olive et al. Measurement of oxygen diffusion distance in tumor cubes using a fluorescent hypoxia probe. *Int J Radiat Oncol Biol Phys*. 22(3):397-402. 1992. [Pubmed](#) PMID: 1735668

Torres Filho et al. Noninvasive measurement of microvascular and interstitial oxygen profiles in a human tumor in SCID mice. *Proc Natl Acad Sci USA* 91(6): 2081-5. 1994. [Pubmed](#) PMID: 8134352

- 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, which is assumed to render all of the tumor cells radiobiologically hypoxic. Thus, the estimate for the fraction of hypoxic cells would be  $0.001/0.1 = 0.01$ .

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

Wouters BG and Brown JM. Cells at intermediate oxygen levels can be more important than the "hypoxic fraction" in determining tumor response to fractionated radiotherapy. *Radiat Res*. 147(5): 541-50. 1997. [Pubmed](#)

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. However it is important to know that as LET increases, OER values decrease.

An oxygen partial pressure greater than about 2-3% during irradiation will result in essentially full radiosensitization (Answer Choice B).

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 (Answer Choice C).

Both acutely and chronically hypoxia cells can reoxygenate (Answer Choice D).

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 (Answer Choice E).

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. [Pubmed](#)

IX-6) C Since the X-ray OER is typically about 3 and the OER for 15 MeV neutrons is about 1.6, 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-1 $\alpha$  (HIF-1 $\alpha$ ) (HIF1A).

Under normoxic conditions, HIF-1 $\alpha$  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-1 $\alpha$  and targets it for degradation.

Oxygen acts as a radiosensitizer principally through its ability to “fix” radiation-induced DNA damage but does not inhibit DNA repair (Answer Choice A).

The OER decreases with increasing LET, whereas the RBE increases with LET until reaching a maximum at approximately 100 keV/ $\mu$ m, and then decreases (Answer Choice B).

Measurements with pO<sub>2</sub> microelectrodes and bioreductive probes have demonstrated that hypoxic cells are often present in human tumors (Answer Choice C).

The  $K_m$  of radiosensitivity for cells (i.e., the concentration at which there is 50% radiosensitivity compared to oxic conditions) is about 3mm Hg (about 0.5%), not 30mm Hg.

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 in tumors has been detected using both imaging and direct electrode measurements. The other statements are true.

Moulder JE and Rockwell S. Hypoxic fractions of solid tumors: experimental techniques, methods of analysis, and a survey of existing data. *Int J Radiat Oncol Biol Phys.* 10(5):695-712, 1984. [Pubmed](#)

- IX-9) C Amifostine is a drug whose active metabolite contains a sulfhydryl moiety and acts as a free radical scavenger. It has been studied as a radioprotectant in several clinical and preclinical settings. As a radioprotectant, it does not sensitize hypoxic cells.

Carbogen is a mixture of 95% oxygen and 5% carbon dioxide and has been used to mitigate chronic hypoxia. Nicotinamide, used concurrently with carbogen, is intended to mitigate acute intermittent hypoxia seen in tumor vessels by preventing intermittent vessel closure (Answer Choice A).

Perfluorocarbons such as perflubron have been shown to improve tumor oxygenation in preclinical cancer models but have not yet shown clinical utility

Both misonidazole and nimorazole are nitroimidazoles that have radiosensitizing properties in hypoxia cells (Answer Choice E).

- IX-10) D OER for energized ions should be 1.0. By definition, OER cannot be smaller than 1.0.
- IX-11) D There is no difference in the uptake of the chemical by aerobic and hypoxic cells, but there is an obvious difference in the action of cell kill due to the amount of oxygen available in the cells.

Tirapazamine (TPZ) is a benzotriazine di-N-oxide which is reduced (i.e. gaining an electron) preferentially under hypoxia with the help of an intracellular reductase forming the reactive, TPZ radical as well as the very potent hydroxyl radical downstream. Hence Answer A is correct.

In contrast, under aerobic conditions, the initial TPZ radical is rapidly back-oxidized (i.e. losing electrons) to the parent compound losing its reactive potency, ultimately driving the selectivity for hypoxic cells (answer B is correct). A byproduct of this reversal is superoxide which may be responsible for the muscle cramps seen in patients.

Srini B Reddy & Stephen K Williamson (2009) Tirapazamine: a novel agent targeting hypoxic tumor cells, *Expert Opinion on Investigational Drugs*, 18:1, 77-87, DOI: 10.1517/13543780802567250

Carmen Avendaño, J. Carlos Menéndez (2008) Anticancer Drugs Acting via Radical Species, *Photosensitizers and Photodynamic Therapy of Cancer*, Medicinal Chemistry of Anticancer Drugs.

Brown JM. SR 4233 (tirapazamine): a new anticancer drug exploiting hypoxia in solid tumors. *Br J Cancer*. 67(6):1163-70, 1993.

[Pubmed](#)

- IX-12) C In the SWOG 0222 trial, tirapazamine was associated with higher rates of esophagitis compared to historical estimates. Tirapazamine was developed as a hypoxic cytotoxin to potentially enhance tumor responses and showed promise in single-arm phase II trials in several disease sites. However, the phase III trials failed to show efficacy above the control arms. These include GOG 219, which examined the efficacy of the drug when added to standard chemoradiation for locally advanced cervical cancer. The trial failed to accrue due to lack of drug availability. Of a planned accrual of 750 patients, only 379 were eligible and evaluable; no difference in overall or progression-free survival was seen, but there was

an increase in grade 3+ leukopenia, GI toxicity, and hepatic/renal dysfunction. The TROG examined tirapazamine's utility and safety in head and neck cancers in the HeadSTART TROG 02.02 Trial. There was no overall survival or failure-free survival benefit of tirapazamine when added to cisplatin-based chemoradiation in this trial. The authors reported more frequent muscle cramps, diarrhea, and skin rash in the experimental arm.

Le QT, et al. Phase II study of tirapazamine, cisplatin, and etoposide and concurrent thoracic radiotherapy for limited-stage small-cell lung cancer: SWOG 0222. *J Clin Oncol.* 27(18):3014-9, 2009. [Pubmed](#)

DiSilvestro PA, et al. Phase III randomized trial of weekly cisplatin and irradiation versus cisplatin and tirapazamine and irradiation in stages IB2, IIA, IIB, IIIB, and IVA cervical carcinoma limited to the pelvis: a Gynecologic Oncology Group study. *J Clin Oncol.* 32(5):458-64, 2014. [Pubmed](#)

- IX-13) A The DAHANCA 5-85 Trial examined the addition of nimorazole to conventionally fractionated radiotherapy for pharyngeal and supraglottic larynx cancers. The trial demonstrated that nimorazole improved locoregional control and disease-specific survival compared to placebo but did not significantly improve overall survival.

Nimorazole was reasonably well-tolerated in the DAHANCA 5-85 Trial, although only 60% of patients completed the treatment, and it was associated with a higher rate of nausea/vomiting, flushing, dizziness, and skin rash; patients also had trouble swallowing the large capsules of the drug. There was no increase in mucositis or late complications with the addition of nimorazole (Answer Choice D).

Additional subset analyses of the trial showed that **high** osteopontin concentration was associated with **worse** disease-specific mortality, but also improved response to nimorazole in terms of locoregional control and disease-specific mortality (Answer Choice B).

The benefit of nimorazole also appears to be isolated to p16-negative tumors, whereas p16-positive tumors did not appear to benefit (Answer Choice C).

Overgaard J, 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(2):135-46, 1998. [Pubmed](#)

Overgaard J, et al. Plasma osteopontin, hypoxia, and response to the hypoxia sensitiser nimorazole in radiotherapy of head and neck cancer: results from the DAHANCA 5 randomised double-blind placebo-controlled trial. *Lancet Oncol.* 6(10):757-64, 2005. [Pubmed](#)

Lassen P, et al. HPV-associated p16-expression and response to hypoxic modification in head and neck cancer. *Radiother Oncol.* 94(1):30-5, 2010. [Pubmed](#)



## 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 experimental manipulations would potentiate cell killing following radiotherapy as measured 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 the DNA prior to irradiation
  - E. Addition of cysteine to the cellular growth medium prior to 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) Compared to the surviving fraction of cells maintained in a non-cycling state for several hours after irradiation, decreased cell survival observed in cells forced to re-enter the cell cycle immediately following 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  $\alpha/\beta$  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 following treatment at the high dose rate
  - D. The difference in the surviving fractions between the two protocols results primarily from repopulation
  - E. The total number of ionizations produced is decreased with treatment at the high dose rate
- X-5) Exponentially growing cells were maintained at 37°C in 95% air/5% CO<sub>2</sub> 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 $\alpha$  (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 between the two doses increases
  - 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
- X-10) The survival of mammalian cycling cells at 37°C increases dramatically if a X-ray dose is split into two fractions, given within 1 hour of one another and reaches its maximum if the time between each fraction is about 2h. What is the mechanism driving this phenomenon?
- A. Enhanced survival in split-dose experiments such as this indicate recovery from potentially lethal damage.
  - B. The main mechanism driving this is the repair of sublethal damage in the G1 cell cycle phase.
  - C. The mechanism that enhances survival is sublethal damage repair that in cycling mammalian cells increases to a maximum between about 1-2h after the first exposure.
  - D. Potentially lethal damage repair tends to be complete by 1h and survival does not increase further by increasing time allowed beyond 1h.
  - E. The initial, dramatic benefit in survival is due to sublethal damage repair while potentially lethal damage repair drives the survival benefits at intervals of about 2h.

## X. Modifiers of Cell Survival: Repair Answers & Explanations

- X-1) D Bromodeoxyuridine, a synthetic nucleoside analogue, when incorporated into cellular DNA in place of thymidine acts as a radiation sensitizer, so cell killing would be enhanced.

S-phase is the most radioresistant phase of the cell cycle, so cell killing would be decreased relative to that for an asynchronous population (Answer Choice A).

Oxygen is a radiation sensitizer, so cell killing would decrease in cells made hypoxic before irradiation (Answer Choice B).

Splitting the dose into two fractions separated by 24 hours would allow sublethal damage recovery and possibly enable cellular proliferation to take place between fractions. Cell killing would therefore be less than if the total dose had been delivered acutely (Answer Choice C).

Cysteine is a sulfhydryl-containing compound that scavenges radiation-induced free radicals; it therefore acts as a radioprotector and reduces cell killing (Answer Choice E).

- X-2) A Therapeutic radiation at a low dose-rate of  $\sim 1$  Gy/hr is associated with increased cell survival compared to higher dose-rates primarily due to DNA repair - especially the repair of DNA double-strand breaks produced as a result of radiation.

- X-3) E Potentially lethal damage recovery is operationally defined as an increase in cell survival under environmental conditions not conducive to progression of cells through the cell cycle for several hours after delivery of a large, single radiation dose. 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, i.e. splitting the dose, as opposed to a single exposure (Answer Choice B).

Repair of DNA damage and rejoining of chromosome breaks presumably underlie both the sublethal and potentially lethal damage recovery (Answer Choice A).

Cell cycle reassortment has a sensitizing effect on a population of cells receiving multi-fraction or protracted irradiation regimens. This is

because surviving cells that were in a resistant phase of the cell cycle during the initial irradiation may progress through the cell cycle between fractions and reassert into a more sensitive phase of the cell cycle by the time of delivery of the next fraction. This process is irrelevant under the conditions described here, in which only a single radiation dose was administered (Answer Choice C).

Translesion DNA synthesis is an error-prone process during which certain DNA polymerases synthesize DNA using a damaged DNA strand as a template, resulting in error-prone DNA synthesis (Answer Choice D).

- 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 will not be able to occur during high dose rate irradiation. Essentially, the chance for separate lesions to interact with one another forming more complex, lethal lesions increases at the higher dose rate. As a result, the  $\beta$  component of cell killing will *increase*. More cell killing would therefore 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,  $\alpha/\beta$  ratio (Answer Choice A).

Treatment with an agent that inhibits DNA repair would have little impact during the 5-minute period of irradiation that would occur at the high dose rate (Answer Choice B).

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 (Answer Choice D).

The total number of ionizations produced is a reflection of the total dose delivered and does not vary with the dose rate (Answer Choice E).

- 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 sublethal damage repair (SLDR). Although SLDR also occurred when the interfraction interval was 8 hours, cells surviving the first dose start progressing through the cell cycle and reassert 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<sub>2</sub> and M), thereby 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 well-aerated 95% air environment.

- 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 (Answer Choice B). 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 by using an oxygen electrode or by treating with a hypoxia maker, such as pimonidazole, that is metabolized and incorporated exclusively into hypoxic cells (Answer Choice A).

Cell cycle age response is best demonstrated by performing cell synchronization followed by irradiation of cohorts of cells in particular cell cycle phases and then performing the clonogenic survival assay as a readout (Answer Choice C).

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 (Answer Choice E).

- X-7) B Ataxia Telangiectasis Mutated (ATM) serves as the central orchestrator of the signal transduction response to DSBs. Cells deficient in ATM activity display cell cycle checkpoint defects and sensitivity to ionizing radiation.

- X-8) C PLD is believed to be complex double strand breaks (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 Sublethal damage (SLD) is the term used to describe the increase in cell survival that is observed if a given radiation dose is split into two fractions

separated by a time interval, i.e. allowing time for repair. However, this benefit in survival can be offset when the time between doses extends so much that reassortment, reoxygenation, and repopulation kick in (answer C). Ultimately, the outcome of split-fraction experiments depends on the interplay between Repair of DNA damage, Reassortment through the cell cycle and Repopulation (requires a split time period greater than the cell cycle length).

S-phase, especially the latter part of S-phase is the most radioresistant cell cycle phase therefore most survivors of the first fraction are generally in S phase. SLD is seen to a far higher-degree in low-LET irradiated cells than in high-LET irradiation.

The fraction of cells surviving a split dose increases with increasing time between the two doses because of the repair of SLD, hence B is false.

- X-10) C The effect of a given dose of radiation is less if it is split into two fractions, delivered a few hours apart which is due to the repair of sublethal damage. Most drastically this is observed within the first hour when most damage is repaired, starting to plateau after 2h for most proliferating mammalian cells.

The majority of surviving cells will come from S-phase as this is the most radioresistant cell cycle phase.

Potentially lethal damage repair is not assessed in split dose experiments but is more about post-exposure conditions and the time available for repair in arrested populations before cell cycle resumes. (single-dose experiment)

## XI. Solid Tumor Assay Systems

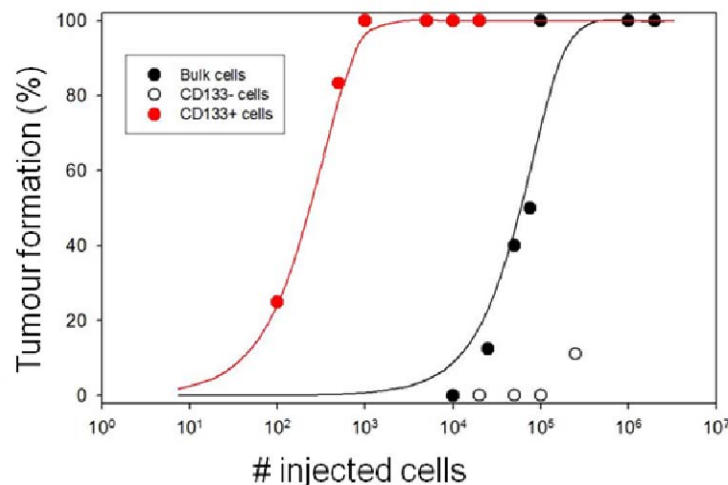
XI-1) Which of the following assays would NOT be useful for the purpose of quantifying the response of a tumor to irradiation?

- A. Lung colony assay
- B. 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<sub>50</sub> 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 tumor formation in mice is indicated in the graph below for a glioblastoma cell line (bulk) and two sub-lines derived from it (CD133<sup>+</sup> and CD133<sup>-</sup>). Which of the following statements would best explain the experimental findings?



- A. CD133<sup>-</sup> 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<sup>-</sup> cells are more radiosensitive than CD133<sup>+</sup> cells
- E. CD133<sup>+</sup> cells are more radiosensitive than CD133<sup>-</sup> 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 following 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. Solid Tumor Assay Systems Answers & Explanations

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. The lung colony assay is a technique to measure the radiosensitivity of tumor cells that are irradiated *in situ*. Following irradiation, the tumor is removed, made into a preparation of single cells, and then injected into the mouse tail vein. Cells that retain clonogenic capacity form colonies in the lung that are countable. This allows comparison of clonogenic survival in various *in situ* experimental scenarios (Answer choice A). Similarly, colony forming ability of cells explanted from a tumor are radiated *in situ*; however, radiation response is tested by counting the surviving colonies grown *in vitro*, also known as the *in vivo/in vitro* assay (Answer choice E). Lastly, tumor growth assays (Answer choices C and D), quantifies radiation response through the measurement of tumor diameter at various time points following radiation compared to untreated tumors.

XI-2) E The TCD<sub>50</sub> assay quantifies the dose required to cure 50% of a group of matched tumors and is therefore a highly relevant endpoint for extrapolation to the clinic. The assay can be conducted using mouse tumors or human tumor xenografts (Answer Choice B), although suppression of the host immune system when using xenografts is crucial in order to minimize misleading results due to rejection of implanted cells (Answer Choice D). The TD<sub>50</sub> assay can be 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 (Answer Choice C).

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, supporting CD133 as a potential cancer stem cell marker. The unsorted bulk cells contain cancer stem cells; given, however, that 100-1,000 more cells are required in order to form the same number of tumors as that seen when purified CD133+ cells are injected, 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 *de novo*. In this experiment, no data are provided regarding the sensitivity of the cell lines (or sublines) to radiation.

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

demonstrate the capacity of self-renewal. In analogy to *in vitro* assays, tumor cells that demonstrate the ability to achieve a local recurrence following radiotherapy have been termed “clonogenic cells” and correspond to putative “cancer stem cells,” or “tumor initiating cells.” The existence of cancer stem cells, defined by the ability for self-renewal and generation of the heterogeneous lineage of cells within a tumor, has been hypothesized.

Baumann M, Zips D, Appold S. Radiotherapy of lung cancer: technology meets biology meets multidisciplinary. *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 (< 1%) of all cells in a tumor are cancer stem cells. If correct, this hypothesis suggests that all cancer stem cells must be killed or lose replicative potential in order to achieve permanent local tumor control. In theory, one surviving cancer stem cell would be sufficient to cause a local recurrence following 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, as well as doomed cancer stem cells, might undergo a number of cell divisions before they permanently stop proliferating and ultimately die. Determination of proliferating cells will therefore not provide information regarding the radiation response of cancer stem cells. Cancer cells can die following exposure to radiation in different ways, including interphase death (i.e. apoptosis) and mitotic catastrophe (apoptosis, autophagy, or 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 most 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](#)

## 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. As a tumor increases in size, the hypoxic fraction of cells 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) Bevacizumab (Avastin) is a monoclonal antibody that targets:
- A. Basic fibroblast growth factor (bFGF; FGF2)
  - B. Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ; 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 chronically 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 with certain bioreductive drugs
  - B. Are resistant to hyperthermia
  - C. Are located within 10  $\mu$ m 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. Even without angiogenesis, tumors can grow up to 2 cm in diameter
- B. For most tumor types a high microvessel density has been negatively correlated with metastatic spread
- C. Vascular endothelial growth factor (VEGF) is induced under hypoxic conditions
- D. Angiostatin and endostatin are stimulators of angiogenesis
- E. Basic fibroblast growth factor (bFGF) is a negative regulator of angiogenesis

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

- A. Under hypoxic conditions, HIF-1 $\alpha$  transcription and translation are upregulated as well as translocation of HIF-1 $\alpha$  from the cytosol to the nucleus
- B. Under aerobic conditions, the HIF-1 $\alpha$  heme moiety becomes oxygenated. This drives a conformational change in the protein that limits DNA binding and prevents upregulation of target genes
- C. Under hypoxic conditions, HIF-1 $\alpha$  is activated by bioreduction, thereby promoting the up-regulation of target genes
- D. Under hypoxic conditions, the HIF-1 $\alpha$  heme moiety becomes deoxygenated. This induces a conformational change in the protein that leads to enhancing DNA binding and subsequent upregulation of target genes
- E. Under aerobic conditions, HIF-1 $\alpha$  is hydroxylated by HIF prolyl hydroxylases that target 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 eliminates this protection and restores endothelial cell sensitivity to chemotherapeutic agents
  - 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 thereby reducing tumor vessel leakiness, dilation, and tortuosity as well as increasing pericyte coverage
- XII-8) At a distance of 150  $\mu\text{m}$  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
  - E. Increased metabolic acids
- XII -9) Paclitaxel appears to be effective in radiosensitizing tumors *in vivo* for all the following mechanisms, EXCEPT:
- A. Induction of apoptosis
  - B. Upregulation of HIF-1
  - C. Oxygenation of radioresistant hypoxic cells
  - D. Arrest of cells in the radiosensitive G<sub>2</sub>/M phase
  - E. Decrease of interstitial fluid pressure

- XII-10) Which of the following statements regarding the tumor microenvironment is FALSE?
- A. Blood vessel supply is heterogeneous and irregular
  - B. Blood flow through micro-vessels may be sluggish
  - C. There tends to be an increase in vessel density compared to normal tissue
  - D. There are a greater number of hypoxic regions within the microenvironment of a tumor compared to that seen in normal tissue
  - E. Nutritional support to the tumor microenvironment is adequate and homogeneous
- XII-11) Which of the following statements regarding angiogenesis is FALSE?
- A. For a multi-cellular organism to grow, it must have the capacity to recruit new blood vessels via angiogenesis
  - B. Angiogenesis is normally regulated by pro-angiogenic, but not anti-angiogenic, molecules
  - C. Angiogenesis is dysregulated in a neoplastic environment
  - D. Without a nearby blood vessel or effective angiogenesis, a tumor cannot grow beyond a critical size or metastasize to other organs
  - E. Tumor cell viability decreases beyond 70 $\mu$ m of blood vessels due to the diffusion limits of oxygen
- XII-12) Which of the following statements regarding anti-angiogenic therapy strategies is FALSE?
- A. Anti-angiogenic therapies interfere with activators of angiogenesis
  - B. Anti-angiogenic therapies target receptor tyrosine kinases and related signal transductions
  - C. Anti-angiogenic therapies seek to amplify endogenous suppressors of angiogenesis.
  - D. Anti-angiogenic therapies use colchicine as an anti-angiogenic agent.
  - E. Anti-angiogenic therapies ultimately target VEGFR-1 in order to achieve inhibition of angiogenesis



XII-13) Which of the following statements concerning vasculogenesis is TRUE?

- A. The process of vasculogenesis is specific to the developing embryo
- B. Vasculogenesis refers to a subset of angiogenesis, in that it describes the formation of only venous vessels as tumors grow beyond 1-2 mm<sup>3</sup>
- C. Tumors use vasculogenesis or angiogenesis in a mutually exclusive fashion
- D. Vasculogenesis is critical for a tumor to achieve local tumor recurrence following radiotherapy
- E. Vasculogenesis utilizes pre-existing blood vessels during the early stages of tumor development to facilitate tumor growth

## XII. Tumor Microenvironment Answers & Explanations

- XII-1) D In the absence of reoxygenation it is unlikely that all hypoxic cells would be eliminated following a typical course of radiotherapy from a tumor possessing even a small percentage of hypoxic cells because hypoxic cells demonstrate approximately 3-fold greater radioresistance compared with aerated cells.

Hypoxic regions in tumors can be detected using a labeled nitroimidazole compound. Bortezomib (Velcade) is a proteasome inhibitor and would not label hypoxic regions within tumors (Answer Choice A).

Although not without exceptions, as tumors increase in size the hypoxic fraction generally also increases. This is because the typically abnormal tumor vasculature is insufficient to maintain oxygen demand (Answer Choice B).

Regions of *acute* or transient hypoxia may develop in tumors due to intermittent blood flow via the intermittent closing down of blood vessels. Chronic hypoxia, on the other hand, is defined as diffusion-limited hypoxia due to the inability of oxygen to diffuse farther than 100  $\mu\text{m}$  from a blood vessel (Answer Choice C).

Acutely hypoxic cells tend to exhibit rapid reoxygenation, whereas chronically hypoxic cells generally reoxygenate more slowly (Answer Choice E).

Dewhirst MW. Relationships between cycling hypoxia, HIF-1, angiogenesis and oxidative stress. *Radiat Res* 172:653-665, 2009. [Pubmed](#)

- XII-2) E Bevacizumab (Avastin) binds to and neutralizes vascular endothelial growth factor (VEGF)-A *ligand*, thereby preventing its interaction with cell surface receptors, including the VEGF receptor (VEGFR).

The fibroblast growth factors (FGFs) are a family of pluripotent growth factors that stimulate proliferation of mesodermal or neuroectodermal cells and can play a role in angiogenesis (Answer Choice A). FGFs have yet to be successfully targeted pharmaceutically.

Hypoxia-inducible factor (HIF)-1 $\alpha$  is a transcription factor that detects hypoxia and enhances angiogenesis (Answer Choice B).

The Von Hippel-Lindau (VHL) protein belongs to a complex that is involved in the ubiquitination and degradation of HIF (Answer Choice C).

The Ras proteins are a family of small GTPases involved in the activation of signaling cascades following activation of receptors. Ras is frequently mutated in human cancers but is difficult to target pharmacologically (Answer Choice D).

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, hypoxia-inducible-factor (HIF)-1 (Answer Choice A).

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 (Answer Choice B).

Pre-clinical studies with animal models as well as clinical studies have linked increased hypoxia in tumors to increased tumor aggressiveness and metastatic potential (Answer Choice C).

Exposure to severe hypoxia halts 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.

Chronically hypoxic cells tend to be *sensitive* to hyperthermia. This is because they exist in an acidic (*low pH*) microenvironment (Answer Choices B and D).

*Acutely* hypoxic cells are a consequence of intermittent blood flow in tumors (Answer Choice E).

It has been shown via model calculations of oxygen consumption rates in respiring tissues and through the use of hypoxia markers that chronically hypoxic cells rarely appear closer than about 70  $\mu\text{m}$  from capillaries (Answer Choice C).

Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer* 4: 437-47, 2004. [Pubmed](#)

- XII-5) C Expression of vascular endothelial growth factor (VEGF) and downstream angiogenesis is induced under hypoxic conditions via hypoxia-inducible transcription factors that bind to the *VEGF* promoter to stimulate its transcription.

In the absence of angiogenesis, tumors would only be expected to reach a diameter of about 2 mm, not 2 cm (Answer Choice A).

Microvessel density, a measure of angiogenesis, has been correlated *positively* with metastatic spread for most tumor types (Answer Choice B).

Angiostatin and endostatin are *inhibitors* of angiogenesis while basic fibroblast growth factor (bFGF) is a *positive* regulator of angiogenesis (Answer Choice D and E).

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 (VEGF). 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 (PDGF). Under aerobic conditions, HIF-1 $\alpha$  is hydroxylated by HIF prolyl hydroxylases. Hydroxylation at two prolyl residues targets HIF-1 $\alpha$  to the von Hippel-Lindau (VHL) E3 ubiquitin ligase and results in HIF-1 $\alpha$  ubiquitination and subsequent proteosomal degradation, thereby limiting upregulation of target genes. Because the hydroxylation catalyzed by prolyl hydroxylases requires molecular oxygen, HIF-1 escapes inactivation under hypoxic conditions.

Kaelin WG Jr. The von Hippel-Lindau tumour suppressor protein: O<sub>2</sub> sensing and cancer. *Nat Rev Cancer* 8:865-873, 2008. [Pubmed](#)

Bertout JA, Patel SA, Simon MC. The impact of O<sub>2</sub> 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 a single agent, several anti-angiogenic drugs have not yielded a long-term survival benefit. In contrast, delivery of 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 were destroying tumor vasculature in combination regimens, one would expect decreased tumor blood flow and compromised delivery of chemotherapy to the tumor. The survival benefits produced by the addition of an anti-angiogenic drug to traditional chemotherapeutic regimens therefore 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 normalized. As a result, the resultant vascular bed achieves greater organization by being less leaky, dilated, and tortuous. 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. Sustained or high-dose anti-angiogenic therapy, however, may drive an imbalance favoring anti-angiogenic factors leading to inadequate tumor blood supply and compromise of the efficacy of systemic therapies.

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<sub>2</sub>), decreased pH, decreased glucose, and *increased* (not decreased) interstitial fluid pressure. Low oxygen levels shift glucose metabolism from oxidative phosphorylation to glycolysis, which increases the production of metabolic acids. Oxygen can diffuse about 70 μm from the arterial end of a capillary.

XII-9) B Paclitaxel stabilizes microtubule polymers and protects them from disassembly. As a result, mitosis is consequently blocked and apoptosis is activated.

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<sub>2</sub>/M phase of the cell cycle, and decreasing interstitial fluid pressure. Some of these studies have been conducted in animal models while others have been performed in clinical trials of human breast cancer patients. No studies have demonstrated upregulation of HIF-1 following treatment with paclitaxel; indeed, one might expect HIF-1 to be degraded *more rapidly* following reoxygenation.

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) E Tumor masses exhibit abnormal blood vessel networks that fail to provide adequate and homogeneous nutritional support.

XII-11) B Since oxygen is unable to diffuse more than approximately 70 μm from the arterial end of a capillary, tumors require the ability to develop new blood vessels in order to grow (Answer Choice A). This process is normally regulated by a balance of both pro-(including VEGF, FGF, PDGF) and anti-(thrombospondin, angiostatin, endostatin) angiogenic molecules.

- XII-12) D Colchicine is an anti-inflammatory agent that binds tubulin. Colchicine itself induces vascular damage but only at doses that are limited by toxicity and therefore not used in the clinical setting for this purpose.
- XII-13) D Whereas angiogenesis is the sprouting of endothelial cells from nearby blood vessels, vasculogenesis is the formation of blood vessels from circulating endothelial progenitor cells (i.e. the bone marrow). Vasculogenesis is of particular importance following treatment with anti-angiogenic agents as well as following irradiation. During early tumor development, both vasculogenesis and angiogenesis are likely utilized. Because tumor irradiation abrogates local angiogenesis, the tumor must rely on the vasculogenesis pathway for re-growth following irradiation. Tumor irradiation produces a marked influx of CD11b+ myeloid cells into tumors and are critical in order for a tumor to achieve formation of blood vessels after irradiation as well as for tumor recurrence.

Brown JM. Vasculogenesis: a crucial player in the resistance of solid tumours to radiotherapy. *Br J Radiol*, 87(1035):20130686, 2014.

[Pubmed](#)

Reale A, et al. Function and biological role of endothelial precursor cells in tumor progression: a new potential therapeutic target in haematological malignancies. *Stem Cells Int* 2016:7954580, 2016.

[Pubmed](#)

### 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<sub>2</sub> into M
  - B. CDK4 – S into G<sub>2</sub>
  - C. cyclin A – G<sub>2</sub> into M
  - D. cyclin B – S into G<sub>2</sub>
  - E. cyclin D – M into G<sub>1</sub>
- 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<sub>0</sub>
  - B. G<sub>1</sub>
  - C. S
  - D. G<sub>2</sub>
  - E. M
- XIII-3) The typical cell cycle time (T<sub>C</sub>) 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 ( $\Phi$ ) 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<sub>D</sub>) is 60 days and the potential doubling time (T<sub>pot</sub>) is 3 days, then the cell loss factor is 5%
  - D. T<sub>pot</sub> has proven useful in predicting tumor response to accelerated radiotherapy
  - E. Typically, the cell loss factor is not of major 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,  $\lambda$ , is 0.7, what is the approximate length of mitosis ( $T_M$ )?
- 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<sub>2</sub>-checkpoint in 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 estimate of relative DNA content?
- A. Bromodeoxyuridine (BrdU) 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 (T<sub>c</sub>) and tumor doubling time (T<sub>d</sub>) for human tumors?
- A. T<sub>c</sub>, 1 to 5 days and T<sub>d</sub>, 20 to 30 days
  - B. T<sub>c</sub>, 1 to 5 days and T<sub>d</sub>, 40 to 100 days
  - C. T<sub>c</sub>, 0.5 to 1 days and T<sub>d</sub>, 20 to 30 days
  - D. T<sub>c</sub>, 0.5 to 1 days and T<sub>d</sub>, 40 to 100 days
  - E. T<sub>c</sub>, 1 to 2 days and T<sub>d</sub>, 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 (pO<sub>2</sub>)
  - B. Growth fraction
  - C. Cell loss factor
  - D. Body temperature where the tumor grows
  - E. Extra- and intra-cellular acidity (pH)

- XIII-14) The cell loss factor represents the ratio of the rate of cell loss to the rate of new cell production. Which of the following is not a dominant cause of cell loss in tumors?
- A. Death from inadequate nutrition
  - B. Apoptosis (programmed cell death)
  - C. Death from immunologic attack
  - D. Metastasis
  - E. 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
- XIII-16) 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<sub>2</sub>
  - B. Short T<sub>pot</sub>
  - C. Slow reoxygenation
  - D. Large number of tumor clonogens
  - E. Early onset of repopulation
- XIII-17) 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. Prevention of cell cycle redistribution
  - B. Induction of G<sub>2</sub> phase arrest
  - C. Inhibition of reoxygenation
  - D. Radioprotection of normal tissues
  - E. Stimulation of DNA repair

- XIII-18) They are great variations among the mammalian cell cycle times, varying for about 10 hours for hamster cells grown in vitro to hundreds of hours for stem cells. This is a result of dramatic variation in which of the cell cycle phases?
- A. M phase
  - B. G1
  - C. S phase
  - D. G2
  - E. Equally split between all phases
- XIII-19) There are three principal checkpoints in the cell cycle: a) G<sub>1</sub>/S b) S-phase and c) G<sub>2</sub>/M. What is the purpose of these checkpoints?
- A. Maintain cell membrane integrity.
  - B. Decrease tumor cell antigenicity
  - C. Enable cells to survive low-pH environment.
  - D. Maintain genomic integrity.
  - E. Decrease REDOX stress

### XIII. Cell and Tissue Kinetics Answers & Explanations

- XIII-1) A CDK1 (and cyclin A/B) is associated with the G<sub>2</sub> to M cell cycle phase transition.

The other CDKs and Cyclins are appropriately paired as follows:

- G<sub>1</sub> phase → S phase: CDK4 and Cyclin D1; CDK2 and Cyclin E
- S phase → G<sub>2</sub> phase: CDK2 and Cyclin A
- G<sub>2</sub> phase → M phase: CDK1 and Cyclin B/A

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. Radiosensitivity across the cell cycle is ranked as follows from least to most sensitive: Late S, Early S, G<sub>1</sub>, G<sub>2</sub>≈M.

Sinclair R. Steady-state suspension culture and metabolism of strain L mouse cells in simple defined medium. *Radiat Res.* 41(1):20-33. 1966. [Pubmed](#)

- XIII-3) B The typical T<sub>c</sub> for tumor cells *in vivo* is generally in the range of 1-5 days.

Terz et al. Analysis of the cell kinetics of human solid tumors. *Cancer.* 28(5):1100-10, 1971. [Pubmed](#)

- XIII-4) A The cell loss factor ( $\Phi$ ) is the percent of newly produced cells that die or fail to continue dividing. Most human tumors have a  $\phi$  of around 77 % and explains why tumors grow *in-vivo* far more slowly than would be expected based upon cell doubling time.

$$CLF=1-T_{pot}/T_d$$

Where:

- T<sub>pot</sub>= potential doubling time = the time necessary to double the number of proliferating tumor cells in the absence of spontaneous cell loss.
- T<sub>d</sub>= volume doubling time = the actual doubling time observed.

Cell loss factor (CLF,  $\phi$ ) 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 (Answer Choice B).

If the observed tumor volume doubling time ( $T_D$ ) is 60 days and the potential doubling time ( $T_{pot}$ ) calculated from the cell cycle time and the growth fraction is 3 days, then the cell loss factor is 95% (Answer Choice C).

Although  $T_{pot}$  (as measured from a tumor biopsy derived from patients previously given bromodeoxyuridine) 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 (Answer Choice D).

For carcinomas, the cell loss factor is usually the *major* determinant of the discrepancy between a tumor's potential doubling time and its overall volume doubling time (Answer Choice E).

- XIII-5) E The question refers to the 'percent-labeled mitoses technique' using tritiated thymidine. The radiolabeled thymidine is added to the growth medium of cells for about 20 minutes (flash labeling). Only those cells in S-phase take up the label. 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 the  $G_2$  phase ( $T_{G2}$ ). 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 ( $T_M$ ). The time to reach 50% of the maximum point, therefore, corresponds to  $T_{G2}$  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  $T_M = (MI)(T_C/\lambda) = (0.05)(14 \text{ hours})/0.7 = 1 \text{ hour}$ . Of note, the  $T_M$  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 a high cell loss factor.

Formation of metastases represents only one of many reasons for cell loss, and usually is only a minor contributor (Answer Choice B).

Human tumor cells typically have cell cycle times of a few days whereas tumor volume doubling times are generally on the order of months (Answer Choice C).

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. In addition, non-viable cells (whether hypoxic or aerobic) have similar effects on the tumor volume doubling time and the potential doubling time (Answer Choice E).

- XIII-8) E The cell loss factor ( $\Phi$ ) is equal to  $1 - (T_{\text{pot}}/T_D)$ . Therefore, if the cell loss factor were zero, then the  $T_{\text{pot}}$  would equal the  $T_D$ .

The mean  $T_C$  is *shorter* than the  $T_{\text{pot}}$  because  $T_{\text{pot}}$  also considers the presence of quiescent cells, and the growth fraction in tumors is generally less than 100% (Answer Choice A).

For solid tumors the  $T_{\text{pot}}$  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  $T_{\text{pot}}$ , so it does not affect the relationship between the  $T_{\text{pot}}$  and the  $T_D$  (Answer Choice B).

$T_{\text{pot}}$  can be calculated from the labeling index (LI) and the duration of S phase ( $T_S$ ) using the equation  $T_{\text{pot}} = \lambda T_S / \text{LI}$ . (where  $\lambda$  is a constant ranging from about 0.6 to 1.0; Answer Choice C).

It has been suggested that tumors with *short* pretreatment values for  $T_{\text{pot}}$ , (suggesting the presence of rapidly proliferating cells and a high growth fraction), would be most likely to benefit from accelerated radiotherapy, but this has not been confirmed in clinical trials performed to date (Answer Choice D).

- XIII-9) C Regulation of the G<sub>2</sub> checkpoint by ATM is thought to occur via the activation of CHK2, which phosphorylates CDC25C phosphatase thereby preventing it from dephosphorylating CDK1 (CDC2), a step necessary for the progression from G<sub>2</sub> into M phase. The remaining proteins listed are all targets for phosphorylation by the ATM kinase, and, consequently, are implicated in various cell cycle control pathways although not the G<sub>2</sub> checkpoint. CHK2 and MDM2 are involved in control of the G<sub>1</sub>-S phase transition (Answer Choice A). ATM also phosphorylates MDM2, which reduces the ability of MDM2 to negatively regulate p53. NBS1 and CHK2 are implicated in S phase progression (Answer Choice B). Upon phosphorylation by CHK2, p53 is stabilized and causes cell cycle arrest in G<sub>1</sub> (Answer Choice D). 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: signaling pathways and their organization in space and time. *DNA Repair (Amst)* 3: 997-1007, 2004. [Pubmed](#)

- XIII-10) A Bromodeoxyuridine (BrdU) 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 volume doubling time ( $T_D$ ) of human tumors is characteristically 40 to 100 days, while the cell cycle time is relatively short, generally between 1 to 5 days. This has important implications, which often are overlooked, in the use of cell cycle-specific chemotherapeutic agents or radiosensitizing drugs for which it is the cell cycle time that is relevant.
- XIII-13) C The growth of a tumor depends on the fraction of cycling or proliferating cells (growth fraction), the cell cycle time, and cell loss (the number of newly produced cells that die or fail to continue dividing). Physical stresses such as low intra-tumoral oxygen concentration, low pH and high temperature both lengthen the cell cycle and slow down tumor growth. The high rate of cell loss in human tumors largely accounts for the great disparity between  $T_c$  and the volume doubling time ( $T_D$ ). Values for the cell-loss factor vary from 0% to more than 90% for 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  $\mu\text{m}$  (the interspace between microbeams), the cells still stay in the tumor mass without affecting cell loss from the tumor.

Crosbie JC, et al. 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.



XIII-15) D The cell loss factor ( $\Phi$ ) is equal to  $1-(T_{pot}/T_D)$ . Rearranging this,  $T_D = T_{pot}/(1-\Phi)$ .  $T_D = 3 \text{ days}/(1-0.8) = 15 \text{ days}$ .

XIII-16) 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.

The potential doubling time ( $T_{pot}$ ) is the time required for a tumor to double in size, taking into account the number of cells in the cell cycle and the speed of progression through the cell cycle. A short  $T_{pot}$  would be deleterious to tumor control because it suggests a high potential for vigorous repopulation during the course of treatment (Answer Choice B).

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 (Answer Choice C). A large number of clonogenic cells would require a higher total dose for eradication; this might increase the probability of adverse normal tissue effects (Answer Choice D).

Early onset of repopulation would also be deleterious as the tumor cells would 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 (Answer Choice E).

XIII-17) B An agent that arrested cells in the *radiosensitive*  $G_2$  phase of the cell cycle could increase tumor response to a fractionated treatment protocol if provided prior to each dose of radiation.

Prevention of cell cycle redistribution would diminish response to fractionated radiotherapy because surviving cells would remain in a radioresistant portion of the cell cycle rather than being permitted to traverse into a more radiosensitive phase of the cell cycle (Answer Choice A).

Inhibition of reoxygenation would reduce tumor response to fractionated radiotherapy due to the prevention of the conversion of surviving radioresistant hypoxic cells to more sensitive aerated cells (Answer Choice C).

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 (Answer Choice D).

Stimulation of DNA repair would *reduce* tumor response to fractionated radiotherapy because a greater proportion of tumor cells may survive if treated with an agent with enhanced capacity for DNA repair.

XIII-18) B Variations in the duration of G1 phase are responsible (Hall chapter 4)

XIII-19) D The purpose of the cell cycle checkpoints is to slow down the cell cycle following DNA damage, hence facilitating DNA repair to maintain genomic integrity.

#### 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- $\kappa$ B 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- $\kappa$ B to ionizing radiation exposure is FALSE?
- A. NF- $\kappa$ B is a transcription factor
  - B. The Inhibitor of Nuclear factor (NF)- $\kappa$ B, I $\kappa$ B, is phosphorylated by ATM and subsequently degraded, allowing NF- $\kappa$ B to move from the cytoplasm into the nucleus
  - C. NF- $\kappa$ B 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- $\kappa$ B
  - E. NF- $\kappa$ B is sequestered as an inactive form in the cytoplasm by interaction with an inhibitory subunit of the I $\kappa$ B
- XIV-6) Based on transcriptomic 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 and varies 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<sub>2</sub> 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 PI(3)K-Akt. Inhibitors of the PI(3)K pathway are in early clinical trials, while androgen-deprivation therapy (ADT) via inhibition of the AR is able to confer a clinical response in most patients. Which of the following statements most CORRECTLY describes the relationship between these two pathways and explains mechanistically why single inhibition of AR or the PI(3)K-Akt pathways rarely induces tumor regression in preclinical models?
- A. ADT represses an AR gene program governing DNA repair and inhibits repair of ionizing radiation-induced DNA damage
  - B. AR and PI(3)K pathways regulate each other by reciprocal negative feedback, such that inhibition of one activates the other
  - C. ADT represses the PI(3)K/Akt/target of rapamycin (TOR) pathway
  - D. ADT activates the unfolded protein response
  - E. All of the above
- XIV-9) The phenomenon of “oncogene addiction” is most clearly represented in which of the following clinical scenarios.
- A. A Chronic Myeloid Leukemia (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 FLT3 mutated Acute Myeloid Leukemia (AML) patient treated with interferon.
- XIV-10) 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

#### XIV. Molecular Signaling Answers & Explanations

- XIV-1) C ATM is a kinase that is activated in response to the presence of DNA double-strand breaks, such as following exposure to ionizing radiation. Activated ATM phosphorylates multiple distinct target proteins, including histone H2AX, p53, BRCA1, and Artemis. Phosphorylation of H2AX (to  $\gamma$ -H2AX) results in chromatin modification that facilitates the recruitment of factors needed for DNA repair (Answer Choice A). The tumor suppressors, p53 and BRCA1, activate cell cycle checkpoint and/or DNA repair processes in response to genotoxic stress (Answer Choices B and D). VEGF is a secreted factor that promotes angiogenesis and is not a direct target of ATM phosphorylation (Answer Choice C).

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 an allele, at a different locus, for a separate and 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 via the activation of vascular endothelial growth factor (VEGF; Answer Choice A).

Cyclin D1 repression is associated with anti-proliferation effects. Its overexpression has been observed in human cancers, including pancreatic, lung, and esophageal (Answer Choice B).

Release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria into the cytoplasm is a primary mitochondrial apoptogenic activity (Answer Choice C).

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 a variety of human cancers, including B-cell chronic lymphocytic leukemia, colorectal cancer, and breast cancer.

Esquela-Kerscher A and Slack FJ. Oncomics --- 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 but has not been shown to modulate *BRCA2*.

HIF-1 is a hypoxia inducible factor known to regulate the expression of the *VEGF* gene and thus the regulation of angiogenesis (Answer Choice A).

p53 is a transcription factor that induces expression of p21 (Answer Choice B).

E2F is known to regulate a large number of proteins involved in cell cycle progression, including *CDC25A* (Answer Choice D).

*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 (Answer Choice E).

Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nature Med* 10; 789-799, 2004. [Pubmed](#)

- XIV-4) D Cytokines are proteins released by cells, including irradiated cells, that stimulate tissues to mount a biological response.

NF- $\kappa$ B is a transcription factor (not a cytokine). TGF- $\beta$ 1 (TGFB1) is an important example of one of the cytokines that has been associated with the development of lung fibrosis following irradiation (Answer Choice A).

A paracrine response is the result of a cytokine that acts upon a cell, other than itself, within a tissue or organ (Answer Choice B). In contrast, an autocrine response is the result of a cytokine targeting the cell from which it was produced (Answer Choice E).

Cytokines generally do not have tyrosine kinase activity (Answer Choice C).

- XIV-5) C Nuclear factor (NF)- $\kappa$ B generally exerts a pro-survival influence through interference with apoptotic signals. It accomplishes this via 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. In some cell types, however, this may not occur, since it may be opposed through the parallel triggering by TNF of a signaling pathway that activates NF- $\kappa$ B via the TRADD and TRAF adaptors. Active NF- $\kappa$ B induces transcription of a set of genes that encode the anti-apoptotic IAPs (“inhibitors of apoptosis”). NF- $\kappa$ B 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. I $\kappa$ B binds to NF- $\kappa$ B 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 I $\kappa$ B, targeting it for ubiquitination and degradation, which allows NF- $\kappa$ B to translocate to the nucleus from the cytoplasm where it can act as a transcription factor. NF- $\kappa$ B 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- $\kappa$ B response. Competition between p53 (TP53) and NF- $\kappa$ B 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<sub>1</sub> 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 (AR) signaling as well as frequent activation of PI(3)K signaling. AR transcriptional output is decreased in human and murine tumors with *PTEN* deletion. In addition, PI(3)K 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. These two oncogenic pathways therefore cross-regulate each other by reciprocal feedback. Inhibition of one pathway leads to activation of the other thereby maintaining tumor cell survival. Combined pharmacologic inhibition of PI(3)K and AR signaling causes near complete prostate cancer regression in a *PTEN*-deficient murine prostate cancer model and in human prostate cancer xenografts, indicating that both pathways coordinately support survival.

Carver BS, et al. Reciprocal feedback regulation of PI(3)K and androgen receptor signaling in *PTEN*-deficient prostate cancer. *Cancer Cell* 19(5):575-86. [Pubmed](#)

- XIV-9) A “Oncogene addiction” was first coined by Bernard Weinstein and refers to the dependence of some tumors on a single dominant oncogene for continued growth and survival and that inhibition of this specific oncogene product is sufficient to halt the neoplastic phenotype. Answer Choice A is correct because imatinib is correctly paired with its target, BCR-ABL. The other answers are examples of oncogene-addicted cancers that are incorrectly paired with agents that do not target the dominant oncogene product.

*EGFR*-mutant lung adenocarcinoma patient can be treated with tyrosine kinase inhibitors such as gefitinib or erlotinib. A *BRAF*-mutant melanoma patient can be treated with a BRAF inhibitor such as vemurafenib

or dabrafenib. An *EML4-ALK* positive lung adenocarcinoma patient treated with an ALK inhibitor such as crizotinib or alectinib. Lastly, a FLT3-mutated AML patient can be treated with FLT inhibitor, midostaurin, along with induction chemotherapy.

Torti D and Trusolino L. Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils. *EMBO Mol Med.* 3(11):623-36. [Pubmed](#)

- XIV-10) C The promoter region is the regulatory portion of a gene that plays a critical role in directing whether a 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 a cell surface tyrosine kinase receptor that is activated by epidermal growth factor (EGF) ligand, among others, and is important for cell proliferation. Loss of heterozygosity is a common mechanism by which tumor suppressor genes are inactivated. Oncogenes are generally activated by mechanisms including deletion/point mutation, chromosome rearrangement, retroviral integration, or gene amplification.

## XV. Cancer

- XV-1) Which of the following statements is TRUE concerning the retinoblastoma protein (RB1)? RB1:
- A. Is an important downstream effector controlling the G<sub>2</sub> 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 of the following pairs of cancer type and corresponding genetic alterations in that cancer is FALSE?
- A. Pancreatic — *K-RAS*
  - B. Lung adenocarcinoma — *ALK*
  - C. Colon — *PTCH*
  - D. Thyroid — *RET*
  - E. Melanoma - *BRAF*
- XV-3) 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 — post-translational regulation
  - E. BRCA1 — DNA damage repair
- XV-4) 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 activated by acute cellular hypoxia
  - 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-5) Oncogenes were first discovered from the study of:
- A. Chicken Retroviruses
  - B. Bacteria
  - C. Yeast
  - D. Mice
  - E. Human cells in culture
- XV-6) Which one of the following is NOT a tumor suppressor gene?
- A. *PTEN*
  - B. *BRCA2*
  - C. *WT1*
  - D. *NF1*
  - E. *ABL*
- XV-7) 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<sub>2</sub> to M phase transition
  - D. p53 stimulates the activity of BAX and BID in irradiated cells, resulting in apoptosis
- XV-8) Which of the following statements is TRUE concerning the products of the *INK4A/ARF* locus?
- A. p16<sup>INK4A</sup> (CDKN2A) stimulates the hyper-phosphorylation of the RB (RB1) protein resulting in release of the E2F transcription factor
  - B. p14<sup>ARF</sup> is induced by the RAS/MEK/MAPK pathway and stimulates cell growth
  - C. p16<sup>INK4A</sup> is encoded by a proto-oncogene
  - D. p16<sup>INK4A</sup> is activated by the PI(3)K/AKT pathway and increases synthesis of cyclin D
  - E. p14<sup>ARF</sup> inhibits the MDM2-mediated degradation of p53
- XV-9) 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 EGFR in non-small cell lung carcinoma.
  - D. Translocated ALK in small cell lung carcinoma.
  - E. Mutated Notch1 in chronic myeloid leukemia.

## XV. Cancer Answers & Explanations

- XV-1) B RB1 is the product of the *RB1* tumor suppressor gene (not an oncogene). Once phosphorylated by CDK4/6, RB1 releases E2F, which then activates genes associated with the G<sub>1</sub> checkpoint. RB1 is functionally inactivated in virtually all human cancers, either directly or indirectly, via p53 (TP53). 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 the majority of 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) 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. 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. Alk mutation is present in ~5% of lung adenocarcinomas and provides a target for crizotinib.

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-3) 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-4) 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 HPV, 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-5) A Retroviruses, viruses with genomes composed of RNA instead of DNA, can cause cancers in animals (example: Rous sarcoma virus [RSV] in chickens). Usually, this occurs because the retroviruses contain modified

(often mutated) proto-oncogenes captured from the genomes of their vertebrate hosts.

- XV-6) 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-7) 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<sub>2</sub> to M phase transition. Lymphocytes and thymocytes with a mutant p53 tend to be more radioresistant than their normal counterparts.

- XV-8) E p14<sup>ARF</sup> inhibits the MDM2-mediated degradation of p53. p16<sup>INK4A</sup> is a cell cycle inhibitor that prevents phosphorylation of RB by CDK4. p14<sup>ARF</sup> is an MDM2 inhibitor thereby causing p53 levels to increase, resulting in greater cell cycle inhibition. p16<sup>INK4A</sup> is encoded by a tumor suppressor gene.

Sharpless NE. INK4a/ARF: a multifunctional tumor suppressor locus. *Mutat Res* 576:22-38, 2005. [Pubmed](#)

- XV-9) 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 context. Each of the listed oncogene products in this question is an addictive oncoproteins in human cancer, however Choices B-E are incorrectly paired to the listed cancer. 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 (Answer Choice B); mutated, not amplified, EGFR in non-small-cell lung carcinoma (Answer Choice C); translocated ALK in non-small cell lung carcinoma (Answer Choice D); mutated Notch1 in T-cell acute lymphoblastic leukemia (Answer Choice E).

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 molecular 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) An employee working in a nuclear power plant is accidentally exposed to a total body  $\gamma$ -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  $^{210}\text{Po}$  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<sub>50</sub> (no medical intervention) – 3.5 Gy
  - C. LD<sub>50</sub> (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<sub>50</sub> (no medical intervention) 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 in one fraction, 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, the majority of those affected experience nausea and vomiting within 30 minutes
  - 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
- XVI-10) With recent advances in supportive measures to manage acute radiation exposure, what syndrome has been seen following exposure to 8-10 Gy in modern nuclear reactor accidents?
- A. Pulmonary
  - B. Renal
  - C. Endocrine
  - D. Musculoskeletal
  - E. Hepatic

## XVI. Total Body Irradiation Answers & Explanations

- 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](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 Radiological 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 his 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 listed 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 Estimated human LD<sub>50</sub> (dose to result in lethality in 50% of an irradiated population) is 7 Gy with the best medical intervention and 3.5 Gy without any medical intervention. The dose thresholds for the hematopoietic, gastrointestinal, and cerebrovascular syndromes are roughly 2 Gy, 8 Gy and 20 Gy, respectively.

Condition	Threshold dose	Days until Death
Hematopoietic Syndrome	2 Gy	30-60 days
Gastrointestinal Syndrome	8 Gy	5-16 days
Cerebrovascular Syndrome	20 Gy	Hours – 2 days

- XVI-4) B The death of a person 30-60 days following a total body radiation dose close to the LD<sub>50</sub> 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<sub>50</sub> since it requires higher doses to 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<sub>50</sub>; 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 even 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. The majority of those who receive greater than 10 Gy would be expected to show prodromal symptoms by about 30 minutes after exposure. Symptoms of the prodromal syndrome after a sublethal exposure to total body irradiation include fatigue, anorexia, nausea and vomiting. The onset of these symptoms are inversely proportional to the dose of the exposure. In individuals who receive a supralethal dose of total body irradiation, additional prodromal symptoms include fever, immediate diarrhea, apathy, headache and hypotension.

Finch, Stuart C. JAMA August 7, 1987

<https://pubmed.ncbi.nlm.nih.gov/3302322/>

- XVI-9) D Systemic corticosteroids are not recommended in management of patients with gastrointestinal syndrome from total body irradiation..Supportive care (including psychological support), symptomatic treatment (5HT3 antagonist antiemetics, etc.), and fluid/electrolyte replacement (PO and/or IV) should be the earliest goals of medical management.

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](#)

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3863169/>

XVI-10

A Pulmonary syndrome has been found to occur in nuclear reactor workers who were exposed to 8-10 Gy and survived hematopoietic syndrome due to modern supportive care with isolation, IV hydration, transfusions, antibiotics and hematopoietic colony stimulating factors. These patients died at approximately 130 days after exposure due to pneumonitis.

## 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. Radiation associated valvular disease is rare in patients receiving  $\geq 35$  Gy to the heart.
  - B. Pericardial effusion is the most common Grade 3+ toxicity after definitive lung 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 a short segment 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) Acute radiation esophagitis presents as dysphagia or a substernal burning sensation as early as 2 weeks after the start of conventionally fractionated radiation therapy. Medical management most often involves:
- A. Angiotensin converting enzyme inhibitors
  - B. Gene therapy with manganese superoxide dismutase
  - C. Non-steroidal anti-inflammatory drugs
  - D. Pentoxifylline
  - E. Vitamin E
- XVII-4) One type of radiation-induced bone injury is mandibular osteoradionecrosis (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-5) Which of the following types of blood cells is most radioresistant?
- A. Granulocyte/monocyte colony forming cells (GM-CFC)
  - B. Spleen-colony forming units (CFU-S)
  - C. Macrophages
  - D. Unprimed T-helper cells
  - E. B-cells
- XVII-6) 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-7) Which of the following statements concerning radiation-induced damage to the eye is TRUE?
- A. The threshold radiation dose for cataract formation is approximately 10 Gy
  - B. It is often possible to distinguish between a radiation-induced cataract and an age-induced cataract
  - C. The neutron RBE for cataract formation is about 5 for low total doses
  - D. The tolerance dose for the development of blindness is lower than the tolerance dose for cataract formation
  - E. The length of the latency period for cataract formation is independent of radiation dose
- XVII-8) Which of the following statements is TRUE concerning radiation effects on the bone marrow?
- A. The absolute lymphocyte count rate of decrease over 2 days may estimate the severity of total body irradiation induced injury.
  - 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 bone marrow syndrome usually die of severe anemia
  - E. There is no late effect pathology associated with bone marrow irradiation

- XVII-9) 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-10) 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. Radiation pneumonitis is a characteristic late effect of lung radiotherapy that occurs 6-12 months after treatment completion.
  - 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 carboplatin-paclitaxel.
  - E. Several cell types are involved in the development of pulmonary late effects, including the type II pneumocyte, fibroblasts, the alveolar macrophage and vascular endothelial cells.
- XVII-11) 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-12) 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-13) With respect to the morphologic changes associated with radiation-induced liver disease (RILD), 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-14) 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-15) 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-16) 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-17) 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

## XVII. Clinically Relevant Normal Tissue Responses to Radiation Answers & Explanations

- 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 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. With current treatment methods, however, a much smaller heart volume is irradiated, so radiation pericarditis is now infrequently observed.

Radiotherapy-induced valvular disease may occur in greater than 80% of patients receiving  $\geq 3$  Gy to the heart (Answer Choice A).

Pericardial effusion is the most common cardiac Grade 1-2 toxicity after definitive lung radiotherapy, but the most common Grade 3+ cardiac toxicities are acute coronary syndrome, new congestive heart failure, and arrhythmia. (Answer Choice B).

Following mediastinal radiotherapy for treatment of Hodgkin's Lymphoma, 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 (Answer Choice C). Similarly, an increased risk of myocardial infarctions has also been 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 (Answer Choice E).

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](#)

Brosius FC 3<sup>rd</sup>, Waller BF, Roberts WC. Radiation heart disease. Analysis of 16 young (aged 15 to 33 years) necropsy patients who received over 3500 rads to the heart. *Am J Med.* 70(3):519-30. PMID: 6782873

Dess R, Sun Y, Matuszak M, et al. Cardiac Events After Radiation Therapy: Combined Analysis of Prospective Multicenter Trials for Locally Advanced Non-Small-Cell Lung Cancer. *J Clin Onc* 35(13): 1395-1402. PMID: 28301264

Spetz J, Moslehi J, Sarosiek K. Radiation induced cardiovascular toxicity: mechanisms, prevention and treatment. *Curr Treat Options Cardiovasc Med.* 20(40):31, 2018. PMID: 29556748

- 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 (Answer Choices A-B). In children, growth retardation is a concern after irradiation of growth plates (Answer Choice C). Bone sarcoma is the most common secondary neoplasm following irradiation of bony structures (Answer Choice D).

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) C NSAIDs can help prevent esophagitis by decreasing inflammation. Although ACE-inhibitors have proven effective in the treatment of radiation nephropathy and pneumopathy, there are no data supporting their use in the treatment of radiation-induced esophagitis (Answer Choice A). 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 translated to a phase I clinical trial, but a benefit of this approach has not been proven in humans (Answer Choice B). Both pentoxifylline and vitamin E have been shown, in combination, to prevent as well as induce 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-4) 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 1960s and 1970s 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-5) 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.

Heylmann D, Rödel F, Kindler T, *et al.* Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. *Biochim Biophys Acta* 1846(1): 121-9, 2014. PMID: 24797212

Falcke S, Rühle P, Deloch L, *et al.* Clinically Relevant Radiation Exposure Differentially Impacts Forms of Cell Death in Human Cells of the Innate and Adaptive Immune System. *Int J Mol Sci* 19(11): 3574, 2018. PMID: 30428512

- XVII-6) C In both animal studies and real-world accidental exposures (e.g., the Chernobyl disaster) to whole-body radiation, the small intestine has typically shown the most sensitivity among components of the gastrointestinal tract. At a histologic level, the small intestine experiences significant denudation due to loss of radiosensitive crypt cells; this ultimately

Hall and Giaccia. *Radiobiology for the Radiologist*, 7<sup>th</sup> Edition. Chapter 8.

- XVII-7) 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 (Answer Choice A). Several recent studies, which included early lens opacities as well as cataracts that interfere with vision, have longer follow-up times than that presented in previous research as well as greater statistical power. This work suggests a low threshold (<1 Gy) for cataract development and is statistically consistent with no threshold for cataract induction.

The low-dose neutron RBE for cataract formation is greater than 20 (Answer Choice C).

The tolerance dose for the production of blindness is greater than that for cataract formation (Answer Choice D).

The latency period for the induction of a radiation-induced cataract decreases with increasing dose (Answer Choice E).

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-8) A 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 accidental exposure. The Andrews lymphocyte nomogram can be used to estimate the severity of injury following total body irradiation. Individuals suffering from 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.

Andrews GA, Auxier JA, Lushbaugh CC. The importance of dosimetry to the medical management of persons exposed to high levels of radiation. Personal dosimetry for radiation accidents. Vienna, International Atomic Energy Agency, 1965. pp 3-16.

- XVII-9) A Older women are more sensitive to the induction of radiation-induced sterility than younger women, presumably due to the diminished number of oocytes compared with that seen in 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-10) B Radiation pneumonitis is a characteristic late effect of lung radiotherapy that occurs approximately 2-3 months after treatment completion.

In patients receiving concurrent chemoradiation therapy for non-small cell lung cancer, the risk of fatal pneumonitis for  $V_{20} = 20-29.9\%$  is 1%,  $V_{20} = 30-39.9\%$  is 2.9%, and  $V_{20} \geq 40\%$  is 3.5%. The volume of lung irradiated has been shown to be a particularly critical factor with respect to the degree of pulmonary toxicity observed (Answer Choice A). Many radiation oncologists are using the  $V_{20}$  or  $V_{30}$ , 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 (Answer Choice C). 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 (Answer Choice D).

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 (Answer Choice E).

Roberts KB, Rockwell S. Radiation pneumonitis. In: *Fishman's Pulmonary Diseases & Disorder, 4<sup>th</sup> 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](#)

Palma DA, Senan S, Tsujino K, *et al*. Predicting radiation pneumonitis after chemoradiotherapy for lung cancer: an international individual patient data meta-analysis. *Int J Radiat oncol Biol Phys*. PMID: 22682812.

- XVII-11) 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-12) 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 transforming growth factor beta 1 (TGF- $\beta$ 1). Many other cell types within the heart contribute to the development of radiation-induced heart disease (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 treatment completion. 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-13) D The Kupffer cells, hepatic-specific phagocytes, often increase in size during the progression of veno-occlusive disease (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 lumens 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-14) 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 (Answer Choice B). 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 (Answer Choice C). 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 (Answer Choice D).

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-15) D There are typically no distinct pathognomonic characteristics of CNS injury that would unambiguously identify radiation as the causative agent.

XVII-16) 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-17) 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.

Adams MJ, Hardenbergh PH, Constine LS, Lipshultz SE. Radiation-associated cardiovascular disease. *Crit Rev Oncol Hematol* 45(1):55-75, 2003. PMID: 12482572

## 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 $\alpha$ )
- 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 of the mucosa does not produce a more severe acute reaction compared to mucosa that has not been previously irradiated
  - 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 initial 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 transforming growth factor beta 1 (TGF- $\beta$ 1) and basic fibroblast growth factor (bFGF/FGF2) is TRUE?
- A. The pro-fibrotic activities and role in radiation-induced fibrosis of TGF- $\beta$ 1 are mediated by SMAD3
  - B. Stimulation of TGF- $\beta$ 1 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- $\beta$ 1 always decreases following lung irradiation
  - E. TGF- $\beta$ 1 promotes the radiation-induced inflammatory response
- 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 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



## XVIII. Mechanisms of Normal Tissue Radiation Responses Answers & Explanations

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 $\alpha$ ) are all considered pro-inflammatory. There is considerable overlap between the activities of TNF $\alpha$  and IL-1. TNF $\alpha$  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 $\alpha$ , IL-1 induces several secondary cytokines, including IL-6 and IL-8. Upon stimulation by IL-1 and/or TNF $\alpha$ , 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. Manipulation of the gut ecosystem through administration of probiotics has been demonstrated to prevent radiation-induced enteritis in animals.

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](#)

Seal M1, Naito Y, Barreto R, *et al.* Experimental radiotherapy-induced enteritis: a probiotic interventional study. *J Dig Dis.* 2007 Aug;8(3):143-7.

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 biologically effective tolerance dose (BED<sub>t</sub>) showed that the lungs could be re-irradiated with doses equivalent to the BED<sub>t</sub> 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 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 while radiotherapy is still ongoing. 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 Transforming growth factor beta 1 (TGF- $\beta$ 1) plays a central role in radiation-induced fibrosis as it causes epithelial to mesenchymal cell trans-differentiation and promotes the influx of fibroblasts as well as the production of extracellular matrix. TGF- $\beta$ 1 activates SMAD proteins, including SMAD3, which modulates the transcription of target genes with pro-fibrotic activities. It is thought that stimulation of TGF- $\beta$ 1 synthesis causes fibrosis and would therefore *decrease* the therapeutic ratio.

Basic fibroblast growth factor (bFGF/FGF2) has been shown to protect (not sensitize) endothelial cells from radiation-induced apoptosis. In addition, TGF- $\beta$ 1 has anti-inflammatory activity. The clinical interpretation of serum TGF- $\beta$ 1 levels during thoracic irradiation is complex; on the one hand, levels are elevated in patients who develop radiation pneumonitis while, on the other hand, lung tumors may self-generate TGF- $\beta$ 1 causing levels to fall during treatment).

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](#)

Lierova A, Jelicova M *et al.* Cytokines and radiation-induced pulmonary injuries. *J Radiat Res.* 2018 Nov; 59(6): 709–753.

XVIII-10) C Fibrosis is one of the most common late radiation effects and can be noted in a majority of irradiated tissues and organs. Although the appearance of fibrosis is both time- and dose-dependent, its extent and severity can vary not only within a single organ, but also across different individuals. Bone marrow is one of the few tissues where fibrosis is rarely seen; in general, fibrosis only appears within the marrow 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- $\beta$ 1.

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 Radiation-induced cognitive impairment is marked by decreased verbal memory, spatial memory, attention, and novel problem-solving ability. The incidence and severity of radiation-induced cognitive impairment increases over time. The hippocampus houses neuronal stem cells and is one of only two areas where neurogenesis continues after birth. The hippocampus plays an important role in learning and memory consolidation.

The importance of sparing the hippocampus was demonstrated in the phase III NRG-CC001 trial of whole-brain radiotherapy plus memantine, with or without hippocampal avoidance. Patients were randomly assigned to memantine plus whole-brain radiotherapy (30 Gy in 10 fractions) vs memantine plus hippocampal-avoidant whole-brain radiotherapy (30 Gy in 10 fractions). Hippocampal-avoidant whole-brain radiotherapy plus memantine reduced the risk of cognitive function failure by 26% (P = .033).

Gondi V, Tomé WA, Mehta MP. *Radiother Oncol*. 2010 Dec;97(3):370-6. [Pubmed](#)

Greene-Schloesser D and Robbins ME Radiation-induced cognitive impairment-from bench to bedside, *Neuro Oncol*. 2012 Sep; 14(Suppl 4): iv37–iv44.

## **XIX. Therapeutic Ratio**

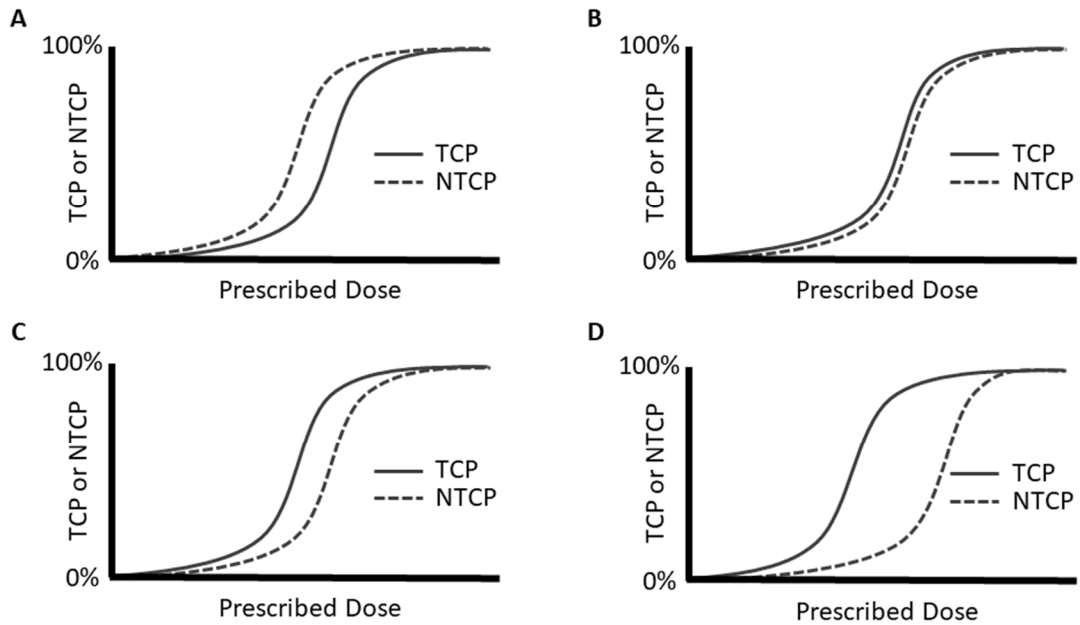
- XIX-1) For tumors exhibiting central hypoxia, what strategy is most likely to improve the therapeutic ratio of treatment?
- A. Stimulation of repair in hypoxic tumor cells
  - B. Inhibition of reoxygenation in tumor cells
  - C. Use of a systemic radioprotector drug that reaches both tumor and normal cells
  - D. Inhibition of DNA repair in tumor and normal cells
  - E. Use of a systemic radioprotector drug that preferentially accumulates in normal tissues
- XIX-2) 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-3) 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. Unchanged in normal tissues and increased in tumors
  - B. Increased in normal tissues but decreased in tumors
  - C. Decreased in normal tissues and in tumors
  - D. Unchanged in normal tissue but decreased in tumors
  - E. Increased in normal tissues and unchanged in tumors
- XIX-4) Which of the following statements is CORRECT? Normal tissue regeneration/repopulation:
- A. Occurs in acutely responding normal tissues during the course of a standard course of radiotherapy
  - 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



- XIX-5) A prostate cancer patient is planned to go through a moderately hypofractionated IMRT treatment. Your clinic's standard for planning has been to use 10 mm margins in every dimension except 7 mm posteriorly without daily image guidance. Which of the following strategies would NOT improve the therapeutic ratio of the treatment?
- A. The addition of a hydrogel spacer between the rectum and prostate
  - B. The use of daily cone-beam CT for image guidance
  - C. The use of smaller PTV margins (i.e., 5 mm except 3 mm posteriorly) without daily cone-beam CT
  - D. The use of daily cone-beam CT plus smaller margins
  - E. The use of implanted gold fiducials and intrafraction stereoscopic x-ray monitoring and targeting adjustment

- XIX-6) Hypofractionation is being used to definitively treat many different malignancies including breast and prostate cancer. Which of the following is TRUE about its use in prostate cancer?
- A. Treatment of the whole prostate using the same total dose but higher dose per fraction allows safe dose escalation while sparing normal tissues
  - B. Treatment of the whole prostate to the same biologically effective dose (BED) to tumor but higher dose per fraction allows shorter treatment time and little to no change in the therapeutic ratio
  - C. Treatment of the whole prostate to the same BED to tumor but higher dose per fraction allows a shorter treatment time and selective sparing of the adjacent normal tissues
  - D. The BED to normal tissues is significantly higher with hypofractionated regimens and therefore stereotactic methods must be used to treat the prostate

XIX-7) Your clinic has a fancy new treatment planning system that uses radiobiologic tumor control probability (TCP) and normal tissue complication probability modeling (NTCP) to help select the best treatment plan. Your dosimetrist has come up with 4 plans to review. Based on the TCP/NTCP curves below, which of the following plans would you choose?



## XIX. Therapeutic Ratio Answers & Explanations

- XIX-1) E Normal tissues are typically well-oxygenated, but some tumors may contain a fraction of radioresistant hypoxic cells. Larger tumors are more likely to harbor hypoxic regions. In order for a radioprotector to be efficacious and improve the therapeutic ratio, it must have a preferential effect on normal tissues either through increased selectivity for normal tissues or decreased selectivity for tumor cells. Amifostine is the prototypical radioprotector with Phase III data and meta-analyses demonstrating reduction in mucositis and xerostomia without affecting progression-free survival or overall survival. The disadvantages of amifostine include the need for IV infusion, moderate rates of nausea/vomiting, and risk of hypotension.

Answers A and B describe strategies that would protect tumor cells.

Answer C describes radioprotection of both normal tissues and tumor, which is next expected to change the therapeutic ratio.

Answer D describes inhibition of DNA repair in both normal and tumor cells and would be expected to sensitize both populations but not change the therapeutic ratio.

Bourhis *et al.* Effect of amifostine on survival among patients treated with radiotherapy: a meta-analysis of individual patient data. *J Clin Oncol.* 29(18): 2590-7, 2011. [Pubmed](#)

Sasse *et al.* Amifostine reduces side effects and improves complete response rate during radiotherapy: results of a meta-analysis. *Int J Radiat Oncol Biol Phys.* 64(3): 784-91, 2006. [Pubmed](#)

- XIX-2) 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-3) 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 typically not compromised in normal tissues. However, since many tumors contain hypoxic cells, increasing blood flow to the tumor could result in radiosensitization. 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-4) 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 repopulation were to take place, then this would reduce, not increase, late effects, irrespective of the fractionation schedule. Repopulation/regeneration plays no role in reoxygenation.

- XIX-5) C All of the proposed interventions would improve the therapeutic ratio except the use of smaller PTV margins without image guidance. Image guidance increases the fidelity of setup and reduces the risk of geometric miss, particularly in the setting of variability in bladder and rectal filling. The use of reduced PTV margins can improve the therapeutic ratio by limiting the volume of normal tissue treated, but only if the dose to tumor is not compromised. The margins proposed in answer C would not be sufficient to cover tumor without image guidance. Lastly, hydrogel spacers have been shown to displace the rectum posteriorly and reduce GI toxicity.

Beltran *et al.* Planning target margin calculations for prostate radiotherapy based on intrafraction and interfraction motion using four localization methods. *Int J Radiat Oncol Biol Phys* 70(1): 289-95, 2008. [Pubmed](#)

Hamstra *et al.* Continued Benefit to Rectal Separation for Prostate Radiation Therapy: Final Results of a Phase III Trial. *Int J Radiat Oncol Biol Phys* 97(5): 976-985, 2017. [Pubmed](#)

- XIX-6) B The goal of hypofractionation is to provide more convenient (shorter) treatments to patients while maintaining or improving the therapeutic ratio. Hypofractionation for low- and intermediate-risk prostate cancer typically involves treatment of the prostate cancer to an EQD<sub>2</sub> of 74-78 Gy (assuming  $\alpha/\beta$  of 1-3 for tumor). The most thoroughly tested regimens include 70 Gy in 28 fractions (RTOG 0415) and 60 Gy in 20 fractions (UK CHHIP and PROFIT). In general, these regimens were tested for non-inferiority against roughly biologically equivalent conventional regimens (73.8 Gy/41 fractions for RTOG 0415, 74 Gy/37 fractions for UK CHHIP, and 78 Gy/39 fractions for PROFIT). A major concern about hypofractionation was the possibility of significantly increased acute and late toxicity not predicted by linear quadratic modeling. The above trials have demonstrated this not to be the case, so long as appropriate dose constraints are achieved. There is some evidence, however, of a small increase in acute GI toxicity across trials. Answer A is incorrect, as

maintaining the same total dose (e.g., 78 Gy) while hypofractionating (e.g., 20 fractions) would increase the BED to both tumor and normal tissues. While this would improve the likelihood of tumor control, it also would move rightward along the normal tissue probability curve with a disproportionate increase in NTCP, thereby decreasing the therapeutic ratio.

Answer C is incorrect, as hypofractionation regimens maintaining an equivalent BED compared to an effective conventional regimen are not expected to significantly spare normal tissues (although the BED to normal tissues may be marginally less depending on the alpha/beta used for tumor).

Answer D is incorrect, as the BED to normal tissues for the regimens mentioned above is not significantly different from conventional regimens. As a result, both predicted toxicity and measured toxicity are not significantly different. Daily image guidance was not mandatory on UK CHHIP (~30% treated with it) but was required on PROFIT and RTOG 0415; none of these trials used real-time tracking methods. However, daily image guidance is typically preferred, especially if treating according to the respective protocol.

Dearnaley *et al.* Conventional versus hypofractionated high-dose intensity-modulated radiotherapy for prostate cancer: 5-year outcomes of the randomised, non-inferiority, phase 3 CHHiP trial. *Lancet Oncol* 17(8):1047-1060, 2016. [Pubmed](#)

Lee *et al.* Randomized Phase III Noninferiority Study Comparing Two Radiotherapy Fractionation Schedules in Patients With Low-Risk Prostate Cancer. *J Clin Oncol.* 34(20): 2325-32, 2016. [Pubmed](#)

Catton *et al.* Randomized Trial of a Hypofractionated Radiation Regimen for the Treatment of Localized Prostate Cancer. *J Clin Oncol.* 35(17): 1884-1890. 2017, [Pubmed](#)

- XIX-7) D Tumor control probability (TCP)/Normal tissue complication probability modeling (NTCP) curves are a convenient way to assess the therapeutic ratio of a treatment as it relates to the prescribed dose. The probability of either tumor control or tissue complication is typically sigmoidal. The therapeutic ratio can be estimated by the widest vertical separation of the TCP and NTCP curves. The ideal treatment plan (i.e., a high therapeutic ratio) demonstrates a high likelihood (e.g., >90%) of tumor control while maintaining TCP as low as possible.

The example in A is the worst case scenario, in which normal tissue toxicity is nearly certain at doses that would provide meaningful tumor

control. Such is sometimes the case in situations where reirradiation is being considered.

Examples B and C show suboptimal scenarios in which NTCP is still highly likely if trying to achieve curative doses. Of the depicted curves, example D demonstrates the highest therapeutic ratio.

## 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  $\alpha/\beta$  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  $\alpha/\beta$  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  $\alpha/\beta$  ratio of 2 Gy and a  $T_{pot}$  of 30 days. For the dose-limiting normal tissue, the  $\alpha/\beta$  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  $\alpha/\beta = 2$  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  $\alpha/\beta$  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  $\alpha/\beta$  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
  - E. The isoeffect curve for skin acute effects is steep, indicating a high sensitivity to fractionation



- 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. 6-8 hours
- 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  $\alpha/\beta$  ratio of 10 Gy and the normal tissue has an  $\alpha/\beta$  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 and safety of conventionally fractionated radiation in the clinic compared to single or hypofractionated treatment, 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 these 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
- XX-14) Considering our current knowledge of typical alpha/beta values and basic radiobiological concepts, which of the following organ sites would be most likely to gain therapeutic benefit with hypofractionation?
- A. Prostate
  - B. Head and Neck
  - C. Breast
  - D. Bladder
  - E. Brain

## XX. Time, Dose, Fractionation Answers & Explanations

- XX-1) C This can be calculated using the linear-quadratic formula that allows comparison of two different fractionation schedules and the resulting relative biological effective dose (BED).

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

n – number of fractions

d – dose per fraction

$\alpha$  and  $\beta$  – tissue specific dose response curve parameters

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 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 (Answer Choice B).

Tissues with steep isoeffect curves have low, not high,  $\alpha/\beta$  ratios (Answer Choice C).

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 (Answer Choice D).

Isoeffect Curve Slope	$\alpha/\beta$ ratio	Total Dose Required	Features
Steep	Low	Higher	<ul style="list-style-type: none"> <li>• Greater sparing with increased fractionation</li> <li>• Greater repair capacity</li> <li>• Increased proliferation</li> </ul>
Shallow	High	Lower	<ul style="list-style-type: none"> <li>• Reoxygenation</li> </ul>

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 if treating with either standard or small fraction sizes (i.e., hyperfractionation) there would be greater sparing of this tumor ( $\alpha/\beta$  ratio = 2 Gy) than for the critical dose-limiting normal tissue ( $\alpha/\beta$  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  $\alpha/\beta$  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<sub>2</sub>, 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 can be used:

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

Thus, the standard treatment results in a BED of:

$$BED = 30 \times 2 \left[ 1 + \frac{2}{(10)} \right] = 72 \text{ Gy}_{10}$$

Therefore, in order to determine the number of fractions to be used if the fraction size is reduced to 1.3 Gy:

$$72 \text{ Gy}_{10} = nd \left[ 1 + \frac{1.3}{(10)} \right]$$

$$nd = 64 \text{ Gy}$$

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  $\alpha/\beta$  ratio than that of 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 (Answer Choice A).

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 (Answer Choices B, C, and D).

- 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 (Answer Choice A).

Hyperfractionation would *reduce* the severity of late effects if the total dose was titrated to maintain the same level of early effects (Answer Choice B).

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 (Answer Choice D).

The isoeffect curve for skin acute effects is shallow, indicating a lower sensitivity to fractionation; in other words, fractionation does not greatly alter the severity of acute dermatitis (Answer Choice E).

Withers HR. Biologic basis for altered fractionation schemes. *Cancer*. 55(9 Suppl):2086-95. 1985. [Pubmed](#)

- 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.

Cox JD, et al. ASTRO plenary: interfraction interval is a major determinant of late effects, with hyperfractionated radiation therapy of carcinomas of upper respiratory and digestive tracts: results from Radiation Therapy Oncology Group protocol 8313. *Int J Radiat Oncol Biol Phys*. 1991. [Pubmed](#)

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<sub>10</sub> and 100 Gy<sub>2</sub>, respectively, for the tumor and late-responding normal tissue, as determined from the equation:

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

Assuming the BED of 100 Gy<sub>2</sub> 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<sub>10</sub> for the standard treatment, to 70 Gy<sub>10</sub> for the hyperfractionated treatment.

The therapeutic index (TI),  $BED_{\text{tumor-hyperfractionated}}/BED_{\text{tumor-standard}}$  divided by  $BED_{\text{normal-hyperfractionated}}/BED_{\text{normal-standard}}$ , equals  $70 \text{ Gy}_{10}/60 \text{ Gy}_{10}/100 \text{ Gy}_2/100 \text{ Gy}_2 = 1.2$ .

- XX-10) E Sublethal damage repair (SLDR) and repopulation in normal tissues treated with fractionated radiation therapy may contribute to reduced toxicity associated with treatment. Reoxygenation in tumors and possible redistribution of proliferating tumor cells into more sensitive phases of the cell cycle may 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 for the treatment of head and neck squamous cancers and non-small cell lung cancer. 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.

Long-term results showed no differences in disease outcomes for head and neck cancer patients, but there was an improvement in late morbidity with CHART compared to conventional fractionation. For NSCLC, there was an improvement in local progression and overall survival. Despite these promising results, CHART has had limited adoption due to the resource intensive nature of the treatment as well as the more widespread use of concurrent chemotherapy.

Saunders MI, et al. Randomised multicentre trials of CHART vs conventional radiotherapy in head and neck and non-small-cell lung cancer: an interim report. CHART Steering Committee. Br J Cancer. 73(12):1455-62. 1996. Pubmed

Dische et al. A randomised multicentre trial of CHART versus conventional radiotherapy in head and neck cancer. Radiother Oncol. 44(2):123-36. 1997. Pubmed

Saunders et al. Continuous, hyperfractionated, accelerated radiotherapy (CHART) versus conventional radiotherapy in non-small cell lung cancer: mature data from the randomised multicentre trial. CHART Steering committee. Radiother Oncol. 52(2):137-48. 1999. Pubmed

XX-13) A 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.

Chapter 23, Time, Dose, and Fractionation in Radiotherapy, in *Radiobiology for the Radiologist*, 8<sup>th</sup> Ed. Hall EJ and Giaccia AJ, Eds. Lippincott Williams & Wilkins, Philadelphia, 2018.

- XX-14) A Because of the low  $\alpha/\beta$  ratio of prostate cancer cells, a hypofractionated protocol would not result in greater normal tissue damage than tumor kill. In the case of other tumors, the higher  $\alpha/\beta$  ratio of the tumor than that of critical dose-limiting normal tissue would result in hypofractionation causing greater recovery in the tumor versus the normal tissue.



## 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

$\alpha$  and  $\beta$  – 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

$\mu$  – repair rate constant =  $0.693/t_{1/2}$

$t_{1/2}$  – tissue repair half-time

$R_0$  – initial dose-rate

$R$  – dose-rate

$\lambda$  – radioactive decay constant =  $0.693/T_{1/2}$

$T_{1/2}$  – radioactive half life of isotope

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

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

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

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

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

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.
- XXI-6) The principal reason for choosing brachytherapy rather than external beam radiation is:
- A. Brachytherapy is associated with lower risk of exposure to hospital staff.
  - B. Implant of a brachytherapy source within the tumor provides a distinct geometrical advantage for sparing the surrounding normal tissues.
  - C. Brachytherapy from a single dwell position provides a uniform dose within the implanted tissue.
  - D. The dose rates that can be achieved with brachytherapy are more cytotoxic compared to that with external beam in all tumor types.
  - E. A precise brachytherapy implant delivers similar dose rate to both normal tissues and the tumor.

- XXI-7) Brachytherapy:
- A. Allows for a low dose rate treatment minimizing toxicities to surrounding normal tissues.
  - B. Is used predominantly for retinal tumors.
  - C. Requires the use of antibodies for targeting the radionuclide to the tumor.
  - D. Can be used to treat larger tumors than can be treated with external beam irradiation.
  - E. Can be activated with microwave radiation.

## XXI. Brachytherapy Answers & Explanations

- 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 fixed dose of radiation due to repair of sublethal damage (SLDR). 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 fixed dose is delivered. This effect relates to reassortment/redistribution of cells into the radiosensitive phase of the cell cycle by progressing through the DNA damage-induced G<sub>2</sub> block. It would not be anticipated that SLDR, the accumulation of cells in S phase, proliferation, or repair of potentially lethal damage (PLDR) 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-5) C Iridium-192 is the most widely used radionuclide in part because of its convenience, 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 is not used for permanent implants.

- XXI-6) B The principal reason for choosing brachytherapy rather than external beam radiation is that a brachytherapy implant within the tumor provides a distinct geometrical advantage for sparing the surrounding normal tissues.

Brachytherapy is associated with higher risk of exposure to hospital staff.

There is a steep dose rate gradient around an implanted radioactive source that results in a variation in the dose and associated cell killing (Answer Choice C).

Dose rates associated with brachytherapy are equal to or less than external beam radiation and would not be more cytotoxic (Answer Choice D).

The cell killing is highest immediately adjacent to the source, within the tumor. This has the advantage in a well-placed implant of lower dose rate to the normal tissues that are at a greater physical distance from the sources (Answer Choice E).

- XXI-7) A One major advantage of brachytherapy is that it is able to treat the tumor and reduce toxicities to normal tissues because of the dose-rate effect. It is used predominantly for prostate and cervical carcinomas. While antibodies CAN be used for targeting, more often the brachytherapy is delivered as a seed therapy or interstitial implant. Brachytherapy is not activated in general, and microwave would not do it in any case.

## 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
- XXII-2) Which of the following statements concerning intensity-modulated radiation therapy (IMRT) is CORRECT?
- A. IMRT employs significantly higher energy photon beams than unmodulated radiation dose-delivery techniques.
  - B. IMRT results in fewer radiation therapy-induced second cancers in the pediatric population as compared to adults.
  - C. IMRT is most conformal if used in the conventional 1.8-2.0 Gy/fraction format
  - D. IMRT 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 the head and scatter from the collimator.
- XXII-3) Which one of the following statements concerning radiolabeled immunoglobulin therapy is FALSE?
- A. One disadvantage associated with the use of  $^{90}\text{Y}$ -labeled antibodies is that the relatively low energy ( $<100\text{keV}$ ) and short range of the  $\beta$ -particles emitted limit the so-called “crossfire effect.”
  - B. Both ibritumomab tiuxetan (Zevalin) and tositumomab (Bexxar) target CD20.
  - C. Radiation safety is an important issue regarding the use of  $^{131}\text{I}$ -labeled compounds because this isotope emits  $\gamma$ -rays that may pass through the patient.
  - D. The dose-limiting organ associated with the use of tositumomab (Bexxar) is the bone marrow.
  - E. The dose-limiting organ associated the use of tositumomab (Bexxar) is the thyroid gland

- 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
- XXII-5) Which of the following are TRUE about particle therapy?
- A. Protons have a Bragg peak and are considered high LET radiation
  - B. C-ions have no Bragg peak and are considered high LET radiation
  - C. Boron-neutron capture therapy delivers high LET radiation to the tumor
  - D. Si-ions have a Bragg peak and are considered low LET radiation.
  - E. Photons are considered as low LET radiation and do have a Bragg peak associated with them.

## XXII. Radiobiological aspects of alternative dose delivery systems Answers & Explanations

- 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 (Answer Choice A).

Two centers, one at the HIMAC in Chiba, Japan, and the other at the HIT in Heidelberg, Germany, have been treating with carbon ions using a gantry for over a decade and a number of other centers have come online since then. 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 (Answer Choice B).

An additional advantage of treatment with carbon ions is the reduction in lateral and longitudinal scatter (Answer Choice C).

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  $^{12}\text{C}$  to the positron emitting isotopes  $^{11}\text{C}$  and  $^{10}\text{C}$ . 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 (Answer Choice E).

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 intensity modulated radiation therapy (IMRT) technique because, in addition to leakage from the head, there is scatter from the collimator.



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 (Answer Choice 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 (Answer Choice B).

IMRT is most conformal if all target volumes are treated simultaneously using different fraction sizes (Answer Choice 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 modified fractionation on acute and late toxicity of normal tissue is taken into account during treatment planning (Answer Choice 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  $\alpha/\beta$  ratios and tumor doubling time) for the isodose calculations for various tissues components in the 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, et al. Second cancer risk after 3D-CRT, IMRT and VMAT for breast cancer. *Radiother Oncol* 110:471-476, 2014. [Pubmed](#)

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. [Pubmed](#)

- XXII-3) A  $^{90}\text{Y}$  emits  $\beta$ -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  $\beta$ -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):  $^{90}\text{Y}$ -ibritumomab tiuxetan (Zevalin) and  $^{131}\text{I}$ -tositumomab (Bexxar; Answer Choice B). 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).

$^{90}\text{Y}$  is a pure  $\beta$ -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  $^{111}\text{In}$ , must be incorporated into the Zevalin framework to allow positional localization. Bexxar incorporates  $^{131}\text{I}$ , which is a medium-energy, mixed-spectrum  $\beta$ - and  $\gamma$ -emitter with a  $\gamma$  emission at 364 keV that can be detected using a gamma camera. Because of the penetrating  $\gamma$ -rays of  $^{131}\text{I}$  and eight-day half-life, more rigorous radiation safety precautions must be used with Bexxar (Answer Choice C).

Hematologic toxicity is the major dose-limiting toxicity for RIT (Answer Choice D). Thyroid uptake of  $^{131}\text{I}$  is not uncommon but is not dose-limiting. To prevent thyroid uptake of  $^{131}\text{I}$ , oral iodine is given a day before Bexxar administration and continued for 14 days after the therapeutic dose.

Hernandez MC and Knox SJ. Radiobiology of Radioimmunotherapy with  $^{90}\text{Y}$  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-4) 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, et al. Stereotactic body radiotherapy using a radiobiology-based regimen for stage I nonsmall cell lung carcinoma: a multicenter study. *Cancer* 118: 2078-2084, 2012. [Pubmed](#)

- XXII-5) C. BNCT uses neutrons to activate Boron-containing compounds to release alpha particles in the tumor. These alpha particles are high LET. Protons have a Bragg peak but are considered to be low LET radiation with an RBE of near 1.1. C-ions have a Bragg peak and are considered high LET radiation. Si-ions have a Bragg peak and deliver high LET radiation. Photons are low LET but have no Bragg peak associated with them.

### 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 $\kappa$ B
  - B. FDA-approved for use in the treatment of renal cell carcinoma
  - 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. Generates DNA crosslinks
  - E. Inhibits DNA synthesis through depletion of nucleotides
- XXIII-4) Sorafenib is FDA approved for use in the treatment of which cancer?
- A. Hepatocellular carcinoma
  - B. Thyroid cancer
  - C. Pancreatic cancer
  - D. Non-small cell lung cancer
  - E. Kidney cancer
- XXIII-5) Which of the following molecularly-targeted agents is an epidermal growth factor receptor inhibitor?
- A. Bevacizumab
  - B. Nivolumab
  - C. Imatinib
  - D. Cetuximab
  - E. Rituximab

- XXIII-6) 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-7) 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 deacetylase
  - E. 5-fluorouracil – glutathione
- XXIII-8) 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-9) 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-10) Which of the following statements concerning photodynamic therapy is INCORRECT? Photodynamic therapy:
- A. Reduces tumor burden via direct tumor cell killing rather than indirectly via 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-11) Cisplatin has all 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 isomer, trans-platinum
  - D. It causes both inter-strand and intra-strand crosslinking
  - E. It is used as a radiosensitizer with concurrent radiation therapy.
- XXIII-12) Which of the following is associated with cardiac toxicity?
- A. Doxorubicin
  - B. Cisplatin
  - C. Bleomycin
  - D. Methotrexate
  - E. Docetaxel
- XXIII-13) 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-14) Which of the following targeted agents is an immune checkpoint inhibitor?
- A. Bevacizumab
  - B. Ipilimumab
  - C. Imatinib
  - D. Cetuximab
  - E. Crizotinib

- XXIII-15) Which of the following pairs of a targeted agent and its potential target is CORRECT?
- A. Crizotinib – anaplastic lymphoma kinase (ALK)
  - B. Imatinib – programmed cell death 1 (PD-1)
  - C. Cetuximab – ABL kinase
  - D. Sunitinib – Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4)
  - E. Sorafenib – CD20
- XXIII-16) Which of the following is an example of synthetic lethality?
- A. Retroviral overexpression of p53 in tumor cells to induce apoptosis
  - B. Infusion of chimeric antigen receptor-expressing T cells to induce an anti-tumor immune response
  - C. The use of taxane-containing nanoparticles to take advantage of leaky tumor vasculature and cause improved tumor cell kill while limiting normal tissue toxicity
  - D. The use of poly-ADP ribose polymerase inhibitors to abrogate repair of DNA damage in BRCA1/2-mutant tumors and thus promote lethal DNA damage
  - E. The infusion of liposomal siRNA targeting K-ras in pancreatic cancers
- XXIII-17) Panitumumab is now FDA-approved for the management of *k-ras* wild-type metastatic colorectal cancer that has progressed through primary chemotherapy. In regards to mechanism of action, panitumumab is most similar to which of the following agents?
- A. Rituximab
  - B. Cetuximab
  - C. Bevacizumab
  - D. Infliximab
  - E. Sunitinib
- XXIII-18) 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 types of non-Hodgkin lymphoma.
  - 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.

### XXIII. Chemotherapeutic Agents and Radiation Therapy Answers & Explanations

XXIII-1) B Erlotinib (Tarceva) is a small molecule inhibitor of the epidermal growth factor (tyrosine kinase) receptor (EGFR). It reversibly binds to the ATP binding site of the receptor, which prohibits the formation of phosphotyrosine residues and subsequent downstream signaling cascades.

Trastuzumab (Herceptin) is a monoclonal antibody against the Her2/neu receptor (Answer Choice A). It binds to domain IV of the extracellular segment of the Her2/neu receptor, leading to arrest during the G1 phase of the cell cycle and decreased proliferation, downregulation of Akt, and suppression of angiogenesis.

Bevacizumab (Avastin) is a recombinant humanized monoclonal antibody that blocks angiogenesis via the inhibition of the vascular endothelial growth factor (VEGF)-A ligand (Answer Choice C).

Sirolimus (Rapamycin) binds to the FKBP12 complex and inhibits mTOR (FRAP1), a downstream target of the PI(3)K/AKT pro-survival signaling pathway that is activated by radiation exposure (Answer Choice D).

Cetuximab is a chimeric (mouse/human) monoclonal antibody against EGFR (Answer Choice E).

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 (Velcade) is a proteasome inhibitor approved for the treatment of multiple myeloma and mantle cell myeloma (Answer Choices D and B). In normal cells, the proteasome facilitates the degradation of abnormal or misfolded proteins or those tagged via ubiquitylation. Bortezomib acts by inhibiting the activity of the 26S proteasome and, therefore, the degradation of proteins.

NF- $\kappa$ B is not directly targeted by proteasome inhibitors, however, proteasome inhibitors indirectly promote NF- $\kappa$ B being kept in its inactive form by blocking the ubiquitin-mediated degradation of its repressor, I $\kappa$ B (Answer Choice A).



EGFR signaling pathways are not a target for bortezomib (Answer Choice C).

Bortezomib is an N-protected dipeptide, not a monoclonal antibody (Answer Choice E).

Richardson PG, Mitsiades C, Hideshima T, *et al.* Bortezomib: proteasome inhibition as an effective anticancer therapy. *Ann Rev Med* 57:33-47, 2006

- XXIII-3) C Irinotecan (Camptosar) is an inhibitor of the topoisomerase I enzyme. Its active metabolite, CPT-11, is a camptothecin analog that specifically inhibits DNA replication and transcription.

Gemcitabine inhibits the ribonucleotide reductase enzyme (Answer Choice A).

5-Fluorouracil (5-FU) stimulates thymidylate synthase (Answer Choice B).

Cisplatin is an example of an exogenous agent that causes formation of a covalent linkage (crosslink) between nucleotides of DNA (Answer Choice D). Other choices can include carmustine and mitomycin C.

Methotexate is an example of an antimetabolite that exerts its antifolate effects by competitively inhibiting dihydrofolate reductase (DHFR) with much higher affinity than folate, thus reducing tetrahydrofolate synthesis which is necessary for amino acid and nucleic acid synthesis.

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 advanced renal cell carcinoma, hepatocellular carcinoma, and radioactive iodine resistant advanced thyroid carcinoma.

Sorafenib 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, PI(3)K, 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) D 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.

EGFR signaling regulates 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 is a therapeutic strategy in several tumor types.

Bevacizumab (Avastin) targets VEGF ligand and inhibits angiogenesis (Answer Choice A).

Nivolumab (Opdivo) is a human IgG4 monoclonal antibody that binds to the programmed death (PD)-1 receptor and blocks its interaction with its ligands, PD-L1 and PD-L2 (Answer Choice B).

Imatinib (Gleevec) 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 (Answer Choice C).

Rituximab is a monoclonal antibody against CD20, which has a direct anti-tumor effect in CD20-positive lymphomas by inducing apoptosis and cell lysis (Answer Choice E).

Bonner JA, Harari PM, Giralt J, 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-6) E Gefitinib is a small molecule tyrosine kinase inhibitor approved in the US for use in non-small cell lung cancer (NSCLC) harboring deletion of EGFR exon 19 or mutation of EGFR exon 21 (L858R).

Glutathione is a cellular sulfhydryl compound that acts as an antioxidant (Answer Choice A).

Nimorazole is a hypoxic cell radiosensitizer, while sulfhydryls are considered radioprotectors (Answer Choice B).

Tirapazamine is a hypoxic cell cytotoxin and therefore a radiosensitizer (Answer Choice C).

Amifostine is a radioprotector that acts by detoxifying reactive metabolites of platinum and alkylating agents, and also scavenges free radicals (Answer Choice D).

- XXIII-7) A Etoposide targets topoisomerase II. Topoisomerases are enzymes that participate in the overwinding or underwinding of DNA. Topoisomerase I catalyzes the transient breaking and rejoining of a single strand of DNA which lets the broken strand rotate around the intact strand, whereas Topoisomerase II cuts both strands of the DNA helix simultaneously in order to manage DNA tangles and supercoils. Topoisomerase I inhibitors include irinotecan and topotecan; Topoisomerase II inhibitors include etoposide (VP-16), doxorubicin, daunorubicin, and mitoxantrone.

Topotecan targets topoisomerase I (Answer Choice B).

Bevacizumab targets the VEGF ligand (Answer Choice C).

Sunitinib is a tyrosine kinase inhibitor with multiple targets including EGFR, FLT3, VEGFR and KIT (Answer Choice D).

5-fluorouracil targets thymidylate synthase (Answer Choice E).

Atkins M, Jones CA, Kirkpatrick P: Sunitinib maleate. *Nat Rev Drug Discov* 5: 279-80, 2006. [Pubmed](#)

- XXIII-8) 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 a different protein that non-specifically effluxes xenobiotics from cells. These multidrug 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 (Answer Choice A).

Radiation exposure does not cause multi-drug resistance (Answer Choice B).

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 (Answer Choice D).

Multidrug resistance represents a permanent change in the cell phenotype and is not transient (Answer Choice E).

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 formation of radicals. Similarly, an increase in the activity of a DNA repair pathway could lead to the 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 in 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-9) D The bioreductive properties of mitomycin C make it more toxic to many cells under hypoxic conditions.

Bleomycin induces DNA breaks and is dependent on the presence of oxygen (Answer Choice A).

Tirapazamine is activated to a toxic radical only at a very low levels of oxygen, such as in human tumors. Tirapazamine has been shown to produce hydroxyl or benzotriazinyl radicals to damage DNA.

Misonidazole is a hypoxic radiosensitizer (Answer Choice E).

- XXIII-10) A Photodynamic therapy (PDT) requires a photosensitizer, oxygen, and visible light to produce the cytotoxic highly reactive singlet oxygen radical, which ultimately achieves tumor cell killing indirectly via damage to the tumor vasculature (Answer Choices A, C, D). 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 results in damage to these endothelial cells, which leads to the indirect killing of tumor cells as a result of the vascular damage.

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 (Answer Choice B).

Because oxygen is required for the PDT reaction, PDT is ineffective in hypoxic conditions (Answer Choice E).

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-11) C Cisplatin is a chemotherapeutic agent that causes DNA synthesis inhibition by causing both interstrand and intrastrand crosslinking (Answer Choices A and D).

It is cell-cycle non-specific (Answer Choice B).

It is used as a radiosensitizer with concurrent radiation therapy (Answer Choice E).

It is much more effective than its isomer, trans-platinum.

- XXIII-12) A Doxorubicin (Adriamycin) is associated with dose-related cardiomyopathy. Cumulative lifetime doses greater than 450 mg/m<sup>2</sup> are associated with increased risk of heart failure similar to dilated cardiomyopathy. The mechanism behind cardiac toxicity is unclear, but appears to be associated with oxidative stress and is quite distinct from its antineoplastic actions.

Cisplatin has been linked to ototoxicity and renal toxicity (Answer Choice B).

Bleomycin is associated with an increased risk of pulmonary toxicity (Answer Choice C).

Methotrexate is associated with mucositis and gastrointestinal toxicity (Answer Choice D).

Docetaxel and other microtubule poisons are associated with peripheral neuropathy (Answer Choice E).

Kanu Chatterjee A, et al. Doxorubicin Cardiomyopathy. *Cardiology*. 115(2): 155–162. 2010. [Pubmed](#)

Swain et al. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer*. 97(11): 2869-79, 2003. [Pubmed](#)

- XXIII-13) C Data suggests that patients treated with definitive radiation and cetuximab who develop a Grade 2 or greater acneiform rash experience have increased overall survival compared to patients who develop either no rash or grade 1 rash (HR 0.49).

Bonner JA et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. *Lancet Oncol*. 11(1):21-8, 2010. [Pubmed](#)

- XXIII-14) B Ipilimumab is an antibody against the immune checkpoint molecule CTLA-4.

Bevacizumab is a humanized monoclonal antibody that inhibits vascular endothelial growth factor A (VEGF-A) ligand (Answer Choice A).

Imatinib is a small molecular inhibitor of receptor tyrosine kinases. It is most selective for BCR-ABL, but also targets c-kit and PDGF-R (Answer Choice C).

Cetuximab is a monoclonal antibody against EGFR (Answer Choice D).

Crizotinib is a small molecular inhibitor of ALK and ROS1 kinases (Answer Choice E).

- XXIII-15) A Crizotinib is a small molecular inhibitor of ALK and ROS1 kinases; it is FDA approved from non-small cell lung carcinomas bearing the EML4-ALK fusion gene.

Imatinib is a small molecular inhibitor of receptor tyrosine kinases. It is most selective for BCR-ABL, but also targets c-kit and PDGF-R (Answer Choice B).

Cetuximab is a monoclonal antibody against EGFR (Answer Choice C).

Sunitinib and sorafenib are “dirty” multi-targeted receptor tyrosine kinase inhibitors (Answer Choices D and E).

Inhibitors of programmed cell death 1 (PD-1) receptor include pembrolizumab and nivolumab. Inhibitors of programmed death ligand 1 (PD-L1) include atezolizumab, durvalumab, and avelumab.

Ipilimumab is an example of a Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) inhibitor. Ipilimumab functions by disinhibiting the native ability of T cells to recognize and destroy cancer cells.

Rituximab is an example of a CD20 inhibitor

- 16) D Synthetic lethality is the situation whereby a defect in one of a pair of genes has little to no effect on cell survival, while defects in both genes results in death. A clinically relevant example of this is the use of poly-ADP ribose polymerase (PARP) inhibitors such as olaparib in BRCA1/2 mutated breast cancers. Because BRCA1/2-mutated cancers have defective homologous recombination repair (HRR), they depend on error-prone alternative DNA repair pathways for genome maintenance and survival. PARP binds to single-strand DNA breaks to initiate non-homologous repair. Inhibition of PARP by compounds such as olaparib interrupt this process, promote stalled replication forks, and result in double strand breaks. BRCA1/2-wild type cells with intact HRR do not depend on this pathway and are therefore not sensitive to PARP inhibition.

Lord CJ and Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. *Science*, 355(6330):1152-1158. 2018. [Pubmed](#)

XXIII-17) B Panitumumab is a humanized IgG2 monoclonal 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; Answer Choice A).

Bevacizumab targets the VEGF-A ligand (Answer Choice C).

Infliximab is a monoclonal antibody against tumor necrosis factor (TNF)- $\alpha$  and is used in the treatment of autoimmune disorders such as rheumatoid arthritis and psoriasis (Answer Choice D).

Sunitinib is a multi-tyrosine kinase receptor inhibitor (Answer Choice E).

XXIII-18) D Ibritumomab tiuxetan is an anti-CD20 antibody tagged with yttrium-90 (Y90) used for the systemic radioisotope based-treatment of widespread B-cell lymphomas (Answer Choice A).

Y90 containing resin microspheres are FDA-approved for the treatment of hepatic metastases from colorectal cancer (Answer Choice B).

Y90 containing glass microspheres are approved for the treatment of primary hepatocellular carcinomas (Answer Choice C).



## 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 neurological toxicity 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) Temozolomide improves survival in patients with glioblastoma that receive radiation therapy, particularly if the tumor demonstrates:
- A. epigenetic silencing of O6-methylguanine-DNA methyltransferase (MGMT)
  - 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 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
  - E. Inhibit the proteasome
- XXIV-10) What is the function of the LAG-3 molecule?
- A. LAG-3 is a tyrosine kinase receptor located on the cell surface.
  - B. Acetylation of histones.
  - C. A negative regulator of the immune system.
  - D. Regulates p53 function.
  - E. Suppress NHEJ repair
- XXIV-11) Which of the following about alectinib is FALSE?
- A. It is an anaplastic lymphoma kinase (ALK) inhibitor and is FDA approved for the treatment of anaplastic lymphoma and ALK-fusion gene positive non-small cell lung cancer (NSCLC).
  - B. Alectinib improves disease-free survival in first-line treatment of ALK fusion-positive NSCLC compared to the 1<sup>st</sup>-generation ALK-inhibitor crizotinib.
  - C. Alectinib has lower rates of Grade 3+ toxicity compared to crizotinib.
  - D. Alectinib has improved efficacy for brain metastases compared to crizotinib.
  - E. Alectinib is more effective when combined with gefitinib
- XXIV-12) Which of the following metastatic cancers is predicted to have the LOWEST response rate from checkpoint blockade?
- A. Melanoma
  - B. Lynch syndrome-associated endometrial cancer
  - C. Non-small cell lung cancer
  - D. Microsatellite-stable colorectal cancer
  - E. Human papilloma virus induced head and neck squamous cell carcinoma

- XXIV-13) Which of the following is FALSE regarding superoxide dismutase (SOD) mimetics?
- A. The chemical structure of a SOD mimetic contains a redox active metal ion that is oxidized in the presence of superoxide ( $O_2^{\bullet-}$ )
  - B. Their mechanism of action involves converting superoxide ( $O_2^{\bullet-}$ ) into hydrogen peroxide ( $H_2O_2$ )
  - C. Their ability to protect against radiation- and chemotherapy-related oral mucositis in head and neck cancer is currently being evaluated in clinical trials
  - D. An SOD mimetic has yet to be identified that protects against radiation toxicity in normal tissues without also protecting tumor tissue
  - E. Their mechanism of action involves inhibition of catalase enzymatic activity
- XXIV-14) Which of the following compounds CANNOT be used to protect mammalian cells from radiation damage?
- A. WR-2721
  - B. WR-638
  - C. Taxanes
  - D. Cysteine
  - E. Amifostine
- XXIV – 15) Superoxide is a reactive oxygen species involved in the biologic cascade resulting in radiation induced severe oral mucositis. Which of the following is INCORRECT about superoxide dismutase and superoxide dismutase mimetics?
- A. The active site of superoxide dismutase or superoxide dismutase mimetics contains a redox active metal ion
  - B. Superoxide dismutases involve both the reduction and reoxidation of the redox active metal
  - C. Two different superoxide dismutase isoforms have been identified in mammals: SOD1 and SOD2
  - D. Avasopasem is a superoxide dismutase mimetic that reduced the incidence of cisplatin and radiation induced severe oral mucositis in a phase 2b clinical trial
  - E. Superoxide dismutases reduce the free radical to hydrogen peroxide, which is subsequently converted to water by a catalase.

## XXIV. Radiosensitizers, Radioprotectors and Bioreductive Drugs Answers & Explanations

- XXIV-1) D Meta analysis 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 (Answer Choice A).

Bioreductive drugs are compounds that are metabolically-*reduced* under hypoxic conditions to yield cytotoxic species (Answer Choice B).

Because the bioreduction occurs preferentially under hypoxic conditions, these drugs are selectively toxic to hypoxic cells and not aerobic ones (Answer Choice C).

In laboratory studies, hypoxic cell radiosensitizers are most effective when given in high doses and with large radiation doses; their effectiveness in model tumor systems decreases with increasing fractionation (Answer Choice E). One would expect from these laboratory studies that radiosensitizers would be more effective in combination with hypofractionated radiotherapy regimens or radiosurgery, rather than 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 the synthesis of both ribosomal and messenger RNA. This accounts 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. Amifostine, the only FDA approved radioprotector, is a prodrug that is hydrolysed *in vivo* by alkaline phosphatase to the active sulfhydryl compound, WR-1065.
- 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 (Answer Choice A).

Hypotension, nausea/vomiting, fatigue, and fever/rash are the main toxicities associated with amifostine (Answer Choice B).

Amifostine should be administered 15-30 minutes before radiotherapy, not after (Answer Choice D).

It is a pro-drug that is metabolized by alkaline phosphatase to the free thiol metabolite that acts as the direct radioprotective agent (Answer Choice E).

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

- XXIV-6) B Cisplatin binds to DNA to create adducts, leading to intrastrand and, at a lower frequency, interstrand cross-links. One mechanism that has been proposed to account for the radiosensitizing effect of cisplatin is through the 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](#)

Boeckman H. J. et al. Cisplatin sensitizes cancer cells to ionizing radiation via inhibition of non-homologous end joining. *Mol Cancer Res.* 2005 May; 3(5): 277–285. [Pubmed](#)

- XXIV-7) A Methylation of the promoter for MGMT (O<sup>6</sup>-methylguanine-DNA methyltransferase) via an epigenetic mechanism (not via a gene mutation) decreases expression of this DNA repair gene. When tumor cells do express MGMT they are able to 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, temozolomide is able to achieve significant DNA damage via alkylation, which ultimately increases its 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

- B Rapamycin, everolimus and temsirolimus are all inhibitors of the mTOR (mammalian target of rapamycin) protein. mTOR functions downstream of PI(3)K 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 Ionizing radiation induces a G2/M checkpoint arrest, thereby allowing sufficient time for the repair of double strand breaks (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 typically enforces it, would lead to significant radiosensitization. The Wee1 inhibitor, MK1775, has been tested in diffuse intrinsic pontine glioma (DIPG) and glioblastoma as a radiosensitizer

- XXIV-10) C LAG-3 (Lymphocyte-activation gene 3, CD223) is a cell surface molecule present on T cells and other various immune system cells. LAG3's main ligand is MHC class II. LAG-3's physiological function appears to be as an immune checkpoint receptor (i.e. negatively regulation of T cell

proliferation and activation) in a similar fashion to CTLA-4 and PD-1. Ongoing trials suggest that LAG-3 and PD-1 synergistically regulate T-cell function in such a way as to allow an anti-tumoral immune response to be blunted effectively by inhibiting both pathways.

- XXIV 11) A Alectinib received full FDA approval in 2017 for the treatment of ALK-fusion positive NSCLC [not anaplastic lymphoma] after the J-ALEX and ALEX trials showed improved PFS compared to crizotinib (Answer Choice B).

In both trials, alectinib had a lower rate of Grade 3+ adverse effects compared to crizotinib (Answer Choice C). The most common side effects with either drug are GI upset, transaminitis, and anemia.

In the ALEX trial, 122 patients had brain metastases at baseline, of whom 46 had received prior radiation therapy. Time to CNS progression was longer with alectinib compared to crizotinib in both patients with brain metastases at baseline as well as those without brain metastases at baseline (Answer Choice D).

Hida T, et al. Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): an open-label, randomised phase 3 trial. *Lancet* 390(10089):29-39. 2017. [Pubmed](#)

Peters S, et al. Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. *N Engl J Med*, 377(9):829-838. 2017. [Pubmed](#)

Gadgeel S, et al. Alectinib versus crizotinib in treatment-naive anaplastic lymphoma kinase-positive (ALK+) non-small-cell lung cancer: CNS efficacy results from the ALEX study. *Ann Oncol*, 29(11):2214-2222. 2018. [Pubmed](#)

- XXIV 12) D The somatic mutation load of cancers is thought to be a major determinant of response to immune checkpoint blockade through the generation of neoantigens targetable by the immune system. Mismatch repair (MMR) deficiency and microsatellite instability (MSI) are essentially synonymous for the purposes of mutagenicity, as the former leads to the latter. MMR deficiency is seen in 10-20% of patients with sporadic colorectal cancer and approximately 20% of sporadic endometrioid endometrial adenocarcinomas. MMR deficiency is also the hallmark of Lynch syndrome, which predisposes patients to these tumors as well as others. Although MSI is not a hallmark of melanoma and NSCLC, these diseases are typically associated with high mutational burden attributed to the instigating mutagenicity of ultraviolet light and tobacco exposure,



respectively. Tumor mutational burden has been associated with response to anti-PD1 in these diseases.

Le DT, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 372(26):2509-20. 2015. [Pubmed](#)

Le DT, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 357(6349):409-413. 2017. [Pubmed](#)

Koopman M, et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. *Br J Cancer* 100(2):266-73. 2009. [Pubmed](#)

Lawrence MS., et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Science* 499(7457):214-218. 2013. [Pubmed](#)

Rizvi NA, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348(6230):124-8. 2015. [Pubmed](#)

Hugo W, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* 165(1):35-44. 2016. [Pubmed](#)

XXIV 13) D One SOD mimetic, GC4419, that is currently in clinical trials is able to selectively protect normal cells from radiation- and chemotherapy-related toxicity without protecting tumor cells.

Superoxide dismutase (SOD) mimetics contain a redox active metal ion as part of their chemical structure (Answer Choice A).

In the presence of superoxide ( $O_2^{\bullet-}$ ), the redox active metal ion is oxidized (loses an electron) and the superoxide is reduced to hydrogen peroxide (Answer Choice B).

SOD mimetics have been evaluated in phase 1 and 2 clinical trials regarding their ability to protect against radiation- and chemotherapy-induced oral mucositis; a phase 3 clinical trial is currently underway (Answer Choice C).

Anderson CM, Sonis ST, Lee CM, et al. Phase 1b/2a Trial of the Superoxide dismutase mimetic GC4419 to reduce chemoradiotherapy-induced oral mucositis in patients with oral cavity or oropharyngeal carcinoma. *IJROBP* 100(2):427-435. 2018. [Pubmed](#)

Mapuskar KA, Anderson CM, Spitz DR, et al. Utilizing superoxide dismutase mimetics to enhance radiation therapy response while protecting normal tissues. *Semin Radiat Oncol* 29(1):72-80. 2019. [Pubmed](#)

Mapuskar KA, Flippo KH, Schoenfeld JD. Et al. Mitochondrial superoxide increases age-associated susceptibility of human dermal fibroblasts to radiation and chemotherapy. *Cancer Research* 77(18):5054-5067. 2017. [Pubmed](#)

XXIV-14) C Taxanes bind to microtubules and adversely affect their function by enhancing and preventing disassembly. Taxanes act as mitotic inhibitors by blocking cells in the G<sub>2</sub>/M phase of the cell cycle and, if the concentration is sufficient, killing them in this phase.

WR-2721, also known as Amifostine or Ethyol, is the only FDA-approved radioprotective drug used for the prevention of xerostomia in head and neck cancer patients treated with radiation (Answer Choices A and E).

WR-638, also known as Cystaphos, has been proposed as a radioprotector (Answer Choice B).

Cysteine as well as cysteamine are radioprotective sulfhydryl (SH) compounds but unfortunately associated with debilitating toxicity (Answer Choice D).

XXIV-15) C Superoxide dismutase and superoxide dismutase mimetics catalyze the conversion of superoxide to hydrogen peroxide. Superoxide dismutase has redox active metals (Mn, Cu, Zn) at its active site. Three superoxide dismutases have been identified in mammals: Cu/Zn SOD, MnSOD, and ECSOD. The redox active metal reacts with superoxide forming molecular oxygen and the reduced redox active metal. The reduced redox active metal reacts with a second superoxide molecule forming hydrogen peroxide and re-oxidizing the redox active metal (see Figure 2 of PMID: 21473702). In a phase 2b clinical trial, Avasopasem reduced the incidence of severe oral mucositis in head and neck cancer patients receive cisplatin and ionizing radiation (PMID: 31618127)

## XXV. Hyperthermia

- XXV-1) Which of the following statements concerning the Arrhenius analysis of mammalian cell killing by heat is TRUE?
- A. An Arrhenius curve plots the log of the slope ( $1/D_0$ ) of heat survival curves as a function of temperature
  - B. The break point in the Arrhenius plot is defined as the temperature at which the slope of the plot significantly increases.
  - C. The Arrhenius relationship has been used to define the temperature dependence of mechanisms of cell killing
  - D. The results of these analysis suggested the nuclear matrix may be a target of heat-induced cell killing
  - E. The break point in the Arrhenius plot is different between rodent and human cancer cells.
- 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. Hyperthermia can restore aeration to hypoxic tumor cells by increasing blood flow, thereby increasing radiosensitivity.
  - C. Heat increases the number of ionizations produced by a given dose of radiation
  - D. Hyperthermia induces radiosensitization by potentiating radiation damage to DNA.
  - 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 directly damages DNA
  - B. Following exposure to hyperthermic conditions, heat shock proteins (HSPs) bind to and activate the heat shock transcription factor (HSF1).
  - C. Hyperthermia leads to the activation of HSF1, which subsequently binds to HSE and increases expression of HSPs.
  - D. Hyperthermia increases expression of HSPs, which bind to the HSE, leading to increased expression of HSF1.
  - E. Heat-induced radiosensitization occurs secondary to HSP-mediated aggregation of nuclear proteins.

- 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. Thermotolerance limits the clinical utility of hyperthermia
  - E. Thermotolerance results in decreased likelihood of subsequent thermotolerance.
- XXV -5) Concerning the effects of heat on cells, which of the following statements is FALSE?
- A. Cells of low  $pH_e$  (extracellular pH) or low  $pH_i$  (intracellular pH) are sensitive to heat.
  - B. Cycling cells are more sensitive to heat than non-cycling cells.
  - C. Both chronic and acutely hypoxic cells have similar sensitivity to heat.
  - D. Initial shoulder of hyperthermic survival curve suggests the repair of sublethal damage.
  - E. Hyperthermia does not affect the repair of radiation-induced DNA damage
- 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 induce compression and occlusion of tumor blood vessels.
  - E. Tumors get hotter than surrounding normal tissues because of ineffective dissipation of heat.

XXV-7) Hyperthermia and radiotherapy perform complementary actions in achieving tumor cell killing. Which of the following statements is FALSE?

- A. Hyperthermia causes protein damage while radiotherapy causes DNA double strand breaks in cells
- B. Hyperthermia preferentially kills cells in S phase and radiotherapy cells in G<sub>2</sub>/M phases of the cell cycle
- C. Hyperthermia causes damage preferentially in hypoxic regions, while radiotherapy primary affects aerobic regions of tumors
- D. Hyperthermia above 43°C inhibits repair of radiotherapy-induced damage
- E. Long-duration mild hyperthermia (42°C) cannot inhibit the repair of sublethal radiation damage because of the low temperature

## XXV. Hyperthermia Answers & Explanations

- XXV-1) C A linear Arrhenius plot is used for first order rate kinetics of a chemical reaction. Although cell killing is not a likely a simple chemical reaction, the plot for cell killing is useful for theorizing the mechanism of cell killing by heat. The Arrhenius plot demonstrates the temperature at which the mechanisms underlying cell killing changes, potentially reflecting different targets for cytotoxicity above the break point (43°C)

The Arrhenius analysis plots survival data of cell cultures exposed to increasing temperatures. The X-axis plots  $1/D_0$ , where  $D_0$  represents the time at a given temperature required to reduce the fraction of surviving cells to 37% of the initial population. The Y-axis plots  $1/T$ , where T is the absolute temperature (Answer Choice A).

The slope of the Arrhenius plot suggests the activation energy of the chemical process involved in the cell killing. At some point ( $1/T$ ), corresponding to approximately 43°, there is a significant and abrupt decrease in the slope (Answer Choice B).

The results of these analysis suggested that the target for heat cell killing may be a protein.

The break point in the Arrhenius plot occurs at a temperature of approximately 43°C, and is the same across mammalian cells (Answer Choice E).

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 Tumor cells are more likely to be hypoxic and demonstrate a low pH compared to normal cells, which can contribute to their relative radioresistance. Hyperthermia can increase the radiosensitivity of tumor cells by increasing blood flow, leading to increased aeration and radiosensitivity.

Tumor cells are not intrinsically more sensitive to heat than normal cells but are often found in a low pH environment which makes them more sensitive (Answer Choice A).

Heat does not affect the number of ionizations produced by a given dose of radiation (Answer Choice C).

Hyperthermia induces radiosensitization by targeting proteins (likely repair proteins), not DNA (Answer Choice D).

If hyperthermia is administered a long time following radiation there is decreased heat-induced radiosensitization because the majority of radiation-induced DNA damage has already been repaired (Answer Choice E).

- XXV-3) C At normal body temperature, heat shock proteins (HSPs), such as Hsp90 or Hsp70, are bound to heat shock transcription factor (HSF1), thereby keeping it in its inactive state. Following exposure to higher temperatures, HSPs dissociate from HSF1 in order to stabilize degenerated proteins. HSF1 is then activated and translocates into the nucleus to bind to Heat Shock Element (HSE), the promoter for HSPs, leading to enhanced transcription of the HSP gene.

HSPs are molecular chaperones that bind to non-native or (partially) unfolded proteins and assist in their correct assembly by preventing their non-productive aggregation. An additional major mechanism for heat-induced radiosensitization is inhibition of the re-polymerization step in the repair of radiation-induced base damage. Heat therefore does not cause DNA damage directly (Answer Choices A and E).

Hyperthermia leads to HSPs dissociating from HSF1, thereby leading to its activation (Answer Choice B).

Hyperthermia leads to HSP dissociation from HSF1, which is then activated and binds to the HSE, leading to increased expression of HSPs (Answer Choice D).

Heat-induced cell death may additionally 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 An initial brief high temperature treatment followed by heating at a lower hyperthermic temperature is called Step-down heating. Step-down heating results in greater *sensitivity* to a subsequent heat treatment at a

lower temperature due to inhibition of the development of thermotolerance following the initial 43°C treatment (Answer Choices E and B). Once the protein damage is removed by HSPs after heat treatment, the HSPs rebind HSF1, thereby decreasing the level of HSPs in an autoregulatory loop and restoring normal heat sensitivity.

Thermotolerance is an acquired transient resistance to heat that is *not* heritable by the progeny of the treated cells (Answer Choice A).

Thermotolerance develops *during* the heating of tissues at temperatures *lower* than 43°C (Answer Choice 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 (Answer Choice D).

- 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.

Hypoxic cells are sensitive to heat due to their likely low pH. In this respect there are no apparent differences between chronic and acute hypoxia (Answer Choice C)

By its effect on proteins, hyperthermia can alter repair of radiation damage (Answer Choice E)

- XXV-6) A Tumor vasculature often lacks normal vascular structure. As a result of poor oxygen delivery by tumor neo-vasculature, tumor capillaries with functioning vasomotor control generally are open and used to capacity. In normal tissues, however, vasomotor activity is related to demand. When demands for oxygen delivery and homeothermy are normal many capillaries in normal tissue are closed.

- XXV-7) E Hyperthermia and radiation have different mechanisms of cell killing where heat affects proteins and radiation causes strand breakage in DNA. Cells are most resistant to radiation in S phase while most sensitive to heat in S phase. Studies have shown that hyperthermia at lower temperatures (42C) can have Thermal Enhancement Ratios above 1.0 (thus increasing radiation damage) and this increases with higher temperatures.



## 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
  - E. Children who receive cranial irradiation as part of their treatment for leukemia have a significantly increased risk for developing meningiomas
- 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) Of the fatal cancers that develop among patients previously treated with total body irradiation, approximately what percentage are leukemias?
- A. 0.1%
  - B. 2%
  - C. 15%
  - D. 40%
  - E. 80%
- XXVI-4) For children who, historically, were treated for tinea capitis using ionizing radiation, 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

- XXVI-5) Which of the following statements is CORRECT? Cancers induced in humans following exposure to low-dose whole-body irradiation:
- 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 exposure
- 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, wholebody 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 choices is considered to be a general conclusion from 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 3-4 in order for these estimates to be properly applicable to chronic, low dose, and low dose-rate exposures
  - B. Analyses of the Japanese A-bomb survivor data indicate that radiation risk is not dependent on gender
  - C. For solid tumors in A-bomb survivors, a linear fit to the data is significantly better than a linear-quadratic fit
  - D. Studies of populations living near nuclear power plants and exposed to elevated background radiation form our primary quantitative estimates of risk following exposure to radiation.
  - 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 5% per 100 mSv

- XXVI-8) Which of the following is an example of a stochastic effect of exposure to high-dose radiation:
- A. Mental retardation following exposure of the fetus *in utero*
  - B. Acute mucositis
  - C. Development of breast cancer 20 years following exposure to radiation as a teenager
  - D. Cardiac toxicity
  - E. Cataracts
- XXVI-9) Which of the following is TRUE about the thyroid carcinomas that occurred secondary to radiation exposure following the Chernobyl nuclear power plant accident?
- A. The carcinomas were induced by <sup>137</sup>Cesium 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 potassium iodide (KI) to the population.
  - D. Initial cancers were induced predominantly in adults that had been exposed.
  - E. The peak in incidence was approximately 30 years after exposure.
- XXVI-10) Which of the following tumors are NOT considered to be highly radiogenic?
- A. breast
  - B. leukemia
  - C. thyroid
  - D. cervical
  - E. bladder
- XXVI-11) Which of the following is TRUE regarding radiation exposure to the United States population?
- A. Medical exposure is the leading contributor to the average annual effective dose.
  - B. Exposure related to nuclear reactors has resulted in the doubling of the average annual effective dose over the past 40 years.
  - C. Natural background radiation contributes very minimally to the average annual effective dose.
  - D. The average annual effective dose secondary to medical procedures is much lower in the United States compared to that seen in other developed countries.
  - E. Of the available diagnostic radiographic procedures in the United States, computed tomography (CT) scans contribute the least to the average annual effective dose.

- XXVI-12) 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
  - E. Targeted alpha-particle radionuclide therapy

## XXVI. Radiation Carcinogenesis Answers & Explanations

- 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 patients diagnosed with soft tissue sarcoma patients has been reported, with a particularly high risk of developing a new soft tissue sarcoma (Answer Choice B).

Radiotherapy to the breast or chest wall of young women is associated with long-term cardiotoxicity and an increased risk of second breast cancers (Answer Choice C).

Genetic factors, as well as the potential carcinogenic effects of treatment, can affect the probability of second cancers in survivors. Patients with the BRCA2 mutation demonstrate an 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 (Answer Choice D).

Children who receive cranial irradiation as part of their treatment for leukemia are at a significant increased risk for developing meningiomas.

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](#)

Goshen Y, Stark B, Komreich, et al. High incidence of meningioma in cranial irradiated survivors of childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer* 49:294-297, 2007. [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 following treatment with ionizing radiation.

Boice JD. Radiation-induced thyroid cancer -- what's new? *J Natl Cancer Inst* 97:703-705, 2005. [Pubmed](#)

Cardis E, Kesminiene A, Ivanov V, *et al.* Risk of thyroid cancer after exposure to <sup>131</sup>I in childhood. *J Natl Cancer Inst* 97:724-32, 2005. [Pubmed](#)

- XXVI-3) C Approximately 15% of the fatal cancers diagnosed among patients previously treated with total body irradiation 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 Among the population of children who were treated for tinea capitis (ringworm) using ionizing radiation, an excess incidence was not detected for head and neck cancers. Brain cancers, thyroid cancers, adenomas, (non-CLL) leukemias, and late development of breast cancer were all observed at an excess incidence among children that were previously treated with X-Rays compared with children who only received topical medications.

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 B, *et al.* Increased risk of breast cancer after low-dose irradiation. *Lancet* 1(8639):629-31. 1989. [Pubmed](#)

- XXVI-5) E The susceptibility to radiation-induced cancer decreases with increasing age at the time of irradiation.

Radium dial painters ingested significant quantities of radium-containing paint by repeatedly licking the paint brushes they used. These women subsequently developed an excess number of osteosarcomas due to the incorporation of radium into their growing bones and the continuous low-dose-rate irradiation received by these tissues over the next decades (Answer Choice A).

At this time, cancers induced by radiation cannot be distinguished from cancers that occur naturally, although molecular markers for radiation exposure may eventually be identified (Answer Choice B).

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 without a

dose threshold (linear no-threshold or LNT model). This hypothesis, however, has been challenged by those who believe that exposure to low radiation doses may be less harmful than what is 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 an increased risk at low doses over those predicted by the LNT model (Answer Choice C).

Hematological malignancies have shorter latency periods compared to solid tumors (Answer Choice D).

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.

Survivors who received less than 5 Gy demonstrate an increased risk of heart disease (Answer Choice A).

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 (Answer Choice B).

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 (Answer Choice C).

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 and die from a fatal cancer (Answer Choice E).

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* 659:109-117, 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 used a value of 1.5 for its own risk estimations, the factor of 2.0 has historically been applied to adjust for lower doses and dose-rates (Answer Choice A).

RERF data clearly indicate that radiation risk is dependent on gender, as well as age at exposure and time since exposure (Answer Choice B).

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 (Answer Choice D).

The BEIR VII estimates the lifetime additional cancer risk is about 1% following 100 mSv (Answer Choice E).

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 Development of cancer after high dose radiation is an example of a stochastic effect.

A stochastic effect fulfills two criteria:

- 1) the probability of an outcome of interest increases with increasing dose, typically without a threshold dose; and
- 2) the severity of an outcome of interest is not altered by dose (all or none)



In contrast, deterministic effects, such as mental retardation, cardiac toxicity, cataracts and acute toxicity, have a threshold dose below which it does not occur and have increased severity with increased dose.

	Stochastic	Deterministic
<b>Threshold dose</b>	No	Yes
<b>Effect of increasing dose</b>	Increased <u>probability</u> of outcome	Increased severity of outcome
<b>Severity of outcome</b>	All or none	Continuous

- XXVI-9) C Administration of potassium iodide (KI) to the children that had been exposed to 131 Iodide (<sup>131</sup>I) as a result of the accident would have reduced the number of thyroid cancers by decreasing exposure to the radioactive iodide.

While <sup>137</sup>Cesium (<sup>137</sup>Cs) was released as part of the accident, <sup>131</sup>I was the cause of the thyroid cancers that occurred (Answer Choice A).

Most of the tumors demonstrated rearrangements of the RET and PTC genes (Answer Choice B).

The majority of the tumors occurred predominantly in children within 7-10 years following exposure (Answer Choices D-E).

- XXVI-10) D Data from the atomic bomb survivors demonstrate that breast cancer, thyroid cancer, bladder cancer, and non-CLL leukemia were all significantly induced following exposure to radiation (Answer Choices A, B, C, and E).

Cancer of the cervix is tightly linked to HPV viral infection and is not considered to be a highly radiogenic tumor (Answer Choice D).

- XXVI-11) A Medical exposure contributes more than 50% of the average annual effective radiation dose, slightly higher than that contributed by environmental exposures, and has significantly increased since the 1980s.

The average annual effective dose related to population and occupational exposure from nuclear reactors remains minimal, at 0.0005 mSv to 0.005 mSv (Answer Choice B).

Following medical exposures, natural background radiation is the next largest contributor to the average annual effective dose, contributing approximately 37% of all radiation exposure (Answer Choice C).

The US population has a higher average annual effective dose from medical procedures compared to that of other developed countries. CT scans represent the greatest contributor followed by nuclear medicine procedures.

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](#)

- XXVI-11) C Alpha-particles are emitted during the decay of radionuclides, such as radon, that occur in nature. Radon gas escapes from the soil and builds up inside homes where it is inhaled and can cause lung cancer.

## 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 Sievert (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 the children of patients who had been treated with ionizing radiation prior to conception demonstrate 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 to be 1-2 Sievert (Sv)
  - E. The absolute mutation rate in humans is approximately 8% per Sv
- XXVII-3) Which of the following statements is CORRECT regarding studies of the Japanese A-bomb survivors by the Radiation Effects Research Foundation (RERF)?
- A. Significantly more mutations were not noted in children who had at least one parent who was exposed to ionizing radiation prior to conception.
  - B. The RERF Life Span Study provides the basis for the estimated doubling dose estimates for radiation-induced genetic mutations in humans.
  - C. More than 60% of the survivor cohort received acute exposures greater than 100 mSv
  - D. A significant limitation of the RERF study is the lack of available dosimetric data
  - 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 CORRECT?
- A. The dose response curve for radiation-induced mutagenesis was linear with a threshold
  - B. Radiation dose-rate was found to significantly affect mutagenesis.
  - C. Males were less susceptible to radiation-induced mutagenesis than females
  - D. Mutation rates at the different loci studied did not vary widely
  - E. The estimated doubling dose for mutations was approximately 2 Gy
- XXVII-5) Which of the following statements is TRUE regarding effects of radiation exposure on the male and female reproductive systems?
- A. The dose to induce temporary sterility in the female is 2 Gy
  - B. The latent period for temporary sterility in the female is 1 year
  - C. Radiation sterility in the male affects hormone balance, libido, and physical capability
  - D. The dose that will cause oligospermia and reduced fertility in the male is 0.15 Gy
  - E. The dose that will cause permanent sterility in the premenopausal (post-pubertal) in the female is 1 Gy
- XXVII-6) Which of the following statements is TRUE regarding ionizing radiation-induced mutagenesis?
- A. Mutations that are induced by ionizing radiation can be identified by T to A nucleotide transitions
  - B. High LET radiation tends to cause small deletions, while low LET radiation tends to cause large deletions
  - C. The spectrum of mutations observed following exposure to ionizing radiation is similar to the spectrum of mutations observed following exposure to ultraviolet (UV) light.
  - D. Exposure of sperm to low dose-rate radiation usually results in fewer mutations than exposure of sperm to the same dose but at a higher dose-rate.
  - E. The relative dose to double the rate of mutagenesis is 5 Gy.
- XXVII-7) Which of the following statements is true about the dose-rate effect?
- A. A dose-rate effect is described as occurring when an increased response (mutation induction, for example) is observed with a decreasing dose rate.
  - B. The testis is considered to be a dose-rate sensitive organ.
  - C. Exposure of the fetus in utero to radiation causes more damage if a high dose rate is used compared to when a low dose-rate is used.
  - D. An inverse dose-rate effect occurs in all mammalian tissues except gut.
  - E. FLASH technology is based on using a low dose rate to induce tumor cell killing in vivo.

## XXVII. Heritable Effects of Radiation Answers & Explanations

- 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.

Exposure to radon does not contribute significantly to the GSD because the decay products of radon are deposited almost entirely in the lung (Answer Choice A).

The GSD resulting from medical procedures performed annually in the United States is estimated to be 0.3 mSv, not 1 Sv (Answer Choice B).

Although the GSD can be utilized 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 (Answer Choice D)

The GSD is an annual population dose, not an individual lifetime dose (Answer Choice E).

- XXVII-2) D The dose required to double the incidence of mutations in humans has been estimated to be approximately 1-2 Sv.

Radiation does not induce characteristic mutations; it only increases the incidence of mutations that are known to occur spontaneously (Answer Choice A).

A higher incidence of genetic abnormalities was *not* found in the children with at least one parent who previously received treatment with ionizing radiation prior to conception (Answer Choice B).

The best estimates are that no more than 1-6% of spontaneous mutations in humans are due to exposure to background radiation (Answer Choice C).

The absolute mutation rate for humans has been estimated to be approximately 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 is that there has NOT been found to be a statistically significant increase in mutations identified in the F1 generation (approximately 70,000 individuals), despite the original expectation that there might be based on animal experiments.

The doubling-dose estimate for radiation-induced genetic mutations in humans is therefore based on mouse data coupled with estimates of human spontaneous mutation rates (Answer Choice B).

A majority of the survivor cohort received relatively low radiation exposure of less than 100 mSv (Answer Choice C).

A recently revised dosimetry model (DS02) provides improved estimates of individual exposures received by individuals who survived the Japanese A-bomb (Answer Choice D).

The Adult Health Study cohort members even today continue to undergo a thorough clinical exam every two years. By providing data and biological samples these participants remain an important resource for future analyses (Answer Choices D and E).

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. Interestingly, this is different than what was observed in the fruit fly study, which demonstrated that dose rate had no effect on the mutagenesis rate.

The dose response curve for radiation-induced mutagenesis was found to be linear WITHOUT a threshold (Answer Choice A).

Males were found to be MORE susceptible to radiation-induced mutation than females (Answer Choice C).

Mutation rates at the different loci studied DID vary widely (Answer Choice D)

The estimated doubling dose for mutations was approximately 1 Gray (Answer Choice E).

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](#)

Reference: Muller HJ, Advances in radiation mutagenesis through studies on drosophila. *Prog Nucl Energy 6 Biol Sci* 2:146-60, 1959. [Pubmed](#)

XXVII-5) D The dose that will lead to oligospermia and reduced fertility in the male is estimated to be 0.15 Gy.

There is neither a latent period nor temporary sterility in the female following exposure to radiation (Answer Choices A and B).

Radiation sterility does not affect hormone balance, libido, or physical capability in the male, but can induce permanent ovarian failure and menopausal symptoms in the female (Answer Choice C).

The dose that will lead to permanent sterility in the female is 12 Gy in the prepubertal woman and 2 Gy in the premenopausal (mature) woman (Answer Choice E).

XXVII-6) D Low dose-rate exposure usually results in fewer mutations than the same dose given at a high dose rate.

T to A transitions are usually found following exposure to ultraviolet (UV) light, but not to ionizing radiation (Answer Choice A).

High LET radiation tends to cause large deletions, while low LET radiation tends to cause small deletions (Answer Choice B).

The types of mutations observed following exposure to ionizing radiation can differ from the spectrum of mutations observed following exposure to UV radiation (Answer Choice C).

The relative dose to double the rate of mutagenesis is estimated to be 1 Gy (Answer Choice E).

- XXVII-7) B Testis is a dose-rate sensitive organ with low dose rates causing more damage than the same dose at a high dose rate. A dose-rate effect is described as occurring when a decreased response is observed following a decreased dose rate. Exposure of the fetus in utero causes more damage if the exposure is at a low dose rate than if the same dose is given at a high dose-rate; this is likely due to the fact that more stages of gestation are affected at a low dose-rate. FLASH technology is based on using an ultra-fast dose-rate to enhance tumor killing and somehow lowering normal tissue toxicity.



## 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 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 during which weeks of gestation?
- A. 0-4 weeks
  - B. 5-8 weeks
  - C. 8-15 weeks
  - D. 16-25 weeks
  - E. 26-40 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 potential risks associated with the exposure of the embryo or fetus in utero to ionizing radiation?
- A. Exposure to ionizing radiation in utero between weeks 8 and 15 of gestation is associated with the highest risk of development of mental retardation, while there is a lower risk at 15-25 weeks of gestation.
  - B. Exposure to ionizing radiation in utero has not been demonstrated to be associated with increased risk of carcinogenesis to the fetus.
  - C. Exposure to ionizing radiation during the preimplantation phase has been shown to result in permanent growth retardation.
  - D. The LD50 for oocyte killing in humans is approximately 5 Gy.
  - E. Exposure of the fetus in utero has not been associated with changes in school performance or intelligence quotient (IQ).
- XXVIII-8) Radiation exposure in utero is associated with an increased risk of childhood cancers. Which of the following statements is INCORRECT?
- A. The Oxford Survey of Childhood Cancers determined the relative cancer risk estimate of 1.5 for children developing leukemia exposed to x-rays in utero.
  - B. An obstetric x-ray examination has approximately a 40% increase in the risk of childhood cancer over the spontaneous level
  - C. Low dose radiation in the first trimester causes an increased risk of childhood cancer
  - D. Leukemias are possible childhood cancer resulting from *in utero* x-ray exposure.
  - E. 10 mSV dose to the fetus can cause a quantifiable increased risk of childhood cancer.

## XXVIII. Radiation Effects in the Developing Embryo and Fetus Answers & Explanations

XXVIII-1) A The most sensitive period during gestation is when radiation exposure may cause embryonic lethality. Based on animal studies, this period of time occurs immediately following conception but prior to implantation within 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, organogenesis, 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.

Gestational Period	Gestation (Weeks)	Main Risk	Additional Risk
Preimplantation	0-1.5 weeks	Prenatal Death	Microcephaly
Organogenesis	1.5-6 weeks	Congenital Malformations	
Early Fetal Period	6-8 weeks		
	8-15 weeks	Mental Retardation ( <b>High Risk</b> ) <i>Risk ~0.4 per Gy</i> <i>~25 IQ points per Gy.</i>	
Late Fetal Period	16-25 weeks	Mental Retardation (Lower Risk) <i>Risk ~0.1 per Gy</i>	Growth Retardation Carcinogenesis
	26-40 weeks		

Adapted from Figure 12.9, Hall EJ and Giaccia AJ, Eds. *Radiobiology for the Radiologist*, 8<sup>th</sup> Ed. Lippincott Williams & Wilkins, Philadelphia, 2018.

XXVIII-3) C Radiation exposure during weeks 8-15 of gestation is mostly likely to cause mental retardation (MR) compared to the other periods of gestation. The incidence of severe MR has been found to be linear during this period of time, with a risk coefficient of 0.4 per Gy. During weeks 16-25, the risk has been found to be lower (by approximately 4-fold). A dose threshold of 0.3 Gy to appreciate development of MR has previously been reported. Mental retardation is thought to be secondary to effects of radiotherapy on neural cell migration.

Otake M, Schull WJ. Radiation-related small head sizes among prenatally exposed A-bomb survivors. *Int J Radiat Biol.* 63:255–270. 1993.  
[Pubmed](#)

Otake M, Schull WJ. In utero exposure to A-bomb radiation and mental retardation: a reassessment. *Br J Radiol.* 57:409–414. 1984.  
[Pubmed](#)

XXVIII-4) C Once a pregnancy is declared, the maximum permissible dose to the fetus is 0.5 mSv per month subsequent to this declaration. 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 to ionizing radiation in utero has been shown to be associated with a higher risk for mental retardation (MR), and can be accompanied by lower intelligence quotient (IQ) and poor school performance (Answer Choices A and E). The risk of development of MR has been shown to be greatest between weeks 8 and 15 of gestation, while this risk decreases by approximately 4-fold between 15 and 25 weeks of gestation. Ultrasound is generally utilized for monitoring the fetus in utero due to concerns regarding radiation carcinogenesis (Answer Choice B).

Exposure to ionizing radiation during the pre-implantation phase has been shown to be associated with pre-natal death (Answer Choice C).

The LD50 for oocytes has been shown to be approximately 0.5 Gy (Answer Choice D).

- XXVIII-8) C Low dose radiation of the fetus during the third trimester, not the first trimester, causes an increased risk of childhood cancers, especially leukemias. The Oxford Survey of Childhood Cancers suggested an association between childhood cancer and in utero exposure to diagnostic x-rays. This was a retrospective case-controlled study of 7,649 children who died of leukemia of childhood cancers. The relative cancer risk estimate assuming radiation to be the causative agent was estimated at 1.52 (Lancet 1:1185, 1970).

## XXIX. Radiation Protection

- XXIX-1) A woman begins working at a nuclear power plant on her 18th birthday. According to current NCRP guidelines, once she reaches her 20th birthday, she will 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 21<sup>st</sup> birthday, the same radiation worker was 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. 50 mSv to the eye and 150 mSv to the skin
  - D. 50 mSv to the eye and 500 mSv to the skin
  - E. 500 mSv to the eye and 150 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) Of the following pairs of individuals and maximum annual effective dose equivalents permitted, which is CORRECT? (These values exclude doses from exposure to background radiation, both natural and man-made)
- A. A radiation oncologist – 200 mSv per year
  - B. A member of the general public – 1 mSv per year
  - C. A sixteen-year-old high school student who works part time in a laboratory – 0 mSv per year
  - D. A nuclear power plant worker – 10 mSv per year
  - E. A patient's relative who transports a radiotherapy patient to and from treatment – 20 mSv per year
- 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. Computed Tomography
  - D. Industrial activity
  - E. Consumer products
- XXIX-9) Fluoroscopy for medical purposes can contribute the most dose to and cause the greatest clinical effects within which organ?
- A. Skin
  - B. Brain
  - C. Lungs
  - D. Heart
  - E. Bone
- XXIX-10) The radiation protection value that is used to take the dose-rate into effect when examining risk for cancer from radiation exposure is:
- A. DDREF
  - B. LNT
  - C. HSR
  - D. LDRR
  - E. There is no such value

## XXIX. Radiation Protection Answers & Explanations

- 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 20<sup>th</sup> birthday. 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: recommendations of the National Council on Radiation Protection and Measurements, 1993. [Link to Report](#)

- 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 mother's fetal monitor worn at the mother's waist, under any protective apron worn by the woman.

- XXIX-3) D A radiation worker is currently permitted 50 mSv to the eye and 500 mSv to the skin in any given year. This is a recent change from the previous NCRP recommendations for which the annual dose equivalent limit for the lens of the eye was 150 mSv. These limits are based on risk estimates for the production of radiation-induced deterministic effects.

The ICRP recommends an annual equivalent absorbed dose limit for the lens of the eye to be 15 mSv for the public. 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.

- 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 imaging 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 of time, 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 (Answer Choice A).

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 (Answer Choice C).

A patient’s relative transporting a patient to and from radiotherapy 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 (Answer Choice E).

- XXIX-6) C The Maximum Permissible Dose (MPD) defines the recommended occupational exposure dose limits and does not include any dose received from medical procedures or natural background radiation (Answer Choices C and D). Radiation workers (including residents) are considered subject to occupational exposure limits. Medical students are considered subject to the education and training exposure limits.

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” (Answer Choice A).

The MPD recommendations for radiation workers are typically 10-50 fold higher than for members of the general public (Answer Choice B).

The NCRP and ICRP guidelines treat age differently in establishing the MPD. The effective dose limit for occupational exposure per the NCRP guidelines is 10 mSv per year of age or 50 mSv per year and per the ICRP guidelines is 20mSv per year (averaged over 5 years) or 50mSv per year. 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 (Answer Choice E).

- XXIX-7) B The “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 Per the National Council on Radiation Protection and Measurements (NCRP), Report No. 160, the average annual radiation dose per person in the United States is approximately 6.2 millisieverts (mSv) or 620 millirem (mrem). The majority (37%) of this dose can be attributed to background radiation sources including Radon and Thoron. These gases are created when other naturally occurring elements undergo radioactive decay.

Cosmic radiation contributes 5% of the average annual dose (Answer Choice B).

An additional 48% of the average dose to an individual in the United States is from medical procedures (not including dose received during therapeutic radiation). Of these, Computed Tomography (CT) scans comprise approximately 24% of radiation dose (Answer Choice C).

Industrial activity contributes only a very small amount of the average annual dose to the average American (<0.1%, or 0.003 mSv).

Consumer products contributes approximately 2% of the average annual dose (Answer Choice E)

Radiation Sources and Doses. United States Environmental Protection Agency. <https://www.epa.gov/radiation/radiation-sources-and-doses>

- 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.

- XXIX-10) A Dose and dose-rate effectiveness factor is used to take into consideration dose and dose-rate when considering exposures for cancer risk. LNT is the linear non-threshold model. HSR is the hypoxia sensitizer radio. LDRR is a usual abbreviation for low dose rate radiation.

### 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) Which of the following statements concerning gel electrophoresis is TRUE?
- A. DNA molecules are negatively charged so they 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.  $^{18}\text{F}$ -2-deoxy-2-fluoro-D-glucose ( $^{18}\text{F}$ -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  $^{18}\text{F}$ -FDG is typically lower in areas of inflammation
  - D. An important advantage to using  $^{18}\text{F}$ -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  $^{18}\text{F}$ -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  $\gamma$ -H2AX foci
  - B. western blot
  - C. alkaline comet assay
  - D. southern hybridization
  - E. northern hybridization

- XXX-5) Which statement regarding next generation sequencing (NGS) is FALSE:
- A. Unlike capillary sequencing, NGS requires the cloning and amplification of DNA sequence-containing phage libraries
  - B. NGS is a massively parallel process with a million or more simultaneous DNA sequence reads.
  - C. NGS is not hampered by homopolymer repeat sequences.
  - D. NGS generally performs short DNA reads of less than 100 bases.
  - E. In NGS, bases are read by sequential computer-mediated image analysis.
- XXX-6) Pulsed-field gel electrophoresis can be used in order 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) A scientist is planning an experiment in which he wants to determine whether his exponential cultures of human HCT116 colorectal cancer cells express wild-type p53 protein. Which of the following experimental assays would NOT be an effective readout for this purpose?
- A. Western blotting with the p53 protein-specific antibody
  - B. Performing flow cytometry in order to analyze the cycling characteristics of HCT116 cells 12 h after exposure to 6 Gy X-Rays
  - C. Northern blotting to measure WAF1/CIP1 mRNA expression in HCT116 cells 3 h after exposure to 6 Gy X-Rays.
  - D. Immunoblotting with a cyclin E-specific antibody in order to detect cyclin E activity 12 hours after exposure to 6 Gy X-Rays.
  - E. Immunoblotting with an antibody against p53, phosphorylated at the serine 15 residue, 1 hour after exposure to 6 Gy X-Rays.

- XXX-9) Clonogenic survival assays are commonly used to assess *in vitro* cellular response to ionizing radiation and cytotoxic agents. Which of the following statements is INCORRECT about the clonogenic survival assay?
- A. A feeder layer consists of irradiated cells that no longer divide but still produce growth-stimulating factors for the colony forming cells
  - B. Trypsinizing donor culture cell is necessary to produce a single-cell suspension
  - C. Plating efficiency is the ratio of the number of formed colonies to the number of cells plated
  - D. The survival fraction is the number of colonies formed multiplied by the plating efficiency and divided by the number of cells plated
  - E. Survival curves are plotted with surviving fraction on the Y-axis and therapeutic dose (radiation or cytotoxic agent) on the X-axis

### XXX. Molecular Techniques used in Radiation and Cancer Biology Answers & Explanations

- XXX-1) E The use of microRNAs (miRNAs) and small interfering RNAs (siRNAs) 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  $^{18}\text{F}$  is 110 minutes, not 10 days. PET imaging cameras detect the 0.51 MeV photons produced by the annihilation resulting from the interaction of a positron and electron. The uptake of  $^{18}\text{F}$ -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  $\gamma$ -H2AX is currently considered the most sensitive assay for the repair of DNA double-stranded breaks, although there is some controversy regarding whether  $\gamma$ -H2AX foci are also formed in response to other types of DNA changes. The *neutral* comet assay can also be used to measure DNA double-strand breaks, although it is generally considered less sensitive than  $\gamma$ -H2AX.

- XXX-5) A Capillary sequencing requires *in vivo* cloning and amplification whereas next generation sequencing (NGS) utilizes adaptor ligation of DNA fragments and binding to a matrix for DNA sequencing.

Metzker ML. Sequencing technologies – the next generation. *Nat Rev Genet.* 11(1):31-46. 2010. [Pubmed](#)

- XXX-6) D Pulsed-field gel electrophoresis is a technique in which a gel is subjected to electrical fields that are alternating in orientation, thereby allowing 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 its mRNA is short-lived ( $T_{1/2} = 8$  min) in unstressed cells. The induction of DNA double strand breaks by X-ray irradiation initiates a p53-dependent signal transduction cascade. One downstream target of this cascade includes the induction of WAF1/CIP1 mRNA, which encodes the p21 protein. Upregulation of WAF1/CIP1 protein inhibits the cyclin E/cyclin-dependent kinase 2 complex, an event that is able to stop cells from progressing through G<sub>1</sub>. 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. [Pubmed](#)

El-Deiry WS, Harper JW, O'Connor PM, et al. WAF1/CIP1 is induced in p53-mediated G<sub>1</sub> arrest and apoptosis. *Cancer Res* 54(5):1169-1174, 1994. [Pubmed](#)

Waldman T, Kinzler KW, Vogelstein B. p21 is necessary for the induced G<sub>1</sub> arrest in human cancer cells. *Cancer Res* 55(22):5187-5190, 1995. [Pubmed](#)

Pandita TK, Lieberman HB, Lim DS, et al. Ionizing radiation activates the ATM kinase throughout the cell cycle. *Oncogene* 19(11):1386-1391, 2000. [Pubmed](#)

- XXX-9) D A clonogenic survival assay is an *in vitro* cell survival assay based on the ability of a single cell to grow into a colony (~50 cells). This approach is commonly used by radiation biologists to assess

reproductive death after ionizing radiation or treatment with other cytotoxic agents. Following treatment with radiation and/or cytotoxic agents, only a fraction of cells retain reproductive capacity and can form colonies. A feeder layer consists of cells (fibroblasts) that have been irradiated (typically ~30 Gy) and can no longer divide but still produce growth factors for the colony forming cells. Trypsin is a protease that breaks down cellular adhering proteins to facilitate release of cells from each other and detach cells from a tissue culture dish. Plating efficiency is the ratio of the number of colonies after treatment to the number of cells plated. The survival fraction is:

$\text{#colonies after treatment} \div (\text{number of cells plated} * \text{plating efficiency})$ .

Survival curves are semi-log plots, with the survival fraction (logarithmic scale) on the Y-axis and therapeutic dose on the X-axis (linear scale).



### XXXI. Molecular Imaging

- XXXI-1) The most commonly used biologically active molecule for positron emission tomography (PET) scanning is a fluoridated 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 using positron emission tomography (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 via CT scanning.
  - C. Water has an X-ray attenuation of 0 Hounsfield units (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 a 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 the time between radioisotope injection and completion of the PET scan.
  - E. Typical doses of FDG for the clinically useful PET imaging are in a range of 15 Ci.
- XXXI -6) Which of the following statements is correct regarding PET-PSMA scans?
- A. Physiological uptake is seen in the brain, heart and kidneys.
  - B. PSMA stands for Phosphatidyl Serine Membrane Antagonist.
  - C. PSMA scans are a good method of detecting micrometastases from prostate cancer
  - D. The tracer is based upon the peptidomimetic Glu-NH-CO-NH-Lys(Ahx)-HBED-CC combined with the radionuclide  $^{68}\text{Ga}$ .
  - E. The radionuclide gallium-68 ( $^{68}\text{Ga}$ ) decays through electron emission to the stable isotope zinc-68 ( $^{68}\text{Zn}$ )

## XXXI. Molecular Imaging Answers & Explanations

XXXI-1) B Secondary to the predominant use by tumors of glycolysis instead of oxidative phosphorylation for energy production, 18-Fluorodeoxyglucose is the most helpful and therefore most commonly used metabolic radiotracer for PET scanning for malignancies...

XXXI-2) D 18-Fluorine labeled thymidine has been used to image DNA synthesis in humans in vivo. The other nucleosides would not differentiate DNA from RNA.

Salskov A, Tammisetti VS, Grierson J, Vesselle H. FLT: measuring tumor cell proliferation in vivo with positron emission tomography and 3'-deoxy-3'-[18F]fluorothymidine. *Semin Nucl Med* 37(6):429-39. 2007. [Pubmed](#)

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 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 ( $\mu$ ) to X-ray attenuation in water  $\mu(\text{water})$  through the equation:

$$\text{HU} = 1000 \times \frac{\mu - \mu(\text{water})}{\mu(\text{water})}$$

Based on the above equation, the HU of water is 0. 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 1 cGy per scan and as much as 10 cGy from multiple CT scans. For example, cumulative doses from 2-3 head CTs to the brain are 5-6 cGy. For comparison, X-ray doses from chest radiography are 0.01 cGy (0.1 mGy) and X-ray doses from mammography are 0.04 cGy (0.4 mGy). The Nuclear Regulatory Commission (NRC) is responsible for the regulation of radioactive materials used medically; this includes the diagnostic radionuclides used in positron emission tomography (PET) imaging. On the other hand, CT scanners, image reconstruction software, as well as other “medical devices” such as linear accelerators and associated

treatment planning software are regulated by the United States Food and Drug Administration (FDA).

See Appendix 1.

<b>Diagnostic Scan</b>	<b>Estimated maximum Organ Dose</b>	<b>Rounded</b>
<b>X-Ray films</b>		
Chest (PA and Lat)	0.014 cGy	<i>0.01 cGy</i>
Dental panoramic	0.07 cGy	<i>0.07 cGy</i>
Lumbar-Sacral spine	0.2-0.3 cGy	<i>0.3 cGy</i>
Mammogram	0.2-0.4 cGy	<i>0.4 cGy</i>
<b>Radiotracer Imaging</b>		
Heart Stress (Tc-99m)	0.6-1.2 cGy	<i>1 cGy</i>
Bone (Tc-99m)	0.4-1.5 cGy	<i>1 cGy</i>
Dual Isotope Stress Test	4.0-4.5 cGy	<i>4 cGy</i>
PET (F-18 FDG, Bladder)	5.5-8.0 cGy	<i>8 cGy</i>
<b>CT Scans (X-Ray) – Multiple scan average dose</b>		
Chest CT	2.0-3.0 cGy	<i>3 cGy</i>
Head CT	3.0-5.0 cGy	<i>5 cGy</i>
Abdominal CT	2.2-6.0 cGy	<i>6 cGy</i>
Full Body CT	5.0-10.0 cGy	<i>10 cGy</i>
<b>Fluoroscopy/Procedures</b>		
Barium Contrast (GI)	1.0-2.2 cGy	<i>2 cGy</i>
Cardiac Catheterization	1.2-4.0 cGy	<i>4 cGy</i>
TIPS Procedure	40-140 cGy	<i>100 cGy</i>

*Adapted from Appendix 1, Metting, NF. "Orders of Magnitude," Office of Science of the Department of Energy, 2010. <http://www.lowdose.energy.gov>*

Additional 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.

McCullough 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 The standard uptake value (SUV) is a standard method of quantifying the radioactive uptake observed in a positron emission tomography (PET) scan image. As a cancer detection method, fluorodeoxyglucose (FDG) PET is based on the observation that in normoxic conditions tumor cells primarily use glycolysis for energy production instead of mitochondrial oxidative phosphorylation as normal cells do. This phenomenon is known as the Warburg effect. SUV scores of >15 g/mL usually indicate a tumor that is highly dependent on glucose metabolism and is therefore more aggressive rather than indolent. It therefore follows that SUV scores inversely correlate with local tumor control (Answer Choice A)

The SUV is calculated as follows:

$$\text{SUV [g/ml]} = (\text{Tissue activity (mCi/ml)}) / (\text{injected dose (mCi)} \times \text{patient's body weight (g)}).$$

SUV score values are therefore proportional to a patient's body weight (Answer Choice B)

SUV values increase in value if the PET scan is delayed after FDG injection (Answer Choice D).

The tissue activity (mCi) per lesion volume (mL) is derived from pixel intensities of PET (or PET/CT) images. This quantity is then divided by the amount of radioactive tracer injected into the patient (mCi) per unit of his/her body weight (g) (specific activity). The typical dose of FDG administered to adult patient is approximately 10-18 mCi (Answer Choice E).

**References:**

Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324(5930):1029-33, 2009. [Pubmed](#)

Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death and Disease* 4:e532, 2013. [Pubmed](#)

- XXXI-6) D PSMA is expressed in certain non-prostate tissues, such as the kidneys, small intestine and salivary glands, consequently these tissues also demonstrate uptake on PET-PSMA scan. Conversely, PET-FDG

scans demonstrate the uptake and subsequent secretion of labelled glucose. On PET-FDG scans physiological uptake is seen in the brain, heart and urinary system and other metabolically active tissues. PSMA stands for Prostate-specific membrane antigen. PSMA is a membrane-bound antigen that is highly specific for benign and malignant prostatic epithelium, although endothelial cells in multiple organs are also immunoreactive. PET PSMA is not reliable for lesions less than 5 mm in size. The radionuclide gallium-68 ( $^{68}\text{Ga}$ ) decays through positron emission to the stable isotope zinc-68 ( $^{68}\text{Zn}$ ). The tracer is indeed based upon the peptidomimetic Glu-NH-CO-NH-Lys(Ahx)-HBED-CC combined with the radionuclide  $^{68}\text{Ga}$ .

## XXXII. Cancer Immunology

- XXXII-1) Which of the following organ systems may be affected by an immune adverse event in a lung cancer patient receiving radiotherapy plus immune check point blockade?
- A. Pulmonary
  - B. Endocrine
  - C. Gastrointestinal
  - D. Skin
  - E. All of the above
- XXXII-2) Which of the following is an example of adaptive immune resistance?
- A. Process whereby a patient is tolerant to a tumor associated antigen (i.e. NY-ESO or PSCA) before starting immunotherapy but develops immunity to it once beginning treatment.
  - B. Process by which tumor cells change phenotype in response to an immune response (cytotoxicity or inflammation) in an attempt to avoid recognition (i.e. the induction of PD-1, PD-L1, and IDO following antigen recognition and the production of IFN $\gamma$ ).
  - C. Process by which tumors, following radiotherapy, undergo accelerated proliferation with an increased incidence of failure.
  - D. Process by which there is increased resistance to radiotherapy due to reactive response by the immune system.
  - E. Process by which pattern recognition receptors detect damage-associated molecular patterns (DAMPs) induced by radiation.
- XXXII-3) Which of the following may increase a patient's susceptibility to experience an immune related adverse event?
- A. History of autoimmune disease
  - B. Abnormal thyroid function
  - C. Previous use of checkpoint blockade therapy
  - D. Previous radiation therapy
  - E. All of the above
  - F. A, C and D

- XXXII-4) Immune related adverse events (irAEs) describe a range of immune-mediated toxicities that can result from treatment with immune checkpoint inhibitors. Which statement regarding irAEs is NOT correct:
- A. Skin, gut, endocrine, lung and musculoskeletal irAEs are relatively common, whereas cardiovascular, hematologic, renal, neurologic and ophthalmologic irAEs occur much less frequently.
  - B. irAEs typically have a delayed onset and prolonged duration compared to adverse events from chemotherapy.
  - C. irAEs are discrete toxicities caused by tissue-specific inflammation and activation of the immune system and can affect almost any organ system.
  - D. The overall incidence of irAEs following treatment with anti-CTLA-4 monotherapy tends to be lower than those with anti-PD-1/PD-L1 agents
  - E. The incidence of irAEs with ipilimumab and pembrolizumab is dose-dependent, with greater toxicity at higher dose levels;
- XXXII-5) Which of the following agents does not target the PD-1/PDL-1 axis?
- A. Pembrolizumab
  - B. Avelumab
  - C. Ipilimumab
  - D. Durvalumab
  - E. Nivolumab
- XXXII-6) Which of the following statements is CORRECT when comparing the abscopal effect versus the bystander effect in the context of radiation responses?
- A. The abscopal effect describes a response that occurs distant from the irradiated site (in the same organism), whereas the radiation-induced bystander effect refers to general biological effects in unirradiated cells that are in close proximity to irradiated cells (*in vivo* or *in vitro*).
  - B. The abscopal effect describes a phenomenon where an irradiated tumor regresses while another unirradiated metastatic site (in the same organism) accelerates in tumor growth, whereas the radiation-induced bystander effect refers to general biological effects in unirradiated cells that are in close proximity to irradiated cells (*in vivo* or *in vitro*).
  - C. During an abscopal response, one irradiated tumor regresses with at least two unirradiated tumor metastatic sites responding as well (in the same organism), whereas the radiation-induced bystander effect refers to the phenomenon when only half of a tumor is irradiated but the whole tumor regresses.
  - D. Both phenomena essentially go hand in hand because non-irradiated bystander cells travel to non-irradiated distant tumors sites and cause an immune-mediated regression.



- XXXII-7) Which of the following statements best describes the “abscopal effect?”
- A. Localized irradiation of a tumor is associated with improved disease-related symptoms that are not associated with the treated tumor.
  - B. Localized irradiation of a tumor is associated with a decrease in size not only of the irradiated tumor but also of a tumor that is far from the irradiated area
  - C. Localized irradiation of a tumor is associated with a decrease in size not only of the irradiated tumor but also of a tumor that is far from the irradiated area when given with concurrent immunotherapy
  - D. Localized irradiation of a tumor is associated with a decrease in size not only of the irradiated tumor but also of a tumor that is far from the irradiated area when given with concurrent chemotherapy
- XXXII-8) Antigen recognition by T cells is imperative for the development of cellular adaptive immunity. How does a T cell recognize an antigen?
- A. T cells recognizes antigens via pattern recognition receptors
  - B. T cells recognizes antigenic determinants presented in the MHC cleft by the T cell receptor
  - C. T cells recognize antigens via the F<sub>c</sub> receptor binding to membrane-bound IgD antibodies
  - D. T cells recognize antigens via PD-1 binding
- XXXII-9) Which of the following molecules is NOT an immune checkpoint receptor protein?
- A. LAG3
  - B. PD-1
  - C. TIM3
  - D. OX40
  - E. CTLA-4
- XXXII-10) Which radiation-induced immune effect would be counterproductive to effective anti-tumor immunity?
- A. Radiation-induced release of danger signals
  - B. Radiation-induced increase in regulatory T cells
  - C. Radiation-induced increase in MHC class I expression
  - D. Release of pro-inflammatory cytokines
  - E. Radiation-induced epitope spreading

XXXII-11) Tumor cells may escape the host's immune response by a plethora of innate and adaptive mechanisms. Which of the following would NOT be considered such a mechanism?

- A. Loss of  $\beta$ 2-microtubulin expression leading to decreased MHC class I expression
- B. Tumor cell intrinsic alterations in signaling pathways such as WNT/ $\beta$ -catenin, loss of PTEN, and  $\text{IFN}\gamma$  that inhibit T cell priming and infiltration
- C. Recruitment of myeloid suppressor cells
- D. Loss of antigen expression through immune selection
- E. Increased expression of immune inhibitory factors such as Indoleamine 2,3-Dioxygenase (IDO) and PD Ligand 1 (PD-L1)

XXXII-12) Which immune-mediated mechanism plays a role in cancer prevention?

- A. Detection and elimination of tumor cells
- B. Allergic responses
- C. Prevention of chronic inflammation
- D. Protection against viral infection and integration
- E. A, C and D

XXXII-13) What does PD-1 stand for?

- A. Programmed cell death 1 receptor
- B. Presentation determinant 1
- C. Pre-determinant molecule 1
- D. Pattern determinant 1
- E. Principal determinant 1

## XXXII. Cancer Immunology Answers & Explanations

- XXXII-1) E Immune related adverse events (irAEs) are a distinctive range of immune-mediated toxicities which may affect any body system. irAEs can occur at any time during or after treatment and they can be life-threatening. Early identification and swift management (typically corticosteroids) are key in avoiding life-threatening severity.

Puzanov et al, Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother of Cancer*. 5(1):95. 2017. Pubmed

- XXXII-2) B The innate immune system is the body's first line of defense against foreign substances entering the body. It responds in the same way to all foreign substances, which is why it is sometimes referred to as the "nonspecific" immune system. It acts very quickly, and any memory it has is short-lived (this is sometimes referred to as the trained immune response). On the other hand, the adaptive immune response is specific, takes longer to develop, lasts longer, and has a distinct of long-term memory. A prototypical innate immune response involves the recognition of pathogen associated molecular patterns (PAMPs, in case of a pathogenic infection) or damage associated molecular patterns (DAMPs, in the case of radiation injury for instance) by pattern recognition receptors (Choice E). A prototypical adaptive immune response involves T cells with specificity for the foreign antigen allowing recognition and targeted (specific) destruction when there's a match of the T cell receptor (with a recognition complex) and the foreign antigen presented to it. The process by which tumor cells change phenotype in response to an immune response (cytotoxicity or inflammation) in an attempt to avoid recognition (i.e. the induction of PD-1, PD-L1, and IDO following antigen recognition and the production of IFN $\gamma$ ) is characteristic of an adaptive immune response (Choice B). Choice A refers to immune tolerance where an initial immune response to a foreign antigen is lost over time. Choice C is not related to immune response and Choice D is a non-specific choice with no correlation to the adaptive immune response.

Ribas A and Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*, 359 (6382), 1350-1355. 2018. Pubmed

Ribas A. Adaptive Immune Resistance: How Cancer protects from Immune Attack. *Cancer Discovery*,. 5(9):915-9. 2015. Pubmed

- XXXII-3) F Abnormal thyroid function does not increase susceptibility to experience an immune related adverse event

XXXII-4) D Most common toxicities from targeting immune checkpoints relate to breaking tolerance. Targeting of PD-1 or PD-L1 tends to be less toxic than targeting CTLA-4 on average, presumably because they regulate different components in the evolution of an immune response. The cytotoxic T lymphocyte-associated antigen 4 (CTLA4)-mediated immune checkpoint is induced in T cells at the time of their initial response to antigen. The level of CTLA4 induction depends on the amplitude of the initial T cell receptor (TCR)-mediated signaling. High-affinity ligands induce higher levels of CTLA4, which dampens the amplitude of the initial response. The key to the regulation of T cell activation levels by the CD28–CTLA4 system is the timing of surface expression. Naive and memory T cells express high levels of cell surface CD28 but do not express CTLA4. Instead, CTLA4 is sequestered in intracellular vesicles. After the TCR is triggered by antigen encounter, CTLA4 is transported to the cell surface. The stronger the stimulation through the TCR (and CD28), the greater the amount of CTLA4 that is deposited on the T cell surface. Therefore, CTLA4 functions as a signal dampener to maintain a consistent level of T cell activation in the face of widely varying concentrations and affinities of ligand for the TCR. By contrast, the major role of the programmed cell death protein 1 (PD1) pathway is not at the initial T cell activation stage but rather to regulate inflammatory responses in tissues by effector T cells recognizing antigen in peripheral tissues. Activated T cells upregulate PD1 and continue to express it in tissues. Inflammatory signals in the tissues induce the expression of PD1 ligands, which downregulate the activity of T cells and thus limit collateral tissue damage in response to a microorganism infection in that tissue. The best characterized signal for PD1 ligand 1 (PDL1; also known as B7H1) induction is IFN $\gamma$ , which is predominantly produced by T helper 1 (TH1) cells, although many of the signals have not yet been defined completely. Excessive induction of PD1 on T cells in the setting of chronic antigen exposure can induce an exhaustive or anergic state.

Postow et al. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. *N Engl J Med*, 378(2):158-168. 2018.  
Pubmed

Pardoll D.M. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*, 12(4):252-64. 2012.  
Pubmed

XXXII-5) C Inhibitors of programmed cell death 1 (PD-1) receptor include pembrolizumab and nivolumab. Inhibitors of programmed death ligand 1 (PD-L1) include atezolizumab, durvalumab, and avelumab.

Ipilimumab is an example of a Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) inhibitor. Ipilimumab functions by disinhibiting the native ability of T cells to recognize and destroy cancer cells.

- XXXII-6) A The abscopal effect describes a situation whereby a patient being treated with radiation therapy to a site of metastatic disease experiences concurrent regression of a distant site of metastatic disease that is not being directly irradiated. The abscopal hypothesis was first described in 1953 to refer to the effects of ionizing radiation occurring "at a distance from the irradiated volume but within the same organism."

In contrast, the bystander effect describes the induction of biologic effects in cells that are in close proximity to cells that are directly traversed by a charged particle.

- XXXII-7) B The abscopal response is defined as the influence of ionizing radiation on cell killing outside of a field of radiation. For example, an abscopal response would be considered if an unirradiated tumor is shown to have decreased in size following the irradiation of a tumor located in a distant area.

Ehlers G, Fridman M. Abscopal effect of radiation in papillary adenocarcinoma. *Br J Radiol* 46:220–2, 1973. Pubmed

Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med* 366:925-931, 2012. Pubmed

- XXXII-8) B The surface of each T cell has approximately 30,000 antigen-receptor polypeptide chains, T-cell receptor  $\alpha$  (TCR $\alpha$ ) and  $\beta$  (TCR $\beta$ ), that are linked by a disulfide bond. The TCR is very similar in structure to the F<sub>ab</sub> fragment of an immunoglobulin molecule and account for antigen recognition by most T cells. A minority of T cells has an alternative, but structurally similar, receptor made up of a different pair of polypeptide chains designated  $\gamma$  and  $\delta$ . Unlike B cells, which can recognize a protein antigen in its native state, T cells recognize an antigen via the TCR only after it has been processed into peptides and loaded onto major histocompatibility complex (MHC) molecules. During the endogenous antigen presentation pathway (performed by the majority of cells), an intracellular antigen is degraded by the proteasome into peptides (typically 9-10 amino acids long) and then loaded onto MHC class I molecules in the Endoplasmic Reticulum (ER). In the exogenous pathway, an extracellular antigen is taken up by antigen presenting cells (APCs) and degraded into peptides (typically 11-19 amino acids long) within endosomes and then bound to MHC class II molecules. In both pathways, full MHC-peptide complexes are transported to the cell surface

for recognition by the TCR on CD8<sup>+</sup> cytotoxic T cells (MHC I) or CD4<sup>+</sup> T helper cells (MHC II). Cross-presentation is the display of peptides from extracellular antigens on MHC class I and is only performed by APCs..

Pattern recognition receptors (PPRs) such as Toll-like receptors (TLRs) are predominantly found on APCs and other innate immune cells and are used for the detection of danger signals such as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The engagement of PPRs initiates the maturation of APCs, especially dendritic cells, thereby allowing them to stimulate T cells by providing the first signal (signal 1: antigen) to the TCR and the second signal (signal 2: co-stimulation) to CD28, which then amplifies signal 1. PD-1 is an immune checkpoint that inhibits proximal signaling of the TCR by sequestering Src Homology Region-Containing Protein Tyrosine Phosphatase-2 (SHP-2) and facilitating Csk-mediated inhibitory phosphorylation of Lck.

- XXXII-9) D Lymphocyte Activating 3 (LAG3) is a cell surface immune checkpoint receptor protein that is expressed on activated T cells and negatively regulates cellular prol and is activated by Major Histocompatibility Class (MHC) II. Programmed cell death protein (PD)-1 is a cell surface immune checkpoint receptor protein that is bound by its two ligands, PD-L1 and PD-L2 and functions to suppress T cell inflammatory activity and promotes self tolerance. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is another immune checkpoint receptor protein that is activated by CD80 and CD86 and functions to downregulate the immune response. More recently identified as a potential immune checkpoint target, T-cell immunoglobulin and mucin domain-3 (TIM-3) is activated by Galectin-9 (Gal-9), Phosphatidylserine, HMGB-1, and Cecam-1 and plays a role in T cell exhaustion. LAG3, PD-1, CTLA-4, and TIM3 all act as co-inhibitory receptors that limit or inhibit the activation of T cells even if the TCR is engaged. In contrast, OX40 (OX40L) is a co-stimulatory receptor that does *promotes* T cell activation by driving T-cell proliferation, memory, cytotoxic effector function, and cytokine production. Other examples of co-stimulatory molecules are 4-1BB, CD40L, GITR, ICOS, and CD27.

Of these, CTLA-4 and PD-1 are probably the most studied to date. CTLA4 counteracts the activity of the T cell co-stimulatory receptor, CD28, both sharing identical ligands: namely, CD80 (B7.1) and CD86 (B7.2). Although CTLA-4 is active on CD8<sup>+</sup> T cells, it seems that most of its effects are derived from down-modulation of helper T cell activity and enhancement of T<sub>reg</sub> immunosuppressive activity. On the other hand, PD-1 limits the activity of T cells in peripheral tissues at the time of an inflammatory response to infection and functions to limit autoimmunity

by, for example, suppressing immune system activation within the tumor microenvironment.

PD-1 expression is induced following T cell activation. When engaged by one of its ligands, PD-1 inhibits kinases that are involved in T cell activation through the phosphatase SHP250, although additional signaling pathways are also likely induced. The general concept is that blocking CTLA-4 affects *early* T cell activation whereas blockade of PD-1 signaling is more relevant *later*, at the tissue site, thereby explaining why the CTLA-4 inhibitors are associated with more significant toxicity.

Pardoll D.M. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*. 12(4):25-264, 2012. Pubmed

Mahoney et al. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. *Clin Ther*. 37(4):764-82, 2015. Pubmed

XXXII-10)B Danger signals, MHC class I expression, pro-inflammatory cytokines and epitope spreading would support the development of an immune response with the purpose of achieving anti-tumor immunity, while activation of regulatory T cells would not. Regulatory (suppressor) T cells are a subset of CD4<sup>+</sup> T cells that express the transcription factor forkhead box P3 (FOXP3). FOXP3 is a potent suppressor of immune responses to self and non-self and is essential to the maintenance of peripheral immunological tolerance. Tregs suppress the activation, proliferation, and cytokine production of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and are additionally thought to suppress B cells and dendritic cells. They exert their suppressive activity through cell-to-cell contact and via the production of soluble suppressive/inhibitory messengers (i.e. TGF-beta, IL-10 and adenosine). Loss of function mutations in the *Foxp3* gene underlie the lymphoproliferative disease of the Scurfy mouse and the homologous autoimmune lymphoproliferative disorder in man, termed Immune dysregulation Polyendocrinopathy Enteropathy-X (IPEX) linked syndrome. Of note, despite the immune suppressive function, the infiltration of tumors by Tregs doesn't necessarily indicate worse prognosis as it is also an indicator for a T cell inflamed tumor phenotype, (i.e. a (positive) sign for immune reactivity).

Recognition of danger signals such as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by pattern recognition receptors on APCs initiates cellular maturation and confers to these cells the ability to process and present antigens to T cells (signal 1) as well as achieve co-stimulation (signal 2), both of which are necessary to induce T cell activation. Antigen presentation without signal 2 can cause T cell anergy. Antigenic peptides are presented within

the MHC cleft on the cell surface with the assumption is that an increase in MHC class I expression would be supportive of better antigen presentation thereby making tumor cells more 'visible' to T cells. Tumor cell death in response to immunotherapy may lead to the release of secondary (nontargeted) tumor antigens that prime subsequent immune responses.

Epitope spreading (or antigen cascade, antigen spread, determinant spread) describes a phenomenon where the immune response evolves and expands from focusing on a single antigenic epitope, into a multi-epitopic response be it naturally or following therapeutic intervention e.g. vaccination or radiotherapy. This process is dynamic and may continue to expand over time. Antigen spreading of the anti-tumor immune response from one antigen to another antigen has been linked to superior clinical outcome with the assumption that it counteracts tumor immune evasion.

Medzhitov R. Approaching the Asymptote: 20 Years Later. *Immunity*. 30(6):766-75, 2009. Pubmed

Pulendran B. Immune Activation: Death, Danger and Dendritic Cells. *Current Biology*, 14(1):R30-2, 2004. Pubmed

Butterfield LH, et al. Determinant Spreading Associated with Clinical Response in Dendritic Cell-based Immunotherapy for Malignant Melanoma. *Clin Can Res*. 9(3):998-1008, 2003. Pubmed

Butterfield LH. Cancer vaccines. *BMJ*. 350:h988, 2015. Pubmed

Gulley JL, et al. Role of Antigen Spread and Distinctive Characteristics of Immunotherapy in Cancer Treatment. *JNCI*. 109(4), 2017. Pubmed

Noval Rivas M and Chatila TM. Noval Rivas and Chatila Regulatory T cells in allergic diseases. *J Allergy Clin Immunol*. 138(3):639-65, 2016. Pubmed

XXXII-11)A Beta-2-microglobulin ( $\beta_2$ -microglobulin, B2M; not  $\beta_2$ -microtubulin) is a crucial component of major histocompatibility complex (MHC) class I molecules. MHC class I molecules are heterodimers made of two, non-covalently linked polypeptide chains,  $\alpha$  and B2M. The conformation of the MHC class I protein is highly dependent on the presence of B2M. B2M is essential for proper MHC class I folding and transport to the cell surface. Its deficiency has long been recognized as a genetic mechanism of acquired resistance to immunotherapy.



Spranger and Gajewski Mechanism of Tumor cell-intrinsic immune evasion. *Annu. Rev. Cancer Biol.* 2018. 2:213–28

Spranger S and Gajewski TF. Impact of oncogenic pathways on evasion of antitumor immune responses. *Nat Rev Cancer.* 18(3):139-147, 2018. Pubmed

Zaretzki JM, et al. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *N Engl J Med.* 375(9): 819–829, 2016. Pubmed

XXXII-12)E Schreiber RD, Old LJ, Smyth MJ, et al. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 331 (6024):1565-70, 2011\_Pubmed

XXXII-13)A

## 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 textbooks used in radiation biology are:

Hall EJ and Giaccia AJ, Eds. *Radiobiology for the Radiologist*, 8<sup>th</sup> Ed. Lippincott Williams & Wilkins, Philadelphia, 2018.

Joiner M and van der Kogel A, Ed. *Basic Clinical Radiobiology*, 5<sup>th</sup> Ed. Arnold, London, 2018.

Additional useful textbooks include:

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*, 5<sup>th</sup> Ed. McGraw-Hill, Medical Pub. Division, New York, 2013

Mettler FA and Upton AC, Eds., *Medical Effects of Ionizing Radiation*, 3<sup>rd</sup> 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, Wazer DE, Perez CA, Brady LW, *et al.*, Eds., *Principles and Practice of Radiation Oncology*, 7<sup>th</sup> Ed. Lippincott Williams & Wilkins, Philadelphia, 2018

Gunderson LL and Tepper JE, Eds., *Clinical Radiation Oncology*, 4<sup>th</sup> Ed. Churchill Livingstone, New York, 2016

Leibel SA and Phillips TL, Eds., *Textbook of Radiation Oncology*, 3<sup>rd</sup> Ed. W.B. Saunders, Philadelphia, 2010

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

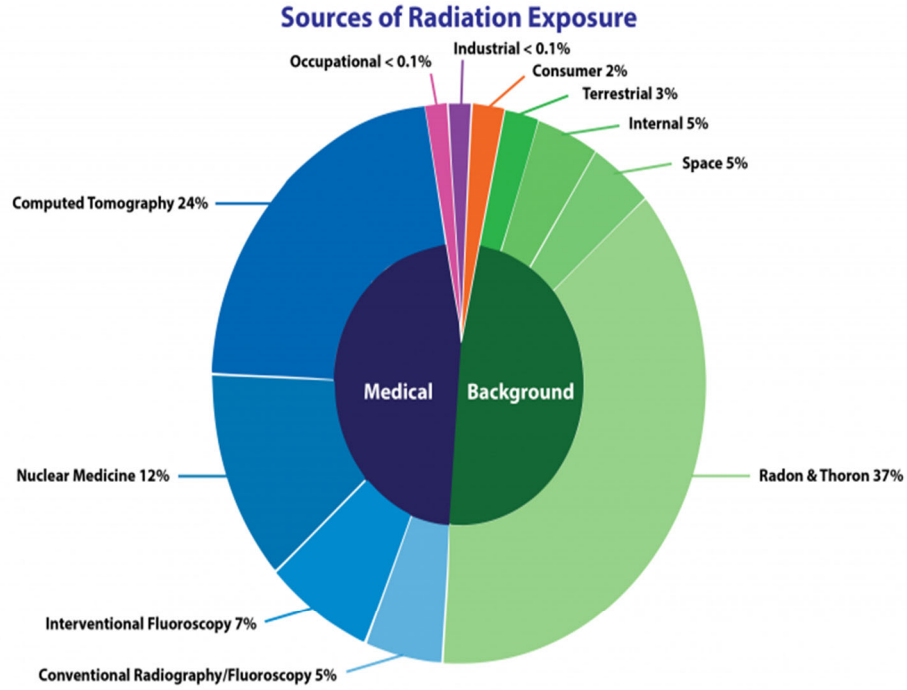
Rosenstein BS, Chapter 3. Molecular Radiobiology: in *Handbook of Radiation Oncology: Basic Principles and Clinical Protocols*. Haffty B and Wilson L, Eds. Jones and Bartlett Publishers, Sudbury MA, 2009

- Baumann M, Kurth I, Cordes N, Krause M, and Linge A, Chapter 2. Molecular Cancer and Radiation Biology. pp. 71-86, in *Principles and Practice of Radiation Oncology*, 7<sup>th</sup> Ed. Halperin EC, Wazer DE, Perez CA, Brady LW, *et al.*, Eds., Lippincott Williams & Wilkins, Philadelphia, 2018.
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- 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](#)
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- Part 2: Tumor Biology, Chapters 3-17, pp 19-230 in *Holland-Frei Cancer Medicine*, 9<sup>th</sup> Ed. Bast Jr RC, Croce CM, *et al.*, Eds., John Wiley & Sons, Inc, Hoboken, NJ., 2017.

# APPENDICES



## Appendix II: Average United States Doses and Sources



Average Annual Radiation Dose											
Sources	Radon & Thoron	Computed Tomography	Nuclear Medicine	Interventional Fluoroscopy	Space	Conventional Radiography/Fluoroscopy	Internal	Terrestrial	Consumer	Occupational	Industrial
<b>Units</b>											
mrem (United States)	228 mrem	147 mrem	77 mrem	43 mrem	33 mrem	33 mrem	29 mrem	21 mrem	13 mrem	0.5 mrem	0.3 mrem
mSv (International)	2.28 mSv	1.47 mSv	0.77 mSv	0.43 mSv	0.33 mSv	0.33mSv	0.29 mSv	0.21 mSv	0.13 mSv	0.005 mSv	0.003 mSv

(Source: National Council on Radiation Protection & Measurements, Report No. 160)

<https://www.epa.gov/radiation/radiation-sources-and-doses>