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RADIATION/CANCER BIOLOGY
STUDY GUIDE

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Contents

Radiation-Matter Interactions

1. Which one of the following sequences correctly orders portions of the electromagnetic spectrum in terms of increasing photon energy?
    a. Radiowaves, infrared, visible light, UV, X-rays
    b. UV, X-rays, microwaves, infrared, radiowaves
    c. Visible light, UV, X-rays, radiowaves, infrared
    d. Radiowaves, UV, X-rays, visible light, infrared radiation
    e. UV, infrared, radiowaves, visible light, X-rays

2. Which of the following ionization processes represents the principal interaction with tissue for X-rays used in radiotherapy?
    a. Pair production
    b. Photoelectric effect
    c. Compton process
    d. Photodisintegration
    e. Coherent scattering

3. An atom or molecule that has an unpaired electron in its outer shell is referred to as a(n):
    a. Spallation product
    b. Heavy ion
    c. Ion pair
    d. Recoil proton
    e. Free radical

4. Which of the following statements concerning photons is correct?
    a. Exposure to a particular dose of 1 MeV γ-rays compared with mono-energetic 1 MeV X-rays will produce significantly different biological effects
    b. Compton scattering results in the release of characteristic X-rays
    c. Electromagnetic radiations travel at less than the speed of light
    d. The annihilation reaction involves an interaction between a positron and an electron
    e. Higher energy photons have longer wavelengths than lower energy photons

5. Which of the following statements concerning the interaction of radiation with matter is TRUE?
    a. Both X- and γ-rays are produced by nuclear disintegration
    b. Auger electrons are a product of pair production
    c. Free radicals have half-lives on the order of seconds
    d. Free radicals carry a net electrical charge
    e. There is complete photon absorption in the photoelectric effect

6. Which one of the following particles has the smallest mass?
    a. Neutron
    b. Positron
    c. α-particle
    d. Proton
    e. Carbon ion

7. Which of the following statements concerning photons is TRUE?
    a. Ideally, photons used for radiotherapy should interact with matter through the photoelectric effect
    b. Photons can be produced by the annihilation reaction, which involves an interaction between a positron and an electron
    c. X-rays travel faster than visible light
    d. The probability of a photoelectric interaction is inversely proportional to the atomic number of the absorber
    e. Compton scattering results in the production of Auger electrons

8. The minimum energy needed to ionize an atom is of the order of:

a. 1 eV
b. 10 eV
c. 100 eV
d. 1 keV
e. 10 keV

9. Ultraviolent radiation consists of photons with a wavelength in the range of 100 nm - 400 nm. Ultraviolet radiation is not a form of ionizing radiation because:
    a. Ultraviolet photons have insufficient charge to produce free electrons in materials that absorb them
    b. Ultraviolet photons have insufficient mass to produce free electrons in materials that absorb them
    c. Ultraviolet photons have insufficient energy to produce free electrons in materials that absorb them
    d. Ultraviolet photons are special and can't be absorbed by any material
    e. Ultraviolet radiation has a higher wavelength than ionizing radiation

10. Under what circumstances is the photoelectric effect most likely to occur when photons interact with matter?
    a. When the photon energy is low and close to the binding energy of an orbital electron
    b. When the photon energy is more than the mass of two electrons (1.022Mev)
    c. When the photon energy is sufficient to allow for a deviation in path of travel and diminution of energy
    d. When the photon is generated after the interaction between an electron and a positron
    e. When the photon energy is less than 1 MeV

11. The deposition of energy in matter from proton beams differs from photon beams in several ways. The majority of energy deposition from proton beams occurs:
    a. Near the beginning of the track, forming the Bragg peak
    b. Near the middle of the track, forming the Bragg peak
    c. Near the end of the track, forming the Bragg peak
    d. Throughout the entire track, forming the Bragg peak
    e. None of the above

12. Select the best description of the pair production process.
    a. Photon interacts with a free electron, and this electron as well as a positron is set in motion
    b. In contrast to Compton and photoelectric processes, pair process is independent of atomic number
    c. No ionizations are produced in pair process
    d. The process is independent of photon energy above the 1.02-MeV threshold
    e. For 10 MV-photon, the total amount of energy transferred to a positron – electron pair is 8.98 MeV

13. Through a Compton scattering event with water, a photon will produce: 1) a free electron with high energy, 2) an ionized water molecule, and 3) the original photon with reduced energy. Which of the following subsequent reactions is considered an indirect effect of the original photon?
    a. The high-energy free electron further interacts with a different water molecule to cause another ionization event
    b. The ionized water molecule encounters another water molecule, and produces a hydroxyl free radical
    c. The original photon continues to ionize other water molecules
    d. Czerenkov radiation is given off
    e. All of the above

14. A researcher is testing the effects of different conditions on the kinetics of an enzymatic reaction. The reaction is known to occur faster in the presence of free radicals. In which of the following conditions is the reaction expected to occur most quickly?
    a. Anhydrous enzyme irradiated with 100 Gy
    b. Anhydrous enzyme with free radical scavenger drug irradiated with 100 Gy
    c. Hydrated enzyme irradiated with 100 Gy
    d. Hydrated enzyme with free radical scavenger drug irradiated with 100 Gy
    e. No difference from one treatment to another

DNA Damage Mechanisms

1. The biological effects resulting from exposure to ultraviolet (UV) radiation are due primarily to the formation of:
    a. Thymine glycols
    b. Ionizations
    c. Pyrimidine dimers
    d. Heat
    e. Oxidized guanine

2. Which type of radiation-induced DNA damage is most important for cell killing caused by exposure to ionizing radiation?
    a. Base damage
    b. DNA double-strand break
    c. DNA single-strand break
    d. DNA-protein crosslink
    e. DNA-DNA inter-strand crosslink

3. Which of the following is NOT produced by exposure to ionizing radiation?
    a. Thymine glycol
    b. Single-strand break
    c. 6-4 photoproduct
    d. 8-oxo-guanine
    e. DNA-histone crosslink

4. The yield of initial DNA double-strand breaks produced in an irradiated mammalian cell will be influenced by all of the following, EXCEPT:
    a. Radiation dose
    b. Lack of oxygen during irradiation
    c. Presence of amifostine during irradiation
    d. Absence of histone proteins
    e. Absence of RAD51

5. Non-targeted, radiation-induced bystander effects are associated with:
    a. Production of pyrimidine dimers
    b. Effects in non-irradiated cells co-cultured with irradiated cells
    c. Induction of mutations in *BCL2*
    d. Radiation-induced heat
    e. Induction of miRNA

6. The DNA damage produced by ionizing radiation causes:
    a. Double strand breaks as the exclusive form of damage
    b. Irreparable damage
    c. Single strand breaks more frequently than double strand breaks
    d. Death for all cells exposed to ionizing radiation
    e. T-T dimers

7. A researcher is able to introduce various compartments of a eukaryotic cell with the radioisotope I-125, an emitter of short ranged gamma-rays and low-energy Auger electrons that deposit energy locally. When using the same amount of I-125 in each compartment, which is most likely to achieve cell kill?
    a. Cytoplasm
    b. Nucleus
    c. Endoplasmic reticulum
    d. Mitochondria
    e. Golgi apparatus

DNA Repair Mechanisms

1. Which of the following is NOT a characteristic of DNA-dependent protein kinase (DNA-PK)?
    a. Consists of a catalytic subunit and two smaller accessory proteins, Ku70 (XRCC6) and Ku80 (XRCC5)
    b. Participates in the repair of DNA double strand breaks primarily through homologous recombination
    c. Loss in mice results in altered radiation sensitivity
    d. Phosphorylates histone H2AX at sites of double strand breaks
    e. Belongs to the phosphatidyl inositol 3-kinase-like protein kinase (PIKK) family

2. Which of the following proteins is NOT directly involved in repairing DNA double strand breaks?
    a. Artemis
    b. RAD51
    c. DNA-PKcs
    d. CDK4
    e. BRCA1

3. SCID mice are often used in cancer research because they:
    a. Are radioresistant
    b. Exhibit high levels of non-homologous end joining
    c. Have efficient immune systems
    d. Are better able to repair radiation damage
    e. Are useful hosts for growing human tumor xenografts

4. Cells derived from individuals diagnosed with xeroderma pigmentosum are deficient in:
    a. Nucleotide excision repair
    b. Methyl-guanine transferase
    c. Mismatch repair
    d. Base excision repair
    e. Homologous recombination

5. Homologous recombinational repair of DNA double strand breaks is most likely to occur:
    a. In G_0
    b. In G_1
    c. In early S phase
    d. In late S phase
    e. Throughout the cell cycle

6. Which syndrome is caused by a deficiency in the repair-associated protein MRE11?
    a. Werner's syndrome
    b. Ataxia-telangiectasia-like disorder
    c. Xeroderma pigmentosum
    d. Bloom's syndrome
    e. Cockayne's syndrome

7. All the following statements are true concerning homologous recombinational repair of DNA double strand breaks, EXCEPT:
    a. H2AX phosphorylation represents an important step in the formation of nuclear foci of DNA repair proteins
    b. The BLM protein serves to coat single stranded DNA regions to prevent their degradation
    c. The MRN complex relocates to sites of DNA double strand breaks to process DNA resulting in production of single stranded ends
    d. RAD51 is a recombinase and forms a nucleoprotein filament that facilitates strand invasion for homologous recombination
    e. ATM is activated following irradiation by auto-phosphorylation and conversion from an inactive dimer to an active monomer

8. All of the following statements about non-homologous end joining (NHEJ) are true, EXCEPT:
    a. Artemis is primarily responsible for ligating broken DNA ends
    b. DNA ligase IV forms a tight complex with XRCC4
    c. DNA-PKcs associates with Ku70/80 to form the DNA-PK holo-enzyme
    d. The Ku heterodimer has a high affinity for DNA ends and forms a close-fitting asymmetrical ring that threads onto a free end of DNA
    e. NHEJ is an error-prone process

9. Which of the following is NOT a known substrate for ATM?
    a. Ku70/80 (XRCC6/XRCC5)
    b. BRCA1
    c. NBS1
    d. p53 (TP53)
    e. CHK2 (CHEK2)

10. Which of the following statements is TRUE concerning DNA repair processes?
    a. Between 10-20% of the population is thought to be heterozygous for the types of mutations that are responsible for causing ataxia telangiectasia (AT)
    b. Non-homologous end-joining requires the involvement of a sister chromatid
    c. Mutations in the genes that encode proteins involved in translesion DNA synthesis are typically present in people who develop hereditary non-polyposis colon cancer
    d. The most common types of DNA damage induced by ionizing radiation are repaired through base excision repair
    e. Sublethal damage repair is significant for both x-rays and neutrons

11. Normal tissues from which of the following syndromes shows the highest level of sensitivity to ionizing radiation?
    a. Ataxia telangiectasia
    b. Systemic lupus erythematosus
    c. Bloom's syndrome
    d. Xeroderma pigmentosum
    e. Fanconi's anemia

12. Repair of DNA double-strand breaks can be accomplished by which one of the following pathways?
    a. Mismatch repair
    b. Non-homologous end joining
    c. Base excision repair
    d. Nucleotide excision repair
    e. Photoreactivation

13. RAD51 and BRCA2 function together:
    a. As inhibitors of cyclin dependent kinases
    b. To phosphorylate H2AX and NBS1
    c. To enhance apoptosis by inhibiting p53 (TP53)
    d. In the initial steps of homologous recombination
    e. To play a central role in nucleotide excision repair

14. Which of the following proteins localizes to and is used as a marker of DNA double strand breaks?
    a. p53
    b. CHk1
    c. γH2AX
    d. RB
    e. ATM

15. Which of the following is first involved in one of the first step of non-homologous end joining?
    a. KU70
    b. LIG4
    c. DNA-PKcs
    d. BRCA2
    e. ARTEMIS

16. Which of the following is the most proximal signaling event in the DNA damage response in cells?
    a. BRCA2 loading onto a double-strand break (DSB)
    b. Gamma-H2AX phosphorylation
    c. DNA-PK catalytic subunit autophosphorylation
    d. BRCA1 recruitment via RNF8 to a DSB
    e. CtIP-induced resection of DSB ends

17. What are the 2 most common DNA double-strand break repair pathways in mammalian cells?
    a. Single-strand annealing (SSA) and Homologous Recombination (HR)
    b. Mismatch repair (MMR) and Nucleotide Excision Repair (NER)
    c. Non-homologous end joining (NHEJ) and Microhomology mediated end joining (MMEJ)
    d. Non-homologous end joining (NHEJ) and Homologous Recombination (HR)
    e. Base excision repair (BER) and Homologous Recombination (HR)

18. Non-homologous end joining is thought to be most active in which phase of the cell cycle?
    a. Early S-phase
    b. G0/G1-Phase
    c. G2-phase
    d. Late S-Phase
    e. Mitosis

19. DNA double strand break repair occurs through several mechanisms. Defects in these pathways may alter cellular radiosensitivity. In mammalian cells, relative to homologous recombination (HR), defects in non-homologous end joining (NHEJ) are likely to lead to:
    a. Decreased radiosensitivity.
    b. Increased radiosensitivity
    c. Decreased radiosensitivity in quiescent cells only
    d. No difference in radiosensitivity in quiescent cells only
    e. No difference in radiosensitivity in dividing cells

20. Which of the following reflects a poor repair of DNA double strand breaks (DSB) after irradiation?
    a. Phosphorylation of H2AX 15 minutes after irradiation
    b. Phosphorylation of H2AX 24 hours after irradiation
    c. Persistent arrest at G1 phase 4 hours after irradiation
    d. Phosphorylation of ATM within 15 minutes after irradiation
    e. Phosphorylation of ATM within 24 hours after irradiation

21. Which of the following statements is TRUE about PARP (Poly-ADP Ribose Polymerase)?
    a. PARP is modified by glycosylation.
    b. Activation of PARP is an early step in double-stranded DNA break repair.
    c. PARP is involved as an important component of single-stranded DNA break repair.
    d. Necrosis is dependent upon PARP.
    e. PARP is phosphorylated by ATM and DNA-PK.

22. The photons in UV lights do not have sufficient energy to cause ionizing events, but it can still cause DNA damage because …
    a. UV lights can break up chemical bonds between atoms without an ionization event.
    b. UV lights usually have photon wavelength longer than 100 nm, which has a photon energy of more than 12.4 eV.
    c. UV lights cannot be absorbed by DNA molecules.
    d. UV lights cannot generate free radicals.

e. UV lights cannot be detected.

23. The following statements about measuring radiation-induced DNA damage are true, EXCEPT:
    a. Pulse Filed Gel Electrophoresis (PFGE) can detect fragmented DNA to assess level of DNA double strand breaks.
    b. Neutral comet assay can measure DNA double strand breaks.
    c. Alkaline comet assay can only detect DNA double strand breaks.
    d. Specific antibodies can be used to detect oxidated bases, such as 8-Oxoguanine (8-OxoG).
    e. Phosphorylated H2AX (gH2AX) can be used as a surrogate marker for DNA double strand breaks.

24. When a typical mammalian cell is exposed to a D_0 dose of γ-rays, a typical mammalian cell would produce the following number of DNA damages, EXCEPT:
    a. >1000 base damages
    b. ~1000 single strand breaks
    c. 30-40 double strand DNA breaks
    d. A large amount clustered DNA damage especially at low does rate.
    e. Some DNA-protein cross-links.

25. The following methods can be used to detect chromosome aberrations or rearrangements, EXCEPT:
    a. A metaphase spread assay can stain the condensed chromosomes at metaphase, and then used to score chromosome aberrations.
    b. Fluorescence *in-situ* hybridization (FISH) can be used to visualize a specific chromosome(s) so that the aberration of a given chromosome(s) may be detected.
    c. SKY (Spectral Karyotyping) FISH can be used to paint individual chromosomes with unique color patterns to detect chromosome aberrations.
    d. Copy number variation (CNV) analysis can be used to identify regional chromosome loss or gain.
    e. Image analysis of the interphase nucleus.

26. Which of these repair proteins is involved in both base excision repair and single-strand DNA break repair and confers a radiosensitive phenotype when mutated?
    a. DNA polymerase β
    b. Apurinic endonuclease I
    c. Xeroderma pigmentosum A (XPA)
    d. X-ray cross complementing factor 1 (XRCC1)
    e. Flap endonuclease 1 (FEN1)

27. The following is a TRUE statement about the effect of dose rate on sublethal damage repair:
    a. There is little variability in the impact of dose rate on the repair of DNA damage among different cell types.
    b. The dose rate effect on sublethal damage repair is most pronounced with reduced cell killing between 1 Gy per minute to 0.3 Gy per hour.
    c. As dose rate decreases, the slope of the survival curve becomes steeper.
    d. Increased survival when doses are split compared to delivered in a single fraction is most significant following exposure to high LET radiation.
    e. The inverse dose rate effect is most pronounced when HeLa cells are treated with a single low dose exposure.

28. Small molecule inhibitors of the following DNA repair proteins would be expected to induce robust radiosensitization, EXCEPT:
    a. ATM
    b. DNA-PK
    c. KU70/80
    d. ATR
    e. MGMT

Chromosome Damage

1. Radiation-induced anaphase bridges generally result from:
    a. Dicentric chromosomes
    b. Ring chromosomes
    c. Acentric fragment
    d. Isochromatid breaks and subsequent union between the sister chromatids
    e. A single chromosome break

2. The minimum whole body radiation dose that can be detected through the measurement of dicentric chromosomes in peripheral blood lymphocytes is approximately:
    a. 0.0005 Gy
    b. 0. 015 Gy
    c. 0.25 Gy
    d. 3.5 Gy
    e. 10 Gy

3. Which one of the following radiation-induced chromosome aberrations is a "single hit" type?
    a. Terminal deletion
    b. Acentric ring
    c. Dicentric
    d. Interchromosomal translocation
    e. Inversion

4. Which of the following types of chromosomal aberrations is **most likely** to cause lethality?
    a. Insertion
    b. Dicentric
    c. Translocation
    d. Inversion
    e. Anaphase bridge

5. An accidental exposure to a radiation source is reported one month following irradiation of a person not wearing a dosimeter. Which of the following assays would represent the best method to estimate the radiation dose received by this person?
    a. Alkaline elution
    b. Staining with a monoclonal antibody to γ-H2AX
    c. Karyotyping peripheral blood lymphocytes
    d. Pulsed-field gel electrophoresis
    e. Neutral comet assay

6. Ionizing radiation induced chromatid-type aberrations are a consequence of failed or mis-repaired DNA double strand breaks produced during which phase of the cell cycle?
    a. G1 and G2 phase
    b. G1 and S phase
    c. S and G2 phase
    d. Only in M phase
    e. G0 phase

7. Chromosomal translocations produced by ionizing radiation are generated:
    a. After the formation of at least 2 DNA double strand breaks
    b. In linear proportion to the radiation dose
    c. Only during S phase of the cell cycle
    d. So that they can be detectable by the comet assay
    e. Produced only following duplication of the genetic material

8. Which of the following statements is FALSE concerning chromosome aberrations in irradiated cells?
    a. The yield of dicentric chromosomes in X-irradiated cells follows a linear function of dose
    b. Spectral karyotyping (SKY) may be useful for the detection of translocations
    c. Acentric fragments and micronuclei often result from asymmetrical exchanges
    d. Ring chromosomes can be detected through staining and karyotyping
    e. It is possible to detect symmetrical exchanges using fluorescence **in situ** hybridization (FISH)

9. Which of the following statements concerning chromosome aberrations is FALSE?
    a. Ring chromosomes are induced as a linear function of dose for high LET radiation
    b. The induction of radiation-induced terminal deletions is a linear function of dose
    c. An anaphase bridge is a chromatid aberration
    d. For a given dose of X-rays, the yield of dicentrics decreases with decreasing dose rate
    e. Symmetrical translocations are unstable chromosome aberrations

Cell Death Mechanisms

1. Which of the following would NOT be a useful assay for the detection of cells undergoing apoptosis?
    a. TUNEL
    b. DNA ladder formation
    c. Annexin V labeling
    d. DAPI
    e. Staining with pimonidazole

2. Which of the following methods would represent the best way to assess the ability of radiation to decrease the survival of actively dividing cells following irradiation?
    a. Clonogenic assay
    b. Division delay
    c. Apoptosis levels at 24 hours
    d. Giant cell formation
    e. Detection of necrotic cells

3. The primary reason for cell death in most solid tumors following ionizing irradiation treatment is due to:
    a. Activation of apoptosis by the DNA damage response
    b. DNA damage induced senescence
    c. Mitotic catastrophe following incorrect segregation of genetic material
    d. Oxidative damage to cellular proteins
    e. Generation of ceramide through the action of sphingomyelinase

4. Following radiotherapy-relevant doses of ionizing radiation, apoptosis:
    a. Is the main mechanism of cell death for most cell types
    b. Is manifested primarily in cells of myeloid and lymphoid lineage and in some epithelial cell types
    c. Takes place when p53 blocks BAK and BAX
    d. Generally only happens during mitosis
    e. Occurs only in tumor cells, not in normal tissue cells

5. Which of the following would be the least likely to contribute to reduced colony-forming ability of irradiated cells?
    a. Presence of chromosomal inversions
    b. Senescence
    c. Autophagy
    d. Apoptosis
    e. Necrosis

6. Which of the following statements concerning cells undergoing radiation-induced apoptosis is TRUE?
    a. Loss of plasma membrane integrity is one of the first steps in the apoptotic process
    b. Caspases become active, move to the nucleus and degrade DNA
    c. Cells susceptible to undergoing apoptosis tend to be radioresistant
    d. Annexin V is able to bind to phosphatidyl serine on the outer membrane
    e. Apoptotic cells usually appear in clusters in irradiated tissues

7. Which of the following statements regarding radiation-induced cell death is TRUE?
    a. The majority of cells undergoing radiation-induced cell death do so following mitotic catastrophe
    b. The cells that will undergo mitotic catastrophe can be identified immediately post-irradiation by their characteristic morphological features
    c. Apoptosis occurs exclusively through a p53-dependent pathway
    d. Cells that undergo necrosis can be identified by blebbing of their cell membrane, shrinking of the cytoplasm and development of specific DNA fragmentation patterns
    e. At sublethal doses, most cells undergo permanent growth arrest

8. Cells undergoing apoptosis following irradiation:
    a. elicit a strong inflammatory response
    b. display enhanced expression of the gene encoding MSH2
    c. exhibit nuclear fragmentation
    d. rapidly swell and burst
    e. only initiate this process upon entry into mitosis

9. Which of the following statements concerning apoptosis is TRUE?
    a. Caspase 8 is an important downstream effector once apoptosis is initiated
    b. p53 activation down-regulates apoptosis
    c. The extrinsic apoptosis mechanism involves stimulation of TNFR family members.
    d. BAD is an anti-apoptotic protein
    e. A distinguishing feature of the extrinsic mechanism is the release of mitochondrial cytochrome c

10. Bcl-xL (BCL2L1) inhibition of apoptosis takes place at the:
    a. Mitochondrion
    b. Ribosome
    c. Cell membrane
    d. Nucleus
    e. Lysosome

11. Which suggests a classic synthetic lethal interaction between two genes, genes X and Y?
    a. X null mutation and Y null mutation=cell death
    b. X null mutation and Y null mutation=cell survival
    c. X wild-type and Y wild-type=cell death
    d. X wild-type and Y null mutation=cell death
    e. X null mutation and Y wild-type=cell death

12. Which of the following pathways has been implicated in cell death after exposure to ionizing radiation:
    a. Autophagy
    b. Apoptosis
    c. Necrosis
    d. Mitotic catastrophe
    e. All of the above

13. The intimate interaction between cell death and repopulation within tumors treated with radiation therapy is best described by which of the following statements:
    a. Caspase 3 serves as a paracrine signal from dying cells to stimulate proliferation of surviving cells
    b. Caspase 7 serves as a paracrine signal from dying cells to stimulate proliferation of surviving cells
    c. Caspase 3 serves as a paracrine signal from surviving cells to accelerate the death of dying cells
    d. Caspase 7 serves as a paracrine signal from surviving cells to accelerate the death of dying cells
    e. None of the above

14. Necroptosis may determine radiotherapy efficacy. What statement best describes this process?
    a. Passive cellular death dependent on caspase 3
    b. Passive cell death dependent on RIP1 kinase
    c. Programmed cellular death dependent on RIP3 kinase
    d. Passive cell death dependent on RIP3 kinase
    e. Programmed cellular death dependent on caspase 3

15. Which of the following statements best describes the “abscopal effect”?
    a. Localized irradiation of a tumor is associated with decreased size of the irradiated tumor and increased size of non-irradiated tumors
    b. Localized irradiation of a tumor is associated with decreased size of the irradiated tumor and decreased size of non-irradiated tumors
    c. Cells growing in the growth media of cells that have been irradiated demonstrate effects of radiation
    d. Sensitivity of low-grade lymphomas to low doses of radiotherapy (eg, 2 Gy x 2)
    e. Ability of transfected cells to transfer death signals to neighboring tumor cells

16. Which of the following statements is TRUE concerning the irradiation of a series of cell lines derived from breast carcinomas with an X-ray dose of 4 Gy?
    a. Most cells will die within several hours
    b. Annexin V staining will be detectable in the majority of cells within minutes
    c. A majority of cells will undergo apoptosis before completing mitosis
    d. Cells derived from tumors with a mutant p53 (TP53) are radioresistant
    e. Many cells will continue to divide for several days

17. Beclin protein is involved in which form of cell death?
    a. Necrosis
    b. Necroptosis
    c. Ferrotoptosis
    d. Pyrropoptosis
    e. Autophagy

Survival Assay

1. Which one of the following is NOT a fundamental assumption underlying the use of the jejunal crypt cell assay to measure cell survival in vivo?
    a. All crypts contain approximately the same number of stem cells
    b. Surviving stem cells (and their progeny) in the irradiated volume do not migrate between crypts during regeneration
    c. Stem cells from outside the irradiated volume do not migrate into the area and contribute to the regeneration of the crypts
    d. Stem cells can be identified morphologically and distinguished from differentiated cells
    e. Stem cells in all crypts proliferate within 3 days after irradiation

2. Till and McCulloch's studies of the radiation response of murine hematological colony forming units (CFU's) represent the first:
    a. demonstration of the presence of rare, pluripotent stem cells in a normal tissue
    b. clonogenic assay of mammalian cell survival after irradiation
    c. attempt at bone marrow transplantation
    d. demonstration that pre-irradiation of a bone marrow recipient could enhance the "take rate" of donated marrow
    e. demonstration that bone marrow transplantation can rescue lethally-irradiated recipients

3. What important feature distinguishes autophagy from other modes of programmed death?
    a. Loss of mitochondrial membrane potential
    b. Influx of extracellular fluid and ions
    c. Leakage of proteases and lysosomes
    d. Reversibility
    e. Wallerian degeneration

4. Consider cells with α/β = 3 Gy. What is the ratio of irreparable to repairable damage at 9 Gy?
    a. 3:1
    b. 1:1
    c. 1:3
    d. 1:9
    e. 9:1

5. Consider tumor cells with α/β = 10 Gy. What approximate percentage of the total amount of damage is repairable at 2 Gy?
    a. 100%
    b. 80%
    c. 60%
    d. 40%
    e. 20%

6. The survival curve is described by D_q = 3 Gy and D_0 = 1 Gy. What is the surviving fraction at 2 Gy (SF_{2Gy}) for the cell line?
    a. e^{-2}
    b. e^{0}
    c. $3 \cdot e^{-2}$
    d. $20 \cdot e^{-2}$
    e. 0.9

7. Assume that a particular tumor contains 10^{10} cells. Find the dose required to ensure that a single cell within the tumor has one chance in 100 to survive. Assume exponential survival and a mean lethal dose of 1 Gy.
    a. 23.4 Gy
    b. 34.5 Gy
    c. 30.5 Gy
    d. 27.6 Gy
    e. 29.7 Gy

8. Which of the following would be the most accurate way to measure reproductive integrity of cells exposed to ionizing radiation?
    a. Caspase cleavage assay
    b. Clonogenic survival assay
    c. 3-day growth assay
    d. Micronucleus assay
    e. Tumor regrowth delay assay

Cell Survival Models

1. A set of data defining the survival of cells irradiated with graded doses of X-rays is well-fitted by the mathematical expression for a single-hit survival curve having an SF2 of 0.37. The best estimate for the α parameter that describes this survival response is:
    a. 0.1 Gy^{1}
    b. 0.01 Gy^{1}
    c. 0.05 Gy^{1}
    d. 0.5 Gy^{1}
    e. 2.0 Gy^{1}

2. According to classical target theory, D0 is a measure of the:
    a. Amount of sublethal damage a cell can accumulate before lethality occurs
    b. total number of targets that must be inactivated to kill a cell
    c. dose required to produce an average of one lethal lesion per irradiated cell
    d. width of the shoulder region of the cell survival curve
    e. total number of hits required per target to kill a cell

3. The D0 for most mammalian cells irradiated with X-rays in vitro under well-aerated conditions falls in the range of:
    a. 0.1 - 0.2 Gy
    b. 0.2 - 1 Gy
    c. 1 - 2 Gy
    d. 2 - 4 Gy
    e. 4 - 8 Gy

4. For a particular cell line characterized by a D0 of 1 Gy and n equal to 1, what would be the approximate percentage of cells killed by a dose of 3 Gy?
    a. 5
    b. 10
    c. 37
    d. 50
    e. 95

5. A multifraction protocol for cells exposed to x-rays produces an effective survival curve that is:
    a. Linear-quadratic
    b. Bell-shaped
    c. Linear
    d. Parabolic
    e. Exponential

6. For a cell line whose single-dose survival curve is characterized by an n of 10, increasing fraction size causes the effective D_0 to:
    a. Remain the same
    b. Increase
    c. Decrease
    d. Decrease over a low dose range, but increase at high doses
    e. Increase over a low dose range, but decrease at high doses

7. Following an X-ray dose of 8 Gy, a clonogenic assay revealed that 20 colonies arose from an initial cell population of 2,000 cells. When 200 unirradiated cells were assayed for clonogenic survival, 40 colonies grew. What is the percent survival following the 8 Gy dose?
    a. 0.1
    b. 0.5
    c. 1
    d. 5
    e. 10

8. What would be the estimated surviving fraction of V79 Chinese hamster cells irradiated with an X-ray dose of 5 Gy delivered acutely? (Assume α=0.2 Gy^{-1} and β=0.05 Gy^{-2})
    a. 0.01
    b. 0.10
    c. 0.37
    d. 0.50
    e. 0.90

9. What would be the approximate surviving of V79 Chinese hamster cells had 5 Gy X-rays been delivered over a 10 hour period? (Assume α=0.2 Gy^{-1} and β=0.05 Gy^{-2})period?
    a. A. 0.01
    b. B. 0.10
    c. C. 0.37
    d. D. 0.50
    e. E. 0.90

10. What is the approximate surviving fraction following 5 doses of 0.5 Gy of carbon ions, assuming that the surviving fraction following one dose is 0.4?
    a. 0.01
    b. 0.10
    c. 0.37
    d. 0.50
    e. 0.90

11. Which of the following is the most plausible explanation for the decreased clonogenic survival observed among the progeny of cells that survived a prior irradiation?
    a. Increased expression of genes which encode repair enzymes
    b. Genomic instability
    c. Increased synthesis of glutathione
    d. Adaptive response
    e. Decreased expression of caspase 8

12. Cells are irradiated with graded doses of x-rays, and clonogenic cell survival is plotted against radiation dose, producing a survival curve. The width of the shoulder of the curve is reflective of:
    a. Delayed cell death after irradiation
    b. Repair of sub-lethal damage
    c. Repair of potential lethal damage
    d. Repopulation of tumors
    e. Response to hypoxia

13. Which of the following statements about cell-cycle dependent radiation sensitivity is correct.
    a. G1 phase cells have the highest DNA non-homologous end-joining capability, thus are most resistant to radiation
    b. S-phase cells are more sensitive to radiation because the DNA replication can be blocked by DNA damages during S-phase
    c. Proliferative tissues are more sensitive than non-proliferative tissues. Therefore, S-phase cells are more sensitive than quiescent G1 or G0 cells
    d. G2 and M phase cells are most sensitive to radiation
    e. G0 phase are very sensitive, almost as sensitive as cells in M phase

14. A researcher has conducted an in vitro radiation experiment with cancer cell lines that grow in colonies, with a doubling time of 2 days. Twelve days after plating a single cell suspension of 100 cells in the absence of any treatment, 60 colonies are counted. In a similar experiment, 400 cells are plated and immediately irradiated with 4 Gy, eventually forming 80 colonies. The surviving fraction after 4 Gy radiation in this cell line is:
    a. 75%
    b. 33%
    c. 12%
    d. 6%
    e. 0%

15. For a population of patients with identical tumors, assuming that: 1) each tumor has $2x10^9$ cells, 2) after treatment with 25 fractions of 2 Gy, the effective survival is 10^9; 3) there is no regrowth of the tumor during the treatment, 4) the tumor may recur even if there is only one viable tumor cell survive. What is a patient's probability to be recurrence-free from the original tumor?
    a. 50.0% (or 1/2)
    b. 13.5% (or e^2)
    c. 0.01 (or 10^2)
    d. 0%, because the each of the patients will have 2 surviving cells
    e. None of the above

16. Assuming that: 1) a tumor has $3x10^9$ cells; 2) a single D0 dose of 1.8 Gy results in 37% survival; 3) there is no tumor re-growth. After 20 fractions of radiation treatment with the D0 dose, average of how many tumor cells will likely to survive?
    a. $6.96x10^7$ [or: $3*10^9x\ (e^{1.8})20 = 3x109xe^{-36}$]
    b. 0.0184 [or 0.37/20]
    c. 6.18 [or: $3x10^9x\ (e^1)20 = 3x10^9xe^{-20}$]
    d. $5.55x10^7$ [or: $3x10^9$ x 0.37/20]

17. The D0 is the dose that results in an average of one lethal event per cell. When there is no repair of sub-lethal damage, exposure of cells with a D0 dose will result in which of the following survival fraction?
    a. 100%
    b. 63%
    c. 50%
    d. 37%
    e. 10%

18. The X-ray survival curve for a particular cell line is characterized by $\alpha = 0.4\ Gy^{-1}$ and $\beta = 0.2\ Gy^{-2}$. What is the dose at which the amount of single-hit cell killing equals the amount of multi-hit cell killing?
    a. 0.08 Gy
    b. 0.16 Gy
    c. 0.4 Gy
    d. 0.6 Gy
    e. 2 Gy

Linear Energy Transfer

1. Which of the following types of ionizing radiation has the highest LET?
    a. 2.5 MeV alpha particles
    b. 75 MeV/nucleon argon ions
    c. 1 GeV/nucleon carbon ion
    d. 18 MeV/nucleon carbon ions
    e. 150 MeV protons

2. The carbon ion RBE for hypoxic cells compared with that for aerated cells is:
    a. Equal
    b. Lower
    c. Greater
    d. Dependent upon the endpoint being measured
    e. The same as the OER

3. Which of the following statements concerning LET is FALSE?
    a. The highest RBE occurs for radiations with LET values of approximately 100 keV/μm
    b. High LET radiations yield survival curves with low D_0 values
    c. The OER increases with increasing LET
    d. High LET radiations often produce exponential survival curves
    e. LET is an average energy (in keV) transferred from a charged particle traversing a distance of 1 μm in the medium

4. What is the effect on both RBE and the α/β ratio as the LET for the type of radiation increases up to 100 keV/μm?
    a. Both remain the same
    b. Both increase
    c. Both decrease
    d. The RBE decreases while the α/β increases
    e. The RBE increases while the α/β decreases

5. Which of the following medically-useful ionizing radiation types is densely ionizing?
    a. Gamma rays from Iodine-125 decay
    b. Gamma rays from Cobalt-60 decay
    c. Gamma rays produced in the annihilation reaction
    d. Protons used in radiation therapy
    e. Beta particles emitted by Yttrium-90

6. Equal doses of densely- and sparsely- ionizing radiations, such as 1-MeV α-particles and 1-MeV γ-rays, produce different levels of biological damage. Which of the following is the most plausible explanation of the difference in biological effectiveness?
    a. Sparsely-ionizing radiation is less effective due to wastage of ionizations in water
    b. Densely-ionizing radiation produces more ionization per unit mass
    c. Densely-ionizing radiations tend to deposit more energy than is needed to produce the effect
    d. The ratio of DNA double- to single-strand breaks is approximately 20:1 per 1 Gy for sparsely ionizing radiation and approximately 1:1 for densely ionizing radiation, independent of radiation quality
    e. Densely ionizing radiations produces more ionizations per unit length of the track

7. Which of the following statements is correct about Relative Biological Effect (RBE)?
    a. RBE is the ratio of doses of two different radiations that produce the same biological endpoint, e.g. 50% survival
    b. RBE is the ratio of survival fractions produced by the same doses of two different radiations
    c. Beta-particles have higher RBE values than alpha-particles

d. High LET radiation have lower RBE values than low LET radiation
e. Stereotactic ablative radiotherapy has a higher RBE than conventional radiotherapy

Oxygen Effect

1. For a given biological system, the D_{37} in the presence of O_2 was determined to be 2 Gy for a particulate radiation of energy A and 1 Gy for the same particle with energy B. Under hypoxic conditions, the D_{37} was 6 Gy for A and 1.5 Gy for B. Which of the following statements best describes the relationship between the two radiations?
    a. Radiation A has a higher LET than type B.
    b. The OER for radiation A is 2.
    c. If a given dose of radiation B was delivered at a low dose rate, the amount of cell killing would not differ markedly from that produced at a high dose rate.
    d. Radiation B likely has a higher energy than radiation A.
    e. None of the above

2. In irradiated cells, oxygen:
    a. acts as a radical scavenger by converting free radicals to non-reactive species
    b. acts as a radioprotector
    c. reacts with hydrogen radicals to form water, thus reducing the number of free radicals formed
    d. modifies the level and spectrum of free radical damage produced in DNA
    e. is unlikely to play a role in the indirect effect of radiation

3. Which one of the following statements regarding radiation and hypoxia is TRUE?
    a. Irradiation under hypoxic conditions yields more DNA strand breaks than under aerated conditions
    b. Irradiation under aerated conditions leads to less overall cellular damage than irradiation under hypoxic conditions
    c. The presence of oxygen reduces radiation toxicity
    d. Oxygen at the time of irradiation must be present either during or within microseconds following irradiation to see maximum OER
    e. The effect of oxygen on radiation-induced damage varies most between 2% and 5% oxygen

4. For single doses of low LET radiation above 2 Gy the OER is typically in the range of:
    a. 0-1
    b. 1-2
    c. 2-3.5
    d. 3.5-5
    e. 5-10

5. Which of the following techniques provides a non-invasive means to assay the extent and location of hypoxia in tumors and correlates with 50% probability of tumor control?
    a. Electron paramagnetic resonance oxygen imaging
    b. Hyperpolarized magnetic resonance imaging of oxygen
    c. Proton resonance shift magnetic resonance imaging
    d. Diffusion tensor imaging of oxygen partial pressures
    e. Photoacoustic imaging of oxy- and deoxy-hemoglobin

6. Which of the following statement is correct about Oxygen Enhancement Ratio (OER) at the time of irradiation?
    a. OER is the ratio of surviving fractions produced by the same radiation doses under two different oxygen conditions
    b. OER is the ratio of radiation doses that produce the same surviving fractions under two different oxygen conditions
    c. Free radical scavengers can increase OER values
    d. High LET and high dose rate radiation may have higher OER values
    e. OER is the SER divided by 2

7. The oxygen enhancement ratio is:
    a. equal to the survival of cells irradiated under hypoxic conditions divided by the survival under aerobic conditions for a fixed radiation dose
    b. greater at low radiation doses than at high radiation doses
    c. the same regardless of radiation quality (LET)
    d. equal to the dose of radiation under hypoxic conditions divided by the dose of radiation under aerobic conditions that results in the same biological effect
    e. equal to the survival of cells irradiated under aerobic conditions divided by the survival under hypoxic conditions for a fixed dose

Cellular Repair

1. The mechanism of the radiation bystander effect is thought to involve all of the following, EXCEPT:
    a. Communication through gap junction
    b. Presence of reactive oxygen species
    c. Aberrant signaling in cancer cells
    d. Extracellular signaling molecules
    e. Involvement of specific cytokines

2. An X-ray dose of 10 Gy delivered at 1 Gy/min has a greater biologic effect than the same dose delivered at 1 Gy/day because:
    a. Fewer free radicals are produced
    b. Apoptosis predominates as the major form of cell death when radiation is delivered at a high dose rate
    c. The normal ATM-mediated inhibition of cell cycle progression is inhibited at the higher dose rate
    d. Cell proliferation may occur during irradiation at the high dose rate
    e. There is less repair of the sublethal damage during the course of irradiation at a high dose rate

3. What would be the expected effect of a drug that inhibits repair of X-ray-induced chromosome breaks? It would:
    a. Decrease the yield of terminal deletions
    b. Increase the dose rate effect
    c. Stimulate the repair of sublethal damage
    d. Enhance repair of potentially lethal damage
    e. Sensitize cells to low dose rate irradiation

4. Generally, the sparing effect during dose fractionation increases with increasing time between fractions. Under certain irradiation conditions however, an increase in the interval between fractions results in **decreased** cell survival. This occurs because of:
    a. Reassortment
    b. Repopulation
    c. Repair
    d. Reoxygenation
    e. Adaptive response

5. Which of the following statements is TRUE concerning SLDR and PLDR?
    a. As the dose rate is reduced and exposure time increased for an X-ray treatment, the biological effectiveness of a given dose of radiation increases
    b. PLDR is best demonstrated with a split dose experiment
    c. There is an inverse correlation between the α/β ratio of an acute dose X-ray survival curve and the amount of SLDR in a fractionated irradiation
    d. The magnitude of PLDR and SLDR is greater following exposure to high LET compared to low LET radiation
    e. PLDR plays an important role in the decreased survival seen with fractionated irradiation to the normal lung as compared to lung cancer cells

6. Sublethal damage recovery is best demonstrated by:
    a. Determining the TCD_{50}
    b. A cell synchronization experiment
    c. A split dose experiment
    d. A delayed plating experiment
    e. The paired survival curve technique

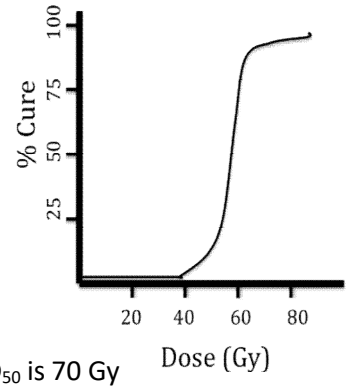
7. The dose rate range over which SLDR most contributes to the dose rate effect for X-rays is:
    a. a.0.001 - 0.01 Gy/min
    b. 0.01 - 1 Gy/min
    c. 1 - 5 Gy/min
    d. 5 - 10 Gy/min
    e. 10 - 20 Gy/min

Tumor Assay Systems

1. Tumor-bearing mice are randomized into a control group and groups treated with localized irradiation of the tumor alone, an anticancer drug alone, or radiation in combination with the drug. Which of the following represents the most rigorous, reliable and informative approach to comparing the effectiveness of the different treatments?
    a. Killing the mice at a predetermined time after treatment, removing and weighing the tumors, and calculating the ratio of the volumes of the treated and control tumors
    b. Measuring three diameters of the tumors with calipers at a predetermined time after treatment, calculating the volume and computing the ratio of the volumes of the treated and control tumors
    c. Measuring the tumors 3x per week until the treated tumors return to their pre-irradiation volume and calculating the mean time needed for each group to reach that volume
    d. Measuring the tumors 3x per week until the control tumors reach 4 times the volume at the time of treatment, and comparing the mean volume of the tumors in each treatment group at that time
    e. Measuring the tumors 3x per week until each tumor reaches 4 times the volume at the time of treatment and calculating the mean time needed for the tumors in each group to reach that volume

2. For a group of tumors identical in size and homogeneous with respect to cellular radiosensitivity, what would be the general shape of the curve in a linear-linear graph defining the increase in tumor control probability with increasing radiation dose?
    a. Step function from 0 – 100% at the dose that kills all of the cells
    b. Linear increase from 0 – 100% over a narrow range of doses
    c. Logarithmic increase from 0 – 100% over a wide range of doses
    d. Sigmoidal increase from 0 – 100% over a narrow range of doses
    e. Exponential increase from 0 – 100% over a narrow range of doses

3. The following graph shows data for the percent of tumors controlled by different doses of radiation therapy. Based upon the data provided in this graph, which of the following statements is correct?



    a. TCD_{50} is 70 Gy
    b. $NTCP_{50}$ is 60 Gy
    c. The additional dose required to increase the probability of tumor control from 50 to 60% is larger than the dose required to increase the probability of tumor control from 90 to 100%
    d. The impact of a radiosenisitizer upon tumor control will be most readily detected for experimental protocols that result in a 50% rate of tumor cure
    e. The TD5/5 is 60Gy.

4. Genetically engineered mouse models (GEMMs) are becoming more popular for preclinical tumor studies with and without radiation. Which of the following is the most correct regarding the advantages of GEMMs over xenograft-based studies?
    a. Intact immune system
    b. Tumors are orthotopic in location
    c. Tumors contain mutations that are relevant for human tumors
    d. Intact extracellular tumor microenvironment
    e. Tumors develop more rapidly

Tumor Biology and Microenvironment

1. Which of the following statements concerning the tumor microenvironment is true?
    a. Hypoxia is found primarily at the core of large tumors
    b. Cellular oxygenation status in solid tumors is expected to remain relatively constant over a 24 hr period
    c. Tumors of a similar size have similar hypoxic fractions
    d. Acute changes in blood flow contribute to tumor hypoxia
    e. Hypoxic tumors are sensitive to irradiation due to expression of HIF1

2. Tumor hypoxia has been specifically associated with all of the following, EXCEPT:
    a. Reduced radiosensitivity
    b. Tumor size
    c. Increased genomic instability
    d. Poor patient prognosis
    e. Increased metastasis

3. In a respiring tissue, the maximum diffusion distance of oxygen from a capillary is:
    a. 1-2 μm
    b. 100-200 μm
    c. 1-2 mm
    d. Independent of cellular respiration rate
    e. Independent of hemoglobin concentration

4. Which of the following statements concerning tumor hypoxia is FALSE?
    a. Chronically hypoxic cells are generally more radiation resistant than acutely hypoxic cells
    b. For a tumor containing only 1% radiobiological hypoxic fraction, essentially all hypoxic cells would be eliminated by the end of a typical course of radiotherapy, even in the absence of any reoxygenation
    c. Hypoxic regions in tumors may be detected using pimonidazole
    d. Regions of acute hypoxia may develop in tumors due to the temporary closing/blockage of a blood vessel
    e. Reoxygenation during fractionation in radiotherapy reduces the influence of hypoxic cells on tumor response

5. HIF-1 activity is increased primarily during hypoxia as a consequence of:
    a. Increased transcription of HIF-1α
    b. Reduced stability of HIF-1β
    c. Increased turnover of HIF-1 α
    d. Reduced hydroxylation of HIF-1α
    e. Increased activity of VHL

6. With regard to the radiobiological influence of oxygen, which of the following statements is FALSE?
    a. Reoxygenation of human tumors during fractionated radiation therapy reduces the impact of both chronically and acutely hypoxic cells on overall response
    b. Metabolically-active hypoxic cells in human tumors can be identified through preferential binding of administered nitroimidazole compounds
    c. Hypoxia induces pro-angiogenic factors such as VEGF
    d. HIF1 a is stabilized under hypoxia
    e. The oxygen enhancement ratio (OER) for X-rays is higher for doses < 2 Gy than for doses > 10 Gy

7. Which of the following markers and imaging approaches would be **least** useful for measuring tumor hypoxia non-invasively?
    a. [^{18}F]-fluorodeoxyglucose (FDG) – PET
    b. [^{18}F]-fluoromisonidazole (FMISO) – PET
    c. [^{123}I] radioiodinated azomycin arabinosides - SPECT
    d. [^{64}Cu]-Cu-ATSM – PET
    e. Electron paramagnetic resonance imaging - EPRI

8. A non-invasive method for the detection of hypoxic regions in tumors and/or measuring oxygen concentration in tumors would allow the:
    a. Identification of patients to receive alternative therapy
    b. Histology of the tumor to be determined
    c. Volume of the tumor to be calculated
    d. Radiation sensitivity of the tumor to be determined
    e. Repair capacity of the tumor to be estimated

9. The expression of which of the following genes is regulated by the stabilization of HIF-1α in the absence of oxygen in tissues?
    a. polε
    b. ATR
    c. PDGFR
    d. VEGF
    e. RAS

10. All of the following have been shown to be affected by HIF1α during hypoxia, EXCEPT:
    a. Glycolysis
    b. Angiogenesis
    c. Epo production
    d. p53 activation
    e. pH regulation

11. Which of the following statements concerning hypoxia is TRUE?
    a. Hypoxic cell radiosensitizers produce a greater increase in the therapeutic index when used with conventional fractionated radiotherapy than for treatment with one or a few large radiation doses
    b. A biphasic survival curve would result from low LET irradiation of a mixed population of both aerated and hypoxic cells
    c. The OER is defined as the dose to produce a given effect in aerated cells divided by the dose to produce the same effect in hypoxic cells
    d. The diffusion distance of oxygen in air is typically less than 100 μm
    e. For low LET radiation, the maximum OER is typically observed only when the tissue oxygen concentration reaches about 20%

12. Which of the following statements is true about lactate:
    a. Lactate is a surrogate for hypoxia in cancer cells
    b. Lactate accumulation via aerobic glycosis under normoxic conditions occurs in cancer cells
    c. Lactate inhibits the secretion of hyaluronan by tumor-associated fibroblasts to prevent migration and metastasis
    d. Lactate concentrations in tumor correlate with radiosensitivity
    e. Lactate inhibits VEGF secretion by tumors to reduce the need for oxygen and nutrient supply

13. Which of the following cells is the dominant contributor to neovascularization following irradiation?
    a. Bone marrow-derived cells that are recruited to irradiated tumors
    b. Pericytes surrounding tumor vasculature that form new endothelial cells
    c. Tumor-associated endothelial cells that survive a course of radiotherapy
    d. Trans-differentiated cancer cells that form new endothelial cells
    e. Trans-differentiated cancer stem-like cells that form new endothelial cells

14. Activation of the immune system by localized radiation of orthotopic pancreatic tumors may be achieved by which of the following methods:
    a. Stereotactic radiation therapy that normalizes aberrant vasculature to aid efficient recruitment of tumor-specific T cells
    b. Low dose irradiation that normalizes aberrant vasculature to aid efficient recruitment of tumor-specific T cells
    c. Stereotactic radiation therapy that programs tumor-associated macrophages to recruit Tregs into the tumor
    d. Low dose irradiation that programs tumor-associated macrophages to recruit Tregs into the tumor
    e. Localized radiation that induces iNOS in tumor-associated macrophages which suppresses TH1 chemokine

15. Which of the following would best be used to estimate the proportion of radiation resistant viable hypoxic cells in an experimental tumor model?
    a. Comparison of radiation response with and without breathing of hyperbaric oxygen
    b. Paired survival curve analysis in vitro following irradiation in vivo under standard conditions and conditions where blood flow to the tumor has been stopped
    c. Extrapolation of the initial exponential portion of the cell survival curve for cells comprising the tumor
    d. Comparison of radiation responses with and without misonidazole administration
    e. Comparison of radiation responses of the tumor treated with high versus low dose-rate radiation

16. Antigen recognition by T cells is imperative for the development of cellular adaptive immunity. How does a T cell recognize antigen?
    a. T cells recognizes antigens via pattern recognition receptors
    b. T cells recognizes antigenic determinants presented in the MHC cleft via the T cell receptor
    c. T cells recognize antigens via Fc receptor binding to membrane-bound IgD antibodies
    d. T cells recognize antigens via PD-1 binding
    e. Comparison of radiation responses of the tumor treated with high versus low dose-rate radiation

17. Which of the following is not an immune checkpoint?
    a. LAG3
    b. PD-1
    c. TIM3
    d. OX40
    e. CTLA-4

18. Which radiation-induced immune effect would be counterproductive to 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

19. Tumor cells can escape the host's immune response using a plethora of mechanisms. Which of the following examples is FALSE?
    a. Loss of beta2-microtubulin expression that decreases MHC class I expression
    b. Tumor cell intrinsic alterations in signaling pathways such as WNT/b-catenin, loss of PTEN, IFN-g that block T cell priming and infiltration and effectiveness
    c. Recruitment of myeloid suppressor cells
    d. Loss of antigen expression through to immune selection
    e. Increased expression of immune inhibitory factors such as IDO, PD-L1

20. Which of the following immune mediated mechanisms facilitates 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

21. 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

22. 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

23. Which of the following agents does not target the PD-1/L1 axis?
    a. Pembrolizumab
    b. Avelumab
    c. Ipilimumab
    d. Durvalumab
    e. Nivolumab

24. Which of the following statements is <u>correct</u> when comparing the abscopal effect versus 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.

25. 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

26. What is an adaptive immune resistance?
    a. Adaptive immune resistance describes a process where a patient is tolerant to a tumor associated antigen, e.g. NY-ESO or PSCA before starting immunotherapy but develops immunity to it once they are on treatment
    b. Adaptive immune resistance is a process in which tumor cells change their phenotype in response to an immune response (cytotoxicity or inflammation), in an attempt to avoid it, e.g. the induction of PD-1, PD-L1 and IDO following antigen recognition and the production of IFN-gamma
    c. Following radiation, tumors undergo accelerated proliferation with an increased incidence of failure
    d. Following radiation, tumors become increasingly more radiosensitive with additional fractions
    e. Adaptive immune resistance occurs when a tumor has a limited set of tumor associated antigens that are recognized by the host's adaptive immune system

27. Which of the following may increase a patient's chance to experience an immune related adverse event?
    a. History of autoimmune disease
    b. Previous use of checkpoint blockade therapy
    c. Previous radiation therapy
    d. All of the above
    e. None of the above

28. Glutathione peroxidases (GPx) play several roles in cells including maintaining H_2O_2 homeostasis. Which of the following statements regarding glutathione peroxidases is false?
    a. Selenocysteine is present at the active site of GPx1-4 and GPx6
    b. Knockdown of GPx1 in human prostate cancer cells enhances the formation of radiation induced micronuclei
    c. GPx2 is upregulated in upregulated in colon cancer and squamous cell carcinomas
    d. All GPx can reduce hydroperoxides within cell membranes
    e. GPx1 can be found in the cytosol in mitochondria and also in peroxisomes

29. Please choose the correct chronologic sequence of cancer therapies, ranked from the earliest first use to the most recent first use:
    a. Coley's toxins; radiation therapy; Bacillus-Calmette-Guérin; interferon; trastuzumab; ipilimumab
    b. Radiation therapy; Bacillus-Calmette-Guérin; Coley's toxins; interferon; trastuzumab; ipilimumab
    c. Bacillus-Calmette-Guérin; radiation therapy; Coley's toxins; interferon; ipilimumab; trastuzumab
    d. Bacillus-Calmette-Guérin; radiation therapy; interferon; Coley's toxins; ipilimumab; trastuzumab

30. Which of the following statements about cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) is true?
    a. CTLA-4 is the target of the immune therapeutic nivolumab
    b. CTLA-4 is exclusively expressed on regulatory T cells (Tregs)
    c. CTLA-4 competes with CD28 for binding to CD80/CD86 to promote T-cell inactivation
    d. Ipilimumab is a small molecule inhibitor of CTLA-4

31. Which of the following is true about PD-1?
    a. It stands for Protein of Differentiation-1
    b. It binds to PD-L1/PD-L12 to induces apoptosis in PD-L1/PD-L2-expressing cells
    c. It down-regulates regulatory T cells
    d. Its expression is upregulated by T cell receptor activation

32. What is the approximate rate of grade 3+ autoimmune toxicity from anti-PD1 immunotherapy?
    a. 5%
    b. 15%
    c. 30%
    d. 50%

Cell and Tissue Kinetics

1. Which of the following statements is FALSE concerning the cell cycle?
    a. Irradiation of cells causes a delay in progression from G_1 into S phase of the cell cycle
    b. Cells in M phase typically have X-ray survival curves with low α/β ratios
    c. Cells are most resistant in late S phase of the cell cycle
    d. G_1 is the cell cycle phase most variable in duration
    e. The G_0 phase of resting cells is within G_1

2. The diameter of a tumor was found to double in 18 days. Assuming that all of the cells in the tumor are proliferating and no cells are lost, the tumor cell doubling time is closest to:
    a. 1 day
    b. 3 days
    c. 6 days
    d. 12 days
    e. 18 days

3. CDK1/cyclin B plays an important role in the transition of cells from:
    a. G_0 into G_1
    b. G_1 into S
    c. S into G_2
    d. G_2 into M
    e. M into G_1

4. Which of the following statements is TRUE concerning the cell cycle kinetics of human tumors?
    a. The growth fraction of a tumor represents the proportion of cells capable of transplanting the tumor
    b. Cell loss is often the major factor that determines the tumor volume doubling time
    c. The growth rate generally increases with increasing tumor size
    d. Volume doubling times are shorter than the value that would be predicted from the cell cycle time of individual cells
    e. The volume doubling time is largely determined by the cell cycle time

5. A tumor is characterized by a cell cycle time of 10 days, a growth fraction of 0.5 and a cell loss factor of 1.0. Assuming these kinetic parameters remain constant over a one month period, how much would the tumor volume have increased during that time?
    a. Increase 2-fold
    b. Increase 3-fold
    c. Increase 4-fold
    d. Increase 5-fold
    e. Remain the same

6. The T_{pot} for a tumor can be calculated from the cell cycle time of the cells comprising the tumor, the tumor's growth fraction and with the assumption that the cell loss factor is:
    a. 0
    b. 1.0
    c. 0.2
    d. 0.6
    e. Nearly 1.0 when the tumor is small, but decreasing exponentially as the tumor grows

7. The T_{pot} for most head and neck tumors is in the range of:
    a. 1-2 days
    b. 2-6 days
    c. 6-24 days
    d. 24-100 days
    e. Greater than 100 days

8. If the T_S, LI and λ (the correction factor for the non-linear distribution of cells through the cell cycle) were determined for a tumor to be 10 hours, 0.2 and 0.7, respectively, then the T_{pot} is:
    a. 2 hours
    b. 10 hours
    c. 18 hours
    d. 25 hours
    e. 35 hours

9. Two patients are diagnosed on the same day with tumors of approximately the same size. However, the T_{pot} for patient A's tumor was determined to be 5 days while the T_{pot} for patient B's tumor was calculated as 20 days. Assuming that there was no cell loss taking place and the tumor's growth fractions did not change, if treatment was delayed for 20 days, the ratio of the number of cells in the tumors of patient A to patient B would have been approximately:
    a. 16:1
    b. 8:1
    c. 1:1
    d. 1:8
    e. 1:16

10. The most likely explanation for why a tumor, composed of cells with short cell cycle times, would have a long volume doubling time is a:
    a. High cell loss factor
    b. Small percentage of cells entering G_0 following mitosis
    c. Large growth fraction
    d. Large hypoxic fraction
    e. Abnormally long S phase

11. The volume doubling time (in days) for a tumor with a cell loss factor of 90% and a T_{pot} of 20 days would be estimated as:
    a. 5
    b. 20
    c. 50
    d. 100
    e. 200

12. Which of the following flow cytometry methods or the combination of methods are used to estimate cell cycle distribution in mammalian cells?
    a. Analysis of annexin V stained cells
    b. Analysis of cells treated with a high dose (2 mM or more) thymidine
    c. Analysis of propidium iodide stained cells
    d. Analysis of cells labeled with an H3 antibody
    e. Analysis of cells pulse-labeled with ^{3}H-thymidine

13. Which of the following observations is most closely associated with G_1/S arrest in wild type-p53 cells following X-irradiation?
    a. Increase in bromouridine incorporation
    b. Increase in the labeling index
    c. Formation of the cyclin E/A-Cdk2 complex
    d. Inreased transcription of the $p21^{Waf1}$ (CDKN1A) gene
    e. Increased transcription of the p53 (TP53) gene

14. Nucleotides can be radiolabeled before they are incorporated into newly forming DNA and can therefore be assayed to track their incorporation. In a set of experiments, a resident-faculty research team used labeled thymidine nucleotides and introduced these into the culture of dividing human cells as a 30-min pulse. Which of the following questions might be answered by such a method?
    a. How many cells are produced by the culture per hour?
    b. What is the length of the S phase of the cell cycle?
    c. What proportion of cells are undergoing autophagy?
    d. How many picograms of DNA are made per cell cycle?
    e. When do spindle fibers attach to chromosomes?

15. If mammalian cells receive a go-ahead signal at the G2/M checkpoint, they will:
    a. Move directly into telophase
    b. Complete the cycle and divide
    c. Exit the cycle and switch to a nondividing state
    d. Show a drop in M-Phase Promoting Factor (MPF) concentration
    e. Complete cytokinesis and form new cell walls

16. What causes the decrease in the amount of cyclin at a specific point in the cell cycle?
    a. An increase in production once the checkpoint is passed
    b. The cascade of increased production once its protein is phosphorylated by a partner Cdk
    c. Assembling new histones into chromatin
    d. Its self-degradation
    e. Covalent modification in the cytoplasm

17. During which phases of the cell cycle can homologous recombination of DNA double strand breaks occur?
    a. G0/G1
    b. G1/Early-S
    c. Late-S/G2
    d. G2/M
    e. M/G1

18. During which phases of the cell cycle does homologous recombination occur as a mechanism of DNA double strand break repair?
    a. G1-early S
    b. Late S-G2
    c. M
    d. G0
    e. All of the above

19. After irradiation, cells with intact checkpoint activation arrest in which phases of the cell cycle?
    a. Only S
    b. G2 and M
    c. G1, S and G2
    d. M and S
    e. G0

20. Which of the following proteins are involved in the G1 cell cycle checkpoint?
    a. EGFR
    b. Stat1
    c. Cyclin D1
    d. Cyclin X
    e. Cdc25

21. Which of the following proteins is involved in the G2 checkpoint?
    a. Cyclin D1
    b. Cdc25C
    c. Cyclin D8
    d. Smad3
    e. Rb

Molecular Signaling

1. Mutations in growth factor receptors are common alterations in cancer that may:
    a. Signal cells to enter senescence
    b. Directly inhibit protein translation
    c. Cause formation of γ-H2AX foci in cell nuclei
    d. Result in constitutive kinase activity that signals cells to proliferate
    e. Stimulate ubiquitination of caspase 3 to induce apoptosis

2. In cancer treatment, there has been clinical interest in targeting the RAS oncogene product using:
    a. HDAC inhibitors
    b. Cyclin-dependent kinases
    c. Farnesyl transferase inhibitors
    d. I-κB
    e. Iressa

3. The transcriptional activity of the tumor suppressor p53 has been shown to be regulated by all of the following, EXCEPT:
    a. Phosphorylation of p53 (TP53) by ATM
    b. Changes in the subcellular localization of p53
    c. Changes in the ubiquitination of MDM2
    d. $p19^{ARF}$-induced changes in acetylation of p53
    e. Binding of FAS ligand (FASLG/CD95-L) to FAS (CD95/APO-1)

4. RAS functions as a:
    a. GTPase
    b. Protein kinase
    c. Phosphatidyl inositol kinase
    d. Phosphatase
    e. Transcription factor

5. Which one of the following is NOT a part of the RAS pathway that stimulates radioprotective and growth promoting signals following irradiation?
    a. RAF1
    b. MEK
    c. MAPK (ERK)
    d. FADD
    e. RHO

6. Epigenetic modification of DNA-associated histones can occur through all of the following mechanisms, EXCEPT:
    a. Phosphorylation
    b. Acetylation
    c. Nitrosylation
    d. Methylation
    e. Ubiquitination

7. Which of the following is the most likely consequence of EGFR activation?
    a. Increased proliferation
    b. Apoptosis
    c. Cell cycle arrest
    d. Stabilization of microtubules
    e. Autophagy

8. The correlation between MET and ionizing radiation is characterized by which of the following:
    a. Radiation-induced MET results in a decrease in clonogenic survival
    b. Radiation-induced MET results in an induction of apoptosis
    c. Radiation induces MET via the ATM-NF-kB pathway
    d. Radiation induces MET via the wnt-b-catenin pathway
    e. Radiation-induced MET inhibits epithelial–mesenchymal transition

9. The phenomenon of "oncogene addiction" most correctly refers to which of the following clinical scenarios?
    a. A 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 CML patient treated with interferon

10. The two most frequently activated signaling pathways in prostate cancer are driven by androgen receptor (AR) and PI3K-AKT pathway. Inhibitors of the PI3K pathway are in early clinical trials and AR inhibitors confer clinical responses in most patients. Which statement most correctly describes the relationship between these two pathways and explains mechanistically why single inhibition of AR and PI3K-AKT pathways rarely induce tumor regression in preclinical models?
    a. ADT represses an androgen receptor gene expression program governing DNA repair and inhibits repair of ionizing radiation–induced DNA damage
    b. AR and PI3K pathways regulate each other by reciprocal negative feedback, such that inhibition of one activates the other
    c. ADT represses the PI3K-AKT-mTOR pathway
    d. ADT activates the unfolded protein response
    e. All of the above

11. Mutations in RAS are associated with which response to radiation?
    a. Severe toxicity
    b. Radioresistance
    c. Senescence
    d. Autophagy
    e. No effect on radioresponse

12. A patient you are seeing as a consult for bilateral vestibular schwannoma has a past medical history of neurofibromatosis type 2. Which of the following statements regarding the NF2 signaling pathway is false?

    a. Merlin is encoded by the gene NF2 and facilitates transports YAP to the nucleus
    b. In schwannomas, YAP stimulates expression of PDGFRβ, Her2, Her 3, and PDGF
    c. PDGFRβ, Her2, and Her3 are cell surface receptors that can stimulate the AKT pathway
    d. The AKT pathway promotes cell survival and is associated with Schwannoma growth
    e. NF2 is a tumor suppressor gene and a transcription factor

13. The Ras proteins are involved in various cellular functions including proliferation, differentiation, and survival. Which of the following statements regarding Ras proteins is false?
    a. Approximately 10-30% of cancer patients have a Ras mutation
    b. HRAS, KRAS, and NRAS have similar DNA sequence homology and function
    c. Ras proteins cycle between an inactive GTP-bound state and active GDP-bound state
    d. Ras proteins may be attached to the cell membrane by the process of prenylation and palmitoylation

Cancer

1. Which of the following statements concerning telomerase is TRUE? Telomerase:
    a. Is activated when telomeres decrease below a critical size
    b. Plays a central role in base excision repair
    c. Is present at high levels in senescent cells relative to normal cells
    d. Adds DNA sequence repeats onto the ends of chromosomes
    e. Therapeutic activation in tumor cells represents a promising cancer treatment strategy

2. Which of the following statements regarding p53 (TP53) is FALSE? p53:
    a. Is targeted by MDM2 for degradation
    b. Mutation in lymphoma cells usually renders these cells radiosensitive
    c. Is a substrate for the ATM protein kinase
    d. Serves as a transcription factor and upregulates p21 (CDKN1A)
    e. Upregulates the pro-apoptotic factors BAX and PUMA

3. Which of the following statements concerning retinoblastoma and the RB (RB1) protein is TRUE?
    a. The RB protein suppresses cell proliferation by binding to the E2F transcription factor, thereby inhibiting gene expression E2F-dependent gene transcription
    b. Cell cycle dependent kinases add hydroxyl groups to the RB gene product causing it to release E2F
    c. A mutant *RB* gene is inherited from one parent in the sporadic form of retinoblastoma
    d. The RB protein product is phosphorylated by CDK1
    e. In the familial form of retinoblastoma, patients are only at elevated risk for retinoblastoma, and not other cancers

4. The importance of DNA repair in preventing carcinogenesis is demonstrated by all of the following clinical/experimental findings, EXCEPT:
    a. People suffering from hereditary non-polyposis colon cancer syndrome often exhibit mutations in DNA mismatch repair genes
    b. Mutations in tumor suppressor genes may play an important role in cancer progression
    c. Xeroderma pigmentosum patients show an elevated incidence of skin cancers
    d. Virtually all tumor cell lines analyzed have been found to have one or more DNA repair deficiencies
    e. Mutations in an oncogene may be an early step in carcinogenesis

5. Oncogenes:
    a. Can be activated by epigenetic silencing
    b. Are inherited in familial cancers
    c. Are induced by gene loss
    d. Can be activated by point mutation
    e. Are important barriers to prevent tumor formation

6. $p16^{INK4A}$ (CDKN2A):
    a. Is an oncogene
    b. Is a CDK inhibitor
    c. Is rarely found mutated in tumors
    d. Over-expression is associated with metastatic potential
    e. Is inactivated in hypoxic cells

7. Which of the following disorders associated with chromosomal instability does NOT predispose to cancer?
    a. Cockayne's syndrome
    b. Bloom's syndrome
    c. Fanconi's anemia
    d. Nijmegen breakage syndrome
    e. Ataxia telangectasia

8. Following irradiation, which of the following events involving ATM occurs? ATM:
    a. Activation is inhibited by the MRN complex
    b. Is phosphorylated by the MRN complex and undergoes monomerization resulting in its activation
    c. Causes phosphorylation of MDM2, stimulating its inhibitory action against p53
    d. Phosphorylates CHEK2 and inhibits CDC25C activity
    e. Dephosphorylates γH2AX

9. All of the following are phosphatidyl inositol 3-kinase like kinases, EXCEPT:
    a. ATM
    b. BRCA1
    c. ATR
    d. mTOR
    e. DNA-PK (PRKDC)

10. Which of the following statements is FALSE concerning NFκB? NFκB:
    a. Inhibits non-homologous end-joining of DNA double strand breaks
    b. Typically inhibits apoptosis
    c. Is a transcription factor
    d. Activation is associated with tumor progression
    e. Can be activated following irradiation

11. Which of the following statements concerning tumor suppressor genes is FALSE?
    a. Loss of heterozygosity is a mechanism for the inactivation of tumor suppressor genes
    b. The products of tumor suppressor genes generally accelerate cell growth
    c. One or more tumor suppressor genes are typically mutated or absent in human cancers
    d. The most commonly altered tumor suppressor gene is *p53* (*TP53*)
    e. The first tumor suppressor gene discovered was RB (RB1)

12. Which one of the following is a tumor suppressor gene?
    a. NEU
    b. RET
    c. BRAF
    d. RAS
    e. PTEN

13. Which "wonder drug" established the concept of oncogene addiction and revolutionized chronic myologenous leukemia (CML) therapy?
    a. Gefitinib
    b. Imatinib
    c. Bevacizumab
    d. Temozolomide
    e. Loperamide

14. Which of the following is NOT a phenotypic characteristic of a person diagnosed with ataxia telangiectasia?
    a. Neurodegeneration
    b. Abnormalities in ocular blood vessels
    c. Immune system defects
    d. Sensitivity to UV induced cancers
    e. Radiosensitivity

15. Which statement regarding oncogenes and tumor suppressor genes is TRUE?
    a. A gain of function mutation of an oncogene would be recessive on a cellular level
    b. A gain of function mutation in a tumor suppressor gene would stimulate malignant progression of a tumor
    c. Loss of function of a tumor suppressor would appear as a dominant inheritance patter on pedigree with respect to cancer susceptibility
    d. A loss of function mutation in a tumor suppressor gene would be dominant on a cellular level
    e. A loss of function mutation in an oncogene would be dominant in a pedigree in regard to cancer susceptibility

16. Which of the following statements is FALSE?
    a. BRCA1 is deleted in the majority of breast cancers
    b. An increased incidence of CLL has not been found in irradiated populations
    c. A translocation between chromosome 9 and 22 is often present in CML
    d. DCC is a tumor suppressor gene that has been found altered in colon cancer
    e. A mutated mismatch repair gene is often found in people with hereditary non-polyposis colon cancer (HNPCC)

17. Which of the following statements is FALSE?
    a. RAS stimulates the MAPK pathway
    b. CDK1/cyclin B constitute the mitosis promoting factor (MPF)
    c. The first oncogene discovered was in a retrovirus (Rous sarcoma virus)
    d. p21 (CDKN1A) levels decrease in irradiated cells
    e. ATM acts upstream of p53

18. Which of the following processes is NOT a typical mechanism for the activation of a proto-oncogene to an oncogene?
    a. Loss of heterozygosity
    b. Point mutation
    c. Retroviral insertion
    d. Chromosomal rearrangement
    e. Gene amplification

19. Defects in mismatch repair proteins have been associated with which one of the following tumors?
    a. Hereditary non-polyposis colorectal cancer
    b. Neurofibromatosis
    c. Ovarian carcinoma of the serous type
    d. Glioblastoma
    e. Retinoblastoma

20. Overexpression of BCL2 promotes tumorigenesis because BCL2 over-expressing cells:
    a. Exhibit diminished levels of apoptosis
    b. Proliferate more rapidly than their normal counterparts
    c. Have increased angiogenesis
    d. Are more likely to be hypoxic
    e. Have a decreased ability to repair DNA double strand breaks

21. Human papillomavirus (HPV) is a significant cause of cancer worldwide; it is associated with cancers of the uterine cervix, penis, anus, vulva/vagina, and oropharynx. The most common oncogenic subtype is:
    a. HPV-16
    b. HPV-6
    c. HPV-11
    d. HPV-55
    e. HPV-77

22. It is believed that viral oncoproteins E6 and E7 are activated and then inactivate both p53 and pRb, thereby leading to the development of cancer. This process occurs after infection with which virus?
    a. Herpes simplex virus 1
    b. Herpes simplex virus 2
    c. Human papillomavirus
    d. Epstein-Barr virus
    e. Cytomegalovirus

23. Which of the following pathways have been implicated in the loss of clonogenic capacity in irradiated tumor cells harboring wild type p53?
    a. Dedifferentiation
    b. Sublethal damage repair
    c. Senescence
    d. Telomere inversion
    e. Oncogene activation

24. Cancer stem cells (CSCs) are most correctly defined by which of the following statements.
    a. Tissue specific cell surface markers, examples include CD133 for lung CSCs or CD34 for AML CSCs
    b. Cells that can self-renew and also give rise to daughter cells that have more limited proliferative potential and are destined to differentiate
    c. Cells following Hoechst 33342 based flow cytometry that reside in the side-population
    d. Cells that express higher levels of the enzyme aldehyde dehydrogenase (ALDH)
    e. Cells that resist chemotherapy treatment

25. What is chromothripsis?
    a. Staining method for individual genes on chromosomes
    b. Shattering of chromosome portions and rejoining together randomly
    c. Translocations and small deletions
    d. Flourescence staining of cells with chromatic dyes
    e. An enzyme induced by radiation

26. What are driver genes in oncogenesis?
    a. An early mutation that drives the cancer and usually involves a dominant or recessive oncogene.
    b. They occur exclusively late in the development of the cancer and drive the development of the metastasis.
    c. Mutations that occur in DNA repair genes
    d. Mutations that are induced by radiation and other mutagens.
    e. Errors introduced during the process of DNA repair

27. Clonal evolution is primarily driven by:
    a. Oncogene activation
    b. Imperfect DNA damage repair
    c. Cell cycle progression
    d. Checkpoint inhibition
    e. Tumor suppressor inactivation

28. Which of the following statements about tumor mutational signatures is true?
    a. Each tumor contains a single mutational signature
    b. Each cancer cell contains a single mutational signature
    c. Mutational signatures are specific to the tissue of origin
    d. Mutational signatures are constant across time
    e. Mutational signatures are caused by specific mutational and DNA repair processes

29. Pathogenic mutations of the *KRAS* oncogene function by:
    a. Recycling GTP
    b. Phosphorylating B-Raf
    c. Preventing GTP hydrolysis
    d. Preventing KRas protein degradation
    e. Localizing KRas to the plasma membrane

30. Which of the following is not a hallmark of cancer, as defined by Hahahan and Weinberg (2011)?

    a. Inducing angiogenesis
    b. Resist treatment
    c. Sustaining proliferative signaling
    d. Enable replicative immortality
    e. Evading growth suppressors

31. The Cancer stem cell hypothesis describes specific characteristics of this population of cells. Which of the following is incorrect?
    a. They are often relatively rare in tumors
    b. They share a common exclusive phenotypic marker
    c. They have high anti-oxidant levels
    d. They often increase after radiation exposure
    e. They express the multiple drug resistant gene product mdr1

32. The tumorigenicity of cancer stem cells is best assessed by what assay?
    a. TD50 assay
    b. TCD50 assay
    c. In vivo - in vitro assay
    d. Tumor regrowth assay
    e. Ability to metastasize

33. Which of the following sets of transcription factors, often called Yamanaka factors, are commonly used to induce pluripotent stem cells (iPSCs) from somatic cells?
    a. Oct4, Sox2, Klf4, c Myc
    b. c-jun, c-fos, K-Ras, B-Raf
    c. NF-□B, Akt, IRF2, Nrf2
    d. BAX, BAM, ATM, TP53
    e. P38, c-Jun, C-Fos, Akt

34. The cancer stem cell theory gained prominence when cell samples from acute myeloid leukemia patients were shown to:
    a. contain one characteristic cell type
    b. have high clonogenicity in vitro and in immune suppressed mice
    c. have a small subset of CD34++CD38− cells with a high capacity for self-renewal and initiated tumors on transplantation into NOD/SCID mice
    d. contain a small subset of CD34++CD38− cells with a high capacity to differentiate in vitro
    e. contain a large number of CD34++CD38- stem cells

35. Which of the following crucial observations was made by Till and McCulloch during bone marrow cell transfer in mice?
    a. Prevention of late hematopoietic lethality in mice after whole body irradiation (WBI)
    b. Bone marrow clones that arise during regeneration after WBI form colonies in the spleen of mice obvious within 10 days after exposure
    c. Bone marrow-derived cells that give rise to colonies within 10 days in the spleen of WBI mice are relatively radioresistant
    d. Repair does not take place over the first 24 hours after bone marrow irradiation
    e. Bone marrow rescue following WBI required more stem cells in smaller compared to larger animals

36. Which of the following is true about jejunal stem cells?
    a. They reside in the villus
    b. They include relatively radiosensitive LGR5- basal columnar epithelial cells that lie interspersed between Paneth cells
    c. They include a quiescent reserve stem cell population that may be activated after injury
    d. They can give rise to regenerating clonogens that can be counted under the microscope within a few hours of exposure >10 Gy
    e. They give rise to rapidly proliferating progenitor/transit cells in the top of villus

37. All of the following are properties of cancer stem cells, EXCEPT:
    a. Self-renewal capacity
    b. Tumorigenicity
    c. Pluripotency
    d. Relative chemoresistancy
    e. Involved in tumor regression

38. Several mechanisms have been proposed as to why cancer stem cells are more radioresistant than non-stem cancer cells. One such example is:
    a. Cancer stem cells have lower levels of free radical scavengers
    b. Cancer stem cells tend to down-regulate developmental pathways such as Wnt/□-catenin, Notch, Hedgehog, and TGF-b
    c. Hypophosphorylation of checkpoint kinases CHK1/2 in cancer stem cells
    d. Hyperactivation of anti-apoptosis pathways in cancer stem cells
    e. Overexpression of MHC class I

39. Which of the following statements about cancer versus normal cell metabolism is TRUE?
    a. Cancer versus normal cell metabolism cannot be used to improve the diagnosis or treatment of cancer with radio-chemotherapy
    b. Cancer versus normal cell metabolism can be visualized with FDG-PET imaging but not spectroscpy
    c. The differences between cancer versus normal cell metabolism are drastically affected by radiation therapy
    d. The differences between cancer versus normal cell metabolism of oxygen have nothing to do with free radical or reactive oxygen species production
    e. Warburg was one of the first scientists to recognize that the regulation of glucose and oxygen metabolism was very different between cancer and normal cell

Total Body Irradiation

1. Which of the following statements concerning whole body effects of radiation is TRUE?
    a. The time to death from the hematopoietic syndrome is 1-2 months
    b. The time to death from the cerebrovascular syndrome is 2-4 weeks
    c. The time to death from the gastrointestinal syndrome is 2-4 months
    d. The threshold dose for the gastrointestinal syndrome is 1 Gy
    e. The threshold dose for the hematopoietic syndrome is 10 Gy

2. The main cause of death from the hematopoietic syndrome is:
    a. Hypotension arising from microvascular destruction
    b. Hemolytic anemia
    c. Infection and hemorrhage resulting from loss of white cells and platelets
    d. Loss of erythrocytes resulting in organ ischemia
    e. Dehydration due to extravasation of fibrin from blood vessels

3. Which of the following statements is TRUE concerning a female worker at a radioactive waste reprocessing facility who accidentally receives an estimated 3 Gy acute whole body γ-ray dose?
    a. Antibiotic treatment should not be initiated until signs of infection.
    b. Tissue typing should be done for a possible bone marrow transplant.
    c. Within one week she will become dehydrated, suffer infections, develop bloody diarrhea and likely die.
    d. She should be sent home and advised to schedule an appointment with a physician about 6 months later, as this represents the minimum latency period prior to the manifestation of radiation injury.
    e. She should be monitored carefully to watch for symptoms of infection

4. Which of the following would probably NOT be noted in an individual who received an acute, whole body dose of 5 Gy of X-rays and received no medical care?
    a. Infection
    b. Nausea
    c. Bleeding
    d. Death within 1 week following irradiation
    e. Epilation

5. A detectable change in blood count would be expected following a minimum whole body dose of approximately:
    a. 0.001 Gy
    b. 0.01 Gy
    c. 0.1 Gy
    d. 1 Gy
    e. 10 Gy

6. Within 4 days of an accidental whole body radiation exposure at a nuclear power plant, 8 workers develop severe diarrhea. Assuming that 3 of the workers are female and 5 male, what is their likely prognosis?
    a. All will live, but will likely develop radiation-induced cancers.
    b. Approximately 50% will survive.
    c. All will live, but with an increased risk of cataracts.
    d. They will all die in less than a month following the irradiation.
    e. The men will be sterilized, but the women will remain fertile.

7. Which of the following radiation-induced effects could be a cause of death one year after total body irradiation of a patient being prepared for a bone marrow transplant?
    a. Hematopoietic syndrome
    b. Gastrointestinal syndrome
    c. Cerebrovascular syndrome
    d. Brain necrosis
    e. Lung fibrosis

8. Immunosuppression observed within 24 hours following exposure to a whole body dose of 5 Gy X-rays would be due primarily to:
    a. Death of hematopoietic progenitor cells
    b. Apoptosis of peripheral blood lymphocytes
    c. A loss of circulating granulocytes
    d. Decreased activity of NK cells
    e. Inactivation of circulating antibodies

9. Which of the following statements concerning the human LD_{50} is TRUE?
    a. The $LD_{50/60}$ associated with an acute whole body irradiation is approximately 3.5 Gy for people who do not receive appropriate medical care following irradiation.
    b. Even with optimal medical care, the $LD_{50/60}$ cannot be increased.
    c. The most common cause of death in people who receive a dose close to the $LD_{50/60}$ is severe anemia.
    d. A person who received a whole body dose close to the $LD_{50/60}$ would exhibit severe diarrhea within 24 hours.
    e. The $LD_{50/60}$ is the dose that leads to death within 50 days of 60% of the population.

10. Which of the following is the correct temporal sequence for the appearance of the stated radiation effect on peripheral blood components?
    a. Lymphocytopenia, granulocytopenia, thrombocytopenia, anemia
    b. Anemia, lymphocytopenia, granulocytopenia, thrombocytopenia
    c. Granulocytopenia, thromobocytopenia, anemia, lymphocytopenia
    d. Lymphocytopenia, anemia, granulocytopenia, thrombocytopenia
    e. Lymphocytopenia, thrombocytopenia, granulocytopenia, anemia

Normal Tissue Response

1. Which of the following statements concerning radiation cataractogenesis is TRUE?
    a. The lens of the eye is capable of eliminating cells damaged by radiation, which has the net effect of decreasing the incidence of cataracts
    b. There is a shorter latency period for the development of cataracts following a large radiation dose than a small one
    c. The neutron RBE for fast neutrons regarding cataract is approximately 3.0
    d. For an acute exposure, the threshold dose for the induction of an X-ray-induced cataract is 15 Gy
    e. As is true for most radiation-induced injuries, there are no pathognomonic characteristics specific for a radiation-induced cataract

2. Which of the following statements concerning the radiation-induced effects of fractionated total body irradiation in children being prepared for a bone marrow transplant is FALSE?
    a. Approximately half of the children develop severe restrictive pulmonary disease
    b. The majority will develop cataracts
    c. Thyroid cancer is the main second malignancy observed
    d. The younger the child at the time of irradiation, the greater the risk for the development of osteochondroma
    e. Manifestations of hypogonadism are common in both boys and girls

3. Which of the following statements is TRUE concerning irradiation of the testes?
    a. Spermatids and spermatozoa are relatively radiosensitive, whereas spermatogonia tend to be radioresistant
    b. A substantial drop in testosterone levels can be detected following a scattered X-ray dose of 0.1 Gy to the testes of an adult man
    c. If sterility in the male is not observed within one month following irradiation, it is unlikely to occur at a later time
    d. Dose fractionation increases the risk for sterility in the male
    e. Full recovery of a normal sperm count following radiation-induced azoospermia caused by exposure to a dose of 6 Gy of X-rays generally occurs within 6 months

4. Which of the following statements concerning complications arising from pelvic irradiation is FALSE?
    a. Diarrhea is the most common manifestation of radiation injury to the bowel
    b. Diarrhea usually does not appear until at least 6 months following the completion of radiotherapy
    c. Late bowel reactions include mucosal atrophy, stenosis, ulceration, obstruction, adhesions and perforation.
    d. Bowel stenosis is a late complication of radiation therapy
    e. Adhesions following irradiation contribute to late bowel injury and usually develop 2-7 months after irradiation

5. Which of the following statements concerning irradiation of the spinal cord is FALSE?
    a. One of the main manifestations of transient demyelination is Lhermitte's sign
    b. Transient demyelination is a strong predictor for later permanent myelopathy.
    c. Irreversible radiation myelopathy typically occurs months after the completion of treatment.
    d. Irreversible radiation myelopathy is characterized by demyelination and white matter necrosis
    e. The spinal cord demonstrates a certain degree of recovery following irradiation, if there is sufficient time between courses.

6. Which of the following statements concerning late radiation effects in the brain is FALSE?
    a. The classical late radiation effect in the brain is localized necrosis generally limited to the involved white matter, with focal coagulative necrosis and demyelination as dominant features
    b. Symptoms of late radiation effects include motor, sensory and/or speech/receptive deficits, seizures and symptoms of increased intracranial pressure
    c. The "somnolence syndrome" is observed 1-6 months post-irradiation
    d. During the 3-6 month period following completion of RT, a general neurologic deterioration may occur that results from transient, diffuse demyelination
    e. Arterial cerebrovasculopathy is commonly observed

7. Which of the following statements is TRUE concerning the response of the kidney to radiation? The kidney:
    a. Is considered a relatively radiosensitive organ because of the marked sensitivity of cells that comprise the nephron
    b. Exhibits little sparing with increasing dose fractionation
    c. Has a relatively low tolerance dose because of the limited number of clonogens within each functional subunit
    d. Displays substantial re-treatment tolerance
    e. Manifests symptoms of radiation nephropathy generally within 3 months following the completion of radiotherapy

8. Concerning radiation induced liver disease (RILD), all of the following statements are true, EXCEPT:
    a. RILD is a clinical syndrome of ascites, elevated liver enzymes, and hepatomegaly in the absence of jaundice
    b. RILD is rarely observed earlier than six months following the completion of radiotherapy
    c. Suprahepatic vein obstruction and veno-occlusive liver disease are seen in RILD
    d. Pathologic changes in RILD include marked venous congestion in the central portion of each lobule – with sparing of the larger veins – and atrophy of hepatocytes adjacent to the congested veins
    e. Killing of vascular endothelial cells appears to be of greater importance than hepatocyte lethality in the pathologic changes observed in RILD

9. Which of the following effects is typically observed within a week following irradiation of the small intestine?
    a. Hypertrophic villi
    b. Lymphocyte infiltration
    c. Atrophic villi
    d. Mucosal atrophy
    e. Bowel stenosis

10. The best way to spare the parotid gland is to:
    a. Use hyperfractionated radiotherapy
    b. Decrease the irradiated volume of the parotid gland
    c. Increase the overall treatment time
    d. Use hypofractionated radiotherapy
    e. Accelerate treatment

11. Of the following, the organ/tissue least able to tolerate re-irradiation is the:
    a. Spinal cord
    b. Oral mucosa
    c. Kidney
    d. Lung
    e. Liver

12. A drug used to treat fibrosis and osteoradionecrosis is:
    a. Amifostine
    b. Tirapazamine
    c. Nicotinamide
    d. Pentoxifylline
    e. Misonidazole

13. The lacrimal gland is comparable to which of the following organs/glands in terms of its radioresponse?
    a. Parotid
    b. Heart
    c. Liver
    d. Sebaceous
    e. Skin

14. Which of the following has the highest radiation tolerance dose ($TD_{5/5}$) for whole organ irradiation?
    a. Kidney
    b. Ureter
    c. Colon
    d. Stomach
    e. Liver

15. Renal irradiation can lead to the development of radiation nephropathy, which is characterized by proteinuria, anemia, hypertension and a chronic, progressive decrease in renal function. The decline in kidney function characteristic of radiation nephropathy can be:
    a. Treated with anti-hypertensive agents such as beta blockers
    b. Prevented using anti-inflammatory agents
    c. Reversed using calcium channel blockers
    d. Mitigated using drugs that block the renin-angiotensin system
    e. Accelerated at lower radiation doses

16. Which of the following is NOT a delayed effect following head and neck radiation therapy?
    a. Stricture
    b. Mucositis
    c. Persistent xerostomia
    d. Telangiectasia
    e. Ulcer

17. Which of the following statements is TRUE concerning radiation effects on lymphoid tissues?
    a. T cells are generally more radiosensitive than B cells
    b. Filter function in lymph nodes is unaffected by radiation
    c. Altered immunity is an important factor in gastrointestinal syndrome following whole body irradiation
    d. Morphologically, the spleen shows few late effects
    e. The thymus appears almost fully functional following irradiation, with doses in the range typically used in radiotherapy, for cancers in which this organ is in the radiation field

18. The Quantitative analysis of normal tissue effects in the clinic (QUANTEC) suggested that prevent of severe xerostomia (long term salivary function <25% of the pre-treatment baseline) requires which of the following dose constraints on the parotid glands:
    a. At least one parotid gland should receive a mean dose <50 Gy
    b. At least one parotid gland should receive a mean dose <20 Gy
    c. Both parotid glands should receive a mean dose <50 Gy
    d. Both parotid glands should receive a mean dose <20 Gy
    e. None of the above

19. Which cell types are implicated as the major course of collagen production in radiation fibrosis?
    a. Myofibroblasts
    b. Keratinocytes
    c. Endothelial cells
    d. Pneumocytes
    e. Macrophages

20. Which of the following pathways has been linked to radiation fibrosis?
    a. TGF-beta signaling
    b. PDGF signaling
    c. Reactive oxygen species
    d. Angiontensin signaling
    e. All of the above

21. Which of the following intermediates is thought to play a critical role in the pro-fibrotic signaling of transforming growth factor-beta (TGF-beta)?
    a. Insulin like growth factor, IGF
    b. Heat shock protein 90, Hsp90
    c. Checkpoint kinase 1, Chk1
    d. Connective tissue growth factor, CTGF
    e. p53

22. Transforming growth factor-beta (TGF-beta) protein levels in the plasma of patients exposed to radiotherapy has been extensively correlated to which of the following?
    a. Acute radiation lung injury
    b. Acute radiation dermatitis
    c. Radiation mucositis
    d. Radiation induced gliosis
    e. Leukemia

23. The tolerance dose for xerostomia resulting from treatment of a head and neck tumor with 3 Gy fractions compared to 2 Gy fractions would be expected to:
    a. Increase substantially
    b. Increase slightly
    c. Decrease substantially
    d. Remain about the same
    e. Either increase or decrease depending upon the particular patient

24. Assuming that the target cells do not have a pro-apoptotic tendency, the time to the expression of radiation damage in early-responding tissues typically correlates best with the:
    a. Radiosensitivity of the cells
    b. Lifespan of the mature functional cells of the tissue
    c. Ability of the cells to perform homologous recombinational repair of DNA damage
    d. Lifespan of the stem cells comprising that tissue
    e. Type of radiation used to irradiate the organ

25. Which of the following statements is FALSE concerning cytokines?
    a. Basic fibroblast growth factor (bFGF or FGF2) enhances radiation-induced apoptosis of endothelial cells
    b. High levels of transforming growth factor-beta (TGFβ1) have been reported to be associated with an increased risk of pulmonary fibrosis following radiotherapy
    c. Interleukin-1 (IL-1) is a bone marrow radioprotector
    d. Vascular endothelial growth factor (VEGF) transcription is stimulated by hypoxia as a result of hypoxia inducible factor (HIF-1) binding to a hypoxia responsive element (HRE) within the VEGF promoter
    e. A paracrine response involves production of cytokines in which the target cells are located in the vicinity of the expressing cell

26. Which of the following growth factors appear to play a role in radiation -induced lung fibrosis?
    a. Transforming growth factor-beta (TGFβ1)
    b. Basic fibroblast growth factor (bFGF or FGF2)
    c. Connective tissue growth factor (CTGF)
    d. Platelet derived growth factor (PDGF)
    e. All of the above

27. As the dose to an organ increases, the latency period prior to the development of a late complication generally:
    a. Increases
    b. Decreases
    c. Remains the same
    d. Increases, but only for an accelerated protocol
    e. Decreases, but only for a hyperfractionated protocol

28. The shape of the dose response curve for the induction of late effects is best described as:
    a. Gompertzian
    b. Linear
    c. Threshold
    d. Sigmoidal
    e. Linear-quadratic

29. Which of the following statements regarding the development of radiation-induced lung damage is TRUE?
    a. The volume of lung irradiated has relatively little effect on the tolerance dose
    b. Radiation-induced pneumonitis is delineated by the treatment field
    c. The majority of patients who develop radiation pneumonitis go on to develop pulmonary fibrosis
    d. The $TD_{5/5}$ for whole lung irradiation with a single dose is approximately 17.5 Gy
    e. Fractionation has little or no effect on lung tolerance

30. In normal tissues, the radiation tolerance dose is hypothesized to depend on the ability of tissue clonogens to maintain an adequate number of mature functioning cells. The relationship between organ function and clonogenic cell survival is dependent on the structural organization of functional subunits (FSUs) within the particular tissue. Which of the following statements concerning FSUs is TRUE? FSUs:
    a. Contain a relatively set number of clonogens
    b. Cannot be repopulated from a single surviving clonogen
    c. Are defined as units with clear anatomical demarcation
    d. Are usually dependent on one another in a functional sense
    e. Cannot be repopulated from an adjacent FSU

31. With the increasingly sophisticated refinements in radiation therapy techniques, more attention is now being paid to normal tissue dose and volume factors as they relate to the probability of treatment- associated late effects. Which of the following statements concerning the volume dependence of late complications is FALSE?
    a. The parameter that best predicts for lung complications after radiotherapy is the V_{20}/V_{30}
    b. Length irradiated is a critical factor in determining the tolerance dose for the esophagus
    c. The percent volume of rectal wall that receives 40-50 Gy positively correlates with the likelihood of rectal bleeding
    d. Late effects are more sensitive to changes in fractionation than early effects
    e. Small volume irradiation of the brain can lead to focal radiation necrosis

32. Which of the following statements about Transforming growth factor-beta (TGFβ1) is FALSE? TGFβ:
    a. Is a chemo-attractant for granulocytes
    b. Is a suppressor of T lymphocytes
    c. Increases proliferation of fibroblasts and smooth muscle cells
    d. Increases proliferation of epithelial cells
    e. Requires activation to be biologically active

33. Which of the following statements concerning the tolerance of normal tissues to re-irradiation is TRUE?
    a. Evidence from animal studies suggests that the spinal cord can be re-irradiated to at least partial tolerance provided at least 6 months have passed since an initial course of treatment
    b. Soft tissue or bone necrosis has not been observed in patients receiving re-irradiation of recurrent or new primary head and neck tumors
    c. Mouse lungs appear incapable of tolerating a second course of fractionated radiation, regardless of the total dose given during the initial course of radiotherapy
    d. Rapidly dividing mucosal tissues cannot be re-irradiated, even several years after completion of the initial treatment
    e. Animal experiments show that the kidney can be re-irradiated to 80-90% of a full tolerance dose as long as 3 months have elapsed since the initial treatment

34. The TD_5 as a function of length of spinal cord irradiated:
    a. Decreases as a linear function of increasing cord length
    b. Initially decreases with increasing cord length, and then remains relatively constant for higher total doses
    c. Increases steeply for lengths greater than approximately 10 cm
    d. Decreases with decreasing cord length
    e. Increases with cord length before reaching a plateau

35. Radiation-induced epilation occurs before dermatitis because:
    a. Basal cells in the epidermis have shorter cell cycle times than the germinal matrix of the hair bulb
    b. Cells in the germinal matrix of the hair bulb have shorter cell cycle times than the basal cells of the epidermis
    c. Of the exquisite sensitivity of sebaceous glands
    d. Of vascular endothelial cell death in the connective tissue at the distal end of the hair follicle
    e. Of keratin synthesis inhibition in the hair follicle

36. All of the following organs can tolerate 70 Gy (delivered in 2 Gy fractions) to 5% of their volume, except the:
    a. Spinal cord
    b. Kidney
    c. Lung
    d. Liver
    e. Heart

37. Neoadjuvant radiation therapy can negatively affect wound healing following surgical resection of a tumor. Which of the following is the last step in the process of wound closure?

    a. Inflammation and fibroblast activation
    b. Fibroblast migration and collagen deposition
    c. Remodeling and collagen degeneration
    d. Keratinocyte activation and wound contraction
    e. Inflammation and stem cell homing

Therapeutic Ratio

1. A tumor contains 10^6 clonogenic cells. Its effective dose response curve has been determined for dose fractions of 2 Gy/day, and is characterized by no shoulder and a D_0 of 2.5 Gy. What is the total dose required to give a 37% chance of tumor cure, assuming sufficient time between fractions to allow full repair of sublethal damage and no cell proliferation between doses?
    a. 5 Gy
    b. 14 Gy
    c. 21 Gy
    d. 28 Gy
    e. 35 Gy

2. Based on the same parameters as provided in the previous question, what additional dose must be added to still achieve a 37% chance of tumor cure, if the clonogens in the tumor went through three cell divisions during treatment (assuming that there is no cell loss)?
    a. 1 Gy
    b. 2 Gy
    c. 5 Gy
    d. 10 Gy
    e. 20 Gy

3. Suppose a chemotherapeutic agent that killed tumor cells, independently of radiation, was also employed during the aforementioned course of treatment. It is known from previous data that this drug regimen results in a surviving fraction of 10^{-4} for the tumor under treatment. Now what is the total radiation dose required to produce a 37% chance of tumor cure (still assuming that the 10^6 cells go through an extra three cell divisions)?
    a. 12 Gy
    b. 17 Gy
    c. 24 Gy
    d. 36 Gy
    e. 48 Gy

4. Tumors A and B have identical single dose TCD_{50} values. However, the cell survival dose response curve for tumor A is characterized by an α/β ratio of 2 Gy, while the curve for tumor B has an α/β ratio of 30 Gy. If these tumors are both treated with a fractionated protocol using daily dose fractions of approximately 2 Gy in the same overall treatment time, the total dose to yield a TCD_{50} for tumor A compared with tumor B will be:
    a. Lower
    b. Greater
    c. Equal
    d. Less for a lower probability of tumor control and greater for a higher probability of control
    e. Impossible to determine from the information provided

5. The cells comprising a patient's tumor are characterized by an SF_2 of 0.3 and a doubling time of 3 days. Due to an unexpectedly severe skin reaction, the patient is put on a 3 week break during treatment to allow some healing to occur. How much extra dose would be required to achieve the same probability of tumor control if the treatment had not been interrupted? (Assume that treatment is given as daily, 2 Gy fractions, the multifraction survival curve for the cells comprising this tumor is exponential, and that radiation-induced cell cycle perturbations are negligible.)
    a. 2 Gy
    b. 4 Gy
    c. 6 Gy
    d. 8 Gy
    e. 10 Gy

6. Assuming that the D_{10} for a tumor cell population is 4 Gy and the extrapolation number n equals 1, the single dose to achieve a TCD_{90} for a tumor containing 100 million clonogenic cells is closest to:
    a. 18 Gy
    b. 24 Gy
    c. 28 Gy
    d. 36 Gy
    e. 44 Gy

7. What is the typical shape of a tumor growth curve?
    a. Gompertzian
    b. Exponential
    c. Parabolic
    d. Linear
    e. Linear-quadratic

8. For conventional fractionation, the tolerance dose for a particular normal tissue complication is found to be 30 Gy. If a patient is treated with a drug that has a dose reduction factor of 1.3, then the new tolerance dose for this tissue should be roughly:
    a. 23 Gy
    b. 30 Gy
    c. 33 Gy
    d. 36 Gy
    e. 39 Gy

9. The TCD_{90} for a series of 0.1 cm diameter tumors receiving fractionated radiotherapy in 1.8 Gy daily fractions was determined to be 56 Gy. Assuming that the tumors each contained 10^6 clonogenic cells, what dose would be necessary to maintain the 90% control rate if the tumors were allowed to continue growing until they reached a 1 cm diameter? (Assume that the growth fraction remained constant during the course of treatment.)
    a. 48 Gy
    b. 56 Gy
    c. 64 Gy
    d. 71 Gy
    e. 80 Gy

Time, Dose, Fractionation

1. Which of the following statements concerning the α/β ratio for tumors and normal tissues is TRUE?
    a. The α/β ratio is generally low for early responding tissues and high for late responding tissues
    b. The α/β ratio corresponds to the dose at which the survival curve begins to bend and deviate from its initial slope
    c. *In vivo*, α/β ratios for normal tissues and tumors are derived from an analysis of isoeffect data derived from multi-fraction experiments
    d. The α/β ratio tends to be low for cells with a pro-apoptotic tendency
    e. The α/β ratio represents the surviving fraction at which the linear and quadratic contributions to cell killing are equal

2. A treatment schedule consisting of 25 daily fractions of 1.8 Gy was found to be biologically equivalent to a schedule consisting of 17 daily fractions of 2.5 Gy with respect to complication probability in a critical normal tissue. The α/β ratio for this tissue is closest to:
    a. 1 Gy
    b. 3 Gy
    c. 6 Gy
    d. 10 Gy
    e. 20 Gy

3. A hyperfractionated protocol is being proposed in an effort to reduce the incidence of late effects following radiotherapy for head and neck cancer. Compared to standard fractionation, it is likely that this alternate schedule will result in a(n):
    a. Comparable probability of tumor control
    b. Increased probability of tumor control
    c. Decreased probability of tumor control
    d. Increased probability of early effects
    e. Decreased probability of early effects

4. The slopes of isoeffect curves for late responding tissues compared to early responding tissues and tumors are typically (assume data are plotted on a log-log scale):
    a. Variable, depending upon the specific tissue
    b. Comparable
    c. Shallower
    d. Steeper
    e. Flat

5. Two isoeffect curves, one corresponding to a given level of tumor control and the other for a given probability of a late complication in a critical normal tissue, are found to intersect. If the curves were plotted as total dose on the Y-axis and dose per fraction on the X-axis, the most important application of this information would be to predict the:
    a. Tumor control probability
    b. Optimal range of fraction sizes to use for treatment
    c. Optimal overall treatment time
    d. Outcomes when split course treatment is used
    e. Normal tissue complication probability

6. If the dose-limiting, normal tissue toxicity of interest is characterized by an α/β ratio of 6 Gy, and the corresponding tumor possesses an α/β ratio of 2 Gy, it is most likely that a patient being treated for this type of cancer would benefit from:
    a. Split course treatment
    b. Accelerated treatment
    c. Hypofractionation
    d. Hyperfractionation
    e. Low dose rate brachytherapy

7. Tumor cell repopulation during treatment causes the BED value to:
    a. Increase
    b. Decrease
    c. No effect
    d. Increase, but only if T_{pot} is greater than 5 days
    e. Increase, but only if the α/β ratio for the tumor is large

8. Accelerated fractionation is used to:
    a. Counteract the inherent radioresistance of some tumor cells
    b. Overwhelm DNA repair processes in tumor cells
    c. Overcome the radioresistance of hypoxic tumor cells
    d. Increase the potential for repopulation by cells in normal tissues
    e. Reduce the potential for tumor cell repopulation

9. A treatment prescription of 72 Gy delivered in 2 Gy fractions is changed to deliver 3 Gy fractions, with the total dose adjusted accordingly so that the new prescription would be isoeffective with respect to late complications in a normal tissue characterized by an α/β ratio of 2 Gy. If the α/β ratio for the tumor is 10 Gy, what is the approximate change in biologically effective dose to the tumor, assuming no change in overall treatment time?
    a. +14%
    b. +7%
    c. 0
    d. -7%
    e. -14%

10. Based on experience in head and neck cancers, accelerated repopulation likely begins how many dates after the initiation of fractionated radiation therapy?
    a. 7 days
    b. 14 days
    c. 21 days
    d. 28 days
    e. None of the above

11. Clinical studies in prostate cancer radiotherapy study whether moderate hypofractionation (2.4-4 Gy/fractions) is superior or at least not inferior to conventional fractionation (1.8-2 Gy/fraction). Which of the following most correctly describes the radiobiological rationale that is used to justify hypofractionation.
    a. Hypofractionated radiotherapy delivers a higher biologically equivalent dose than conventional fractionation
    b. Hypofractionated radiotherapy takes place over a shorter period of time (from first treatment to last treatment) than conventional fractionation
    c. Hypofractionated radiotherapy is useful when cancer cells have an alpha/beta ratio equal to or lower than surrounding tissues and organs at risk
    d. Hypofractionated radiotherapy is more precise than conventional radiotherapy
    e. Hypofractionated radiotherapy does less damage to late-reacting normal tissues

Brachytherapy

1. Which of the following isotopes is most commonly used for HDR brachytherapy?
    a. Ir-192
    b. Pd-103
    c. I-125
    d. Co-60
    e. Y-90

2. Iodine-131 tositumomab (Bexxar) is:
    a. A radiolabeled small molecule tyrosine kinase inhibitor used to treat lung cancer
    b. Used to treat thyroid cancer
    c. Of limited clinical utility because of its high toxicity to the GI tract
    d. A radiolabeled antibody against the CD20 antigen over-expressed in non-Hodgkin's lymphoma cells
    e. Highly effective at cell killing because of the high LET α-particle emissions from the I-131

3. A primary advantage of high dose rate (HDR) brachytherapy for the treatment of prostate cancer is that:
    a. The oxygen enhancement ratio (OER) is expected to be lower for HDR than for low dose rate (LDR) brachytherapy
    b. The probability of late normal tissue damage decreases with increasing fraction size
    c. Tumor response should be improved by using larger fraction sizes because of the lower α/β ratio associated with prostate cancer compared with that for the surrounding normal tissues
    d. Radiation safety issues are generally of less concern for the radioisotopes used for HDR brachytherapy vs/ those for LDR brachytherapy because of their reduced shielding requirements
    e. HDR brachytherapy allows for better coverage of regional lymphatics

4. Brachytherapy has been used to treat ocular melanoma using multiple radionuclides. There is clinical data on the use of all of these isotopes EXCEPT:
    a. Ruthenium-106
    b. Cobalt-60
    c. Iodine-125
    d. Iodine-131
    e. Palladium-103

Alternative Delivery Systems

1. The use of one or a few large radiation doses is generally contraindicated for radiotherapy because of an increased likelihood of late normal tissue complications compared to more conventional fractionation. However, special procedures such as stereotactic radiosurgery and intraoperative radiotherapy employ large doses, apparently without an increase in late effects. The **best** explanation for this finding is that:
    a. These special procedures have not been in use long enough for all of the anticipated late complications to manifest themselves
    b. Normal tissue radioprotectors are usually administered along with the high dose treatments
    c. Radioresistance caused by tissue hypoxia is more pronounced when large doses are used rather than small doses
    d. Extra care is taken in these procedures to produce the most conformal treatment plan possible, so as to minimize the amount of late-responding normal tissue irradiated
    e. DNA repair systems in tumor cells are more easily saturated following one or a few large doses than in the surrounding normal tissue cells incidentally irradiated

2. For which of the following types of radiation is the description provided FALSE?
    a. Carbon ions – have both depth-dose and biological advantages for radiotherapy
    b. Electrons – useful for the treatment of deep-seated tumors
    c. Protons – dose distribution advantages, but with an RBE approximately equal to 1.0.
    d. Photons – most common type of radiation used for radiotherapy
    e. Neutrons – relatively poor dose distributions, but with greater biologic effectiveness

3. Which statement comparing carbon ion with proton beam radiotherapy is FALSE? Both carbon ions and protons:
    a. Provide the type of precision radiotherapy needed to treat certain tumors located near critical structures
    b. Display a lower OER compared with X-rays
    c. Exhibit a Bragg peak.
    d. Represent particulate forms of radiation
    e. Both are positively charged and generated using a cyclotron

4. Protons used for cancer radiotherapy:
    a. Show the greatest potential in the treatment of tumors with high hypoxic fractions and/or poor reoxygenation rates
    b. Are typically in the 1 - 10 MeV range
    c. Exhibit LET values <10 keV/μm
    d. Exhibit an RBE of approximately 5
    e. Are densely ionizing

5. Which of the following correctly describes differences between the dose-depth profiles of therapeutic beams of photons and protons?
    a. Dose in front of the tumor is higher than dose behind the tumor with protons
    b. Entrance dose with photons is always higher than with protons
    c. The Bragg peak can be spread out by changing proton fluence
    d. Photon dosimetry is more affected than proton dosimetry by in-depth tissue inhomogeneities
    e. Lateral proton beam penumbra that is advantageous compared with the penumbra of external beam photon therapy

6. Dose conformality in IMRT is usually achieved by:
    a. Use of multiple monoenergetic pencil beams
    b. Deflecting the beam with multiple collimators so that it conforms to the desired shape of the target volume
    c. “Inverse” treatment planning based on CT images of the target volume
    d. Prompt gamma-based imaging from excitation of oxygen and nitrogen nuclei
    e. Reducing the time to deliver each dose per fraction

7. Which of the following statements is correct? Radioactive microsphere therapy:
    a. Uses radioactive iron nanospheres to improve thermal dose distribution in tumors
    b. Uses α-particle emitters to radiolabel antibodies against HIF in hypoxic tumors
    c. Uses positron emitters to label to radiolabel antibodies against the CD20 antigen
    d. Uses encapsulated yttrium-90 to deliver very high radiation doses to hepatic tumors
    e. Uses encapsulated iodine-131 to deliver very high radiation doses to hepatic tumors

8. Which of the following statements comparing proton with neutron therapy is correct?
    a. Tissues demonstrate higher α/β with neutron compared to proton irradiation
    b. Therapeutic energy protons lose the fractionation dose-sparing effect for early but not late responding tissues
    c. Proton but not neutron doses are described using the term “equivalent dose”
    d. Protons and neutrons transfer energy to tissue via nuclear reactions
    e. “Scanning” the beam describes energy-modulated broad beam neutron irradiation

9. Which of the following ions (or particles) have optimal OER and RBE values for radiation therapy?
    a. Hydrogen
    b. Helium
    c. Carbon
    d. Neutron
    e. Silicon

10. Which of the following pairs of radiation are BOTH high LET and have a Bragg peak?
    a. Gamma rays and X-rays
    b. P+ and C-ions
    c. Neutrons and C-ions
    d. Neon ions and neutrons
    e. Si ions and C-ions

11. Which one of the statements regarding proton beam therapy is TRUE:

    a. Treatments with proton beams typically result in no entrance dose
    b. Proton beams are superior to photon beams because its RBE (relative biological effectiveness) is approximately 3.5
    c. Prescription dose of proton beam treatments is described in CGE (Cobalt Gray Equivalent)
    d. Compared to photon beams, proton beams create more single strand DNA breaks
    e. Compared to proton beams, photon beams are known to have a higher LET (Linear Energey Transfer)

12. Which one of the statements regarding fast neutron beam therapy is TRUE:
    a. Fast neutron beam energies range between 500 to 700 MeV
    b. Fast neutron beams have lower LET (Linear Energy Transfer) compared to photon beams
    c. Fast neutron beams have an RBE (relative biological equivalent) of approximately 250
    d. Fast neutron beams create DNA damage primarily via Compton Effect
    e. Fast neutron beams are associated with a low oxygen enhancement ratio

Chemotherapy

1. Irinotecan:
    a. Acts directly on RNA polymerase
    b. Is activated intracellularly to camptothecin
    c. Is a proteasome inhibitor
    d. Acts by stabilizing the topoisomerase I cleavable complex
    e. Is a derivative of cyclophosphamide

2. The epidermal growth factor receptor (EGFR) is a target of which of the following agents?
    a. Bevacizumab
    b. Cetuximab
    c. Celecoxib
    d. Sirolimus
    e. Bortezomib

3. Herceptin (trastuzumab) is a:
    a. mTOR/FRAP inhibitor
    b. FLT-3 inhibitor
    c. siRNA that targets ATM
    d. Inhibitor of RAS
    e. Anti-HER2 antibody

4. Iressa (gefitinib) is a(n):
    a. Monoclonal antibody against VEGF
    b. Analog of nitrogen mustard
    c. COX-2 inhibitor
    d. EGFR inhibitor
    e. Anti-HER2 antibody

5. Cyclooxygenase (COX)-2:
    a. Tends to be down-regulated in tumors.
    b. Is constitutively produced by most normal tissues.
    c. Inhibits prostaglandin synthesis.
    d. Mediates synthesis of eicosanoids from arachidonic acid.
    e. Is specifically inhibited by erlotinib.

6. Which of the following agents acts in a cell cycle specific fashion?
    a. Cisplatin
    b. Ifosfamide
    c. 5-FU
    d. BCNU
    e. Epirubicin

7. Which of the following drugs is an anti-metabolite?
    a. Melphalan
    b. Gemcitabine
    c. Etoposide
    d. Taxol
    e. Mitomycin

8. Which of the following pairs of chemotherapeutic agents and their mechanism of action is FALSE?
    a. Chlorambucil – DNA alkylator
    b. Gleevec – tyrosine kinase inhibitor
    c. Etoposide – topoisomerase II poison
    d. Doxyrubicin – DNA intercalator
    e. Methotrexate – thymidylate synthase inhibitor

9. Which of the following agents has a mechanism of action similar to that of paclitaxel (Taxol)?
    a. Methotrexate
    b. Camptothecin
    c. Carboplatin
    d. Dactinomycin
    e. Vincristine

10. Cisplatin causes cell lethality due to:
    a. Microtubule depolymerization
    b. Inhibition of thymidylate synthase
    c. Inhibition of ribonucleotide reductase
    d. The formation of cyclobutyl bonds between adjacent bases
    e. Production of DNA crosslinks

11. Bortezomib (Velcade) inhibits the activity of:
    a. Tyrosine kinases
    b. KIT
    c. mTOR (FRAP1)
    d. Proteasomes
    e. VEGF

12. Avastin (bevacizumab) is a monoclonal antibody that targets:
    a. ERBB3
    b. DNA-PK
    c. VEGF
    d. Sphingomyelinase
    e. Caspase 3

13. Enzalutamide is an FDA approved agent for metastatic castration resistant prostate cancer. Clinical trials utilizing this promising agent with radiation therapy are due to commence. What is the mechanism of action of Enzalutamide?
    a. Inhibits 17 α-hydroxylase/C17,20 lyase (CYP17A1), an enzyme which is expressed in testicular, adrenal, and prostatic tumor tissues
    b. Inhibits the ligand for osteoprotegerin and functions as a key factor for osteoclast differentiation and activation
    c. Luteinizing hormone releasing hormone (LHRH) agonist
    d. Bone seeking alpha-particle emitter
    e. Androgen receptor antagonist drug

14. Abiraterone is an FDA approved agent for metastatic castration resistant prostate cancer. Clinical trials utilizing this promising agent with radiation in the localized setting are due to commence. What is the mechanism of action of Abiraterone?
    a. Inhibits 17 α-hydroxylase/C17,20 lyase (CYP17A1), an enzyme which is expressed in testicular, adrenal, and prostatic tumor tissues
    b. Inhibits the ligand for osteoprotegerin and functions as a key factor for osteoclast differentiation and activation
    c. Luteinizing hormone releasing hormone (LHRH) agonist
    d. Bone seeking alpha-particle emitter
    e. Third generation androgen receptor antagonist drug

15. Which of the following targeted agents is an immune checkpoint inhibitor?
    a. Bevacizumab
    b. Imatinib
    c. Crizotinib
    d. Ipilimumab
    e. Cetuximab

16. Rad-223, Sam-153 and Str-89 are radiopharmaceuticals in clinical use for bone metastatic disease. What physical property makes Rad-223 more tolerable than the other two agents?
    a. Alpha particle emitter
    b. Beta and gamma particle emitter
    c. Gamma particle emitter
    d. Alpha, gamma and beta particle emitter
    e. Beta particle emitter

17. Cisplatin is a common chemotherapy agent used to treat a variety of solid-tumor malignancies. A common acute toxicity associated with cisplatin therapy is kidney toxicity. Which of the following statements is false regarding cisplatin induced acute kidney injury?
    a. Cisplatin induces the greatest damage to proximal tubular cells
    b. Serum creatinine increases of ≥ 1.5x baseline is considered acute kidney injury
    c. Cisplatin induces intrastand DNA crosslinks typically between pyrimidine bases
    d. Cisplatin induces superoxide anion formation in the glomerulus and proximal tubules
    e. Acute kidney injury is more commonly associated with cisplatin delivered q3 weeks at 100 mg/m2 compared to weekly at 40 mg/m2

Radiation Modifying Drugs

1. Treatment with an antiangiogenic agent may cause a tumor to exhibit increased sensitivity to a subsequent radiation dose. It has been hypothesized that this reflects the fact that:
    a. Most antiangiogenic agents are also chemical radiosensitizers
    b. Vascular damage decreases tumor perfusion and results in longer retention of the toxic, radiation- induced free radicals
    c. Vascular damage increases hypoxia, which increases expression of HIF-1 in tumor cells, which in turn increases cellular radiosensitivity
    d. Some antiangiogenic agents transiently "normalize" tumor vasculature, resulting in increased oxygenation of the tumor and increased radiosensitivity
    e. Transient normalization of the tumor vasculature can occur after treatment with some antiangiogenic agents, resulting in a more uniform radiation dose delivery

2. Which of the following statements is TRUE concerning the DAHANCA trial testing the effectiveness of nimorazole with radiotherapy for the treatment of supraglottic and pharyngeal tumors?
    a. Nimorazole radiosensitizes through depletion of natural sulfhydryl compounds present in the cell
    b. No significant improvement was noted with respect to either loco-regional tumor control or disease- free survival
    c. Nimorazole has greater radiosensitizing efficiency than other compounds in its chemical class
    d. The toxicity produced by nimorazole was relatively mild.
    e. Due to the negative results, the authors of this trial concluded that nimorazole has no role in the treatment of head and neck cancers

3. A new biological response modifier will be of value in combination with radiotherapy only if it:
    a. Acts synergistically with radiation on the cellular level
    b. Selectively modulates the radiation response of proliferating cells
    c. Selectively modulates the radiation response of the vasculature
    d. Has minimal cytotoxicity to cells in normal tissues
    e. Produces a therapeutic gain

4. Overgaard has published a meta-analysis of clinical trials in which agents such as oxygen and hypoxic cell radiosensitizers were used to address the problem of radioresistant hypoxic cells. He concluded that the overall effect of these hypoxia-directed interventions on tumor control and patient survival was that:
    a. Tumor control remained the same, but survival improved
    b. Tumor control improved, but survival remained the same
    c. Tumor control decreased, but survival improved
    d. Neither tumor control nor survival were affected
    e. There was an improvement in both tumor control and survival

5. One of the mechanisms by which gemcitabine is thought to act as a radiosensitizer is through an effect on:
    a. RAD50
    b. Ribonucleotide reductase
    c. ATM
    d. DNA pol α (POLA1)
    e. DNA topoisomerase II alpha (TOP2A)

6. Which one of the following treatment modifications would NOT be expected to alter the radiation response of normal tissues to fractionated radiotherapy?
    a. Changing the fraction size
    b. Step down in field size
    c. Scheduling a gap
    d. Co-administration of nimorazole
    e. Administration of amifostine

7. Which of the following drugs is used clinically as a radiosensitizer of cancer cells?
    a. Curcumin
    b. Ciprofloxacin
    c. Amifostine
    d. Neomycin
    e. Cisplatin

8. Stereotactic body radiation therapy (SBRT) has proven to be a highly effective treatment for a variety of cancers. Proposals to further optimize SBRT efficacy include the concurrent use of hypoxic cell radiosensitizers. The most correct answer below describes the radiobiologic rationale for the addition of such an agent.
    a. Fractionation of radiation greatly mitigates the protection afforded by tumor hypoxia because of the phenomenon of reoxygenation which could be further augmented with a hypoxic cell radiosensitizer
    b. Tumor hypoxia is a major negative factor in limiting the curability of tumors by SBRT due to loss of the phenomenon of reoxygenation and this negative effect of hypoxia could be overcome by the addition of a hypoxic cell radiosensitizer
    c. Hypoxic cell radiosensitization with SBRT causes acute damage to the endothelial cells of the tumor vasculature
    d. Only a small proportion of tumor cells are clonogenic cancer stem cells and these could be preferentially killed by a hypoxic cell radiosensitizer
    e. Hypoxia cell sensitizers are needed for SBRT to stimulate neovascularization of the tumor between fractions

9. What is the definition of a radiation mitigator?
    a. Agents delivered at the time of irradiation or after irradiation is complete with the intent preventing the manifestation of normal tissue toxicity
    b. Agents delivered prior to irradiation with the intent of preventing or reducing damage to normal tissues
    c. Agents delivered to ameliorate established normal tissue toxicity
    d. Agents delivered at the time of irradiation with the intent preventing the manifestation of normal tissue toxicity
    e. Agents delivered to increase the therapeutic ratio of clinical radiotherapy

10. What is the mechanism of action of the radiation protector/mitigator flagellin that binds to the Toll-like receptor 5?
    a. Activation of the NF-κB pathway
    b. Activation of BCL2 anti-apoptotic pathway
    c. Activation of the EGFR growth factor signaling pathway
    d. Activation of the IL-6 cytokine signaling pathway
    e. Activation of the MnSOD anti-oxidant pathway

11. What is the most correct mechanism by which Hsp90 inhibitors may result in radiosensitization?
    a. Inhibition of the microtubular machinery
    b. Inhibition of DNA damage response and repair pathways
    c. Reassortment of cancer cells into the S-phase of the cell cycle
    d. Inhibition of accelerated tumor repopulation
    e. All of the above

12. What is the most correct mechanism by which the HIV protease inhibitor nelfinavir may result in radiosensitization?
    a. Enhance the effect of ionizing radiation on endothelial cell function
    b. Downregulate VEGF expression in tumor cells via hypoxia-inducible factor 1α
    c. Inhibition of the PI3K-AKT-mTOR pathway
    d. Activation of the unfolded protein response
    e. All of the above

13. Multiple phase III clinical trials have demonstrated a combinatorial beneficial effect of radiation therapy and androgen deprivation therapy (ADT) in localized and locally advanced prostate cancer. What is the most current molecular mechanism for this beneficial combinatorial effect?
    a. ADT represses an androgen receptor gene expression program governing DNA repair and inhibits repair of ionizing radiation–induced DNA damage
    b. ADT downregulates VEGF expression in prostate cancer cells via hypoxia-inducible factor 1α
    c. ADT represses the PI3K-AKT-mTOR pathway
    d. ADT activates the unfolded protein response
    e. All of the above

14. An agent which must be present at the time of irradiation to reduce normal tissue injury is called what?
    a. A radiation sensitizer
    b. A radiation mitigator
    c. A radiation protector
    d. An antifibrotic
    e. A radiation mimetic agent

15. Amifostine selectively radioprotects normal tissue by which mechanism(s)?
    a. Scavenging free radicals
    b. Selective uptake in normal tissues compared to tumor tissues
    c. Reduction of oxygen tension in normal tissues
    d. Stabilizing oxygenated hemoglobin
    e. A and B

16. Palifermnin decreases mucositis after irradiation and stem cell transplant by signaling through which pathway?
    a. Keratinocyte growth factor binding to fibroblast growth factor receptor 2b
    b. Epidermal growth factor (EGF) binding to EGF receptor
    c. Hepatocyte growth factor binding to the MET receptor
    d. TGF-beta binding to TGF-beta receptor
    e. Blocking ATM in mucosal cells

Hyperthermia

1. Which of the following statements concerning methods to produce local tumor heating is FALSE?
    a. Microwaves produce uniform temperature distributions at shallow depths, but treatment of more deeply-seated tumors leads to hotspots on the body surface that can limit treatment
    b. The presence of bone or air cavities during ultrasound heating compromises thermal dosimetry
    c. Uniform temperature distributions may be achieved in soft tissues through use of ultrasound for heating
    d. For readily accessible tumors, the use of implanted microwave or radio-frequency sources results in good temperature distributions
    e. Radiofrequency ablation combined with radiotherapy produces radiosensitization

2. The optimal time to deliver heat (relative to radiation) in order to achieve the greatest radiosensitization is:
    a. Two hours prior to RT
    b. One hour prior to RT
    c. During RT
    d. One hour after RT
    e. Two hours after RT

3. Which of the following statements concerning hyperthermia is TRUE?
    a. There is little or no age response through the cell cycle for hyperthermia
    b. Hyperthermia is thought to enhance the effect of radiation primarily by creating additional DNA damage
    c. Once thermotolerance develops, it becomes a permanent, heritable phenotype in the heated cells
    d. Step-up heating may be useful clinically because it inhibits the development of thermotolerance
    e. The thermal enhancement ratio is the dose of radiation to produce a given effect in cells or tissues irradiated at normal physiologic temperature, divided by the dose of radiation for cells or tissues irradiated at elevated temperature to produce the same effect

4. Which of the following statements concerning hyperthermia is TRUE?
    a. G_2 cells are the most resistant with respect to both heat and X-rays
    b. Cells maintained in a low pH microenvironment tend to be more sensitive to heat than cells maintained at physiologic pH
    c. Acutely hypoxic tumor cells are more sensitive to hyperthermia than chronically hypoxic ones
    d. In laboratory rodents, hyperthermia tends to increase blood flow in tumors, but decrease blood flow in most normal tissues
    e. The amount of killing produced in a population of cells heated at 43ºC for 10 minutes will be greater than for cells heated at 46ºC for 5 minutes

5. Nanoparticle-mediated hyperthermia sensitizes tumors to radiation therapy via:
    a. Decreased perfusion of tumors and resultant increase in necrosis
    b. Vascular disruption and resultant increase in hypoxia
    c. Induction of epithelial-to-mesenchymal transition
    d. Tumor stem cell sensitization
    e. Increased glucose delivery and increased cellular metabolism

Radiation Carcinogenesis

1. Thymic irradiation during infancy has been shown to increase the incidence of:
    a. Breast cancer
    b. Leukemia
    c. Thyroid cancer
    d. Bone tumors
    e. Head and neck cancers

2. Which of the following organs has the highest tissue weighting factor (W_T)?
    a. Breast
    b. Bladder
    c. Brain
    d. Gonads
    e. Kidney

3. What is the most common type of cancer identified in children who were in the vicinity of the Chernobyl nuclear power plant when it exploded in 1986?
    a. Osteosarcoma
    b. Leukemia
    c. Thyroid cancer
    d. Glioma
    e. Mesothelioma

4. In the Childhood Cancer Survivor Study, the incidence of which of the following cancers was NOT elevated in irradiated children compared to those who did not receive radiotherapy as part of their cancer treatment?
    a. Skin cancer
    b. Sarcoma
    c. Meningioma
    d. Pancreatic
    e. Thyroid cancer

5. Which of the following statements is FALSE concerning radiation carcinogenesis?
    a. The use of prenatal X-rays during the 1950's and 1960's increased the risk for the development of childhood cancer among children who received these diagnostic examinations while *in utero*
    b. For radiation protection purposes, it is assumed that the shape of the dose response curve for radiation-induced solid tumors is linear with no threshold
    c. Evidence for radiation-induced leukemia comes from epidemiological studies of children irradiated *in utero* and from the Japanese A-bomb survivors
    d. A radiation oncologist with a lifetime dose equivalent of 250 mSv has about a 10% chance of developing a fatal radiation-induced cancer
    e. In the 1920s the association of radium exposure to development of osteosarcoma was identified in women who worked to detail watch dials with the radioactive paint

6. Approximately how many excess, fatal cancers would be induced by the use of CT scanning if 10 million people receiving this type of radiologic examination got an average effective dose equivalent of 10 mSv?
    a. 25
    b. 150
    c. 800
    d. 5,000
    e. 20,000

7. Which of the following radiation-induced malignancies has the shortest median latent period?
    a. Colorectal cancer
    b. Leukemia
    c. Bone sarcoma
    d. Breast cancer
    e. Lung cancer

8. The EPA estimates that the fraction of the total number of U.S. lung cancer deaths annually caused by indoor radon is approximately:
    a. Zero for non-smokers
    b. 0-0.1%
    c. 1-2%
    d. 10-20%
    e. 40-60%

9. Which one of the following conditions treated with radiation is associated with an increased incidence of leukemia?
    a. Breast cancer
    b. Ankylosing spondylitis
    c. Cervical cancer
    d. Brain tumors
    e. Enlarged thymus

10. The p53 tumor suppressor protein protects against radiation-induced lymphomagenesis by detecting the following radiation-induced lesion.
    a. Radiation-induced single-stranded and double-stranded breaks detected by the DNA damage response machinery
    b. Radiation-induced double-stranded breaks detected by the DNA damage response machinery
    c. Radiation-induced single-stranded detected by the DNA damage response machinery
    d. Sustained mitogenic stimulation detected by the p19ARF machinery from radiation-induced oncogenic mutations
    e. Sustained mitogenic stimulation detected by the p16INK4a machinery from radiation-induced oncogenic mutations

11. Which of the following statements on radiation induced carcinogenesis is correct?
    a. Internally deposited radioactive materials confer equal cancer risk to all organs
    b. External and whole body exposure to radiation confers the equal cancer risk to all organs
    c. Cancer is a stochastic effect of radiation because its severity is not determined by the radiation dose, but the probability to occur is
    d. Cancer is a deterministic (or non-stochastic) effect of radiation because its severity and the probability to occur are determined by the radiation dose
    e. Cancer is a stochastic effect of radiation because its severity and the probability to occur are determined by the radiation dose

12. Patients who are treated for breast cancer are at higher risk than the normal population for developing second malignancies. Approximately what proportion of secondary malignancies in breast cancer survivors are attributable to radiation therapy?
    a. 5%
    b. 20%
    c. 30%
    d. 50%

Heritable Effects

1. The probability of a hereditary disorder in the first generation born to parents exposed to radiation is estimated to be approximately:
    a. 0.02/mSv
    b. 0.2/mSv
    c. 0.002/Sv
    d. 0.02/Sv
    e. 0.2/Sv

2. The genetically significant dose (GSD) resulting from diagnostic radiology procedures performed in the U.S. annually has been estimated to be:
    a. 0.03 mSv
    b. 0.3 mSv
    c. 3 mSv
    d. 30 mSv
    e. 3 Sv

3. A 22-year-old man completed a course of radiation therapy for Hodgkin's lymphoma one year ago. For the previous 6 months, he and his wife tried unsuccessfully to conceive a child. He expressed concern to his radiation oncologist that the radiation exposure (gonadal dose of 0.83 Gy) may have left him sterile. How should the radiation oncologist respond?
    a. The radiation dose likely caused permanent sterility
    b. The dose of radiation should have had no effect on the patient's sperm count and probably isn't the cause of the couple's fertility problems
    c. The patient should not even be attempting to conceive a child due to a significantly increased risk for radiation-induced mutations in the offspring of irradiated individuals
    d. Hormonal dysfunction caused by the radiation, and not lowered sperm count *per se*, probably accounted for the couple's fertility problems
    e. This dose should interfere with fertility for no more than about a year, so the patient should keep trying to conceive a child

Embryonic Effects

1. Midway through the course of a standard external beam treatment for breast cancer, the patient discovered she was pregnant and near the end of her first trimester. Which of the following statements about this situation is TRUE?
    a. The woman should be advised to discontinue treatment until she gives birth
    b. The fetus is quite resistant to radiation during this gestational stage, so there is no need to discuss options with the patient
    c. The scattered dose already delivered to the fetus is sufficiently high that a miscarriage or stillbirth is probable
    d. The fetus will be at an increased risk for the development of a radiation-induced cancer later in life, even if the scattered dose is relatively small
    e. The fetus probably received less than 0.01 cGy, so no remedial action is necessary

2. The thyroid of a developing fetus will incorporate radioactive iodine:
    a. At no point during gestation
    b. At any point during gestation
    c. From about the 10th week of gestation onward
    d. Only during the first trimester of gestation
    e. Only during the third trimester of gestation

3. A young woman is concerned about ovarian irradiation secondary to a screening mammogram she had received with respect to possible deleterious effects on her future offspring. The radiologist should inform her that:
    a. Transient changes in hormonal balance will likely result from the ovarian dose received during mammography, but these should not affect future offspring
    b. Mature ova are highly radiosensitive and those present at the time of irradiation were probably killed, so future offspring cannot be affected
    c. Her ovaries received no scattered dose from screening mammography
    d. Her ovaries received the equivalent of a genetic doubling dose for mutations
    e. Effects on possible future offspring cannot be excluded but are highly unlikely

4. Temporary growth inhibition would most likely be observed for a developing mouse irradiated during which stage of gestation?
    a. Preimplantation
    b. Organogenesis
    c. Early fetal period
    d. Mid fetal period
    e. Late fetal period

5. Many types of congenital abnormalities, and of varying severity, have been noted in laboratory animals irradiated during the organogenesis period of gestation. This wide spectrum of effects is due primarily to:
    a. The sex of the irradiated fetus
    b. Which organs were actively developing at the time of irradiation
    c. The type of ionizing radiation to which the fetus was exposed
    d. Innate differences in radiosensitivity of the different cell types
    e. Maternal age at conception

6. What dose to an embryo or fetus during the 10 day to 25 week period of gestation is considered the threshold above which a physician should discuss with a pregnant patient the risk of radiation-induced birth defects, and possible actions to be taken?
    a. 0.001 Gy
    b. 0.01 Gy
    c. 0.1 Gy
    d. 1.0 Gy
    e. 10 Gy

Radiation Protection

1. Which of the following statements is FALSE concerning exposure to radiation?
    a. The largest contributor to background radiation exposure in the United States is radon
    b. The average annual dose equivalent resulting from the combined exposure to cosmic, terrestrial, and internal background radiation is roughly 1 mSv
    c. The effective dose equals the equivalent dose only under conditions where the whole body is irradiated
    d. Radiation exposure from medical diagnostic tests constitutes about 2% of the average total radiation dose residents of the United States receive each year
    e. Background radiation exposure increases with increasing altitude at which an individual resides

2. The ratio of the human genetic doubling dose to the average annual genetically significant dose (GSD) resulting from diagnostic X-ray procedures performed in the U.S. is closest to:
    a. 1
    b. 20
    c. 3,000
    d. 100,000
    e. 600,000

3. It is important for radiologists to use medical X-rays judiciously and avoid ordering unnecessary tests for all of the following reasons, EXCEPT:
    a. Radiation-induced cancers caused by diagnostic X-ray procedures are thought to account for at least 1% of all cancer deaths each year
    b. There is no dose of radiation that can be considered “safe”
    c. According to Medicare regulations, an order for a diagnostic X-ray examination may be based not only upon medical need, but also for the purpose of limiting legal liability
    d. The use of X-rays for medical diagnosis has been increasing
    e. Diagnostic X-rays are the greatest source of man-made background radiation exposure in the human population

4. The maximum permissible dose per year for a member of the general population includes dose contributions received from:
    a. Storage of radioactive waste material
    b. Radioactive elements in the earth’s crust
    c. A course of radiotherapy
    d. Exposure to radon
    e. Mammography

5. Which one of the following effects that may be caused by irradiation, represents a possible deterministic effect?
    a. Breast cancer
    b. Phenylketonuria
    c. Mental retardation
    d. Leukemia
    e. Galactosemia

6. The term stochastic is used to describe an effect of radiation in which the:
    a. Severity of the effect depends on the dose above a threshold
    b. Severity of the effect depends on the dose without a threshold
    c. Probability of occurrence is a function of dose, with no threshold
    d. Probability of occurrence is a function of dose above a threshold
    e. Dependency is on age at exposure

Molecular Techniques

1. Which of the following statements concerning molecular techniques is FALSE?
    a. Fluorescence *in situ* hybridization (FISH) can be used to identify the chromosome location of a gene of interest
    b. A restriction fragment length polymorphism (RFLP) may result if the copy number of a particular DNA fragment varies
    c. An exonuclease produces a cut in the middle of a DNA strand
    d. A Western blot can be used to detect and characterize a particular protein
    e. A restriction endonuclease typically cuts DNA at a specific sequence

2. Which one of the following reagents is NOT used for a reporter gene assay?
    a. Chloramphenicol acetyltransferase (CAT)
    b. Firefly luciferase
    c. RNA polymerase
    d. β-galactosidase
    e. Green fluorescent protein (GFP)

3. An antibody would be used to screen which type of library?
    a. Genomic
    b. Expression
    c. cDNA
    d. Intronic
    e. Endonuclease

4. The sequence of temperatures (in °C) used in a round of PCR to amplify a particular DNA fragment would most likely be:
    a. 95, 72, 57
    b. 57, 95, 72
    c. 72, 57, 95
    d. 95, 57, 72
    e. 72, 95, 57

5. Which of the following assays would NOT be used for the detection of single nucleotide polymorphisms (SNPs)?
    a. TaqMan assay
    b. Subtractive hybridization
    c. Single-stranded conformation polymorphism (SSCP)
    d. Invader assay
    e. Molecular beacons

6. Which of the following statements is TRUE concerning the structure of eukaryotic genes?
    a. An exon can generally be identified by its lack of stop codons
    b. Introns represent only a small percentage of the total genome
    c. Most human genes do not contain intronic regions
    d. Introns represent the coding sequences of genes
    e. The RNA transcribed from a DNA template is translated directly on the ribosomes

7. A DNA ligase:
    a. Performs the resynthesis step of nucleotide excision repair
    b. Is responsible for the initial step in non-homologous end joining of DNA double strand breaks
    c. Recognizes a particular type of DNA damage and produces single strand breaks on either side of the damaged nucleotide
    d. Recognizes and removes a damaged base from DNA
    e. Rejoins simple strand breaks

8. Which technique would best be used to investigate gene expression?
    a. Western blot
    b. EMSA
    c. Southern blot
    d. DNase I footprinting
    e. Northern blot

9. The best method to locate a gene on a chromosome is:
    a. Promoter bashing
    b. ELISA
    c. Two-hybrid screen
    d. FISH
    e. RFLP

10. Which of the following statements is TRUE?
    a. A mature mRNA contains the information present only in the DNA introns
    b. Sequencing of a cDNA can be used to predict the amino acid sequence of the protein encoded by the gene
    c. A functional complementation assay involves hybridization of a probe to its complementary sequence in genomic DNA
    d. A cDNA library is created using whole genomic DNA
    e. A unique oligonucleotide probe for a particular gene can be ”backwards engineered” from the amino acid sequence of the protein encoded by that gene

11. Which of the following techniques can be used to directly evaluate the induction and repair of DNA double strand breaks?
    a. Northern blotting
    b. Neutral comet assay
    c. Alkaline comet assay
    d. Polymerase chain reaction
    e. Western blotting

12. How does single-cell RNA-seq differ from ChIP-seq?
    a. scRNA-seq provides gene expression data from individual cells whereas ChIP-seq tests for protein-DNA interactions.
    b. Both techniques measure the amount of RNA but only single-cell RNA seq can detect mitochondrial RNA.
    c. Both techniques measure the RNA transcripts, but only ChIP-seq is performed on a micro-chip.
    d. Only ChIP-seq has reverse transcription, amplification, library generation and sequencing as part of the work-flow.

13. What does TCR sequencing measure?

    a. TCR-seq identifies the entire DNA sequence of the TCR on each T cell in a sample.
    b. It identifies and quantifies the variable and constant region of each TCR.
    c. The output of TCR-seq is the sequence and abundance of the Tim-3 complex receptors in a lymphocyte sample and an estimate its diversity.
    d. TCR-seq determines the sequence and abundance of all T cell receptors in a sample and can be used to describe an entire T cell repertoire.
    e. It sequences and quantifies the expression of the T cell receptor mRNA after VDJ gene splicing.

14. What best describes the use of whole exome sequencing (WES) versus genome-wide association (GWAS) studies?

    a. GWAS is based on sequencing the entire genome while WES looks at the non-coding regions of genes.
    b. WES quantifies and sequences mRNAs levels post splicing while GWAS captures disease-specific mRNA prior to splicing.
    c. WES forms a part of GWAS. WES determines the mutations in coding regions of genes which then get aligned with GWAS data on the intron mutations.
    d. WES is a next-generation sequencing test that looks at the coding regions of genes while GWAS is used to identify genetic loci with phenotypic traits.

15. Which statement is true for CyTOF?
    a. CyTOF stands for Cytometry of fluorescence and is a techniques that allows the staining of cells with antibodies similar to flow cytometry only with the added capability of measuring mRNA levels on a per cell basis.
    b. CyTOF is a mass spectrometry technique that is based on staining cells with metal-conjugated antibodies with fewer limitation of spectral overlap than flow cytometry.
    c. CyTOF uses antibodies-coupled with radioisotopes to measure protein levels in and on cells.
    d. CyTOF is a gas chromatography technique that analyses antibody-stained cells on a single-cell basis and is much more powerful than flow cytometry.

16. Which of the following is a gene editing technology?
    a. Luciferase
    b. CRISPR
    c. GWS
    d. RT-PCR
    e. ChIP

17. What does a promoter-bashing assay accomplish?
    a. Identifies regions of genes that are required for gene expression.
    b. Identifies proteins that bind to DNA
    c. Measures DNA double-strand breaks
    d. Identifies miRNA binding regions in mRNA
    e. Quantitates gene expression

18. Which of the following are needed to carry out a successfully CRISPR modification on a target sequence?
    a. gRNA, cas9 nuclease
    b. miRNA, caspase 9
    c. gDNA, repetitive palindromic DNA
    d. RNA primer, telomerase
    e. snRNA, DNase A

19. The CRISPR/Cas9 system is a recent advancement that has revolutionized basic research and has promising potential therapeutic implications. The most proximate host cellular effect immediately downstream of Cas9 protein activity is:
    a. Induction of apoptosis
    b. Activation of the immune response
    c. Suppression of protein expression
    d. Activation of the dsDNA damage response
    e. Suppressing protein activity

20. The first application of the CRISPR/Cas9 system applied to human cells was to induce gene knockout. When the CRISPR/Cas9 system is used for this purpose, it does so by:
    a. Inducing small insertions, deletions, and frameshifts
    b. Excising the target gene from the genome
    c. Preventing RNA synthesis by DNA-dependent RNA polymerase
    d. Inducing the degradation of target mRNA
    e. Inducing target protein degradation

21. Single nucleotide polymorphisms (SNPs) are used in cancer research primarily because:
    a. Alterations in protein sequence always arise from SNPs.
    b. They can be associated with DNA repair defects.
    c. They can be associated with specific clinical outcomes.
    d. They are causal in specific clinical outcomes.
    e. They are due to specific mutational processes.
    f. All of the above.

Molecular Imaging

1. The most commonly used biologically active molecule for PET scanning is a fluoridinated analog of which of the following:
    a. Phosphate
    b. Glucose
    c. Calcium
    d. Albumin
    e. Sphingomyelin

2. The following nucleoside has been radiolabeled in an effort to image DNA synthesis with PET:
    a. Adenosine
    b. Guanosine
    c. Thymidine
    d. Uridine
    e. Cytidine

3. The prostate-specific membrane antigen (PSMA) is a promising, well-characterized biomarker of prostate cancer. Attempts to image PSMA by SPECT using the agent 111In-Capromab Pendetide (ProstaScint™) is approved by the FDA. Which statement best describes the limitations of 111In-Capromab Pendetide?
    a. Limitations of antibody-mediated imaging of quick blood-pool clearance
    b. Rapid tumor penetration
    c. The 7E11-C5.3 antibody on which 111In-Capromab Pendetide is based binds to an intracellular epitope of PSMA
    d. Poor specificity with high-volume disease
    e. Inherent limitations of PET imaging

4. The prostate-specific membrane antigen (PSMA) is a promising, well-characterized biomarker of prostate cancer. Newer agents such as N-[N-[(S)-1,3-dicarboxypropyl]carbamoyl]-4-fluorobenzyl-L-cysteine (DCFBC) is a low-molecular-weight, urea-based inhibitor of PSMA. What statement best describes the principles that make 18F-DCFBC a better molecular tracer than 111In-Capromab Pendetide (ProstaScint™)?
    a. Slower tumor penetration and blood-pool clearance
    b. Faster tumor penetration and slower blood-pool clearance
    c. Higher sensitivity with high-volume disease
    d. Better specificity with high-volume disease
    e. Inherent limitations of SPECT versus PET imaging

5. Which of the following statements in TRUE about targeted radiotherapy strategies?
    a. Targeted radiotherapies can involve the use of both a diagnostic nuclide coupled with therapy nuclides which represent a new class of theragnostic approaches.
    b. Peptide receptors that are differentially expressed on cancer versus normal cells represent easily exploitable targets for theragnostic approaches.
    c. Somatostatin receptors represent a class of targets amenable to theragnostic approaches.
    d. Targeted radiotherapy approaches are FDA approved for selective tumor types.
    e. Targeted radiotherapies can’t be used for bone metastasis.

ANSWER KEY

Radiation-Matter Interactions

1. A Portions of the electromagnetic spectrum show the following order with increasing photon energy: radio waves, microwaves, infrared radiation, visible light, UV and X-rays. This corresponds to decreasing wavelength and increasing frequency. Only x-ray and γ-ray photons have sufficient energy to cause ionization event.

2. C For photons in the energy range used typically in radiotherapy, the Compton process is predominant. In the Compton process, a high-energy photon interacts with an atom to cause ejection of an outer shell electron (referred to as a recoil electron) and a scattered photon. The energy of the incident photon is distributed between the scattered photon and the kinetic energy of the recoil electron. The Compton interaction may occur when photon energies range from 150 keV to 3 MeV although it also occurs to some extent at lower energies of 100-150 keV. Pair production occurs when a photon of greater than 1.02 MeV interacts with a nucleus to form an electron-positron pair. This amount of energy is just sufficient to provide the rest mass of the electron and positron, 0.51 MeV each. Excess of energy above 1.02 MeV will be possessed by these two particles, which produce ionizations as they travel in the material. As the positron comes to rest, it interacts with an electron in an annihilation reaction and is replaced by two photons, each having an energy of 0.51 MeV and moving in opposite directions. Pair production becomes an important form of interaction above about 10 MeV. The photoelectric effect is predominant for photons that have energies less than approximately 100-150 keV, typical of X-rays used in diagnostic radiology. In the photoelectric process, a photon interacts with an inner orbital electron and is completely absorbed. The electron is ejected from the atom becoming a free photoelectron. The kinetic energy of the ejected electron is equal to the energy of the incident photon minus the binding energy of the electron that has been ejected. The vacancy left in the shell by the ejected electron is filled in by the transition of an electron from an outer shell and is accompanied by the emission of a characteristic X-ray, whose energy represents the difference in the energy levels of the shells involved in the electron transition. When the excess energy derived from the transition of the electron from the higher to the lower energy state is transferred to an orbital electron that is ejected, this is referred to as an Auger electron. Photodisintegration occurs at photon energies much higher than those used in either diagnostic radiology or radiation therapy. In this process, a high-energy photon interacts with the nucleus of an atom resulting in the emission of one or more nucleons. An electron is not ejected through coherent scattering and no energy is transferred in this type of interaction, only the direction of the incident photon is altered.

3. E A free radical is an atom or molecule with an unpaired electron, regardless of its charge status, making it highly reactive with other atoms and molecules. Spallation products are the result of nuclear fragmentation; for example, when high energy particles, such as neutrons, strike a target nucleus. Nuclear reaction products include nuclear fragments called spallation products in addition to nucleons (protons and neutrons) and alpha particles. Conventionally, ionized atoms with an atomic number less than or equal to 10 are called light ions, whereas those with an atomic number greater than 10 are termed heavy ions. In the case of water radiolysis produced from an X-ray interaction, an electron is produced in addition to a positively charged water ion radical. This is referred to as an ion pair. For neutrons with energies less than 6 MeV, the main type of interaction is elastic scattering, which in soft tissue involves interaction of the neutron with a hydrogen nucleus causing the formation of a recoil proton that goes on to cause ionizations.

4. D The positron formed through pair production combines with an electron on a separate atom to form two photons, each with energy of 0.511 MeV and moving in exactly opposite directions. This process is termed the annihilation reaction. 1 MeV γ-rays and mono-energetic 1 MeV X-rays are identical as they are both photons with an energy of 1 MeV and will therefore have the same relative biological effectiveness (RBE). The photoelectric effect results in the production of characteristic X-rays. All forms of electromagnetic radiation travel at 3×10^8 m/sec, the speed of light. The probability of a photoelectric interaction is proportional to the atomic number, Z^3. The wavelength is inversely proportional to photon energy.

5. E There is complete absorption of the incident photon during the photoelectric process. Although γ-rays, which represent energy released from the nucleus of an atom, are produced during nuclear disintegration, X-ras are produced from physical processes that occur outside of the nucleus. Auger

electrons may beproduced through the photoelectric effect, not pair production. Free radicals have half-lives on the order of micro- to milliseconds. Free radicals do not necessarily possess charge. An atom with an unpaired electron that is charged is referred to as an ion radical.

6. B A positron has a mass approximately 1,840 times smaller than either a neutron or proton. An α-particle is the nucleus of a helium atom and therefore consists of 2 protons and 2 neutrons. A carbon ion is the nucleus of a carbon atom and therefore consists of 6 protons and 6 neutrons.

7. B The annihilation reaction involves an interaction between a positron and an electron to produce two photons, each with energy of 0.511 MeV and moving in exactly opposite directions. Photon energies in the range that would result in the photoelectric effect are suboptimal for radiotherapy since there would be undesirable, preferential absorption by bone, which contains a disproportionate amount of higher atomic number elements (such as calcium and phosphorus) than soft tissues. This is because, unlike the Compton process, the probability of a photoelectric interaction is proportional to the third power of the atomic number of the absorber. In addition, the relatively low photon energies associated with the photoelectric effect result in poor penetration through tissue and therefore result in large doses to skin and superficial tissues. All forms of the electromagnetic radiation spectrum (i.e. radio waves, infrared radiation, visible light, ultraviolet light, X-rays, etc.) travel at 3×10^8 m/sec in a vacuum. The different types of electromagnetic radiation are categorized not by their speed, but by their frequency or wavelength. The Auger effect is seen as the result of the movement of an electron from an atom's outer shell to a vacant more tightly bound, inner orbital. An Auger electron may be produced through the photoelectric effect because the excess energy that results when an electron moves to a lower energy state during replacement of the ejected photoelectron may cause ejection of a second electron, which is referred to as an Auger electron.

8. B To detach an electron, it is necessary to supply a quantity of energy of the order of 10 eV. Energy losses in water (soft tissue) via ionization range from 10 to 100 eV with a peak at 25 eV. The outmost electron can be moved to one of more external orbits, normally empty, by supplying smaller amounts of energy such as 5eV; this process is called an atomic excitation. Absorption of 5 eV is sufficient to break intramolecular binding; e.g., 4.9 eV for the C=C and 5.2 eV for H—OH (the chemical energy)

9. C The energy of a photon can be calculated as: E (keV) = 1.24/ λ (nm), or E (eV) = 1240/λ (nm), where λ is the wavelength of the photon (in nm), and E is the energy of the photon. The minimum orbit electron binding energy for existing elements on earth is about 12.5 (or 10-25ev). For examples, hydrogen electron has a binding energy of ~13.6 eV, and oxygen of about 41.6 eV. Therefore, for any photon with a wavelength of longer than 100nm, its energy will be less than 12.4eV, which is not enough to produce any free electron from existing element on earth. It is true that photon does not have charges or mass, but it can produce free electrons when there is enough energy to prompt a photoelectric effect, Compton scattering, or electron pair-production.

10. A Photoelectric effect is most efficient when the photon energy is close to the binding energy of an orbital electron. The photon energy needs to be more than 1.022 MeV to be able to produce electron pair. During a Compton scattering event, the entering photon does not vanish, but exit with a reduced energy and likely an altered direction. Annihilation radiation refers to the new photon produced by the interaction between a positively charge electron (a positron or an antielectron) with a negatively charged electron.

11. C For charged particle, they tend to loss their energy near the end of the track. When the amount of energy deposited to the absorbing material (or the energy lost by the particle) is plotted again the distance of the particle traveled, it will result in a peak at the end of the track on the plot, which is also called Bragg peak. Alpha particles are positively charged and most heavy ions), and will have a similar pattern to a proton beam.

12. E Pair production involves the interaction between a photon and the atomic nucleus. The high-energy electron and positron are created as a result; this is an example of energy-to-mass conversion according to $E=mc^2$ where m = 0.51 MeV, the resting mass of electron or positron. The amount of energy carried away by the two particles equals the incident photon energy *minus* two resting masses of electron. For 10 MV photon, the total kinetic energy of the two particles is 10 MeV – 2x0.51 MeV = 8.98 MeV. Both high-energy electrons and positrons are ionizing particles. The positron has small probability of being annihilated until it

slows down. The slowly moving positron will combine with electrons (which are abundant in matter) to produce two 0.51-MeV photons moving in opposite directions from the scene of the annihilation. These two photons will produce ionizations *via* Compton and/or photoelectric interactions as they move through matter. The probability of pair process rapidly increases with photon energy above the threshold (1.02 MeV), and with atomic number of the absorber (tissue).

13. B The interaction of ionized water molecule with another water molecule to produce new species of free radicals is considered the indirect effect. The newly produced electron with high energy and the remaining photon can be considered secondary radiation and their interactions with the absorbing materials are considered part of the physical interactions with matters, or direct effect. However, when the high-energy free electron slows down to form an aqueous electron, it can interact with other water molecules to trigger indirect effects.

14. C This is a classical experiment that demonstrates the indirect effect of ionizing radiation. Due to the presence of water, it further reacts with the ionized molecules and propagates the effect of ionizing radiation. The presence of free radical scavengers may reduce the water mediated free radical reactions to reduce the effect of ionizing radiation.

DNA Damage Mechanisms

1. C Ultraviolet radiation is non-ionizing but its wavelengths are preferentially absorbed by bases of DNA and by aromatic amino acids of proteins. The major types of DNA damage produced in cells by exposure to UV radiation include cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts. In both cases, two pyrimidines, located next to each other, react to form a dimer following excitation of atoms in DNA. DNA-protein crosslinks are also important lesions in cells exposed to UV radiation. Crosslinks are particularly disruptive, as they occur mostly in the area of the chromosome that is undergoing replication. Thymol glycol [5,6-dihydroxy-5,6-dihydrothymine] and oxidized guanine [8-oxo-7,8-dihydroguanine (8-oxo-G)] are DNA base lesions present in clustered DNA damage induced in cells by ionizing radiation. Heat is a form of energy associated with the motion of atoms and molecules.

2. B The type of radiation-induced DNA damage most implicated in cell killing is the double-strand break.

3. C 6-4 photoproducts are produced by UV and not ionizing radiation.

4. E The absence of RAD51, which is a recombinase that plays a role in homologous recombinational repair, may affect the repairability of DNA double-strand breaks, but not their initial yield. The number of double-strand breaks produced increases with radiation dose. A lack of oxygen will decrease the number of initial breaks because the free radicals formed through interactions with oxygen that may result in the formation of DNA double-strand breaks will not be created if oxygen is at a diminished level. In tissues, amifostine is converted to a compound that is a radical scavenger whose presence would decrease the number of breaks induced by a particular dose of radiation. Nuclear proteins play a critical role in protecting DNA from radiation damage. Thus removal of histones greatly enhances the sensitivity of mammalian cells to radiation damage.

5. B Non-targeted, radiation-induced bystander effects are effects that appear in unirradiated cells in the presence of irradiated cells. Pyrimidine dimers are produced in DNA by UV exposure. There is no evidence that the product of the antiapoptotic gene, BCL2, is involved in bystander responses. The average heat input from the absorption of ionizing radiation is very small. For example, the temperature rise in the tissues irradiated with 5 Gy is only about $0.001^{o}C$.

6. C Base damage is greater than single strand breaks is greater than double strand breaks in frequency following exposure to ionizing radiation. This damage can be repaired in some cases if sufficient time is allowed between doses. DNA damage does not always result in cell death. T-T dimers occur following UV, not ionizing radiation.

7. B The introduction of the isotope into the nucleus will be most lethal if the same amount of isotope is used in all labelings. This is because DNA is the most sensitive target for radiation induced cell killing. This is one of the classical experiments that demonstrated the critical target of radiation is in the nucleus.

DNA Repair Mechanisms

1. B DNA-PK is involved with non-homologous end-joining, not homologous recombination. It has a PI3K domain and KO of the gene in mice causes the induction of a SCID-like syndrome that includes radiosensitivity and immunodeficiency.

2. D CDK4 is a cyclin dependent kinase that plays an important role in the progression of cells through G_1 and into S phase. Artemis and DNA-PKcs play important roles in non-homologous end-joining of DNA double strand breaks, whereas RAD51 and BRCA1 are involved in the repair of double strand breaks through homologous recombination.

3. E SCID mice are immune deficient, making them good hosts for growing xenografts of human tumors. SCID mice are deficient in DNA-PK and are therefore radiosensitive. Cells from these mice have low levels of non-homologous end-joining. Nevertheless, it is their lack of an immune system that permits the human tumor cells to survive in a mouse without being rejected.

4. A People with xeroderma pigmentosum are deficient in one of the several proteins involved in nucleotide excision repair. They are therefore extremely sensitive to UV irradiation because they are unable to repair the pyrimidine dimers produced in DNA, but they are not sensitive to ionizing radiation.

5. D Homologous recombinational repair requires the presence of a homologous DNA template, and is therefore most likely to occur following DNA replication in late S phase (when a sister chromatid is available as a template) or G2 phases of the cell cycle.

6. B A deficiency in MRE11 (which makes up the MRE11-RAD50-NBS1 complex) results in an ataxia telangiectasia-like disorder. The protein plays a role in both homologous recombination and NHEJ.
    a. Taylor AM, Groom A, and Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis, DNA Repair 3:1219-1225, 2004. PubMed link

7. B The BLM protein (deficient in people with Bloom Syndrome) is a RecQ helicase that works in the 3' to 5' direction. RPA (Replication Protein A) serves to coat single stranded DNA regions generated during homologous recombination, DNA replication, and other processes to prevent their degradation.
    a. Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis, Nature Reviews Molecular Cell Biology, 11:196-207, 2010.
    b. Branzei D, Foiani M. Maintaining genome stability at the replication fork, Nature Reviews Molecular Cell Biology, 11:208-219, 2010. PubMed link
    c. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability — an evolving hallmark of cancer, Nature Reviews Molecular Cell Biology 11:220-228, 2010. PubMed link
    d. Powell SN, Kachnic LA. Therapeutic exploitation of tumor cell defects in homologous recombination, Anticancer Agents Med Chem 8:448-460, 2008. PubMed link
    e. Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. Cell Res 18:99-113, 2008. PubMed link
    f. O'Driscoll M, Jeggo PA. The Role of Double-Strand Break Repair - Insights from Human Genetics. Nat Rev Genet 7:45-54, 2006. PubMed link

8. A The main role for Artemis is to cleave (through its nuclease activity) any residual DNA loops or hairpins that form during non-homologous end-joining. It prepares the ends for ligation by ligase.
    a. Lieber MR. The mechanism of human nonhomologous DNA end joining, J Biol Chem 283:1-5, 2008. PubMed link
    b. Weterings E, Chen DJ. The endless tale of non-homologous end-joining. Cell Res 18:114-124, 2008. PubMed link
    c. Collis SJ, DeWeese TL, Jeggo PA, *et al.* The Life and Death of DNA-PK. Oncogene 24:949-961, 2005. PubMed link

d. Jeggo PA , Lobrich M. Artemis Links ATM to Double Strand Break Rejoining. Cell Cycle 4:359-362, 2005. PubMed link

9. A All of the proteins listed are substrates for ATM except for Ku70/80.

10. D The most common alterations produced in the DNA by radiation are base damages, which are repaired by base excision repair, a repair process that is usually rapid and accurate. The proportion of the population that is heterozygous for the types of mutations that are found in people with AT – typically protein truncation mutations – is roughly 1-2%. Non-homologous end joining does not require a sister chromatid. Mutation of the genes involved with mismatch repair, primarily MSH2 and MLH1, are often present in people who develop hereditary non-polyposis colon cancer. Homologous recombination is a relatively error-free process. Sublethal damage repair is nearly non-existent following neutron-irradiation.

11. A Radiation sensitivity is greatest in a person with ataxia telangiectasia. People with this syndrome are very sensitive to ionizing radiation due to the absence of functional ATM protein, which plays a central role in the repair of DNA double strand breaks and regulation of the cell cycle following irradiation.

12. B Non-homologous end-joining represents the principal means by which human cells repair DNA double strand breaks. Mismatch repair is primarily responsible for correction of errors made during DNA replication. Base excision repair removes base damages. Nucleotide excision repair mainly removes bulky adducts from DNA such as UV-induced pyrimidine dimers and chemical adducts. Photoreactivation involves the action of DNA photolyase which is activated by long wavelength UV and visible light to split the cyclobutyl bond of a pyrimidine dimer restoring it back to its original state.
    a. Huen, MS, Sy SM, Chen J. BRCA1 and its toolbox for the maintenance of genome integrity, Nat Rev Mol Cell Bio, 11:138-148, 2010. PubMed link
    b. Caldecott KW. Single-strand break repair and genetic disease. Nat Rev Genet,9:619-631, 2008. PubMed link
    c. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy, Nat Rev Cancer, 8:193-204, 2008. PubMed link
    d. Bonner WM, Redon CE, Dickey JS, *et al*. GammaH2AX and cancer, Nat Rev Cancer, 8:957-967, 2008. PubMed link
    e. Pollard JM, Gatti RA. Clinical radiation sensitivity with DNA repair disorders: An overview, Int J Radiat Oncol Biol Phys, 74:1323-1331, 2009. PubMed link
    f. 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 link
    g. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle, Nat Rev Mol Cell Biol, 9:297-308, 2008. PubMed link
    h. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy, Nat Rev Cancer, 8:193-204, 2008. PubMed link
    i. Wang W. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins, Nat Rev Genet, 8:735-748, 2007. PubMed link

13. D RAD51 and BRCA2 function together in homologous recombinational repair of DNA double strand breaks.

14. C γH2AX, the phosphorylated histone variant H2AX, localizes to sites of DNA double strand breaks rapidly after damage and acts as a docking site for other DNA repair proteins. PubMed link

15. A KU70 binding is involved in the initial recognition of a DSB and is one of the first steps of NHEJ repair, followed by DNA-PKcs activation and binding to the ends. LIG4 is involved in sealing the ends by ligation in the NHEJ repair process. ARTEMIS has exonuclease activity and is also involved in NHEJ repair. BRCA2 is an HR protein and may have some NHEJ functions.

16. B One of the first events that occurs at the site of an induced DSB is phosphorylation of the histone variant, H2AX at serine 139. ATM is the primary kinase, which phosphorylates H2AX, although ATR and DNA-PKcs also perform this action. This leads eventually to BRCA1 recruitment which likely is followed by CtIP binding and resection. NHEJ repair is initiated by DNA-PKcs, but likely occurs after H2AX phosphorylation.

17. D DSBs are considered the most serious form of DNA damage, since they eventually lead to cell death if not properly repaired. Thus, complex systems have evolved to rapidly detect and repair these lesions. HR and NHEJ represent the two major pathways by which DSBs are repaired, and cells derived from patients with DSB repair gene mutations are profoundly radiosensitive. While HR utilizes homologous DNA sequences as a template for repair, NHEJ simply processes and re-ligates the exposed DNA termini of DSBs. The NHEJ pathway is considered more error prone than HR and occurs more frequently in cells. NHEJ is the predominant pathway in the G0/G1-phases of the cell cycle, while HR increases during S/G2, when a sister chromatid becomes available as a template for repair. PubMed link

18. B The NHEJ pathway is considered more error prone than HR and occurs more frequently in cells. NHEJ is the predominant pathway in the G0/G1-phases of the cell cycle, when a sister chromatid is not available for HR repair. NHEJ is active other parts of the cell cycle, but its highest activity appears to be in G1. Recent studies suggest it may also be the predominant pathway in the G2/M-phases of the cell cycle after compaction of the chromatin, but further studies are needed to clarify this. PubMed link

19. B In mammalian cells, NHEJ defects confer a higher sensitivity to radiation than HR. It contributes to sensitivity to both proliferative and quiescent cells.

20. B Excessive level of γH2AX is an indication of weak DSB repair. The initial activation of ATM and γH2AX formation are normal cell responses to irradiation. Radiation induced G1 cell cycle arrest usually last for a few hours in human cells, thus the arrest in G1 after 4 hours after irradiation is considered a normal DNA damage response.

21. C PARP plays a major role as one of the first components of the SSBR pathway (which is often considered a part of the BER pathway). The other statements are not true: glycosylation is usually reserved for membrane proteins; PARP does not play a significant role in DSB repair; necrosis is a form of unscheduled cell death where there is no program and no dependence on any proteins; PARP is not phosphorylated.

22. A The energy (E in eV) of a photon can be calculated as: E (eV) = 1240/λ (nm), where λ is the wavelength (nm) of the photon. The minimum energy required to cause an ionizing event in any atoms is about 10-12.5 eV. Thus, the photon wavelength needs to be less than ~100nm to be able to offer sufficient energy to cause an ionization event. Photons with wavelength longer than 100nm should have an energy of less, not more, than 12.5 eV. However, much less amount of photon energy is needed to cause a bond shift. For example, it would only need ~4.9eV to break up a C=C bond between two atoms.

23. C Neutral comic assay can only detect Double strand DNA, thus reflect the only double strand DNA breaks. Alkaline comic assay can detect both double and single stranded DNA, thus theoretically can be used to measure single strand breaks as well as double strand break.

24. D A single exposure to D0 dose (that gives 37% survival) would produce more than 1000 base damage, about 1000 single strand breaks, and 30-40 double strand breaks. Clustered DNA damage are more likely to occur when cells are exposed with high LET radiation. DNA-protein cross-links can be produced by radiation.

25. E A typical chromosome aberration assay would need to block cells at metaphase, and then visualize the condensed chromosomes using conventional staining (A) or fluorescent labeling of a specific set of chromosomes (B and C), and then identify the aberrated chromosomes. Newer technology such as copy number variation (CNV) can also be used to identify the regional chromosome deletion or amplification. An imaging analysis of the interphase nucleus is unlikely able to be identify aberrated chromosomes.

26. D X-ray cross complementing factor 1 (XRCC1) works with Ligase III to mediate the final ligation step in the base excision repair process. XRCC1 is also involved in single-stranded DNA break repair. Unlike all other base excision repair proteins, mutations in this repair protein results in cells being about 1.7-fold more radiosensitive than wild-type cells.

27. B The traditional dose-rate effect is most pronounced between dose rates of 1Gy/min and 0.3Gy/hour. Within this range, as dose rate decreases, cell killing decreases and the slope of the survival cure becomes more shallow, not steeper. The magnitude of dose-rate effect varies significantly among different cell types due to inherent differences in capacity for sublethal damage repair. Split dose experiments show a very little difference between split and single doses after exposure to high let radiation as there is little sublethal damage repair in this setting. Lastly, the inverse dose rate effect is seen in a specific in vitro experiment where HeLa cells are treated with continuous low dose rate irradiation, where an asynchronous cell population becomes synchronized as sensitive G2 cells and there is increased killing at this low dose rate compared with higher dose rates. This effect would not be seen with a single low dose exposure.

28. E Small molecule inhibitors which target key DNA DSB repair pathway proteins would be expected to induce significant levels of radiosensitization. ATM is a key proximal transducer of the DNA damage response (DDR) and loss of protein function leads to persistently unrepaired DSBs, and is associated with hereditary radiosensitivity syndromes. DNA-PK and KU70/80 are key NHEJ proteins and loss of these proteins also results in profound radiosensitivity. ATR recognizes damage associated with stalled replication forms, which induce DSBs upon collapse, and thus inhibition of the protein also results in high levels of radiosensitivity. MGMT removes alkylation damage at the O6 position of guanine, and loss of this protein would not be expected to induce significant enhancement of radiation-induced cell killing.

Chromosome Damage

1. D Anaphase bridges generally result from the induction of isochromatid breaks, which are breaks induced in both chromatids of a sister chromatid pair following DNA replication in late S phase or G_2 of the cell cycle. It is also possible to introduce isochromatid breaks due to replication fork collapse. They undergo an illegitimate union resulting in a bridge-like structure during anaphase of mitosis due to the inability of the sister chromatids to separate normally. Dicentric chromosomes result from breaks induced in two chromosomes in which the two broken chromosomes possessing the centromere join, resulting in a dicentric chromosome. An acentric fragment is produced following the breakage of a chromosome or chromosomes in which a portion of the chromosome that does not include the centromeric region is detached from the remainder of the chromosome. A single chromosome break would result in a terminal deletion.

2. C The minimum whole body dose that can be detected through measurement of dicentric chromosomes in peripheral blood lymphocytes is approximately 0.25 Gy.
    a. 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 link
    b. Rodrigues AS, Oliveira NG, Gil OM*, et al.* Use of cytogenetic indicators in radiobiology. Radiat Prot Dosimetry, 115:455-460, 2005. PubMed link

3. A A terminal deletion is produced when a single chromosomal break results in deletion of a portion of the chromosome, that is, a “one-hit” aberration. An acentric ring results from two chromosomal breaks within the same arm of a chromosome. A dicentric results from breaks in two different chromosomes, while an inversion is produced by two breaks in the same chromosome. While an anaphase bridge can cause lethality, it is not a chromosomal type of aberration and is produced by breaks in two sister chromatids.

4. B The formation of a dicentric chromosome is most likely to trigger the events during mitosis that lead to mitotic catastrophe and the death of the cell (although it should be noted that some dicentrics are stable and long-lived). The other chromosomal aberrations listed are not as likely to result in cellular death (for example, inversions, translocations and insertions do not produce acentric fragments) although they could play an important role in carcinogenesis if the portion of the chromosome altered results in the inactivation of a tumor suppressor gene or activation of an oncogene. The anaphase bridge is a chromatid type lesion.

5. C The most reliable approach to estimate dose one month following a radiation exposure is to karyotype peripheral blood lymphocytes to detect chromosomal aberrations, particularly dicentric chromosomes, which are normally not found in unirradiated people. Alkaline elution would detect single strand DNA breaks while γ-H2AX, pulsed-field gel electrophoresis and the neutral comet assay can all measure DNA double strand breaks. These would not be useful assays to measure a dose that had been received one month prior to tissue being obtained as virtually all DNA single and double-strand breaks would be repaired by this time.

6. C Chromatid type of chromosome aberrations are caused by chromatid breaks in S or G2 phase. The breaks induced in G1 phase often display as chromosome type of aberrations.

7. A The formation of chromosome translocation between two chromosomes needs two DSB. The dose response curve is linear quadratic because the two DSB can be produced by a single event or two independent events, especially when low LET radiation is used. It can be formed in G1 phase, and detected by SKY technology. Comet assay measures strand breaks in agarose but not metaphase chromosome aberrations.

8. A The number of dicentric chromosomes in X-irradiated cells follows a linear-quadratic function of dose because it usually needs mis-ligation of two double strand break to form dicentric chromosome.

9. E Symmetrical translocations are stable chromosome aberrations as they generally do not interfere with the ability of the cell to replicate its DNA nor proceed through mitosis, although they may play a role in carcinogenesis, e.g., such as with the BCR-ABL fusion.

Cell Death Mechanisms

1. E Pimonidazole detects hypoxic cells, whereas all the other assays listed would be useful for the identification of apoptotic cells. During the execution phase of apoptosis, nucleases are activated which cleave DNA into 180-200 base pair increments. Several assays are available to measure this phenotype. The TUNEL method identifies apoptotic cells by using terminal deoxynucleotidyl transferase (TdT) to transfer biotin-dUTP to strand breaks of cleaved DNA. The Annexin V Assay, a classical technique for detecting apoptosis, is the most commonly used method for detecting apoptosis by flow cytometry. Annexin V is a calcium-dependent phospholipid binding protein that has a high affinity for the phophatidylserine (PS), a plasma membrane phospholipid. One of the earliest features of apoptosis is the translocation of PS from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external environment. Annexin V binds to PS exposed on the cell surface and identifies cells at an earlier stage of apoptosis than assays based on DNA fragmentation. DNA ladder formation is detected by gel electrophoresis of pooled DNA. Diamidino-2-phenylindole (DAPI) is DNA-specific dye that displays a blue fluorescence. This dye could be used to assess the nuclear morphology of normal versus apoptotic cells by fluorescence microscopy.

2. A The most appropriate approach to assess cellular survival to radiation for an actively dividing population of cells is to determine what fraction of the irradiated cells is capable of clonogenic survival (colony formation). Division delay would measure the amount of cell cycle perturbation caused by radiation, but occurs in all actively dividing cells regardless of whether they ultimately live or die. Apoptosis is just one form of death, and can occur at many different times after irradiation. The formation of giant cells with multiple nuclei is a manifestation of cells undergoing mitotic catastrophe following the formation of chromosome aberrations, but is not the only mechanism of radiation-induced cell death. Likewise, detection of necrotic cells would only provide the fraction of cells that undergo this form of cell death, and would not give an overall sense of cellular lethality that could also occur through either apoptosis, autophagy, mitotic catastrophe or senescence.

3. C Mitotic catastrophe is caused by the mis-segregation of genetic material into daughter cells resulting from radiation-induced chromosome aberrations and/or damage to the replication machinery of the cell. Apoptosis is a form of programmed cell death and can occur in response to initial radiation induced damage. However, this is rare and limited to specific tumor types such as low-grade lymphoma. Even when cells die by apoptosis, this usually occurs after mitotic catastrophe. In this case mitotic catastrophe is the reason for cell death, and apoptosis is just the mode of cell death. Oxidative damage to proteins can occur, but is not significant at doses that are lethal to cells due to DNA damage. The generation of ceramide through the action of sphingomyelinase plays a role in the intrinsic pathway leading to apoptosis, and may be important in endothelial cells, but is not a major mechanism for the lethality of irradiation in solid tumors.

4. B Apoptosis predominates in some normal tissue cells derived from lymphoid tissues. In addition, radiation-induced apoptosis occurs in some normal epithelial tissues, such as the salivary gland and intestinal epithelium. However, apoptosis is not the most frequent mode of death for most cancer cells following radiation. Instead, mitotic death is more common. Apoptosis often occurs during interphase prior to mitosis. p53 plays a large role in regulation of the apoptotic program by increasing pro-death proteins like PUMA that block anti-death Bcl-2 proteins, which allow pro-death Bcl-2 proteins like BAX and BAK to kill the cell via apoptosis.

5. A Two breaks in a single chromosome can cause inversion, deletion or ring structure. Inversion is a chromosomal abnormality in which the segment between two breakpoints is inverted before sealing the breaks. Chromosomal inversions are stable aberrations and cells may continue to go through many divisions in their presence. Apoptosis and necrosis are forms of cell death and would reduce clonogenic survival. Autophagy, in some but not all circumstances can also lead to cell death. Senescence does not result in lethality per se, however senescent cells do not divide and therefore would not be able to contribute to colony formation.

   Wouters BG. Cell death after irradiation: How, when and why cells die. Chapter 3 in: Basic Clinical Radiobiology. M Joinner and A van der Kogel, Eds, Fourth Edition (2009), Hodder Arnold, London UK.

6. D Annexin V stains phosphatidyl serine, a phospholipid, which is normally located on the inner leaflet of the cell membrane, but flips to the outer portion of the membrane during apoptosis. Plasma membrane

integrity is maintained until the final stages of apoptosis, when the membrane blebs and pinches off to form apoptotic bodies. Cleavage of nuclear DNA at linker regions between nucleosomes is carried out by a DNAase, which is activated by caspases. Cells, such as lymphocytes and serous acinar cells that have a pro-apoptotic tendency, are generally radiosensitive, not radioresistant. In irradiated tissues, apoptotic cells often appear singly and in isolation.

7. A The majority of both normal and tumor cells die by mitotic catastrophe following one, or no more than a few, abortive mitotic cycles. However, until these cells attempt their first division post-irradiation, there is no morphological evidence of injury. In comparison to cells undergoing apoptosis, those undergoing necrosis demonstrate a loss of membrane integrity, a swelling of the cytoplasm and mitochondria, and random degradation of DNA (leading to a smear following agarose gel electrophoresis). An alternate pathway by which cells cease to proliferate following lethal doses of radiation is permanent growth arrest (also called replicative senescence); cells acquire a senescent-like morphology, characterized by increased granularity within the nucleus, accompanied by increased levels of $p16^{INK4A}$ (Cdkn2a) and SA-β-galactasidase. A number of pathways can be activated that lead to apoptosis, only some of which are p53-dependent.
    a. Vandenabeele P, Balluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: An ordered cellular explosion, Nature Reviews Molecular Cell Biolog, 11:700-714, 2010. PubMed link
    b. Cotter TG. Apoptosis and cancer: The genesis of a research field, Nat Rev Cancer 9:501-507, 2009. PubMed link
    c. Eisenberg A, Bialik S, Simon HU and Kimchi A. Life and death partners: Apoptosis, autophagy and the cross-talk between them, Cell Death Diff, 16: 966-975, 2009. PubMed link
    d. Ohtani N, Mann DJ, Hara E. Cellular senescence: Its role in tumor suppression and aging, Cancer Sci, 100:792-797, 2009. PubMed link
    e. Letai AG. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. Nat Rev Cancer, 8:121-132, 2008. PubMed link
    f. Kroemer G, Levine B. Autophagic cell death: The story of a misnomer, Nat Rev Mol Cell Biol, 9:1004-1010 2008. PubMed link
    g. Ow YP, Green DR, Hao Z, *et al.* Cytochrome c: Functions beyond respiration. Nat Rev Mol Cell Biol, 9:532-542, 2008. PubMed link
    h. Taylor RC, Cullen SP, Martin SJ. Apoptosis: Controlled demolition at the cellular level, Nat Rev Mol Cell Biol, 9:231-241, 2008. PubMed link
    i. 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 link
    j. Wouters BG, Brown JM. Apoptosis, p53, and tumor cell sensitivity to anticancer agents, Cancer Res, 59(7):1391-1399, 1999. PubMed link

8. C Cells undergoing apoptosis exhibit nuclear fragmentation. Apoptosis does not induce an inflammatory response, unlike necrosis. Apoptotic cells do not exhibit an increased expression of the *MSH2* gene, whose product is involved in mismatch repair. Apoptotic cells do not swell, but exhibit condensation and fragment into apoptotic bodies. Apoptosis can take place during interphase.

9. C The extrinsic apoptotic pathway involves stimulation of TNFR family members. Caspase 8 is an important initiator caspase for the extrinsic mechanism. p53 upregulates apoptosis. BAD is a pro-apoptotic protein. Leakage of cytochrome c from the mitochondrial membrane is a central aspect of the intrinsic apoptotic pathway.

10. A Bcl-xL prevents apoptosis primarily through inhibition of cytochrome c release from the mitochondria.
    a. 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 link

11. A The concept of synthetic lethality has gained significant attention in adult oncology in recent years. Two genes or pathways are considered to be synthetically lethal if inactivation of either does not kill cells, while simultaneous inactivation of both is lethal. Thus, if a tumor harbors a mutation in one gene of a “synthetic lethal pair”, targeting the corresponding gene in the pair will selectively kill cancer cells while sparing normal cells without the cancerous mutation. This concept has also been extended to include oncogenic mutations and unique translocations. One of the most well-known examples of synthetic lethality is the interaction

between the two double-strand break (DSB) repair genes, BRCA1 and BRCA2, and poly (ADP-ribose) polymerase-1 (PARP-1). BRCA1 and BRCA2 play key roles in homologous recombination repair, while PARP-1 is involved in base excision and single-strand break (SSB) repair. Inhibition of PARP activity is thought to increase the number of SSBs in cells, which are then converted to DSBs during replication. As replication-associated DSBs appear to require the homologous recombination repair pathway for repair almost exclusively, PARP inhibition induces significant cell death in tumors with loss-of-function mutations in BRCA1 or BRCA2. Importantly, normal cells are not affected by PARP inhibition because BRCA1 or BRCA2 function is intact. This discovery was ultimately translated to the clinic, where PARP inhibitors were shown to have single agent activity in metastatic patients with BRCA-deficient tumors. PubMed link

12. E Cell death occurs via number of mechanisms after exposure to IR, including mitotic catastrophe, autophagy, apoptosis, and necrosis. PubMed link

13. A A novel discovery that cell death induced by radiation therapy is a key trigger for tumor cell repopulation and a vital component of this signaling is mediated by caspase 3 positively regulating paracrine signaling from dying cells to stimulate proliferation of surviving tumor cells was recently made. PubMed link

14. C Necroptosis is a programmed form of necrotic cell death. Necrotic cell death has been considered a form of passive cell death. However, the discovery that TNF-alpha mediated necrosis can be inhibited by a specific inhibitor of RIP1 kinase, necrostatin-1, led to the concept of necroptosis. Necroptosis has now been established as a regulated necrotic cell death pathway controlled by RIP1 and RIP3 kinases. Under conditions that are insufficient to trigger apoptosis, TNF-alpha activates TNFR1 and in turn induces the recruitment of RIP1 kinase and other proteins to form complex I. Subsequently, these proteins dissociate from TNFR1 and RIP1 can be found in the cytosol in complex IIb, which includes RIP1 and RIP3. The formation of complex IIb leads to necroptosis (Refs. 20045303 and 22136818). The correct answer is “c”. The other answers are incorrect.

15. B The abscopal effect is a phenomenon in the treatment of metastatic cancer where localized irradiation of a tumor causes, not only a shrinking of the irradiated tumor, but also a shrinking of tumors far from the irradiated area. While this phenomenon is extremely rare, its effect on the cancer can be stunning, leading to the disappearance of malignant growths throughout the entire body. Such success has been described for a variety of cancers, including melanoma, lymphoma, and kidney cancer. Studies in mice suggest that the effect may depend upon activation of the immune system (Refs. 4706791 and 22397654). The correct answer is “b”. Answers “c” and “e” are the bystander effect.

16. E It is likely that following a dose of 4 Gy, many cells that may be reproductively dead will still be able to divide for several days following irradiation until they undergo mitotic catastrophe. It would be anticipated that a minority of carcinoma cells would undergo apoptosis and exhibit annexin V staining. Possession of a mutation in p53 would likely not substantially affect the radiosensitivity of carcinoma cells. It is only tumor cells, such as lymphomas that have a pronounced pro-apoptotic capacity, for which a p53 mutation results in radioresistance since the apoptotic pathway is inhibited in these mutant cells.

17. E Beclin is a protein important in the development of the autophagosome for autophagy. It is also important and used in some pathways of apoptosis. It plays no role in necrosis (which is an unscheduled death and not programmed in any way) or the other forms of death listed.

Survival Assays

1. D This and other *in vivo* clonogenic assays do not require that the investigator be able to unambiguously identify the stem cell or distinguish it from its differentiated progeny. Instead, the stem cell is identified functionally, by its ability to produce progeny; its survival is assayed by the ability to repopulate the depleted crypt after irradiation. Crypt turnover time is approximately 3-5 days. There are two populations of intestinal stem cells: rapidly active intestinal stem cells (LGR5+) that are very radiation sensitive, and the more quiescent, and radioresistant reserve intestinal stem cells that have proliferative potential and can re-enter the cell cycle to help repopulating the crypt. All of the other factors would compromise the accuracy of the assay. A wide variation in the number of stem cells (e.g., 1 in some crypts, 10 or 50 in others) would result in large variations in the extrapolation number, n, of the radiation survival curve, and therefore in the vertical position of the exponential region of the survival curve. Such variability would make the assay unusable. The clonogenic assay also assumes that the presence of one (or more) surviving stem cells in an irradiated crypt leads to the regeneration of that crypt, and that a crypt where no stem cells survive does not regenerate. The migration of surviving stem cells from one regenerating crypt into a neighboring crypt that had no surviving stem cells, or the repopulation/survival of dying crypts as a result of the migration of unirradiated stem cells from outside of the irradiated volume, would result in an overestimation of the survival of the irradiated crypt stem cells. Conversely, if some stem cells survived, but did not proliferate for several days after irradiation, their crypts would not regenerate during the relatively short observation period used in this assay and the stem cells would erroneously be scored as dead. Stem cell survival would be underestimated in this case.
    a. Kim CK, Yang VW, Bialkowska AB. The Role of Intestinal Stem Cells in Epithelial Regeneration Following Radiation-Induced Gut Injury [published correction appears in Curr Stem Cell Rep. 2018;4(1):95]. *Curr Stem Cell Rep*. 2017;3(4):320-332. doi:10.1007/s40778-017-0103-7

2. A During the early 1960s, Till and McCulloch performed a series of experiments in which bone marrow cells were injected into lethally-irradiated mice, some of which went on to form colonies/nodules of bone marrow cells in the spleens of the recipient mice 9-10 days later. Only a small fraction of injected bone marrow is able to form spleen colonies because most bone marrow cells are too differentiated upon harvest and have lost that ability to produce progeny. This was the first demonstration that normal tissues possess pluripotent stem cells. In addition, for some experiments, they irradiated the donor mice and showed that with increasing dose, greater numbers of cells were necessary to produce spleen nodules in the recipient mice. This represented the first *in vivo* radiation dose response curve for a normal tissue (although radiation survival assays for cells *in vitro* had been developed a few years earlier by Puck & Markus in 1956). In recognition of this work, they were awarded the 2005 Albert Lasker Prize for Basic Medical Research.
    a. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells, Radiation Res, 14:213-222, 1961. PubMed link

3. D Autophagy is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules and as such it is a survival mechanism. Autophagy allows a starving cell, or a cell that is deprived of growth factors to survive up to a point. Cells that do not receive nutrients for extended periods ultimately digest all available substrates and die through autophagic death. Supplying nutrients before this critical point would restore the cell's health. Loss of mitochondrial membrane potential occurs during apoptosis. Choices B and C occur during necrosis. Wallerian degeneration occurs after axonal injury in both the peripheral and central nervous systems..
    a. RS Hotchkiss, A Strass, JE McDunn, PE Swanson, Cell death. N Eng J Med 2009;361: 1570-1583. PubMed link

4. C The fraction of cell killing due to single-track damage (a*D) does not change with fractionation whereas as the multi-track portion (b* D^2) does. This is because in the latter case individual DNA breaks can interact forming complex lesion, which can be separated in time by splitting the dose and hence they become repairable. Example - alpha=3 and beta=1, so irreparable damage is alpha x dose = 3 x 9 = 27, and repairable damage is beta x dose squared = 1 x 81 = 81. Giving ratio of 27:81 or 1:3

$\beta \cdot D^2$ 9 3

5. E Repairable damage amount is $\beta \cdot D^2$ (let us called it Y)
   Irreparable damage amount is $\alpha \cdot D$ (let us called it X)

   $(\alpha \cdot D)/(\beta \cdot D^2) = X/Y = 10/2 = 5$

   But x+y = 1. Since x = 5y, 6y = 1 → y = $\frac{1}{6}$ = 0.17. This means that approximately 20% of the total amount of damage is repairable, and 80% is irreparable at 2 Gy delivered to tumor cells. Note that at 10 Gy, 50% of damage would be repairable and 50% of damage would be irreparable. This means that the amount of repairable damage increases with dose.

6. B Any number, including the base of natural logarithm $e \cong 2.72$, raised to zero-power equals 1. Multitarget single-hit survival curves described by the expression: SF= N · $e^{-D/D0}$ are characterized by zero slope at D=0 Gy. The graph of this function appears to resemble a hockey stick in that there is a flat portion for D ≤ D_q (SF = 1) followed by a straight line with the final slope 1/D_0, when plotted on semilog paper. A flat portion is described by the semithreshold dose ("the shoulder width") D_q or the related parameter, the extrapolation number, N. The three parameters: a mean lethal dose D_0, N, and D_q are related by the expression: natural log N = $\frac{Dq}{D0}$. In the present example, $\log_e$ N = 3, so N = $e^3 \cong 20$ and SF = 2.7 at 2 Gy (choice D). This paradoxical (and erroneous) result shows that the multitarget single-hit model is not applicable in the range of doses of less than D_q.

7. D The required dose must reduce the cell population by a factor of $10^{10} \times 10^2 = 10^{12}$. The mean lethal dose, usually denoted D_0 and sometimes D_{37} , is the dose required to reduce the population of cells by a factor of 0.37 from its initial value (thus kill 63% of the population). In this example, D_0 =1 Gy. In solving problems similar to this one, it is convenient to use the dose required to reduce the population by a factor of 10 (from 0.1 to 0.01, from 0.01 to 0.001, etc) instead of D_0. It is easy to see that $D_{10} = D_0 \log_e 10 = 2.3 \cdot D_0$. In this example, D_{10} = 2.3 Gy. Since the survival is reduced by a factor of 10 with each dose of 2.3 Gy, required dose = $12 \times 2.3\ Gy = 27.6\ Gy$.

8. B Clonogenic cell survival assays (CSAs) are considered the "gold standard" for the assessment of IR response patterns in vitro. Importantly, these assays can be used to determine whether the effect of a given drug on IR-induced cell killing is additive versus synergistic. Caspase assays measure apoptosis, which is a common mechanisms cell death in lymphoma and leukemia cells, but it is not as predominant in epithelial cancers. The 3-day growth assay and the micronucleus assay may correlate with clonogenic survival, but the don't measure true clonogenicity, which can take many more days to manifest. PubMed link

Cell Survival Models

1. D The formula for a single-hit survival curve is $S = e^{-\alpha D}$. Because the SF_2 (the surviving fraction following a dose of 2 Gy) is 0.37, $0.37 = e^{-\alpha D}$ or $\alpha D = 1 = \alpha$ (2 Gy). Hence, $\alpha = 0.5\ Gy^{-1}$

2. C In classical target theory, the D_0 is the dose that reduces cell survival to 37% of some initial value, as measured on the exponential portion of the radiation survival curve. In essence, D_0 is the dose that produces an average of one lethal lesion per cell in a population of irradiated cells; Because the radiation-induced ionizations are random, discrete events , the probability to be killed follows a Poisson distribution with the assumption that one hit is enough to kill. In this instance (at D_0), 37% of the targets will not receive a lethal hit and will survive. It is the quasi-threshold dose, Dq, which is an approximation of the total amount of sublethal damage that a cell can accumulate before lethality occurs. The extrapolation number, n, represents the total number of targets that must be inactivated (or hits that must be received in a single target) for a cell to be killed. The Dq would be a manifestation of the width of the shoulder of a survival curve.

3. C The D_0 for most oxygenated, mammalian cells falls in the range of 1 - 2 Gy.

4. E A cell survival curve characterized by an extrapolation number equal to 1 is exponential. Therefore, if the D_0 is 1 Gy, then a dose of 3 Gy would yield a surviving fraction of (0.37) x (0.37) x (0.37) or approximately 0.05. Thus, 95% of the cells would be killed. An alternative solution can be obtained by applying the single hit single target equation $S = \exp(-D/D_o)$ with D_o =1 Gy and D = 3 Gy; the surviving fraction will be $S = e^{-3/1} = 0.05$.

5. E The survival curve resulting from a fractionated protocol is referred to as the effective survival curve. Assuming the time intervals between fractions is sufficient to allow for sublethal damage repair without proliferation, the shoulder of the survival curve is repeated with each fraction. It is exponential and therefore appears as a straight line when plotted on a log-linear scale. Thus, the effective survival curve is not literally “linear” mathematically-speaking, but only takes on this appearance when the data are plotted in this manner. Bell-shaped implies that survival first increases with dose and then decreases, which does not occur. A parabolic dose response also does not occur.

6. C For a cell line that exhibits significant curvature of its acute dose survival curve (as suggested by an n of 10), the effective D_0 would decrease with increasing fraction size compared to a multifraction survival curve employing smaller-sized dose fractions.

7. D 20 colonies/2,000 cells plated = 0.01 absolute surviving fraction (1% survival). However, this value must be corrected for the plating efficiency of unirradiated cells, which is 40 colonies/200 cells plated = 0.2 (20% survival). Thus the normalized percent survival is 0.01/0.2 = 0.05 = 5%.

8. B As is typical of most mammalian cell lines, the dose response curve for X-irradiated V79 Chinese hamster cells is linear-quadratic in shape, and can be modeled using the expression S = . Using the parameters provided, the surviving fraction following a dose of 5 Gy would be $S = e^{-[(0.2)(5)+(0.05)(25)]} = e^{-(1+1.25)} = e^{-2.25} \sim 0.1$.

9. C If the 5 Gy dose is delivered over a 10 h period, then the dose rate equals 5 Gy/10 h = 0.5 Gy/h. Assuming that relatively few (human) cells divide during the 10 hour irradiation interval, the surviving fraction will increase due to repair of sublethal damage and the β parameter value will approach zero. ($\beta = 0$ means that all repairable damage has been repaired). Thus, the surviving fraction will equal $e^{-(0.2)(5)} = e^{-1} = 0.37$

10. A Since the survival curve for high LET carbon ions is exponential, the surviving fraction following 5 irradiations with a dose that results in a surviving fraction of 0.4 would be $(0.4)^5 = 0.01$.

11. B Genomic instability can be induced in cells surviving a prior irradiation, and this would be inherited by those cells’ progeny, which may contribute to their showing a decreased clonogenic survival. All of the remaining explanations have the potential to increase, not decrease, survival.

12. B The width of shoulder should reflect the repair of sub-lethal damage. It has significant implications for how effective the dose fractionation can impact the overall survival.

13. D G2 and M phase cells are most sensitive to irradiation among all the phases. It is true that proliferative cells are more sensitive to irradiation than non-proliferative tissue, but this does not mean the S-phase cells are more sensitivity than cells in other phases.

14. B The colony formation efficiency for non-treated cells is 60% (60/100). The expected number of colonies after plating 400 cells would therefore be 240. However, 4 Gy of radiation reduces the number of colonies to 80. The surviving fraction is therefore 33% (80/240).

15. B Among the identical patients treated, there will be a distribution for the actual number of survived cells, although the average number of cells to survive should be 2 (or 2x109 * 10^9). The probability of having no cell to survival (thus recurrence-free) fits the Poison distribution. It can be calculated as: p=e-n, where n is the average number of survived cells. Because the average number of survived tumor cells is 2, the probability to have no tumor cells survived should be: e-2.

16. C The survival fraction for each of the D0 fractionation will be: e-1 (36.78%). Thus the overall survival after 20 fractionations will be: (e^1) 20, or e^{20}. This results in an average number of survived cells of 6.18 (or: $3*10^9*e^{20}$)

17. D D0 is the dose that produces an average one lethal hit on the cell population. The survival fraction at this dose is: e^{-1} or 37%.

18. E The dose at which the level of single-hit equals the multi-hit killing is equal to the α/β ratio, which in this case is 0.4 Gy^{-1}/0.2 Gy^{-2} = 2 Gy.

Linear Energy Transfer

1. B Clinically relevant 75 MeV per nucleon argon ions have LET 250 keV/µm. 1 GeV/nucleon and 18 MeV/nucleon carbon ions have LET values of approximately 10 keV/µm and 108 keV/µm, respectively. 2.5 MeV alpha particles have an LET value of approximately 170 keV/µm. 150 MeV protons are considered low LET, with values in the range of 0.5 keV/µm.

2. C The carbon ion RBE is the dose required to produce a certain effect in X-irradiated cells divided by the carbon ion dose to produce the same biological effect. This ratio will be greater for cells irradiated under hypoxic conditions because of the much greater dose required to produce the effect in the X-irradiated cells where an oxygen effect is present, compared to the high LET irradiated cells where the oxygen effect is absent. The oxygen enhancement ratio, OER, for carbon ions would be lower than that for low LET radiations, but the absolute value of the OER is not related to the value of the RBE.

3. C OER decreases with increasing values of LET. Maximum effectiveness and therefore RBE reaches a peak for radiations whose LET is approximately 100 keV/µm. The RBE of high LET radiations is generally high, resulting in low values for D_0. The survival curves resulting from irradiation of cells with high LET radiations are typically exponential. LET is the term that describes the density of ionization or the average amount of energy lost (in keV) to the medium per unit of track length (µm).

4. B As the LET for different forms of radiation increases to about 100 keV/µm, both the RBE and the α/β ratio for the corresponding cell survival curves increase due primarily to an increase in the α parameter.

5. A For a given charged particle, the density of ionization events along a track (i.e. LET) decreases with increasing energy. The iodine-125 decay mechanism is electron capture to form the nearly-stable tellurium-125. This is followed by gamma decay at 35 keV and Auger electron emission at 50-500 eV. LET values for these low energy gamma rays and Auger electrons is greater than 30 keV/µm. Energy of gamma rays from Cobalt-60 decay and gamma rays produced in the annihilation reaction is in a 1-MeV range and LET ~ 0.2 keV/µm. Yttrium-90 is a high-energy beta-emitting isotope. The maximum energy of the beta particles is 2.27 MeV with a mean of 0.93 MeV. These electrons are sparsely ionizing. Protons used in the clinic have an energy of about 200MeV and are sparsely ionizing for the most part with a high LET component at the Bragg-peak.

6. E Equal doses produce the same number of ionizations per unit mass, regardless radiation quality. 1 Gy = 1 J/kg which corresponds to $2x10^{17}$ ionizations/kg, roughly 10^6 ionizations per mammalian cell. Biological efficiency of ionizing radiation is related to its spatial energy concentration along the track, which is the random localized pattern of the individual ionizing produced by a charged particle or secondary electrons set in motion by the original particle. For fast electrons set in motion by γ-rays, the energy releases are widely spaced and, even though the track passes through DNA, there is a chance that no energy will be released in it. On the other hand, the track left by α-particle is so dense that if it passes through DNA, there will be enough energy released in it to destroy the ability of the DNA to function properly. The LET value of cobalt-60 γ-rays is 0.25 keV/µm. This means that along each micron of track length, 250 eV is released by 1 MeV γ-ray, which would be enough to produce 7-8 ion pairs/µm. In contrast, 1 MeV α-particle (LET = 250 keV/µm) will lose ~ 1000 times as much energy along each micron of track and may produce 7000-8000 ion pairs/µm.

7. A RBE is calculated based on the ratio of isoeffect doses, not the ratio of effect. In other words, RBE values are endpoint specific. Traditionally, 250kVp X-rays is the reference radiation. Generally, high LET radiations have higher the RBE values. Stereotactic ablative radiotherapy involves high dose per fraction radiotherapy, compared to conventional radiotherapy, and does not necessarily use radiation of different energies.

Oxygen Effect

1. C Radiation type B likely has a higher LET than type A since less of an oxygen effect was observed for type B. Thus, if the radiation delivered by type B was delivered at a low dose rate allowing for reoxygenation, the amount of cell killing would not differ substantially from that produced at a high dose rate. The OER for radiation type A is 3.0 since three times the dose was required to produce the same biological effect (D_{37}) for the cells under hypoxic conditions than aerated conditions. Since types A and B are the same form of ionizing radiation, then type B would likely be lower energy than type A since LET is inversely proportional to energy of the particle.

2. D In irradiated cells, oxygen increases the number and/or type of free radicals and thereby acts as a radiosensitizer, effectively increasing the level of damage produced. Oxygen reacts with free radicals resulting in the production of different radical species, which may be longer lived, and therefore more damaging than the original radicals. For example, oxygen may react with hydrogen radicals to produce peroxyl radicals. Through its reaction with free radicals formed from the radiolysis of water, oxygen plays a role in the indirect effect of radiation.

3. D In order for oxygen radiosensitization to be observed, oxygen must be present either during or within microseconds following the irradiation. Irradiation under hypoxic conditions results in fewer DNA strand breaks than irradiation under aerated conditions. Irradiation in air results in more cellular damage and cell killing than irradiation under hypoxic conditions. The effect of oxygen upon radiobiologic response changes most between 0.05%-2%, with a half-maximum effect around 1%

4. C The OER for most forms of low LET radiation delivered acutely is in the range of 2-3.5.

5. A EPR O2 imaging is a good surrogate for TCD50. PubMed link

6. B OER is calculated based on the ratio of isoeffect doses, not the effect. Free radical scavengers will reduce the contribution of indirect effect, thus reduce OER. High LET and high dose rate radiation often rely less on indirect effect to kill cell, thus should have lower OER values.

7. D The OER is calculated as a ratio of doses, not effect. It is equal to the ratio of doses under hypoxic and aerobic conditions that yields the identical level of a biologic effect. For low LET radiation this value is approximately 2.5-3, and is somewhat lower at low doses (high levels of survival). The OER decreases with radiation of increased LET, due to an increased proportion of direct DNA damage.

Cellular Repair

1. C The bystander effect has been documented in both cancer cell lines and normal, untransformed cells, with no indication that abnormal cellular signaling plays a role. The use of chemical gap junction inhibitors and connexin knock-out cells has shown a large inhibitory effect on bystander endpoints, as has the use of radical scavengers. Some aspect of bystander signaling however, is transmissible through medium transfer, and extracellular molecules such as TGF-beta have been implicated.
    a. Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy, Nat Rev Cancer, 9:351-360, 2009. PubMed link

2. E An X-ray dose delivered at a high dose rate results in greater cellular lethality since there is less opportunity (time) for repair of sublethal damage occurs during irradiation at a high dose rate. In contrast, during the course of irradiation at a low dose rate, many sublethal damages will be repaired and therefore will no longer be available to interact and form lethal damage. The fraction of cells undergoing apoptosis is primarily a reflection of the apoptotic tendency of the cell type rather than a reflection of the rate at which the dose was delivered. Activation of ATM, which in turn stimulates the production of molecules that cause inhibition of cell cycle progression, occurs regardless of whether the radiation is delivered at high or low dose rates. Cell proliferation is inhibited in cells irradiated at a high dose. Furthermore, cell proliferation would increase survival.

3. E A drug that inhibits the rejoining of chromosome breaks in irradiated cells would be expected to decrease the amount of sublethal damage repair (itself a manifestation of the rejoining of chromosome breaks), and therefore, to sensitize cells to low dose rate irradiation where sublethal damage would otherwise be repaired. The repair of potentially lethal damage would also be inhibited. The yield of terminal deletions would be expected to increase as there would be less repair of chromosomal breaks. The dose rate effect, manifested as increased cell survival for irradiation at low, compared to high dose rates, would also be diminished.

4. A Generally, increasing the time between fractions in a split dose treatment results in a higher cell surviving fraction due to repair at relatively short interfraction intervals of a few hours, or due to repopulation for longer times between fractions. However, under certain irradiation conditions and depending on the cell line, the initial dose may cause inhibition of progression from G_2 into M phase. Therefore, the second dose may be delivered when the majority of the surviving cells have reassorted into G_2, a radiosensitive phase of the cell cycle. Thus, even though repair of sublethal damages has occurred in these cells, which by itself would lead to a greater surviving fraction, this may be more than counterbalanced by reassortment sensitization, resulting in lower cell survival. Hypoxic conditions would not be expected for cells grown in tissue culture, so reoxygenation, which could lead to greater cell killing if it were to occur, is unlikely. The adaptive response in which cells treated with an initial low “priming” dose of radiation exhibit greater resistance to a second, higher, “challenge” dose, would increase, not decrease, cell survival.

5. C Cell lines whose X-ray survival curves have low α/β ratios generally display a large capacity for SLDR, whereas cells whose X-ray survival curves have high α/β ratios show relatively little SLDR. As the dose rate is lowered and exposure time increased, the biological effect of an X-ray dose diminishes due to SLDR. PLDR is best demonstrated with a “delayed plating” experiment, and is operationally defined as an increase in the surviving fraction resulting from prolonged incubation of cells under non-growth conditions following irradiation. There is little or no SLDR or PLDR following exposure to high LET radiation. Fractionated irradiation would be expected to increase survival (not decrease it) in normal lung tissue compared to lung cancer cells, and this would result from SLDR, not PLDR.

6. C Sublethal damage recovery is operationally defined as an increase in cell survival when a total dose is split into two fractions separated by a time interval compared with delivery of the same dose in one large fraction. The TCD_{50} is the total dose that locally controls, on average, 50% of tumors in laboratory animals, but by itself does not directly demonstrate sublethal damage recovery. A cell synchronization experiment would not demonstrate sublethal damage recovery, although it could be used to show variations in SLDR capacity in cells of different cell cycle “ages”. An increase in cell survival when cells are maintained under a

non-growth state after irradiation, is the operational definition of potentially lethal damage recovery. The paired survival curve technique is used to determine a tumor's radiobiological hypoxic fraction.

7. B As the dose rate decreases from about 1 Gy/min to 0.01 Gy/min, the greatest increase in cell survival due to SLDR is observed for most X-irradiated cell lines. Decreasing the dose rate further may permit an even greater increase in the surviving fraction, but this further increase would be due to repopulation that may take place if the dose is delivered at a very low dose rate over a long interval.

Tumor Assay Systems

1. E The tumor regrowth delay assay measures the average time necessary for a treated tumor to reach a pre-determined size compared to the time it takes for control tumors. Of the assays listed, the tumor regrowth assay is most informative as to the effect of radiation and/or drug treatment. The techniques described in A and B are still used to measure the relative effectiveness of experimental chemotherapy drugs, however they provide only very limited information. The technique described in C is effective only if the treated tumors shrink immediately and dramatically following treatment, but is less effective with agents such as radiation that produce a delayed cell death or with drugs that have cytostatic effects, because these agents will not produce rapid and sizeable shrinkage of the tumors. The approach described in D may not allow meaningful comparisons between the different treatment groups, if some or all treatments have been relatively successful and tumors in several groups have not even begun to regrow by the time the control tumors become large.

2. D Radiation-induced cell killing is random, and the probability follows a Poisson distribution; a tumor will be controlled only when no clonogenic cells remain. The dose at which a specific tumor is controlled will be determined by the probability of killing the last clonogenic cell in that tumor. However, this will not be the same for each tumor because of the random nature of radiation damage and of cell death. The result of this, statistically, is that the tumor control probability plotted as a function of dose on a linear scale will yield a steep, sigmoid-shaped curve that reflects only the random variation in the dose needed to kill the last clonogenic cell in the tumor. Heterogeneity between the tumors (e.g., differences in size/cell number), or heterogeneity within the tumor cell population (e.g., heterogeneity in the radiosensitivity of the cells because of their position in the cell cycle, oxygenation status, or genotype), would broaden the dose range over which the sigmoidal increase in tumor control probability occurred, and the resulting tumor control probability curve would be shallower.

3. D The impact of a radiosenisitizer upon tumor control will be most readily detected for experimental protocols that result in a 50% rate of tumor cure, since even a small level of sensitization will significantly decrease the TCD_{50} in this portion of the curve. This curve demonstrates that 50% of the tumors will be controlled by a dose of 60 Gy (ie. TCD_{50} = 60 Gy), whereas 70 Gy increases tumor cure to >90%. NTCP stands for normal tissue complication probability. The additional dose to increase cures from 50% to 60% is relatively small because the TCD_{50} is on the steep part of the dose-response curve, but to increase cure rates from 90% to 100%, a much larger dose is required. At 50 Gy, approximately 10% of the tumors will be controlled.

4. A In vivo studies are necessary in order to examine agents that act on the tumor microenvironment or other non-cell autonomous cancer cell processes, such as anti-angiogenic agents. The majority of in vivo studies involve immunocompromised mice with mutations in DNA response and repair pathways, including athymic, severe combined immune-deficiency (SCID) or NOD-SCID mice. The abnormal DNA repair mechanisms in these mice limit the applicability of results with radiosensitizers given the integral role of DNA damage to the biologic effect of radiation therapy. Furthermore, anti-tumor effects of radiotherapy may be mediated by the immune system. Therefore, immunocompromised mice are not optimal in this regard given that they lack a functional immune system. As a result of these limitations, genetically engineered mouse models (GEMMs) are becoming more widely used in preclinical studies with and without radiotherapy (24331186 and 23863691). The correct answer is “a”. The other answers are possible for both GEMMs and xenograft tumor models.

Tumor Biology and Microenvironment

1. D Acute changes in blood flow cause acute, or perfusion limited hypoxia. This can be partial or total occlusion of blood vessels that causes hypoxia in cells being fed by the vessel in question. Acute hypoxia can change over a period of 15 min-2 hrs and will thus alter overall tumor oxygenation during a period of 24 hrs between radiation fractions. Hypoxia can be distributed throughout the entire tumor mass, and can potentially be present surrounding any blood vessel in the tumor. No relationship has been observed between tumor hypoxia and tumor size. Tumor hypoxia varies widely amongst patient tumors and is thought to be an important contributor to the overall variation in response to therapy. However, in preclinical models HIF1a has been shown to coordinate a transcriptional program of gene expression that results in radiation resistance. This occurs independently of the lack of oxygen to "fix" DNA damage.
    a. Cao Y. Off-tumor target –beneficial site for antiangiogenic cancer therapy? Nat Rev Clin Oncol, 7:604-609, 2010. PubMed link
    b. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response, Nature Reviews Cancer, 8, 425-437, 2008. PubMed link
    c. https://pubmed.ncbi.nlm.nih.gov/22016813/

2. B Clinical studies indicate that hypoxia does not show a strong relationship with tumor size. This is due to the fact that hypoxia arises through deficiencies in blood vessel perfusion and from a limited ability to diffuse through metabolically active tissue. Hypoxia is observed at distances from 100-200 microns from functional vessels. Thus, even very small tumors may have high hypoxic fractions. Hypoxic cells are radioresistant and exhibit genomic instability and increased probability of metastasis. Each of these factors translates into a worse patient prognosis.
    c. Bristow RG, Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability, Nat Rev Cancer, 8:180-192, 2008. PubMed link

3. B The maximum possible distance that oxygen can diffuse from a capillary before hypoxia is detected, is roughly 150 μm. This distance is dependent on the physical properties of oxygen itself and the tissue through which is it diffusing. Cells residing outside this distance from blood supply will be hypoxic. This is not to be confused with the oxygen diffusion *rate*, which is determined by the diffusion distance and the PO2 difference between hypoxic areas and the blood vessel (i.e., the pressure gradient), as determined by Fick's Law. The PO2 difference is determined by oxygen consumption rate of cells surrounding blood vessels as well as the starting PO2 in the blood, which is influenced most heavily by hemoglobin levels. An increase in oxygen utilization in cells would increase the diffusion rate and decrease the average diffusion distance of oxygen, but not the maximum possible diffusion distance.

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    https://physoc.onlinelibrary.wiley.com/doi/pdf/10.1113/jphysiol.1919.sp001844
    https://pubmed.ncbi.nlm.nih.gov/14833874/

4. B Without reoxygenation, it is unlikely that a tumor comprised of any significant proportion of hypoxic cells (even as low as 1%), would be controlled following total doses used in typical radiotherapy protocols. It has been shown that in addition to the absence of the direct effect of oxygen "fixation" on DNA damage, chronically hypoxic cells upregulate pathways leading to greater radiation resistance than acutely hypoxic cells.

5. D Under normoxic conditions, HIF-1α is hydroxylated by prolyl hydroxylase enzymes in a

reaction that uses oxygen as a co-factor. When hydroxylated, HIF-1α is recognized by VHL and degraded. During hypoxia, hydroxylation is prevented and HIF-1α becomes stabilized and active as a transcription factor.

6. E The oxygen enhancement ratio (OER) is the ratio of radiation doses in hypoxic to aerobic conditions needed to achieve the same degree of cell kill. This is lower (~1.5-2.0) for X-ray doses <2 Gy and higher (~3.0) for doses >10 Gy. Chronic hypoxia develops in regions of a tumor distant from blood vessels, which is mainly due to the limited diffusion distance of oxygen. Acute hypoxia is associated with transient changes in vascular perfusion that could be due to either temporary blockage in a blood vessel, vascular status or other factors. Reoxygenation of both chronically and acutely hypoxic cells has been demonstrated in experimental tumors, however, both the mechanisms and the time course of reoxygenation are different for chronic and acute hypoxia. Hypoxia plays a role in tumor control through the induction of hypoxia-inducible factor alpha (HIF-1α) (HIF1A). HIF-1α is degraded under well-oxygenated conditions, but is stabilized under hypoxic conditions. In hypoxia, stabilized HIF-1α dimerizes with constitutively expressed HIF-1β (ARNT) to form a transcription factor that regulates expression of pro-angiogenic genes, including that for vascular endothelial growth factor (*VEGF*).
   a. Dewhirst MW. Relationships between cycling hypoxia, HIF-1, angiogenesis and oxidative stress, Radiat Res, 172:653-665, 2009. PubMed link
   b. Kaelin WG Jr. The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat Rev Cancer, 8:865-873, 2008.PubMed link
   c. Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer, Nat Rev Cancer, 8:967-975, 2008. PubMed link
   d. Bristow RG, Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability, Nat Rev Cancer, 8:180-192, 2008. PubMed link
   e. 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 link
   f. 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 link

7. A [18F]-fluorodeoxyglucose (FDG) – PET would be most useful to measure glycolytic activity in the tumor, but not hypoxia. The other compounds can all be used to detect hypoxic regions in tumors non-invasively.
   a. Lapi SE, Voller TF, Welch MJ. PET imaging of hypoxia, PET Clin 4:17-38, 2009. PubMed link
   b. Brindle K. New approaches for imaging tumour responses to treatment, Nat Rev Cancer, 8:94-107, 2008. PubMed link
   c. Lee ST, Scott AM. Hypoxia positron emission tomography imaging with 18f fluoromisonidazole, Semin Nucl Med, 37:451-461, 2007. PubMed link

8. A A non-invasive method for the detection of hypoxic regions or measurement of oxygen concentration in tumors would help to select patients to receive drugs or specialized therapies aimed at targeting hypoxia. Studies with tirapazamine and use of carbogen have indicated that the benefit of these therapies are limited to patients with hypoxic tumors. While hypoxia influences sensitivity to radiation in isogenic preclinical systems, other genetic factors seem to affect radiation sensitivity to a greater degree across genetically diverse patient populations than does tumor hypoxia.

9. D Under hypoxic conditions, HIF-1α regulates the transcription of the gene encoding VEGF.

10. D Although p53 is activated by hypoxia, this does not occur as a result of HIF activation. p53 is activated at oxygen concentrations lower than that required to activate HIF. HIF regulates glycolysis (e.g GLUT-1), angiogenesis (e.g. VEGF), erythropoiesis (e.g. EPO), and pH (e.g. CA9).

11. B A biphasic survival curve, in which the survival curve initially displays a steep slope followed by a shallower response, would be predicted for irradiation of a mixed population of aerated and hypoxic cells. This type of dose response would be anticipated since the initial portion of the survival curve reflects the killing of radiosensitive aerated cells; whereas the survival curve obtained at higher doses primarily reflects the killing of radioresistant hypoxic cells. A fractionated protocol would be expected to decrease the effect of a hypoxic cell radiosensitizer to enhance tumor control, compared with an acute treatment, since reoxygenation would cause aeration of many of the hypoxic cells between fractions thereby diminishing the apparent effectiveness of the radiosensitizer. The OER is the dose to produce an effect in hypoxic cells divided by the dose to produce the same effect in aerated cells. The diffusion distance in air for oxygen is much greater than 100 µm. Maximum OER is typically observed only when the oxygen concentration reaches about 3-5%.

12. B Increased glucose uptake and accumulation of lactate, even under normoxic conditions (i.e., aerobic glycolysis or the Warburg Effect), is a common feature of cancer cells. This phenomenon clearly indicates that lactate is not a surrogate of tumor hypoxia. Metastasis of tumors is promoted by lactate-induced secretion of hyaluronan by tumor-associated fibroblasts. Radioresistance has been positively correlated with lactate concentrations. Tumor cells ensure sufficient oxygen and nutrient supply for proliferation through lactate- induced secretion of VEGF, resulting in the formation of new vessels. PubMed link

13. C Tumor response following radiation into has been described to occur in 4 discrete stages where stage I involves massive endothelial apoptosis or at least stopping of endothelial proliferation, stage II involves tumor regression via tumor cell death, stage III involves early regrowth of vessels from remnant endothelial and tumor cells with some growth factor support from myeloid bone marrow-derived cells, and stage IV involves late recurrence due to the tumor bed effect mediated by defective neovascularization. PubMed link

14. B In this model, local low-dose radiation of pancreatic cancers in mice efficiently normalizes aberrant tumor vasculature to recruit effector T cells into tumors for enhanced tumor rejection and improved survival. This is mediated by iNOS induction in tumor- associated macrophages that amplify TH1 chemokines and inhibit angiogenic and immune suppressive cytokines. These effects were not observed with high-dose radiation (possibly because of lack of normalization of vasculature, lymphopenia induced by concomitant irradiation of the spleen, or other nearby immunologic effector organs and tissues). PubMed link

15. B The paired survival curve technique is used to determine the proportion of viable clonogenic cells in a tumor that is hypoxic. In this assay, animals possessing tumors are irradiated while breathing either room air (typical tumor response), or where they are clamped to block blood flow. The ratio of the surviving fractions for the cells, under aerated to fully anoxic conditions, provides an estimate of the fraction of the cells in the tumor that are hypoxic under normal conditions. While misonidazole is a hypoxic radiosensitizer, it's effects on hypoxic tumors are not complete, and it can have antineoplastic activity in some well-oxygenated cancer cells as well. Thus an assay involving administration of misonidazole would be "contaminated" by incomplete activity against hypoxic cell and "off-target" killing of normoxic cells. In addition, any assay utilizing a pharmacologic intervention assumes equal distribution throughout the tumor, which may not be the case due to inconsistent vascularization.

16. B Each T cell has about 30,000 antigen-receptor molecules on its surface, consisting of two different polypeptide chains, termed T-cell receptor α (TCRα) and β (TCRβ) chains, linked by a disulfide bond. α/β TCRs are very similar in structure to the Fab fragment of an immunoglobulin molecule, and they 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 γ and δ. Unlike B cells, that can recognize a protein antigen in its native state, T cells via their TCR recognize antigens only after they have been processed into peptides and loaded onto MHC molecules. During the endogenous antigen presentation pathway (most cells can perform this), intracellular proteins are degraded by the proteasome into peptides (typically 9-10 amino acids long), prior to loading onto MHC class I in the ER. In the exogenous pathway, extracellular antigens are taken up by antigen presenting cells (APCs) and degraded to peptides within endosomes (typically 11-19 amino acids long) and bound to MHC class II. During either pathway, full MHC-peptide complexes are transported to the cell surface for recognition by the TCR on CD8+ cytotoxic T cells (MHC I) or CD4+ T helper cells (MHC II). Cross-presentation (only done by APCs) is the display of peptides from extracellular antigens on MHC class I.
Pattern recognition receptors (PPRs) such as Toll-like receptors are predominantly found on APCs and other innate immune cells and are used for the detection danger signals such as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The engagement of PPRs initiates maturation of APCs, especially dendritic cells who are then, and only then, able to adequately stimulate T cells by providing signal 1 (antigen) to the TCR and signal 2 (co-stimulation) to CD28 that amplifies signal 1. PD-1 is an immune checkpoint, that inhibits TCR proximal signaling by sequestering SHP-2 phosphatase and facilitating Csk-mediated inhibitory phosphorylation of Lck.

17. D LAG3 (binding MHC II), PD-1 (binding PD-L1, PD-L2), TIM3 (binding Gal-9, PtdSer, HMGB1 and Ceacam-1) and CTLA-4 (CD80, CD86) are co-inhibitory receptors, i.e. immune checkpoints 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 the opposite, i.e. it re-enforces T cell activation 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+ T cells, it seems that most of its effects are derived from down-modulation of helper T cell activity and enhancement of Treg 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 to limit autoimmunity, i.e. immune suppression within the tumor microenvironment. PD1 expression is induced when T cells become activated. 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 probably induced. The general concept is that blocking CTLA-4 affects early T cell activation whereas PD-1/L1 blockade is more relevant later on at the tissue site, hence explaining why the former has more side effects.
cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death receptor 1 (PD-1), T cell immunoglobulin and mucin domain 3 (TIM3), galectin-9 (Gal-9), phosphatidylserine (PtdSer); high mobility group protein B1 (HMGB1), carcinoembryonic antigen cell adhesion molecule 1 (Ceacam-1)
Pardoll D.M. The blockade of immune checkpoints in cancer immunotherapy. Nature Reviews Cancer volume 12, pages 252–264 (2012)
Mahoney et al. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma Clin Ther. 2015 April 1; 37(4): 764–782. doi:10.1016/j.clinthera.2015.02.018.

18. B Danger signals, MHC I expression, pro-inflammatory cytokines and epitope spreading would support the development of an immune response including anti- tumor immunity, regulatory T cells would not. Regulatory T cells (or suppressor T cells) are a subset of CD4+ T cells that express the transcription factor Foxp3 and potently suppress immune responses to self and non-self, essential to the maintenance of peripheral immunological tolerance. Tregs suppress activation, proliferation and cytokine production of CD4+ T cells and CD8+ T cells, and are thought to suppress B cells and dendritic cells. They exert their suppressive activity through cell-to-cell contact and through the production of soluble messengers, which have a suppressive function, e.g. 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 linked syndrome (IPEX). 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 antigen presenting cells e.g. dendritic cells (DCs) initiates their maturation and allows these cells to process and present antigens to T cells (signal 1) with the necessary secondary signal (co-stimulation or signal 2), both of which are necessary to induce T cell activation. Antigen presentation without that second signal, i.e. without danger signals and/or maturation can cause T cells anergy. Antigenic peptides are presented within the MHC cleft on the cell surface with the assumption is that an increase in MHC I expression would be supportive of better antigen presentation, making tumor cells more 'visible' to T cells. Tumor cell death in response to immunotherapy may lead to the release of secondary (ie, 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.
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19. A Beta-2-microglobulin (β2-microglobulin, B2M) (not beta2-microtubulin) is a crucial component of major histocompatibility complex (MHC) class I molecules, present on all nucleated cells (excluding red blood cells). MHC I are heterodimers made of two, non-covalently linked polypeptide chains, α and B2M and the conformation of MHC I is highly dependent on the presence of B2M. B2M is essential for proper MHC class I folding and transport to the cell surface, and its deficiency has long been recognized as a genetic mechanism of acquired resistance to immunotherapy.
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20. E *Schreiber et al. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion Science 25 Mar 2011: Vol. 331, Issue 6024, pp. 1565-1570 DOI: 10.1126/science.1203486*

21. A

22. 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 aCTLA-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 on their surface. 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 (PD-1) 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 PD-1 and continue to express it in tissues. Inflammatory signals in the tissues induce the expression of PD-1 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 PD-1 ligand 1 (PD-L1; also known as B7 H1) induction is IFNg, which is predominantly produced by T helper 1 (TH1) cells, although many of the signals have not yet been defined completely. Excessive induction of PD-1 on T cells in the setting of chronic antigen exposure can induce an exhaustive or anergic state.

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    Pardoll D.M. The blockade of immune checkpoints in cancer immunotherapy. Nature Reviews Cancer volume 12, pages 252–264 (2012)

23. C

24. A

25. 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. on behalf of the Society for Immunotherapy of Cancer Toxicity Management Working Group Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group Journal for ImmunoTherapy of Cancer20175:95 https://doi.org/10.1186/s40425-017-0300-z

26. B
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    Ribas Adaptive Immune Resistance: How Cancer protects from Immune Attack. Cancer Discovery 2015 DOI: 10.1158/2159-8290.CD-15-0563

27. E

28. D Glutathione peroxidases (GPx) are enzymes that detoxify peroxides and hydroperoxides. Selenocysteine is present in the active site of many GPx and facilitate the reduction of peroxides into alcohols. Knockdown of GPx1 in human prostate cancer cells enhances the formation of radiation induced micronuclei. GPx2 is upregulated in colorectal cancer, Barrett's esophagus, squamous cell carcinoma, and lung adenocarcinoma's of smokers. GPx4 is the only GPx that is able to reduce hydroperoxides within membranes. PMID: 23201771

29. A Despite its recent attention, cancer immuno-therapeutics have existed since the 19th century. A notable example is the 1893 report from William Coley of the use of erysipelas inoculations to treat sarcomas. In the paper he describes several remarkable tumor responses from both inadvertent and intentional erysipelas infections. He would later develop a heat-inactivated mixture of S. pyogenes and B. prodigious termed "Coley's toxins." While he reported many successes in treating patients, Coley's toxins fell out of favor due to limited reproducibility and supplantation by radiation therapy and then chemotherapy. The first claimed use of radiation to treat cancer was by Emil Grubbe in 1896. Much later, the Bacillus-Calmette-Guérin (BCG) would be shown in the 1950s-1970s to have anti-tumor effects in mice and then in human cancers. However, it too was superseded by other therapeutics except for the treatment of superficial bladder cancers. Interferon was discovered in 1957 but not shown to have anti-cancer effects until 1984 when it demonstrated a response against hairy cell leukemia; it was later used for chronic myelogenous leukemia and melanoma. Soon after, high-dose interleukin-2 was shown effective in renal cell carcinoma and melanoma, with FDA approvals in 1992 and 1998, respectively. Trastuzumab was developed in 1990s as the first antibody-based therapy in breast cancer treatment and was approved for use in 1998. Ipilimumab was the first immunotherapeutic based on immune checkpoint blockade and was FDA-approved in 2011.
    REFERENCES

    1. Coley. The American Journal of Medical Sciences (1893) 105(5): 487-510
    2. Decker et al. Front Immunol (2017) PMID: 28824608
    3. Connell and Hellman. Cancer Research (2009) PMID: 19147546
    4. Herr and Morales. J Urol (2008) PMID: 17997439
    5. Jiang et al. Oncoimmunology (2016) PMID: 27471638

30. C CTLA-4 is a critical molecule in immune regulation. CTLA-4 is upregulated by T-cell activation and allows for a negative feedback loop to prevent an inappropriately prolonged T-cell response. It binds to CD80 and CD86 on antigen-presenting and B cells thus competing for the T-cell costimulatory protein CD28, thus limiting T cell activation. It is also expressed by regulatory T cells to promote immune suppression. Without CTLA-4, mice develop a massive lymphoproliferative disorder and die within 2-3 weeks of birth. CTLA-4 expression is expressed by activated effector T cells and not just Tregs; it can also be expressed variably by tumor cells. Ipilimumab is a monoclonal antibody targeting CTLA-4 and was the first immune checkpoint inhibitor to see clinical use, namely in the treatment of melanoma.
    REFERENCES

    1. Khattri et al. J Immunol (1999) PMID: 10229811
    2. Contardi et al. Int J Cancer (2005) PMID: 15912538

31. D PD-1 stands for programmed cell death protein-1. It is expressed on a variety of cells but most notably activated T cells, and its upregulation is seen after T-cell receptor (TCR) activation.
    REFERENCES

    1. Sharpe et al. Nat Rev Immunol (2018) PMID: 28990585

32. B Checkpoint inhibitor therapy has a different toxicity profile compared to conventional cytotoxic chemotherapy, namely the absence of cytopenia and low-grade nausea. However, there is an approximate 10-20% risk of grade 3+ autoimmune toxicity, mainly thyroid dysfunction, colitis, and pneumonitis. These estimates come from anti-PDL1 monotherapy trials

including KEYNOTE-024 (pembrolizumab/NSCLC), KEYNOTE-006, (pembrolizumab/melanoma), CheckMate-066 (nivolumab/melanoma), and CheckMate-025 trial (nivolumab/renal cell carcinoma).

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1. Reck et al. NEJM (2016) PMID: 27718847
2. Robert et al. NEJM (2015) PMID: 25891173
3. Robert et al. NEJM (2015) PMID: 25399552
4. Motzer et al. NEJM (2015) PMID: 26406148

Cell and Tissue Kinetics

1. B The survival fraction for mitotic cells exposed to radiation is dominated by the linear (or alpha) component of cell killing . Mitotic cells possess little-to-no capacity for sublethal damage repair, which results in low quadratic (or beta) component of cell death. Therefore, mitotic cells are characterized by high α/β ratios.

2. C Assuming that all cells are proliferating, the number of cells in a tumor that doubled in diameter would increase approximately 8-fold as the number of cells can be approximated from the volume of a sphere which is equal to $\pi d^3/6$. An 8-fold increase in the cell number would require three cell doublings. Since it took 18 days to achieve this increase, the cell cycle time can be estimated at 6 days.

3. D The CDK1/cyclin B complex plays an important role in the transition of cells from G_2 phase into mitosis.

4. B Cell loss is often the main factor that determines the tumor volume doubling time, since tumors with a low cell loss factor will grow more rapidly than tumors with a high cell loss factor. The growth fraction of a tumor is the number of proliferating cells in a tumor divided by the number of proliferating and quiescent cells in the tumor. For growth fraction determination, cells are defined as proliferating by the detection of proteins associated with proliferation and not by rigorous tumor transplant assays. The growth rate generally decreases with increasing tumor size. Volume doubling times are longer than the value that would be predicted from the cell cycle time of individual cells because the growth fraction is usually less than one and the cell loss factor may be large. The volume doubling time is a reflection primarily of the growth fraction and cell loss factor.

5. E Since the cell loss factor is equal to 1.0, the tumor would remain the same size since for every new cell produced, one existing cell would die and slough off the tumor.

6. A T_{pot} represents the time it would take a tumor to double its cell number in the absence of cell loss (i.e., $\phi = 0$).

7. B The T_{pot} for most head and neck tumors is in the range of 2-6 days. Overall tumor growth is a combination of T_{pot} (which accounts for growth fraction, $T_{pot} = T_C/GF$ where T_C = cell cycle time, GF = growth fraction) and cell loss factor ($\phi = 1 - (T_{pot}/T_D)$, where T_D = tumor doubling time.

8. E The T_{pot} is equal to λT_S /LI = (0.7)(10 hours)/0.2 = 35 hours.

9. B. If the treatment was delayed for 20 days , then the number of cells in the tumor in patient B would double the number of cells present when the cancer was diagnosed. In contrast, the tumor in patient A is growing more rapidly with a T_{pot} of 5 days. Therefore, if treatment was delayed for 20 days, the cancer in this patient would go through 4 doubling times, leading to 2 x 2 x 2 x 2 = 16 times as many cells. Thus, the ratio of the number of cells in the tumors in patients A and B would be 8:1. Examples like this illustrate how tumors of varying degrees of growth will experience large differences in volume across time.

10. A The most likely reason why a tumor made up of cells with a short cell cycle time would grow slowly is most likely due to a high cell loss factor. A large growth fraction would contribute to a short volume doubling time. A small percentage of cells entering G_0 would increase the fraction of cycling cells and thus promote a short volume doubling time. A large hypoxic fraction may indirectly affect cell cycle time and cell loss factor, but is not the best answer of the choices given. A long S phase is at odds with the premise of the question, given a short cell cycle time. The majority of variation in cell cycle time occurs during G_1, not S phase.

11. E The volume doubling time can be estimated from the equation $\phi = 1-(T_{pot}/T_{vol})$ where ϕ is the cell loss factor, T_{pot} is the potential doubling time and T_{vol} is the measured volume doubling time. Therefore, 0.9 = 1 - (20 days/T_{vol}) or T_{vol} = 200 days.

12. C The cell cycle can be analysed by flow cytometry using a fluorescence-activated cell sorter (FACS). Cells are treated with a DNA-binding dye, such as propidium iodide. The amount of cellular DNA is proportional to the amount of fluorescence detected by dye binding. For a proliferating cell population, a plot of cell number versus DNA content yields the percentage of cells in G1 (with 1 arbitrary unit of DNA or 2N), in G2 + M (with 2 arbitrary unit of DNA or 4N), and in S (cells with more than 1 but less 2 units of DNA. Annexin V conjugates provide a method for studying the externalization of phosphatidylserine, an indicator of intermediate stages of apoptosis, by flow cytometry. Nuclear fragmentation, mitochondrial membrane potential flux, and caspase-3 activation precede phosphatidylserine "in-out flipping" during apoptosis, whereas permeability to propidium iodide occur later. Antibody staining for caspase 8 can also be measured using flow cytometry to detect apoptosis and is not used for cell cycle analysis. Treatment with a high dose of thymidine can stop DNA synthesis, because of deoxyribonucleotide feedback inhibition. This will lead to a build-up of cells at the G1/S checkpoint. Radioactive labeling is not used in flow cytometry.

13. D p21inhibits cyclin A-Cdk2 and cyclin E-Cdk2 activity, which in turn prevents G_1 cells from entering into S phase. The cyclin E/A-Cdk2 complex is required for entry of G_1 cells into S-phase. The induction of p21 in X-irradiated cells is dependent on functional p53. p53 is a transcription factor which is activated in response to a wide variety of genotoxic stresses, frequently via post-translational modification. In response to ionizing radiation-induced DNA damage the existing p53 protein is modified by phosphorylation at multiple sites. The modified p53 becomes more stable (that is, its half-time is significantly increased), which results in increased amounts of the p53 protein, and confers its activity as a transcription factor. The labeling index is the fraction of cells in S-phase, relative to the total number of cells in a proliferating cell population. Choices A and B imply the paradoxical increase in the number of the S-cell population following G_1/S arrest.

14. B The incorporation of radioiotopes into the DNA and autoradiography is the technique to estimate the length of S phase as a fraction of the whole cycle (labelling index) in dividing cells. In 1953, Alma Howard and S.R. Pelc discovered the cell cycle by observing that a radioisotope (^{32}P or ^{35}S) is taken up in the nucleus of plant cells at a distinct interval after mitosis. This led them to conclude that the DNA in the nucleus is replicated during only a limited portion of interphase and called this period of DNA synthesis the S phase of the cell cycle. Howard and Pelc studied the meristem of the bean root tip. They found a 30 h cell cycle for these meristem cells, with 4 h for mitosis (M), 6 h for DNA synthesis (S), a time post-division to DNA synthesis (12 h for G_1), and another time interval from after DNA synthesis to mitosis (8 h for G_2) (G = gap). Variations of this technique, including ^3H thymidine autoradiography and bromodeoxyuridine incorporation, have been used to estimate cell cycle time periods for other species. Cell cycle times in mammalian cells vary widely, from less than 8 h to more than a year in adult animals, with most of the variability being in the length of the G_1 phase.

15. B The checkpoints are accessories of the cell cycle control machinery and inactive under normal physiological conditions. When the cell misbehaves by a set of specific criteria that define what is “normal”, the checkpoint is activated and the cell is forced to halt before its entry into the next phase. While the M checkpoint is best studied, the G_1 checkpoint is arguably more important because it prevents passing defective DNA to daughter cells by initiating repair processes. The M phase consists of nuclear division (mitosis) and cytoplasmic division (cytokinesis). Mammalian cells do not have a cell wall. MPF = the M-phase-promoting factor (former “maturation-promoting factor”). MPF first discovered in mature unfertilized Xenopus eggs (which are arrested in M phase), is a ubiquitous inducer of mitosis.

16. D Transition through the checkpoints are regulated by a combination of cyclins, cyclin dependent kinases (Cdk) and cyclin kinase inhibitors (CKI). CKIs bind to Cdk or to Cdk-cyclin complex and inhibit the kinase activity of CdK. Cyclins are present only during short periods within the cell cycle and are controlled by their own degradation. The Cdks remain throughout the cell cycle but are inactive without their corresponding cyclins. Cdks are activated in a cascade with the cyclins, and have several control CKIs, such as p16, p27 and p21 that serve as regulators and indicators of cell physiological conditions, including nuclear DNA damage. New histones are synthesized along with the new DNA molecule during S-phase. Cyclins are degraded by the ubiquitin proteosome system.

17. C Homologous recombination involves the repair of a damaged section of DNA by having the homologous sister chromatid provide a template for repair. This can only occur at times when a sister chromatid exists (late G2 and late S phases). PubMed link

18. B Homologous recombination occurs predominantly during G2 and S phases, when another copy of DNA is available as a template for error-free repair. Data show that non-homologous end joining (NHEJ) dominates throughout the cell cycle, even during S phase and G2, however during S/G2 the cell also has the additional ability to undergo HR. PubMed link

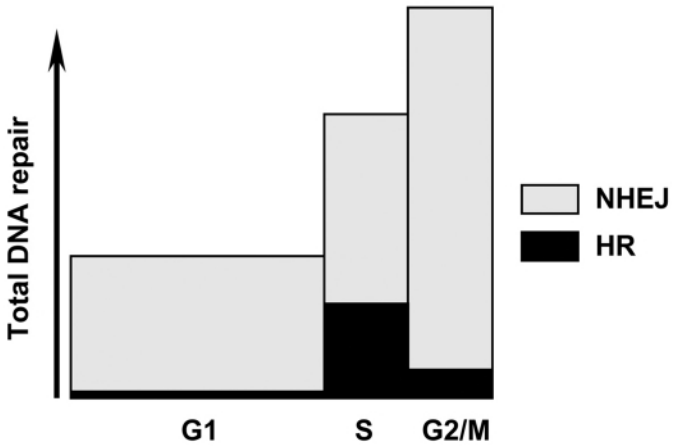

Image from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2754209/

19. C There are checkpoints in G1 (to stop entry into S-phase, within S-phase and in G-2 (to stop entry into M). (Hall 8th Edition Chapter 22)

20. C The G1 checkpoint is controlled by p16, which inhibits CDK 4/6 to prevent it from interacting with cyclin D1. When complexed, cyclin D1/CDK4/6 phosphorylates Rb, which in turn de-represses the E2F transcription factors to allow cell cycle progression. Interrupting any of these intermediates may alter cell cycle progression. PubMed link

21. B Normally Cdc25 acts as a phosphatase to dephosphorylate CDK proteins, thereby promoting CDK activity and cell cycle progression. Cdc25 inactivation occurs after phophorylation by ATM in the setting of DNA damage, resulting in the destruction of Cdc25 and the activation of the G2 checkpoint. PubMed link

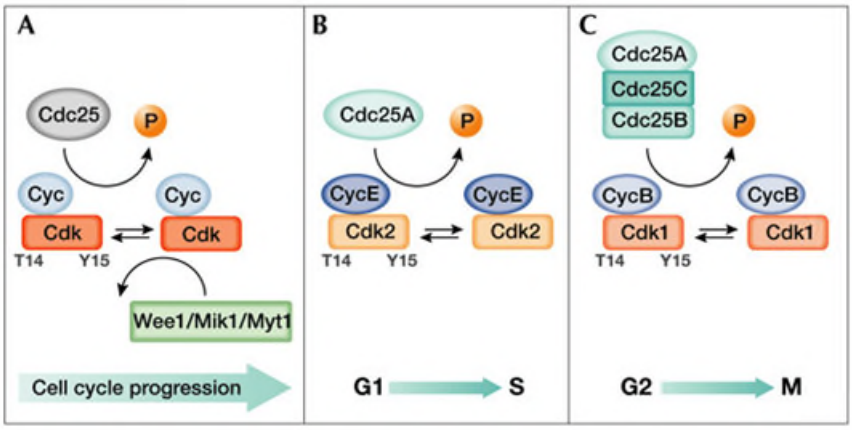

Image from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1326326/

Molecular Signaling

1. D Growth factor receptors generally have three domains: an extracellular ligand-binding domain, a trans-membrane domain that spans the plasma membrane of the cell, and an intracellular kinase domain. Mutations can occur in all three domains in ways that contribute to cancer development. The resultant changes in the protein often lead to constitutive kinase activity, which signals the cell to proliferate, not to undergo senescence. Kinases are proteins that attach phosphate groups to other molecules. Such receptor mutations have not been shown to stimulate general protein translation, cause DNA damage that would stimulate formation of γ-H2AX foci or affect caspase 3, which is normally activated by cleavage, not ubiquitination, to cause apoptosis.

2. C In order to become active, the RAS protein must be prenylated by the action of farnesyl transferases. Hence, RAS activation in cells can be prevented by farnesyl transferase inhibitors (FTIs). It has been postulated that this could decrease the growth of cancer cells and should cause radiation sensitization, since some studies have found a correlation between RAS expression and radioresistance. In clinical studies however, FTIs have had less effect than anticipated on cancer cells because RAS can also be geranylated by geranylgeranyl transferases, which the FTIs do not block. It now appears that FTIs may have additional cellular effects, which are still under investigation. HDAC (histone deacetylase) inhibitors alter chromatin configuration and may be radiation sensitizers; cyclin-dependent kinases are intracellular enzymes involved in regulating the cell cycle; I-κB is an intracellular inhibitory molecule that regulates the transcription factor NF-κB; and Iressa is an inhibitor of ERBB1.

3. E All of the processes listed, except binding of FAS ligand to FAS receptor on the plasma membrane, have been associated with p53 activation. Binding of FAS ligand to FAS receptor activates the extrinsic pathway to apoptosis, which does not appear to involve p53.

4. A RAS is a GTPase. In the active form, Ras is bound to GTP; in the inactive form, it is bound to GDP. The GTPase activity of Ras is activated by GTPase activating proteins (GAPs), which promote Ras to cleave GTP into GDP and inorganic phosphate.
    a. Vigil D, Cherfils J, Rossman KL, Der CJ. Ras superfamily GEFs and GAPs: Validated and tractable targets for cancer therapy?, Nature Reviews Cancer 10:842-857, 2010. PubMed link
    b. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer, Nat Rev Cancer, 7:295-308, 2007. PubMed link

5. D FADD (FAS-associated death domain) protein plays an important role in the extrinsic apoptotic pathway through activation of caspase 8. FADD is associated with the Fas receptor at the plasma membrane. Activated RAS stimulates cellular proliferation through activation of multiple pathways including the RAF, MAPK/ERK, MEK, JNK, RAC/RHO, PLC and PI3K/AKT pathways.

6. C Epigenetic regulation of genes can occur at the level of the histone proteins intimately associated with the DNA. Modification of the histones that surround the DNA can lead to complex signaling that directs the packing and unpacking of the DNA double helix. Epigenetic regulation of histones can occur through acetylation, phosphorylation, methylation and ubiquitination. Nitrosylation does not occur.
    a. Camphausen K, Tofilon PJ. Inhibition of histone deacetylation: A strategy for tumor radiosensitization, J Clin Oncol, 25:4051-4056, 2007. PubMed link
    b. Lohrum M, Stunnenberg HG, Logie C. The new frontier in cancer research: Deciphering cancer epigenetics, Int J Biochem Cell Biol, 39:1450-1461, 2007. PubMed link

7. A EGFR is activated in tumors by overexpression or mutation and functions to induce proliferation. EGFR activation would prevent, not promote, apoptosis, autophagy, and cell cycle arrest. The pathways activated by EGFR may also stimulate DNA repair, and promote angiogenesis. As such, it is an important target for therapy.

8. C MET induction by radiation induces radioresistance via the ATM-NF-kB pathway. MET activation by radiation stimulates invasion through epithelial to mesenchymal transition and makes cells resistant to apoptosis. J Natl Cancer Inst. 2011 Apr 20;103(8):645-61. PubMed link

9. A Oncogene addiction is a term first coined by Weinstein. Oncogene addiction is the phenomenon that despite the diverse array of genetic lesions typical of cancer – some tumors rely on one single dominant oncogene for growth and survival, so that inhibition of this specific oncogene product is sufficient to halt the neoplastic phenotype (21953712). Answer "a" is correct as imatinib targets BCR-ABL. The other answers are all examples of oncogene addicted cancers that are treated with agents that do not target the dominant oncogene product.

10. B The correct answer is "b". Prostate cancer is characterized by its dependence on androgen receptor and frequent activation of PI3K signaling. AR transcriptional output is decreased in human and murine tumors with PTEN deletion and that PI3K pathway inhibition activates AR signaling by relieving feedback inhibition of HER kinases. Similarly, AR inhibition activates AKT signaling by reducing levels of the AKT phosphatase PHLPP. Thus, these two oncogenic pathways cross-regulate each other by reciprocal feedback. Inhibition of one activates the other, thereby maintaining tumor cell survival. However, combined pharmacologic inhibition of PI3K and AR signaling causes near complete prostate cancer regressions in a PTEN-deficient murine prostate cancer model and in human prostate cancer xenografts, indicating that both pathways coordinately support survival. PubMed link

11. B Mutations in RAS are associated with decreased radioresponsiveness. This is likely due to constitutive signaling through the Ras-MAPK pathway. PubMed link

12. A Merlin is a tumor suppressor protein encoded by the *NF2* gene on chromosome 22. Loss of function mutations in *NF2* cause neurofibromatosis type 2. Patients with neurofibromatosis type 2 may develop bilateral vestibular schwannomas, meningiomas, and ependymomas. In Schwann cell, merlin **blocks** YAP nuclear accumulation and thereby inhibits cell proliferation (PMID: 24558021). YAP, or Yes-associated protein 1, is a transcriptional regulator of the cell surface receptors PDGF, PDGFRβ, Her3, and Her2. PDGF, PDGFRβ, Her3, and Her2 receptors can accumulate on the cell surface and promote proliferation and survival. Neurofibromin is a tumor suppressor protein encoded by the *NF1* gene on chromosome 17.

13. C It is estimated that 10-30% of cancers have a Ras mutation with a recent publication demonstrated that 19% of cancer patients have a Ras mutation (PMID: 32209560). HRAS (Harvey sarcoma virus), KRAS (Kirsten sarcoma virus), and NRAS (human neuroblastoma cells) are ubiquitously expressed genes that have similar DNA sequence and function. Ras proteins cycle between an active (GTP-bound) and inactive (GDP-bound state). Ras is attached to the cell membrane by the addition of hydrophobic residues including prenyl groups (prenylation) and palmitic acid (palmitoylation).

Cancer

1. D Telomerase adds specific repeat sequences onto and caps the ends of chromosomes, thereby creating telomeres. This both prevents the ends of chromosomes from shortening with each cell division as well as from unraveling and/or inappropriate identification by the cellular DNA repair enzymes as double strand breaks. Telomerase is generally active in normal stem cells and many tumor cells, but not other differentiated, normal cells, which confers on them unlimited replicative potential, i.e., "immortality". Telomerase does not play a central role in base excision repair and tends to be present at low levels in senescent cells. Inhibition, not stimulation, of telomerase represents a potential means to inhibit proliferation of cancer cells.
    a. O'Sullivan RJ, Karlseder J. Telomeres: protecting chromosomes against genome instability, Nature Reviews Molecular Cell Biology, 11:171-181, 2010. PubMed link
    b. Harley CB. Telomerase and cancer therapeutics, Nat Rev Cancer, 8:167-179, 2008. PubMed link
    c. Gilson E, Géli V. How telomeres are replicated, Nat Rev Mol Cell Biol, 8:825-838, 2007. PubMed link

2. B Since p53-mediated apoptosis is the main way lymphoma cells die following irradiation, possession of a mutation in the p53 gene renders these cells radioresistant, not radiosensitive.
    a. Murray-Zmijewski F, Slee EA, Lu X. A complex barcode underlies the heterogeneous response of p53 to stress, Nat Rev Mol Cell Biol, 9:702-712, 2008. PubMed link
    b. Szumiel I. Intrinsic radiation sensitivity: Cellular signaling is the key, Radiat Res, 169:249-258, 2008. PubMed link
    c. 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 link
    d. Gudkov AV and Komarova EA. The role of p53 in determining sensitivity to radiotherapy, Nat Rev Cancer, 3:117-129, 2003. PubMed link

3. A The RB protein suppresses cell growth by binding to the E2F transcription factor, preventing it from activating the transcription of cell cycle-related proteins that allow the cell to transition from G_1 to S phase. Cell cycle dependent kinases add phosphate, not hydroxyl, groups to the RB gene product causing it to release E2F. A mutant *RB* gene is inherited from one parent in the familial form of retinoblastoma, not the sporadic form. The RB protein product is phosphorylated by CDK4, not CDK1. In the familial form, people who inherit a mutated copy of the *RB* gene exhibit an increased incidence not only of retinoblastoma, but also osteosarcomas, as well as carcinomas of the lung, kidney and bladder.
    a. Mittnacht S. The Retinoblastoma Protein--from Bench to Bedside, Eur J Cell Biol, 84(2-3):97-107, 2005. PubMed link
    b. Massague J. G1 Cell-Cycle Control and Cancer, Nature, 432:298-306, 2004. PubMed link

4. D Although a germline DNA repair deficiency may lead to greater cancer proneness, and somatic mutatons may lead to a higher mutation rate in cancers, it is **not** true that cells derived from all human tumors have such deficiencies.

5. D Oncogenes are frequently activated by point mutations. Examples include single nucleotide mutations in K-ras or in receptor tyrosine kinases that result in constitutive activation. Oncogene activation drives tumor proliferation and carcinogenesis. Epigenetic silencing and gene loss are events that inactivate tumor suppressors. Familial cancers are

caused by inheritance of defective tumor suppressors.

6. B $p16^{INK4A}$ is an inhibitor of CD4 and CDK6. The gene coding for it is a tumor suppressor that is found mutated in many cancers, particularly melanomas and pancreatic cancers. Inactivation of the gene is associated with an increased metastatic potential, but presumably plays no role vis-à-vis tumor hypoxia. Elevations in p16 are seen in HPV-driven cancers due to a failed negative feedback loop where cells become insensitive to the levels of p16 due to E7-dependent inhibition of Rb.

7. A People with Cockayne's syndrome are deficient in transcription-coupled nucleotide excision repair and are characterized by stunting of growth, impaired development of the nervous system, photosensitivity and premature aging. However, there is no evidence for cancer proneness. The other syndromes are associated with the following cancers:
   Bloom's syndrome – leukemia and lymphoma
   Fanconi's anemia – leukemia
   Nijmegen breakage syndrome - lymphoma
   ataxia telangiectasia – leukemia, lymphoma

8. D Following irradiation, ATM activates CHEK2, which then phosphorylates CDC25C phosphatase, preventing it from dephosphorylating CDK1, a step necessary for progression from G_2 into M. Although the mechanism for activation of ATM following irradiation is not clear, it has been suggested that the MRN complex stimulates, not inhibits, its activation. Following irradiation, ATM is autophosphorylated and converted from an inactive dimer to an active monomer. ATM causes phosphorylation of MDM2 and inhibits its inhibitory activity against p53. H2AX is a substrate for ATM kinase activity causing addition of phosphate groups resulting in γH2AX.
   a. Boutros R, Lobjois V, Ducommun B. CDC25 phosphatases in cancer cells: Key players? Good targets?, Nat Rev Cancer, 7:495-507, 2007. PubMed link
   b. Bode AM, Dong Z. Post-translational modification of p53 in tumorigenesis, Nat Rev Cancer, 4:793-805, 2004. PubMed link

9. B BRCA1 is not a phosphatidyl inositol 3-kinase like kinase, whereas ATM, ATR, mTOR and DNA-PK all fall into this category of protein.

10. A NFκB does not inhibit non-homologous end-joining of DNA double strand breaks.

11. B The products of tumor suppressor genes generally inhibit cell growth, not stimulate it.

12. E PTEN is a tumor suppressor gene. . It converts phosphatidylinositol (3,4,5)-triphosphate (PI(3,4,5)P3 into phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2, which directly counteracts the action of PI3K in converting PI(4,5)P2 into PI(3,4,5)P3. NEU, also known as HER2, is a member of the EGFR family. RET is a receptor tyrosine kinase and is mutated in multiple endocrine neoplasias type 2A and 2B. RAS is a family of key proto-oncogenes with at least 32 members involved in many cancers. They are all classified as small GTPases and are mutated in approximately 25% of all human cancers. The most well-known family members are HRAS, KRAS, and NRAS. BRAF, one of 3 RAF genes, is a proto-oncogene that acts downstream of RAS and upstream of ERK.
   Vogelstein B and Kinzler KW, Cancer Genes and the Pathways They Control, Nat Med 10(8): 789-799, 2004. PubMed link

13. B Chronic Myelogenous Leukemia (CML) was once thought to be incurable until the 1990's, until Brian Druker and Charles Sawyers worked together to develop a novel, targeted approach to treat CML. Their therapy, imatinib, is a tyrosine kinase inhibitor which targets the key 9:22 BCR:ABL translocation associated with the disease. A once fatal disease led to 90% cure rates, with survival increasing from 5-6 years to 15-20 years [10]. Their work is important because it further supports the concept that many tumor cells become

dependent on their oncogenes for cell survival, which is referred to as oncogene addiction. PubMed link

14. D People with ataxia telangiectasia do not exhibit an increased sensitivity to UV induced damage which is repaired by nucleotide excision repair. People with ataxia telangiectasia do exhibit progressive ataxia beginning in childhood, telangiectasias of sun exposed skin and the sclera, hypogammaglobulinemia, dysphagia, dysarthria, and chronic lung diseaeses. They also have an increased susceptibility to various cancers, especially leukemias and lymphomas, with an approximate 25% lifetime risk.

15. C A loss of function mutation in a tumor suppressor gene would be dominant in a pedigree. This is highly dependent on gene penetrance but is true for a highly penetrant gene. This is observed because inheritance of a mutated copy of a tumor suppressor would result in the inactivation of one copy of the tumor suppressor gene in all cells in the body. It is likely that, during the course of such an individual's life, the other copy of the tumor suppressor gene would be lost through loss of heterozygosity in at least some cells, thereby creating conditions to promote malignant transformation. A gain of function mutation of an oncogene would be dominant on a cellular level since the protein encoded by the oncogene would then be overexpessed and stimulate malignant progression. A gain of function mutation in a tumor suppressor gene may, if anything, inhibit malignant progression of a tumor since it would likely be inhibitory of cell growth. A loss of function mutation in a tumor suppressor gene is recessive on a cellular level since the remaining normal copy of the gene should encode sufficient protein. A loss of function mutation in an oncogene would probably have either no effect or potentially inhibit cancer susceptibility since there may be a diminished level of the gene product which could reduce cell growth.

16. A Although a mutation in BRCA1 results in a susceptibility for the development of breast cancer, it is not deleted in the majority of breast cancers.

17. D p21 levels **increase** in irradiated cells.

18. A Loss of heterozygosity of a tumor suppressor gene, not an oncogene, often occurs during malignant progression, and involves the loss of a protein that otherwise would play a role in inhibiting cell proliferation.

19. A Hereditary non-polyposid carcinomas of the colon have displayed mutations in mismatch repair genes. Neurofibromatosis and retinoblastoma are associated with the loss of tumor suppressor genes. Ovarian cancers and glioblastomas have been reported to harbor numerous gene defects.

20. A BCL2 is an anti-apoptotic protein that counters the release of cytochrome c from the mitochondria, a necessary step in the intrinsic apoptotic pathway. Therefore, BCL2 over-expressing cells are resistant to apoptosis. BCL2 over-expressing cells do not proliferate rapidly, do not have increased angiogenesis, are not necessarily hypoxic, and do not have decreased DNA double strand break repair.

21. A HPV-16. The estimated worldwide prevalence of infection with HPV in women without a diagnosis is 11-12%. The most prevalent subtype is HPV-16 at 3.2% followed by HPV-18 at 1.4%. HPV-55 is also considered a high-risk subtype, but occurs much less frequently. HPV-6 and HPV-11 are associated with the development of genital warts; they are not considered high-risk subtypes for malignant transformation. (Forman D et al. *Vaccine* 2013;305:F12-23).

22. C Human papillomavirus. Human papillomavirus (HPV) has been linked to cancers of multiple subsites, including the anus, cervix, oropharynx, vulva, vagina, and penis. The oncogenic subtypes are believed to produce the early proteins E6 and E7. E6 goes on to

inactivate p53 and E7 goes on to inactive pRb (Chaturvedi A et al. *Epidemiology, Pathogenesis, and Prevention of Head and Neck Cancer* 2010. These changes in the tumor suppressors then cause subsequent development of cancer through dysregulation of cell cycle checkpoints. Herpes simplex viruses 1 and 2 are not related to cancers. Epstein-Barr virus is linked to the development of nasopharynx cancer, among others, but do not result in E6 and E7 production.

23. C Senescence is a possible response to genotoxic insult that can result in loss of replication capacity. This can be observed in normal tissues and in tumor cells with a wild type p53. Tumor cells that harbor a mutant p53 may be at least partially immune to radiation induced senescence. PubMed link

24. B The correct answer is "b" while the other answers are all surrogate markers or features of CSCs (19249645). Stem cells must be able to self-renew. Importantly, self-renewal and cellular proliferation are not synonymous, since in addition to cell division the former term encompasses both the differentiation and future mitotic potential of the daughter cells. Secondly, stem cells must give rise to daughter cells (i.e. progenitors) that have limited proliferative potential and are destined to differentiate. Through this process stem cells give rise to the mature effector cells that perform a given tissue's biological functions.

25. B Chromothripsis is a catastrophic chromosomal event where portions of the chromosome are broken apart and then joined together in a random way. This leads to several oncogenic changes with a single event. FISH will allow for staining of individual genes on chromosomes. Translocations and small deletions are non-lethal chromosomal aberrations. The other answers are not relevant.

26. A A driver gene is a mutation event that occurs most often early in the development of the cancer and drives the cell to oncogenesis; it is usually in dominant or recessive oncogenes. Other answers are wrong: B drive mutations are most often early in oncogenesis and do not just drive metastasis; C they can but do not necessarily occur in DNA repair genes; D they can be induced by radiation and other mutagens but are not exclusively induced in such a way; E they can be introduced during the process of DNA repair, but they are not exclusively so. None of these answers provides a definition of driver genes except A.

27. B Clonal evolution describes the phenomenon whereby the clonal makeup of a tumor changes over time. This is driven by mutations in individual cells that arise over time. Imperfect DNA damage repair results in mutations, even if a cell contains no overt DNA damage repair deficiencies (e.g., NHEJ). By random chance some of these mutations will be disadvantageous for the cell, while fewer will provide a fitness advantage. Advantageous mutations become enriched in the overall tumor sample over time as they outcompete nearby cells without such advantageous mutations. Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3367003/

28. E Mutational signatures are defined as specific patterns of alterations in DNA bases. This analysis takes into account the alteration of a specific base as well as the base context on the 5' and 3' ends (i.e., the base's immediate neighbors). While many other types of DNA alterations occur in cancer, including but not limited to insertions, deletions, and translocations, mutational signatures refer only to point mutations. While point mutations are randomly distributed throughout the genome, the likelihood of any one specific base to be mutated is heavily dependent on the chemistry of the base itself and its immediate neighbors. This is influenced by 1.) the specific mutagen, and 2.) the DNA damage repair processes at work. There are currently approximately 30 identified mutational signatures, including aging, smoking, APOBEC, mismatch repair deficiency, and UV. Practically, understanding mutational signatures will allow researchers to determine the specific underlying cause(s) of a patient's cancer. Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3990474/

29. C Common mutations in *KRAS* function by preventing GTPase activity. The KRas protein is active in the GTP-bound form and inactive in the GDP-bound form. Guanine nucleotide exchange factors (GEFs) exchange GDP for GTP, thereby activating KRas. GTPase activating proteins (GAPs) are proteins that, when bound to KRas, can induce GTP hydrolysis into GDP. Pathologic mutations of KRAS, including those at positions G12 and G13, prevent KRas from hydrolyzing GTP. Thus KRas remains in the active GTP-bound state, with resultant downstream signaling. Reference:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4869631/

30. B Originally published in 2000, the Halmaks of Cancer represent six fundamental biological characteristics cells acquire in order to transform. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. An update was published in 2011, which added reprogramming of energy metabolism and evading immune destruction. Underlying these hallmarks are the processes of genome instability and inflammation. While all of the hallmarks of cancer can potentially be exploited therapeutically, none of them specifically reference therapy; they only refer to the natural history of cancer. References:

    1. https://www.cell.com/cell/fulltext/S0092-8674(00)81683-9
    2. https://www.cell.com/cell/fulltext/S0092-8674(11)00127-9?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867411001279%3Fshowall%3Dtrue

31. B Cancer stem cells are heterogeneous but are generally more radioresistant and chemoresistant than the more proliferative tumor cell populations, which can be attributed to high anti-oxidant levels, slow cycling, mdr1 expression, and reprogramming. They have characteristic phenotypic markers that can be used to identify and purify them, but there is no single common marker that is shared by all cancer stem cells univocally identifying them. *Rossi, F., Noren, H., Jove, R. et al. Differences and similarities between cancer and somatic stem cells: therapeutic implications. Stem Cell Res Ther 11, 489 (2020).* *https://doi.org/10.1186/s13287-020-02018-6*

32. A Cancer stem cells are highly tumorigenic. This can be shown by injecting graded numbers of different cancer cell populations into mice (also called limiting dilution assay) to determine the number that are needed to grow tumors in 50% of sites, which is the tumor dose 50 (TD50). This is why they are also called “tumor initiating cells”. Human cancer stem cells can be grown in immune deficient mice using the same approach. TD50 tends to be inversely correlated with TCD50 indicating the lower tumor radiocurability with rising levels of cancer stem cells.
*Hill and Milas “The proportion of stem cells in murine tumors.” International Journal of Radiation Oncology*Biology*Physics, Volume 16, Issue 2, 1989, Pages 513-518,* *https://doi.org/10.1016/0360-3016(89)90353-2*.

33. A Yamanaka factors or OKFM induce pluripotent stem cells (iPSCs) from mature cells, such as skin cells or fibroblasts, and earned Yamanaka the 2012 Nobel Prize for Physiology or Medicine.

34. C Bonnet and Dick showed that most of the AML cells were non-tumorigenic but a small population of tumor initiating cells existed that grew in NOD/SCID immune deficient mice. *Bonnet, D.; Dick, J.E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 1997, 3, 730–737.*
35. B Till and McCulloch -considered as the “fathers of stem cell science”- showed that bone marrow-derived colonies arose within 10 days in the spleens of WBI mice (endogenous

assay) or after injection of bone marrow cells (exogenous assay) and prevented acute hematopoietic death. They used this assay to show that the bone marrow was very radiosensitive in general and described in vivo repair and repopulation after radiation exposure. The pluripotent stem cell in the bone marrow is however rare, relatively quiescent and radioresistant and best assessed by cobblestone area forming assay in vitro, or by flow cytometry.
Till and McCulloch "A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells" Radiat. Res. 1961 Vol. 14 Pages 213-222

Ploemacher RE, van der Sluijs JP, van Beurden CA, Baert MR, Chan PL. Use of limiting-dilution type long-term marrow cultures in frequency analysis of marrow-repopulating and spleen colony-forming hematopoietic stem cells in the mouse. Blood. 1991 Nov 15;78(10):2527-33. PMID: 1824250

36. C There are two populations of intestinal stem cells both residing in the crypt: rapidly active intestinal stem cells (LGR5+), and the more quiescent, reserve intestinal stem cells that have proliferative potential and can re-enter the cell cycle to help repopulating the crypt. The LGR5+ stem cells are relatively radiosensitive and depleted after irradiation which is when the Bmi-1+ population comes into play and starts to rapidly proliferate to replenish the LGR5+ population. Cells in the villus are non-proliferating functional cells that are more radioresistant but fairly rapidly lost after irradiation according to their short natural life span. The stem cell compartment regenerates within a few days (crypt turnover time is approximately 3-5 days) before differentiating to replace the functional cells in the villus.
Hans Clevers "The Intestinal Crypt, A Prototype Stem Cell Compartment" Cell, Volume 154, Issue 2, 2013, Pages 274-284, https://doi.org/10.1016/j.cell.2013.07.004.

Kim CK, Yang VW, Bialkowska AB. The Role of Intestinal Stem Cells in Epithelial Regeneration Following Radiation-Induced Gut Injury Rep. Curr Stem Cell Rep. 2017;3(4):320-332. doi:10.1007/s40778-017-0103-7

37. E *Rossi, F., Noren, H., Jove, R. et al. Differences and similarities between cancer and somatic stem cells: therapeutic implications. Stem Cell Res Ther 11, 489 (2020). https://doi.org/10.1186/s13287-020-02018-6*

38. D Cancer stem cells appear to have higher levels of free radical scavengers, abnormal activation of developmental pathways, hyperphosphorylation of checkpoint kinases which collectively tend to drive their superb resistance to radiation.
Claudia Peitzsch, Ina Kurth, Nadja Ebert, Anna Dubrovska & Michael Baumann (2019) Cancer stem cells in radiation response: current views and future perspectives in radiation oncology, International Journal of Radiation Biology, 95:7, 900-911, DOI: 10.1080/09553002.2019.1589023

39. A

Total Body Irradiation

1. A The time to death from the hematopoietic syndrome is about 1-2 months. The latent period before death from the cerebrovascular syndrome is 1-2 days. The threshold doses (the minimum dose at which these syndromes may be detectable in some people in an irradiated population), for hematopoietic and gastrointestinal syndromes are approximately 1 Gy and 5 Gy, respectively. However, it should be noted that doses of approximately 2.5 Gy and 8 Gy are necessary before a substantial portion of an irradiated population would exhibit pronounced symptoms of hematopoietic and gastrointestinal syndromes, respectively. The latent period until death from GI syndrome is about 3-10 days.

2. C Death from the hematopoietic syndrome usually results from infection and hemorrhage due to radiation-induced loss of white cells and platelets.

3. E A person exposed to 3 Gy of γ-rays should be carefully watched for symptoms of infection and hemorrhage resulting from loss of white blood cells and platelets, with the critical period being 2-4 weeks following irradiation. Prophylactic administration of antibiotics should be initiated immediately following the accident, rather than waiting for overt signs of infection. A bone marrow transplant would likely be of no value at this dose, so tissue typing is not necessary, since use of antibiotics and transfusion of blood components, as necessary, would substantially enhance the probability for survival without the use of a transplant. The dose the worker received was too low for her to develop symptoms of the GI syndrome, which include dehydration and bloody diarrhea, likely culminating in death. If the dose received was less than 2 Gy, it would be reasonable to be monitored from home, but following a dose of 3 Gy a person should be hospitalized in reverse air flow isolation with supportive care, including antibiotic administration immediately.
    a. Goans RE, Waselenko JK. Medical management of radiological casualties, Health Phys 89:505-512, 2005. PubMed link
    b. Turai I, Veress K, Gunalp B, *et al*. Medical response to radiation incidents and radionuclear threats, BMJ, 328:568-572, 2004. PubMed link
    c. 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 link

4. D Death from the gastrointestinal syndrome could occur within one week following irradiation, but is unlikely following a whole body dose of 5 Gy. However, a person irradiated with this dose who did not receive appropriate medical care has a greater than 50% chance of dying within a 1-2 month period from bone marrow syndrome. Following a whole body dose of 5 Gy, infections are likely due to loss of white blood cells and lack of treatment with antibiotics. Nausea would be observed during the early prodromal period. Epilation and bleeding would occur during the period before the person dies from hematopoietic syndrome.

5. D A drop in the level of white cells and platelets may be observed following a whole body dose of approximately 1 Gy, although it has been detected at doses as low as 0.5Gy.

6. D A whole body dose that results in severe diarrhea within 4 days of irradiation is likely to be lethal (probably 8 Sv or higher). Therefore, all of the people would be expected to die within 1-2 weeks following irradiation due to GI syndrome.

7. E A person who dies one year following total body irradiation would not die from any of the conventional whole-body radiation syndromes. These syndromes cause death at about 1-2 days (cerebrovascular), 1-2 weeks (gastrointestinal) or 1-2 months (hematopoietic), respectively, following irradiation. Since the dose received was not sufficiently high to cause death from the GI syndrome (i.e., at least 8 Sv), it would likewise not be high enough to cause brain necrosis. However, the treatment dose may have been high enough to cause lung fibrosis, which may result in death, within one year after irradiation.

8. B Immunosuppression observed within 24 hours after irradiation would be the consequence of the rapid death of lymphocytes due to radiation-induced apoptosis. A much longer period than 24 hours would be required for the death of progenitor cells and a loss of granulocytes. Doses much greater than 5 Gy would be necessary to cause decreased activity of NK cells and inactivation of circulating antibodies.

9. A The $LD_{50/60}$ for an acute, whole body irradiation is estimated to be 3.5 Gy without medical intervention and approximately 7 Gy with optimal medical care. The principal causes of death for people who receive a dose close to the $LD_{50/60}$ are infections and hemorrhage. A person who received a dose of about 3.5 Gy would not exhibit the symptoms associated with the GI syndrome, such as severe diarrhea. The $LD_{50/60}$ is the dose that leads to death within 60 days of 50% of the population.

10. A The chronological order for decline of the components of peripheral blood after irradiation is lymphocytes, granulocytes, platelets and lastly erythrocytes.

1. B Increasing the radiation dose decreases the latent period for cataract formation. The lens does not have the ability to eliminate damaged fibers. The RBE for cataract formation following irradiation with a series of small doses is in the range of 50-100 since there is substantial sparing associated with the X-irradiation, thereby substantially increasing the threshold dose to induce a cataract by X-rays. In contrast, the neutron dose to induce a cataract is relatively unaffected by the magnitude of the individual doses. Hence, the RBE, which is the ratio of the X-ray dose divided by the test radiation (neutrons) dose to induce an effect (cataract formation), increases with decreasing fraction size. The threshold dose for the induction of a radiation-induced cataract following an acute X-ray dose is 2 Gy or less. A radiation-induced cataract is one of the few examples of a radiation injury which does have distinct pathognomonic characteristics that identify it as having been induced by ionizing radiation; radiation-induced cataracts typically begin in the posterior portion of the lens, unlike age-related cataracts.
    a. Ainsbury A, Bouffler SD, Dörr W, *et al*. Radiation cataractogenesis: A review of recent studies, Radiat Res, 172:1-9, 2009. PubMed link

2. A Only about 1% of children develop severe restrictive pulmonary disease, although the majority develop some symptoms.
    a. Faraci M, Barra S, Cohen A *et al.* Very late nonfatal consequences of fractionated TBI in children undergoing bone marrow transplant, Int J Radiat Oncol Biol Phys, 63:1568-1575 2005. PubMed link

3. D Dose fractionation increases the risk for sterility in the male; the TD_5 and TD_{50} for sterility are 2 Gy and 8 Gy, respectively, for a single dose of X-rays, whereas these values decrease to 1 Gy and 2 Gy for fractionated irradiation. This effect results from spreading the dose over time permitting reassortment sensitization to occur for spermatogonia, which have a large variation in radiation sensitivity through the course of their cell cycle, and more than compensating for any repair that might occur between fractions. Thus, spermatogonia located in a relatively radioresistant portion of the cell cycle may progress into a more radiosensitive part of the cell cycle at the time of the second and subsequent irradiations. Spermatids and spermatozoa are relatively radioresistant, whereas spermatogonia are radiosensitive. A drop in testosterone levels would not be detectable following a scattered dose of 0.1 Gy to the testes, particularly to an adult. Following a moderate dose of radiation, which kills a large number of spermatogonia, there may be relatively little effect on the levels of spermatocytes, spermatids and spermatozoa initially, since a period of 67 days is required for maturation of a spermatogonial stem cell to a mature spermatozoan. Hence, there may be very little drop in sperm count for the first month following irradiation, although the sperm count will decrease at a later time. Full recovery of a normal sperm count following radiation-induced azoospermia caused by exposure of the testes to a dose of 6 Gy, would require a period of at least 2 years.

4. B Diarrhea usually occurs about 3 weeks after the start of fractionated radiotherapy.

5. A Early myelopathy differs from transient demyelination because it is more severe and progressive, not less so.

6. E Arterial cerebrovasculopathy is an infrequent, not common, occurrence.
    a. Kelsey CR, Marks LB. Somnolence syndrome after focal radiation therapy to the pineal region: Case report and review of the literature, J Neurooncol, 78(2):153-156., 2006. PubMed link
    b. Ryan J. Radiation somnolence syndrome, J Pediatr Oncol Nurs, 17(1):50-53, 2000. PubMed link
    c. 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 link
    d. Tofilon PJ, Fike JR. The Radioresponse of the Central Nervous System: A Dynamic Process, Radiat Res, 153:357-370, 2000. PubMed link

7. C The kidney has a relatively low tolerance dose because of the limited number of clonogens within each nephron, although the cells comprising the functional subunits of the kidney are not particularly radiosensitive. The kidney exhibits substantial sparing with fractionation and displays little or no tolerance to re-irradiation. A much longer latent period than 3 months is required before the appearance of radiation nephropathy.
    a. Cohen EP, Robbins ME. Radiation Nephropathy, Semin Nephro, 23(5):486-499, 2003. PubMed link
    b. 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 link

8. B RILD typically occurs between 2 weeks and 3 months after completion of radiotherapy.
    a. Fajardo LF, Berthrong M, Anderson RE: *Radiation Pathology*. University Press, Oxford, 2001.
    b. 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 link

9. C Atrophic villi would likely be observed within a week following the start of irradiation of the small intestine, since the cells lining the villi have relatively short life spans.

10. B The best way to spare the parotid gland is to decrease the volume of the gland irradiated. The parotid exhibits relatively little sparing with fractionation so use of either a hyperfractionated or hypofractionated protocol would have only a modest impact. Prolongation or acceleration of treatment would have little effect on the parotid.
    a. Konings AW, Coppes RP, Vissink A. On the mechanism of salivary gland radiosensitivity, Int J Radiat Oncol Biol Phys, 62(4):1187-1194, 2005. Review. Erratum in: Int J Radiat Oncol Biol Phys, 64:330, 2006. PubMed link

11. C The kidney exhibits little or no re-irradiation tolerance, whereas the other organs, including the spinal cord, exhibit at least some recovery following irradiation.
    a. Cohen EP and Robbins ME, Radiation Nephropathy, Semin Nephrol, 23, 5:486-499, 2003. PubMed link

12. D There is clinical evidence that pentoxifylline may be helpful for the treatment of radiation fibrosis and osteoradionecrosis.
    a. Delanian S, Lefaix JL. Current management for late normal tissue injury: Radiation-induced fibrosis and necrosis, Semin Radiat Oncol, 17:99-107, 2007. PubMed link

13. A The lacrimal gland is comparable to the parotid in terms of both its structure and the tendency of secreting cells to undergo radiation-induced interphase death.

14. E Temporary epilation can be caused by a 3 Gy acute exposure, and is observed around 3 weeks after irradiation. The doses and times to appearance for the other skin reactions are:

    Temporary erythema - 2 Gy - 1 day
    Permanent epilation - 7 Gy - 3 weeks
    Moist desquamation - 18 Gy - 4 weeks
    Dry desquamation - 14 Gy - 4 weeks

    a. Geleijns J, Wondergem J. X-ray imaging and the skin: Radiation biology, patient dosimetry and observed effects, Radiat Prot Dosimetry, 114(1-3):121-125, 2005. PubMed link

15. D There is an extensive series of laboratory studies that have established a clear role for the renin-angiotensin system in the pathogenesis of radiation nephropathy. Administration of angiotensin-converting enzyme inhibitors (ACEI), such as captopril, and angiotensin type 1 receptor antagonists (AT_1RA), such as L-158,809, have been shown to be effective as prophylactic agents and as mitigators of injury when administered after irradiation. The decline in renal function observed in a patient presenting with radiation nephropathy following TBI was reported to be prevented by administration of losartan, an AT_1RA. At present, there are no randomized clinical studies to suggest that renal function will improve following treatment with ACEI or AT_1RA. The ability of these agents to modulate radiation nephropathy is not due to a reduction in blood pressure; ACEI are effective at doses that do not affect blood pressure. Moreover, administration of antihypertensive agents does not ameliorate radiation nephropathy. The decline in kidney function is not accelerated at low radiation doses.
    a. Cohen EP, Robbins ME. Radiation nephropathy, Semin Nephrol, 23:486-499, 2003. PubMed link
    b. Cohen EP, Hussain S, Moulder JE. Successful treatment of radiation nephropathy with angiotensin II blockade. Int J Radiat Oncol Biol Phys, 55:190-193, 2003. PubMed link
    c. Zhao W, Diz DI, Robbins ME. Oxidative damage pathways in relation to normal tissue injury, Br J Radiol, 80 Spec No 1:S23-31, 2007. PubMed link
    d. 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 link
    e. Robbins ME, Diz DI. Pathogenic role of the renin-angiotensin system in modulating radiation-induced late effects, Int J Radiat Oncol Biol Phys, 64:6-12, 2006. PubMed link

16. B Mucositis is an acute response, not a late effect, and is one of the main dose-limiting toxicities in the management of head and neck and digestive track carcinomas with radiation therapy. The remaining toxicities are some of the chief late complications seen in these patients.
    a. Mantini G, Manfrida S, Cellini F, *et al.* Impact of dose and volume on radiation-induced mucositis, Rays, 30:137-144, 2005. PubMed link
    b. Cooper JS, Fu K, Marks J, Silverman S. Late effects of radiation therapy in the head and neck region, Int J Radiat Oncol Biol Phys, 31:1141-1164, 1995. PubMed link

17. C Although increased permeability of the mucosa in the GI tract is also a key determinant, the altered immunity associated with effects on the lymphoreticular system plays a leading role in the infection that characterizes mortality from the gastrointestinal syndrome. B cells,those that mature in the bone marrow, are more radiosensitive than T cells, due to the sensitivity of the progenitor cells. However, there can be a persistent depression in T cell numbers. Localized radiation to the thymus can predispose a patient to a series of late effects due to the radiation sensitivity of both thymocytes and other thymic cell populations. There is a decrease in spleen size

following radiation, as well as marked fibrosis, thickened capsule, and obliteration of the sinusoids.

18. B At least one parotid gland should receive a mean dose <20 Gy**.** The QUANTEC report provided guidelines on normal tissue toxicity and recommendations on dose constraints to prevent long-term toxicity. For severe xerostomia, the recommendation was that one parotid should receive a mean dose less than 20 Gy or both parotid glands should receive a mean dose less than 25 Gy (Deasy et al. *Int J Radiation Oncology Biol Phys* 2010;76:S58-63). PubMed link

19. A Myofibroblasts and fibroblasts are the predominant cells that elaborate the collagen responsible for radiation fibrosis. Myofirboblasts may originate from a variety of cells including local fibroblasts, epithelial cells, endothelial cells, and other progenitors. To produce collagen these non-fibroblast cells must first undergo epithelial or endothelial to mesenchymal transition. PubMed link

20. E All of these pathways have been implicated in the initiation or perpetuation of radiation fibrosis. TGF-beta is well described as a mediator of radiation fibrosis through downstream signaling and is often considered a drive of the fibrotic process. Reactive oxygen species generated at the time of irradiation and by infiltrating immune cells are also thought to contribute to the perpetuation of injury and can further activate TGF-beta signaling. PDGF has been implicated as a mitogenic signal from multiple cell types that can initiate collagen and extracellular matrix production by myofibroblasts. PubMed link

21. D CTGF, or connective tissue growth factor, is a downstream effector of TGF beta signaling. It is known to be involved in wound repair, fibrosis, and several pathologic states associated with increased extracellular matrix production. PubMed link

22. A Plasma TGF-beta levels have extensively been evaluated as a measure of and predictor for acute and chronic radiation lung injury. Although some controversy exists, newer literature confirms that TGF-beta can play a role in determining which patients are at highest risk of lung injury from radiation. PubMed link

23. D Killing of serous cells in the parotid gland, which causes xerostomia in many head and neck cancer survivors who received radiotherapy, would not be substantially affected by fraction size.
    a. Eisbruch A, Rhodus N, Rosenthal D*, et al.* The prevention and treatment of radiotherapy-induced xerostomia, Semin Radiat Oncol, 13:302-308, 2003. PubMed link
    b. 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 link

24. B Assuming that the mature differentiated cells comprising a tissue do not have a pro-apoptotic tendency, the time to expression of radiation damage in an early-responding tissue correlates best with the lifespan of the mature functional cells. This occurs because tissues with a hierarchical structure (i.e., most early-responding tissues) depend on the constituent stem cells to reproduce and supply new cells to replace the mature ones, when they reach the end of their lifespan. However, because stem cells are likely to be killed by radiation, there is a lack of “replacement” cells when the mature cells reach the end of their lifespan. Therefore, the time scale for the appearance of the radiation injury mimics to a first approximation the lifespan of the mature cells.

25. A bFGF protects against, rather than enhances, radiation-induced apoptosis of endothelial cells.
    a. Brush J, Lipnick SL, Phillips T, *et al.* Molecular mechanisms of late normal tissue injury, Semin Radiat Oncol, 17:121-130, 2007. PubMed link
    b. Fleckenstein K, Gauter-Fleckenstein B, Jackson IL, *et al*. Using biological markers to predict risk of radiation injury, Semin Radiat Oncol, 17:89-98, 2007. PubMed link
    c. 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 link
    d. Bentzen SM. Preventing or reducing late side effects of radiation therapy: Radiobiology meets molecular pathology, Nature Rev Cancer, 6:702-713, 2006. PubMed link
    e. Denham JW, Hauer-Jensen M. The radiotherapeutic injury - a complex "wound", Radiother Oncol, 63:129-145, 2002. PubMed link
    f. Anscher MS, Vujaskovic Z. Mechanisms and Potential Targets for Prevention and Treatment of Normal Tissue Injury after Radiation Therapy, Semin Oncol, 32(2)Suppl 3:S86-91, 2005. PubMed link
    g. 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 link
    h. Paris F, Fuks Z, Kang A, *et al.* Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science. 2001 293(5528):293-7. PubMed link

26. E TGFβ, bFGF, CTGF and PDGF all appear to play a role in radiation- induced lung fibrosis.

27. B The latent period prior to the manifestation of a late effect generally decreases with increasing dose to the irradiated organ.

28. D The dose response for the induction of late normal tissue damage is sigmoidal in shape.

29. C The majority of patients who develop clinically-detectable pneumonitis will progress to fibrosis. It is strongly suspected that many of the patients who develop lung fibrosis in the apparent absence of pneumonitis did, in fact, have pneumonitis, but that it was asymptomatic and had gone unrecognized. Lung is a very sensitive, dose-limiting organ with a steep dose response curve for single dose, whole organ irradiation, characterized by a $TD_{5/5}$ of 7 Gy (the $TD_{5/5}$ for fractionated radiotherapy using a conventional dose per fraction is about 17.5 Gy). Both volume irradiated and fractionation pattern have large effects on the tolerance dose. A number of investigators have identified regions of pneumonitis that extend outside of the treatment field, known as abscopal effects, however the mechanism for their development remains unclear.
    a. Roberts KB, Rockwell S. Radiation pneumonitis. In: *Fishman's Pulmonary Diseases & Disorder*, *4th Ed*, (A.P. Fishman, Ed.) McGraw-Hill, New York, 2009.
    b. 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 link
    c. McDonald S, Rubin P, Phillips TL, Marks LB. 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 link

30. A FSUs contain a relatively constant number of clonogens. FSU's *can* be repopulated from a single surviving clonogen, and, for certain tissues, from clonogens that migrate from an adjacent FSU. For some tissues, FSUs are anatomically discrete structures (such as the nephron in the kidney), although for other tissues, there may not be any clear structural or anatomical unit that corresponds to an FSU (such as in the CNS and skin). FSUs are thought to be functionally independent of each other, even though they may be structurally interdependent.

a. Stewart FA, Van Der Kogel AJ. Proliferative and cellular organization of normal tissues. In: Basic Clinical Radiobiology, Third Edition, Ed. GG Steel, Arnold, London, 2002.
b. 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 link
c. Withers HR, Taylor JM, Maciejewski B. Treatment volume and tissue tolerance, Int J Radiat Oncol Biol Phys, 14:751-759, 1988. PubMed link

31. B The volume of normal tissue included in the irradiation field can have significant effects in the subsequent development of late effects. Despite being a serially arranged tissue like the rectum and spinal cord, several recent studies have shown that increasing the length of the esophagus in the treatment field does not predict the severity or duration of radiation-induced esophagitis. The morphological structure of the lung makes it difficult to define precise threshold limits. However, the best predictor for late effects has been found to be the V_{20}/V_{30}, that is, the percentage of normal lung volume that receives 20 Gy or 30 Gy, respectively. In contrast, in the rectum, it is the percentage of the wall that has received 40-50 Gy that determines the likelihood of rectal bleeding, although the extent of reserve, unirradiated tissue is also a factor. The liver is deemed an organ whose FSU's are arranged in parallel. Early estimates of V_{eff} gave a value of 0.32, but with changes in the standard of care over time, this value has risen to 0.94, emphasizing the importance of treatment volume in the probability of late complications. In the brain, the complex structure and morphology allows for focal radiation necrosis to be distinguished from diffuse white matter changes. The latency period for cerebral necrosis ranges from 6 months to several years post-radiation.
    a. Kong FM, Pan C, Eisbruch A, Ten Haken RK. Physical models and simpler dosimetric descriptors of radiation late toxicity, Semin Radiat Oncol, 17:108-120, 2007. PubMed link

32. D TGFβ generally has an inhibitory effect on epithelial cell proliferation. TGFβ is an important fibrogenic cytokine. It increases proliferation of mesenchymal cells and extracellular matrix deposition, and appears to be mechanistically involved in radiation fibrosis. It is secreted as a biologically inactive (latent) homodimer that is complexed with latency-associated peptide (LAP), and requires activation in order to exert its biological activities. TGFβ is one of the strongest known chemotactic factors for granulocytes, and on a molar basis, has been estimated to be about 1000-fold more potent than cyclosporine as a T-cell suppressor.
    a. Ikushima H, Miyazono K. TGFβ signalling: A complex web in cancer progression, Nature Reviews Cancer, 10:415-424, 2010. PubMed link
    b. Travis EL. Genetic susceptibility to late normal tissue injury, Semin Radiat Oncol, 17:149-55, 2007. PubMed link
    c. Bentzen SM. Preventing or reducing late side effects of radiation therapy: Radiobiology meets molecular pathology, Nat Rev Cancer, 6:702-713, 2006. PubMed link

d. Bierie B, Moses HL. Tumour microenvironment: TGFbeta: The molecular Jekyll and Hyde of cancer, Nat Rev Cancer, 6:506-520, 2006. PubMed link
e. 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 link
f. 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 link
g. 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 link

33. A Evidence from animal studies suggests that at least a partial recovery and re-irradiation tolerance occurs in the spinal cord provided at least 6 months have passed since an initial course of treatment. Soft tissue or bone necrosis *has* been observed in clinical studies involving re-irradiation of recurrent or new primary head and neck tumors. Mouse lungs are capable of tolerating a second course of fractionated irradiation, depending on the total dose given during the first course (the higher the initial total dose, the less tolerance to re-irradiation, and vice versa). Full re-irradiation tolerance for acute damage in rapidly dividing mucosal tissues is generally observed, provided at least a month or two has passed since the initial treatment course. Animal experiments have shown that the kidney does not appear to recover from radiation injury, as it will not tolerate re-irradiation even after a period of several years following the original treatment course.
a. 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 link

34. B The TD_5 (as a function of length irradiated for the spinal cord) decreases with increasing cord length and then remains relatively constant.

35. B Radiation-induced epilation occurs before dermatitis due to the short cell cycle time of the cells in the germinal matrix of the hair bulb, compared to that of the basal cells of the epidermis.

36. A Irradiation of a small volume of the spinal cord to 70 Gy can cause myelopathy because of the serial arrangement of the FSUs in this organ (i.e., inactivation of a single FSU can compromise the function of the entire organ), whereas the FSUs in the other organs are arranged in parallel, meaning that these organs have a large functional reserve and therefore can tolerate high doses provided the irradiated volume is small.

37. C The three main steps of wound healing include inflammation, proliferation, and remodeling. Initially, pro-inflammatory cytokines stimulate angiogenesis and fibroblast activation as well as keratinocyte activation and wound contraction. Fibroblasts then migrate to the wound and granulation tissue formation and collagen deposition. The final step of wound healing is remodeling which includes regression of capillaries and collagen degeneration.

Therapeutic Ratio

1. E In order to achieve a 37% tumor control probability, the total dose delivered must reduce the number of surviving clonogenic cells to an average of 1. This is based on the equation $P = e^{-(M)(SF)}$, where P is the probability of tumor cure (37% or 0.37 in this case), M is the initial number of tumor clonogens (10^6), and SF is the surviving fraction resulting from the irradiation protocol. Thus, for 10^6 clonogenic cells, a total dose that reduces the surviving fraction to 10^{-6} (i.e., 1 surviving clonogen) must be used to achieve a 37% control rate. Since the survival curve is exponential with a D_{10} of 5.75 Gy (D_{10}, = D_0 X ln 10 = 2.5 X 2.3 = 5.75 Gy) it would be necessary to use a dose of 34.5 Gy.

2. C Three cell divisions would result in an 8-fold increase in the number of cells. Therefore, the dose would need to be increased by a dose D, where $e^{(D/D0)} = 8$. Therefore, D = 2.5 x ln 8 = 5.2 Gy of additional dose would be needed to achieve the same level of tumor control. It is also worth remembering that 3.3 times the number of cell doublings corresponds to one $\log_{10}$ of cell kill.

3. B Since the chemotherapy results in a surviving fraction of 10^{-4}, the number of clonogens in the tumor would be reduced from $8x10^6$ to $8x10^2$. Since the D_{10} for this tumor is 5.75 Gy, then a dose of approximately 17 Gy would produce a 37% control rate. Another way to more precisely determine the answer to this problem is to recognize that since the chemotherapy results in a surviving fraction of 10^{-4}, the amount of radiation dose, D, NOT needed is given by $SF = e^{(-D/D0)} = 10^{-4}$. Therefore $-D/D_0 = \ln 10^{-4}$ or $D = -(D_0)(-\ln 10^{-4}) = -(2.5)(-9.2) = 23$ Gy and so the final dose required is 34.5 + 5.2 – 23 = 16.7 Gy. Alternatively, with use of chemotherapy, the number of clonogens is reduced from $8x10^6$ to $8x10^2$, so the dose D now required for 37% cure is given by $D = (2.5)[\ln(8 \times 10^2)] = (2.5)(6.7) = 16.8$ Gy.

4. B Tumor A has a low α/β ratio and therefore this tumor will exhibit a high degree of sparing with dose fractionation. In contrast, tumor B, which has a high α/β ratio will exhibit correspondingly less sparing with fractionation. Thus, the TCD50 for a fractionated protocol will be higher for tumor A compared with tumor B.

5. D During a 3 week (21 day) break, cells with a 3 day doubling time will undergo 7 additional doublings, leading to an increase in the number of tumor cells by a factor of 128. Solving for x in the equation $(0.3)^x = 1/128$, where x is the number of fractions, yields $x \approx 4$. (Taking the logarithm of both sides of the equation gives x log 0.3 = - log 128, so x = 2.10/0.52). Thus, "compensating" for the extra cells produced by proliferation would require an additional 4 fractions of 2 Gy, or 8 Gy.

6. D In order to achieve a 90% tumor control probability, it is necessary to reduce the number of tumor cells to 0.1 (on average). Since the extrapolation number is 1 for the cells comprising the tumor, it can be assumed that there is little or no "shoulder" on the survival curve. Thus, for a tumor with 10^8 cells initially, the surviving fraction would need to be 10^{-9}. This would be achieved by a dose of 4 Gy x 9 logs = 36 Gy.

7. A If a tumor increases its volume by a constant fraction per unit time, then it would display exponential growth as per the equation $V = e^{(.693)(T/Tv)}$, where T is the total elapsed time and T_v is the tumor's volume doubling time. In practice however, this is rarely observed because as a tumor grows, generally the growth fraction decreases and cell loss increases. This type of progressively slowing growth curve is best fit using the Gompertz equation,, where V_0 is the volume at time zero and A and B are growth parameters specific for the

particular tumor. At small times for t, the equation is exponential with $V = V_0e^{At}$. At long times, e^{-Bt} becomes very small, so the volume reaches a maximum of $V_0e^{A/B}$.

a. Joiner M and van der Kogel A, Eds. Basic Clinical Radiobiology, 4th Ed. Hodder Arnold, London, 2009; page 79.

8. E The dose reduction factor (DRF) is a parameter used to measure the effectiveness of a radioprotector. The DRF equals the dose to produce a certain effect in the presence of a radioprotector divided by the dose to produce the same effect in the absence of the protector. Thus, 1.3 = x/30 Gy, so x = 39 Gy.

9. E To produce a TCD_{90} for a series of tumors containing 10^6 clonogenic cells would require a total dose that would reduce the surviving fraction to 10^{-7}. Since 56 Gy produced this level of control, the D_{10} for these cells must be approximately 56 Gy/7 logs = 8 Gy. The relative increase in the number of clonogens resulting from an increase in tumor diameter from 0.1 cm to 1 cm is $(1/0.1)^3 = 10^3$, so the number of cells would increase from 10^6 to 10^9. To produce 90% control, would require 8 Gy x 10 logs = 80 Gy. Depending on the normal tissue(s) of concern in the radiation field, its tolerance dose, and how much of its volume would need to be irradiated, delivering a total dose of 80 Gy may or may not be feasible.

1. C An analysis of multifraction isoeffect data for normal tissues and tumors *in vivo* forms the basis for the determination of the α/β ratio. This is accomplished by generating a so-called reciprocal dose plot ("F_e plot"), a type of isoeffect curve in which the reciprocal of the total dose to produce an isoeffect is plotted as a function of the dose per fraction used in multifractionation experiments. Based on such an isoeffect curve (which should be linear in shape assuming the linear-quadratic model provides a good fit to the data), the α/β ratio would be equal to the intercept of the curve extrapolated to zero dose divided by its slope. The α/β is generally high for early responding tissues and low for late responding tissues. The flexure dose, not the α/β ratio, is the dose at which the survival curve first begins to bend away from its initial slope. The α/β ratio tends to be high, not low, for cell types with a pro-apoptotic tendency. The α/β ratio is the *dose* at which the linear and quadratic contributions to cell killing are equal.

2. D The α/β ratio for this tissue can be determined by setting $n_1d_1\,[1 + d_1/(\alpha/\beta)] = n_2d_2\,[1 + d_2/(\alpha/\beta)]$, where n_1 and n_2 are the number of fractions and d_1 and d_2 are the doses per fractions used for the first and second protocols, respectively. Thus, (25)(1.8 Gy)(1+1.8 Gy/α/β) = (17)(2.5 Gy)(1+2.5 Gy/α/β) = 45 Gy + 81 $Gy^2/\alpha/\beta$ = 42.5 Gy + 106.25 $Gy^2/\alpha/\beta$ or
α/β = 25.25 Gy^2/2.5 Gy= 10.1 Gy.

3. A Since the α/β ratio for head and neck cancers tends to be high, whereas the α/β ratios for late effects are low, it would be anticipated that a hyperfractionated schedule could produce a decrease in late effects while maintaining a level of tumor control similar to that produced by the standard protocol.

4. D When plotted as the log of the total dose to produce a given isoeffect as a function of the log of the dose per fraction (plotted on a reverse scale), most late responding normal tissues are characterized by steep isoeffect curves, whereas those for early responding normal tissues and most tumors tend to be shallow.
    a. Joiner M and van der Kogel A, Eds. Basic Clinical Radiobiology, 4th Ed. Hodder Arnold,
    London, 2009; page 103.

5. B The dose per fraction, at which the isoeffect curves for tumor control and late effects intersect, helps to define the range over which the desired tumor control probability can be achieved while also staying at or below the tolerance dose for the late responding normal tissue. Since the use of smaller-than-conventional fraction sizes generally results in greater sparing of late effects relative to tumor control, treatment protocols involving the use of fraction sizes smaller than the point of intersection between the two isoeffect curves would yield the desired level of tumor control while not exceeding normal tissue tolerance. This type of analysis would *not* provide any information as to the actual extent of tumor control or the extent of normal tissue damage anticipated since these are already specified by the chosen isoeffect. (It would be necessary to determine TCP and NTCP curves to obtain this information, independent of any isoeffect analysis.) Also, these isoeffect curves provide no information as to the effects of changing overall treatment time, since the type of isoeffect curve plot as stated evaluates the influence of dose per fraction and not time (and further, it is assumed that overall time remains fairly constant in this analysis, and that it is only dose per fraction that changes). Likewise, the effect of a split course treatment could not be evaluated in this case, because data as to the tumor's potential doubling time are not provided.

6. C If the α/β ratio is less for a patient's tumor than their dose-limiting normal tissue, such a

patient may benefit from the use of large fraction sizes, because the tumor would be more sensitive to fraction size than the dose limiting normal tissue and would be preferentially damaged by hypofractionation.

7. B Tumor cell repopulation during treatment would cause a decrease in the BED, since the cell divisions that take place during the course of therapy could counteract some, if not all, of the toxicity of the radiation. This can be calculated from the equation BED = $nd[1+d/(\alpha/\beta)] - [(0.693)(T)/(\alpha)(T_{pot})]$, where n is the number of fractions, d is the dose per fraction, α and β are the parameters characterizing the underlying dose response curve for the tumor, T is the length of time during treatment that repopulation occurs and T_{pot} is the potential doubling time (the time it would take the tumor to double its cell number in the absence of cell loss).

8. E An accelerated treatment schedule is used primarily to limit the amount of tumor cell repopulation that may occur before the completion of radiotherapy. The repopulation that may occur, particularly for tumors with short T_{pot} values, can severely limit the effectiveness of treatment.

9. E The BED equation that can be used for this problem is $BED = nd[1+d/(\alpha/\beta)]$, where n is the number of fractions and d is the dose per fraction. (It is not necessary to correct for either tumor cell proliferation, since the regimens are specified as having the same overall treatment time, or incomplete repair, since these are both once-per-day treatments.) Thus, the late effects BED associated with the use of 2 Gy fractions is (36)(2 Gy)(1+2 Gy/2 Gy) = 144 Gy_2. Since it is indicated that the new treatment schedule is isoeffective with respect to late effects, then the BED for the second protocol will also be 144 Gy_2. The number of 3 Gy fractions to use can be calculated using 144 Gy_2 = n(3 Gy)(1+ 3 Gy/2 Gy) = 7.5n or n = 19 fractions. The tumor BED for the first schedule is (36)(2 Gy)(1+2 Gy/10 Gy) = 86 Gy_{10}. The BED for the second protocol is (19)(3 Gy)(1+3 Gy/10 Gy) = 74 Gy_{10}. Thus, there is a decrease of 12 Gy_{10} for the second compared with the first protocol, or a [(86 Gy_{10} – 74 Gy_{10})/86 Gy_{10}] (100%) = 14% decrease. An alternative method to compute the answer to this problem is to use the biologically equivalent in 2 Gy fractions dose (EQD2), which is $EQD2 = D[(d+\alpha/\beta)/(2\ Gy+\alpha/\beta)]$) where D is the total dose and d is the dose per fraction. Thus, the EQD2 for the standard 2 Gy protocol is (72 Gy)[(2 Gy+ 2 Gy)/(2 Gy +2 Gy) = 72 Gy (clearly, the EQD2 equals D for all protocols involving a fraction size of 2 Gy). Since it is indicated that the new treatment schedule is isoeffective with respect to late effects, then the EQD2 for the second protocol will be 72 Gy. With α/β = 2 Gy for late effects, the total dose (D) to use in 3 Gy fractions can be calculated using 72 Gy = D(3 Gy+ 2 Gy)/(2 Gy+ 2 Gy) = 1.25D or D = 57.6 Gy. For the nearest integral number of 3 Gy fractions (19), this is a total dose of 57 Gy. With α/β = 10 Gy for tumor, the tumor EQD2 for the second protocol is (57 Gy)[(3 Gy + 10 Gy)/(2 Gy+ 10 Gy)] = 61.75 Gy. Thus, there is a decrease in EQD2 of 10.25 Gy for the new treatment compared with the standard protocol which had an EQD2 of 72 Gy (once again, the EQD2 is equal to D for all 2 Gy protocols) or a [10.25 Gy/72 Gy] (100%) = 14% decrease. Note, it is critical to recognize the distinction between the BED (biologically **effective** dose) and the EQD2 (biologically **equivalent** dose in 2 Gy fractions), which has also been referred to as the NTD (normalized total dose) or the LQED (linear quadratic equivalent dose). A loss of this distinction can result in miscalculations that may lead to crucial errors in treatment dose calculations.
    a. Joiner M and van der Kogel A, Eds. Basic Clinical Radiobiology, 4th Ed. Hodder Arnold, London, 2009; page 123.
    b. Fowler JF, Dale RG. When Is a "BED" not a "BED"?—When It Is an EQD2: In Regard to Buyyounouski et al. (Int J Radiat Oncol Biol Phys, 76:1297–1304, 2010); Int J Radiat Oncol Biol Phys, 78(2):640-641, 2010. PubMed link

10. D 28 days. Based on studies by Withers and colleagues, clonogen repopulation in head and neck cancer begins approximately 28 days after the initiation of radiation therapy. This suggests that there should be attempts to minimize treatment breaks once therapy is

started. (Withers et al. *Acta Oncol* 1988;27:131-146.) PubMed link

11. C Hypofractionated radiotherapy may be useful when cancer cells (such as prostate cancer) are thought to have an alpha/beta ratio similar to or less than the alpha/beta ratio of surrounding normal tissues. A is incorrect because the biologically equivalent dose depends on dose per fraction and total dose. B is incorrect because hypofractionation refers to fewer fractions than would be delivered in conventional fractionation, not the rate of dose delivery (answer B describes accelerated radiotherapy). D is incorrect because hypofractionation does not specify the delivery technique (although most hypofractionated treatments are delivered using precise delivery techniques to avoid normal tissue toxicity). E is incorrect because hypofractionation generally uses larger than conventional fraction sizes, which are generally associated with more late effects. PubMed link

Brachytherapy

1. A Ir-192 is most commonly used for HDR brachytherapy. Pd-103 and I-125 are used in LDR brachytherapy. Co-60 is used in external beam radiotherapy. Y-90 is used in radioimmunotherapy.

2. D I-131 tositumomab (Bexxar) is a radiolabeled antibody against the CD20 cell surface antigen found in a very high percentage of B cell non-Hodgkin's lymphomas. The β- and γ-emitting (*not* α-emitting) radioisotope I-131 is used for treatment of thyroid cancer, and is administered singly, not attached to any antibody. The primary clinical toxicity from I-131 tositumomab is a dose-related, reversible, hematopoietic suppression.
    a. Macklis RM. Iodine-131 tositumomab (Bexxar) in a radiation oncology environment, Int J Radiat Oncol Biol Phys, 66:S30-S34, 2006. PubMed link
    b. Pohlman B, Sweetenham J, Macklis RM. Review of Clinical Radioimmunotherapy, Expert Rev Anticancer Ther, 6:445-461, 2006. PubMed link

3. C Most clinical evidence now indicates that prostate cancers have unusually low α/β ratios, possibly as low as 1.5 Gy, and significantly less than the α/β ratio of roughly 3 Gy assumed for late complications in the normal tissues surrounding the prostate. This low α/β ratio suggests that prostate tumors should be especially sensitive to the large fraction sizes used for HDR brachytherapy. Since the OER usually increases with dose and dose rate, it would be expected to be greater for HDR than LDR brachytherapy. The probability of late normal tissue complications could increase with HDR because of the high doses per fraction used, but the high conformality of the dose makes this less of an issue compared with the use of external beam irradiation. The radioisotopes such as I-125 and Pd-103 used for LDR brachytherapy require relatively little shielding (HVLs of 0.025 mm and 0.008 mm lead, respectively), and are generally delivered as a permanent seed implant. In contrast, Ir-192, an isotope commonly used for HDR brachytherapy, has an HVL of 2.5 mm lead and is typically administered through a catheter-based after-loading technique.

4. D Iodine-131. Ocular melanoma is the most common intraocular malignancy in adults. Randomized trials have shown that definitive radiation therapy did not alter the risk of melanoma-related mortality (Collaborative Ocular Melanoma Study Group, *Archives of ophthalmology* 2006;124:1684-1693); hence, definitive radiation has become the standard of care for medium sized tumors (Singh AD et al. *Ophthalmology* 2011; 118:1881-1885). There have been clinical reports of the use of Cobalt-60, Iodine-125, and Ruthenium-106 for these treatments. Iodine-131 is used in nuclear medicine applications for treatment of thyroid.

Alternative Delivery Systems

1. D Although stereotactic radiosurgery or intraoperative radiotherapy employ large fraction sizes in which the entire treatment dose may be delivered in one irradiation, the incidence of late complications from these regimens has generally not been significantly elevated compared with a standard protocol because the dose is delivered to avoid irradiation of normal tissue. In addition, the biologic mechanisms for achieving tumor control and production of normal tissue damage may differ substantially for very large dose fractions compared with standard 2 Gy dose fractions. For many trials, a sufficient follow-up period has been realized so that most late effects, if they were to develop, would have appeared. Normal tissue radioprotectors are not routinely used in conjunction with these procedures. Although radioresistance by tissue hypoxia is more pronounced when large doses are used and there is less opportunity for hypoxic tissue to reoxygenate with only one or a small number of fractions, in most instances, normal tissues do not contain hypoxic regions. There is no evidence that DNA repair systems would saturate more readily in tumor cells than normal cells, if at all.

2. B Electrons are useful only for relatively superficial treatments. Based on the energies used for radiotherapy, they are not capable of penetrating very far into tissue.

3. B Although carbon ions exhibit a reduced OER, the OER for protons is high and similar to that for X-rays.

4. C Protons used for radiotherapy must be of a very high energy (> 100 MeV) in order to be sufficiently penetrating and are therefore of relatively low LET, typically less than 10 keV/µm. Since radiotherapy protons are low LET, they exhibit an OER in the range of 2-3 and therefore, like X-rays, would not be particularly effective at eradicating hypoxic tumor cells. Protons are only slightly more biologically effective than X-rays and have an RBE of ~1.1.

5. A The depth dose distribution of proton beams differs significantly from that of photon beams. Protons show an increasing energy deposition with penetration distance leading to a maximum, named the Bragg-peak, near the end of the range of the proton beam. In front of the Bragg-peak, the dose level is modest as compared to photon beams; beyond the Bragg-peak the dose falls practically to zero. By choosing appropriate proton beam energies, the depth of the Bragg-peak can be adjusted according to the depth and extent of the target volume. Hence, excellent conformality can be achieved compared to conventional or intensity modulated radiotherapy. The increased skin dose for proton therapy is known to complicate treatments of tumors that are not deep seated. Less widely realized is that it is difficult to achieve a lateral proton beam penumbra that is clearly advantageous compared with the penumbra of external beam photon therapy. The position of the Bragg peak in tissue is determined by proton energy, not fluence; Fluence = (Number protons)/Area.

6. D Intensity-modulated radiation therapy (IMRT) is a method of radiation therapy which is able to modulate the intensity of the overlapping radiation beams projected on the target and to use multiple-shaped treatment fields. It uses a device (a multileaf collimator, MLC) which, coupled to a computer algorithm, allows for "inverse" treatment planning. The radiation oncologist delineates the target on each slice of a CT scan and specifies the target's prescribed radiation dose, acceptable limits of dose heterogeneity within the target volume, adjacent normal tissue volumes to avoid, and acceptable dose limits within the normal tissues. Based on these parameters and a digitally reconstructed radiographic image of the tumor and surrounding tissues and organs at risk, computer software optimizes the location, shape, and intensities of the beams ports, to achieve the treatment plan's goals. Prompt

gammas from excitation of oxygen and nitrogen nuclei can be used for in-beam proton dosimetry, that is, for defining the proton beam margin in tissue. Choice A and B could describe a "gamma knife" for radiosurgery and a "scanning" method for achieving a desired field size in particle therapy, respectively. The time to deliver a dose fraction by IMRT is much longer than with other technologies.

7. D The transarterial administration of yttrium-90 microspheres is U.S. Food and Drug Administration-approved liver-directed cancer therapy. Yttrium-90 is pure high-energy (~ 1 MeV) beta emitter with an average range of penetration of 2.5 mm in tissue. Iodine-131 is a mixed-spectrum emitter, 10% of radiation dose is via gamma decay and the other 90% of radiation dose is via beta decay. Iodine-131 is the most commonly used gamma-emitting industrial tracer. Non-radioactive iron (oxide) nanospheres and nanocubes have been proposed for magnetic field-induced hyperthermia.

8. A Therapy-relevant protons are low-LET beams, whereas neutrons are high-LET beams. There is much less changes in isoeffective total doses with decreasing dose per fraction for high- LETcompared to low-LET, and correspondingly higher α/β with neutron compared to proton irradiation. Proton doses are described in gray equivalents or GyE. The GyE is equal to the physical dose in grays *times* proton RBE taken to be equal 1.1. Thus, proton dose is 10% lower than photon dose in a radiation therapy setting. The term "equivalent dose" is the term used in radiation protection and measured in Sievert (Sv); 1 Sv = 1 Gy *times* radiation weighting factor (Q), which is 1 for protons and energy-dependent for neutrons (Q=20 for 1-10 MeV neutrons). The ICRP has recommended a new name for this radiation protection dose, "radiation weighted dose" to avoid confusion.

9. E Based on historic in vitro and in vivo data, therapeutic energy silicon ions have the highest RBE of ~ 3.5 and the lowest OER of ~ 1.5, and neutrons are the first runner-ups on the list with RBE of ~ 2 and OER of ~ 2. Hydrogen ions (protons) and helium ions have RBE of 1.1 and OERs between 2.5 and 3.

10. E Gamma-rays and X-rays are low LET and have no Bragg peaks. p+ and C-ions both have Bragg peaks, but p+ are low LET. Neutrons and C-ions are high LET, but neutrons do not have a Bragg peak. Neon ions and neutrons are both high LET, Neon ions have a Bragg peak, but neutrons do not. Both Si and C are high LET and have Bragg peaks.

11. C Entrance Dose in a proton beam plan is typically reduced, but not eliminated. Relative Biological Effectiveness (RBE) of proton beam is 1.1. Proton Beam dose is typically described in Cobal Gray Equivalent, which is RBE x dose in Gy. Proton beams, as well as other charged particle beams, are known to create more double strand DNA breaks. Proton beams are known to have a higher linear energy transfer compared to photons, especially at the Bragg Peak region.

12. E Fast neutron beam energies are typically 50 – 70 MeV. Fast neutron beams have a higher LET compared to photons. RBE of fast neutron beams are typically 2-20. Photons create DNA damage via Compton Effect. Fast neutrons, as well as other charged particles, are associated with low oxygen enhancement ratio.

Chemotherapy

1. D Irinotecan is a synthetic analogue of camptothecin (CPT) and inhibits topoisomerase I by trapping the cleavable complex formed between this enzyme and DNA. CPT is a natural product derived from the bark and stem of Camptotheca (Happy Tree) with remarkable anticancer activity, but also low solubility and high adverse reactions. Because of these disadvantages, synthetic derivatives have been developed. The other CPT synthetic analogue used in cancer chemotherapy is topotecan. Proteasome inhibitors are drugs that block the action of proteasomes, the cellular complexes that break down proteins, such as p53. Examples of proteasome inhibitors include bortezomib, the first proteasome approved for use in the US, and salinosporamide A currently in clinical trials for multiple myeloma. Cyclophosphamide (Cytoxan) is an alkylating agent.
    a. Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA. DNA repair pathways as targets for cancer therapy, Nat Rev Cancer, 8:193-204, 2008. PubMed link
    b. Pommier Y. Topoisomerase I inhibitors: Camptothecins and beyond, Nat Rev Cancer, 6:789-802, 2006. PubMed link

2. B Cetuximab is a monoclonal antibody that blocks the epidermal growth factor receptor. The combination of cetuximab and radiation has been shown to be an effective treatment for cancers of the head and neck. Bevacizumab is a monoclonal antibody against VEGF and acts by interfering with angiogenesis. Celecoxib is a nonsteroidal anti-inflammatory drug that inhibits the cyclo-oxygenase 2 enzyme. Sirolimus is an immunosuppressant whose mode of action is to bind the FK-binding protein 12 (FKBP12), which in turn inhibits the mammalian target of rapamycin (mTOR) pathway. Bortezomib is a proteasome inhibitor that is used to treat multiple myeloma.
    a. 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 link
    b. Atkins M, Jones CA, Kirkpatrick P. Sunitinib maleate, Nat Rev Drug Discov, 5:279-280, 2006. PubMed link
    c. Bonner JA, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck, NEJM 354:567-578, 2006. PubMed link
    d. Chinnaiyan P, Allen GW, Harari PM. Radiation and new molecular agents, part II: Targeting HDAC, HSP90, IGF-1R, PI3K, and RAS, Semin Radiat Oncol, 16:59-64, 2006. PubMed link
    e. Mendelsohn J, et al. Epidermal growth factor receptor targeting in cancer, Semin Oncol, 33: 369-385, 2006. PubMed link
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    g. 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 link
    h. Sabatini DM. mTOR and Cancer: Insights into a complex relationship, Nature Reviews Cancer, 6: 729-734, 2006. PubMed link
    i. Spalding AC, Lawrence TS. New and emerging radiosensitizers and radioprotectors, Cancer Invest, 24:444-456, 2006. PubMed link
    j. 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 link
    k. Sartor CI, Raben D, O'Neil B. Biologicals and their interactions with radiation. In: G. Tepper (ed.), Clinical Radiation Oncology, 2nd edition, pp. 99-109: Churchill Livingstone Elsevier, 2006.

l. Hynes NE, Lane HA. ERBB receptors and cancer: The complexity of targeted inhibitors, Nat Rev Cancer, 5:341-354, 2005. PubMed link

3. E Herceptin is an anti-HER2 antibody. An example of a mTOR/FRAP inhibitor is Rapamycin, which inhibits translation initiation. Activating mutations of FMS-like tyrosine kinase 3 (FLT3) are present in approximately 30% of patients with de novo acute myeloid leukemia (AML) and are associated with lower cure rates from standard chemotherapy-based treatment. Targeting the mutation by inhibiting the tyrosine kinase activity of FLT3 is cytotoxic to cell lines and primary AML cells harboring FLT3 mutations. An example of FLT3 inhibitor is CEP-701. RAS mutations may result in constitutive activation of the RAS/RAF/MEK/ERK kinase signaling pathway, and have been found to occur frequently in human tumors. Multiple kinase inhibitors of this pathway are being evaluated.

4. D Iressa is a small molecule EGFR-tyrosine kinase inhibitor. Monoclonal antibodies directed against vascular endothelial growth factor (VEGF) such as Avastin may benefit some patients with colorectal, breast and lung cancers. Nitrogen mustards are used as antineoplastic agents in cancer therapy as nonspecific DNA alkylating agents. The antitumor activity of nitrogen mustards has been connected with their ability to cross-link the twin strands of DNA which if not repaired, can inhibit DNA replication and transcription, eventually leading to cell cycle arrest, apoptosis, and the inhibition of tumor growth. Cyclooxygenase (COX) inhibitors are compounds that block the action of COX enzymes, which are produced in response to inflammation and by precancerous and cancerous tissues. An example of a COX inhibitor is Celecoxib. Antibodies against HER-2 receptor, which is overexpressed in some breast cancers, include trastuzumab (Herceptin).

a. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer, Nature Reviews Cancer, 10:760-774, 2010. PubMed link

5. D Cyclooxygenase (COX)-2 mediates synthesis of eicosanoids from arachidonic acid. It tends to be over-expressed in tumors, is not constitutively produced in most normal tissues and stimulates, rather than inhibits, prostaglandin synthesis. EGFR is inhibited by erlotinib.

6. C 5-FU affects thymidylate synthase and inhibits the synthesis of nucleotides required for DNA synthesis. Accordingly, it primarily affects cells in S phase of the cell cycle. All of the other agents can create damage throughout the cell cycle, and do not have any phase specificity.

7. B Gemcitabine is a nucleoside analog of deoxycytidine in which the hydrogens at the 2' carbons in the sugar are replaced by fluorines. Once incorporated into DNA, the presence of this analog inhibits further DNA synthesis. In contrast, the other drugs listed cause toxicity either due to the damage they produce or by interfering with normal cellular processes. Melphalan and mitomycin c are alkylating agents. Etoposide is a topoisomerase II poison. Taxol stabilizes microtubule formation.

8. E Methotrexate is a competitive inhibitor of dihydrofolate reductase (DHFR) and thus prevents the formation of reduced folate. Reduced folate is required for transfer of methyl groups in the biosynthesis of purines and in the conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP). Reduced folate becomes oxidized to folic acid in this reaction and its regeneration is dependent on DHFR for reduction to its active form.

9. E Both vincristine and paclitaxel affect microtubules. However, vincristine binds to tubulin dimers, inhibiting assembly of microtubule structures. Taxol affects microtubule formation through hyper-stabilization.

10. E Cisplatin causes cellular lethality due to the formation of crosslinks between the two DNA strands. This prevents normal DNA synthesis.
    a. Kelland L. The resurgence of platinum-based cancer chemotherapy, Nat Rev Cancer, 7:573-584, 2007. PubMed link

11. D Bortezomib is a proteasome inhibitor.
    a. Richardson PG, Mitsiades C, Hideshima T, et al. Bortezomib: Proteasome inhibition as an effective anticancer therapy, Annu Rev Med, 57:33-47, 2006. PubMed link
    b. Schwartz R, Davidson T. Pharmacology, Pharmacokinetics, and Practical Applications of Bortezomib, Oncology, 18:14-21, 2004. PubMed link

12. C Avastin is a monoclonal antibody against VEGF.
    a. Ellis LM, Hicklin DJ. VEGF-targeted therapy: Mechanisms of anti-tumour activity, Nat Rev Cancer, 8:579-591, 2008. PubMed link
    b. Jain RK, Duda DG, Clark JW, et al. Lessons from Phase III Clinical Trials on Anti-VEGF Therapy for Cancer, Nat Clin Pract Oncol, 3:24-40, 2006. PubMed link

13. E All the answers are agents for the clinical management of prostate cancer patients (21577233, 21577234 and 22521546). The correct answer is "e". Answer "a" is abiraterone, "c" is leuprolide, "b" denosumab and "d" is Ra-223.

14. A All the answers are agents for the clinical management of prostate cancer patients (21577233, 21577234 and 22521546). The correct answer is "a". Answer "b" is denosumab, "d" is Ra-223 and "e" is enzalutamide.

15. D Antitumor immunity is often ineffective due to the tight regulation associated with the maintenance of immune homeostasis. One of the major limitations results from chronic exposure to antigens and is characterized by the upregulation of inhibitory immune checkpoint receptors in order to prevent uncontrolled immune reactions. Blocking of one or several of these immune checkpoints with monoclonal antibodies (mAbs) has been shown to rescue otherwise exhausted antitumor T cells, and most importantly, has been associated with objective clinical responses in cancer patients. The first immune-checkpoint inhibitor to be tested in a clinical trial was ipilimumab (Yervoy, Bristol-Myers Squibb), an anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) mAb. CTLA-4 belongs to the immunoglobulin superfamily of receptors, which also includes programmed cell death protein 1 (PD-1), B and T lymphocyte attenuator, T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and V-domain immunoglobulin suppressor of T cell activation. In 2011, the US Food and Drug Administration approved the use of ipilimumab in patients with metastatic melanoma, either as initial therapy or after relapse (24161671). Bevacizumab is the humanized monoclonal antibody that inhibits vascular endothelial growth factor A (VEGF-A). Imantinib is a small molecular inhibitor of receptor tyrosine kinases most selective for Bcr-Abl, but also less so against c-kit and PDGF-R. Cetuximab is a monoclonal antibody against EGFR. Crizotinib is a small molecular inhibitor of ALK and ROS1 kinases.

16. A The use of radium-223 to treat metastatic bone cancer relies on the ability of alpha radiation from radium-223 and its short-lived decay products to kill cancer cells. Radium is preferentially absorbed by bone by virtue of its chemical similarity to calcium, with most radium-223 that is not taken up by the bone being cleared, primarily via the gut, and excreted. Although radium-223 and its decay products also emit beta and gamma radiation, over 95% of the decay energy is in the form of alpha particle radiation. Alpha particle radiation has very short range in tissues, around 2-10 cells, compared to beta or gamma radiation. This reduces damage to surrounding healthy tissues, producing an even more localized effect than the beta-emitter strontium-89, also used to treat bone cancer. Taking account of its preferential uptake by bone and the alpha particles' short range, radium-223 is estimated to give targeted osteogenic cells a radiation dose at least 8 fold higher than other non-targeted tissues (Ref. 23863050). The most correct answer is "a". All other

answers are incorrect because they are either not true (“b”, “c” and “e”) or are not the primary reason for the tolerability of Ra-223 (answer “d”).

17. C Cisplatin induces acute kidney injury (AKI) in 20-30% of treated patients with typical onset occurring 3-5 days following cisplatin dose. Cisplatin forms intrastrand crosslinks between DNA, predominantly between purine bases. The sensitivity of cells to cisplatin correlates both with the density of mitochondria and mitochondrial membrane potential. The proximal tubular cells have the highest density of mitochondria in the kidney and are therefore the most sensitive to ciplsatin injury. (PMID: 16107504; PMID: 17016583) Cisplatin can induce superoxide anion formation in the glomerulus and proximal tubulues but not the distal tubules (PMID: 25388649).

Radiation Modifying Drugs

1. D It has been suggested that the transient increase in radiation response reflects the transient normalization of the tumor vasculature, which results in increased perfusion and increased oxygen delivery, leading to a decrease in tumor hypoxia and decreased hypoxia-induced radioresistance.
    a. Jain RK. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy, Science, 307:58-62, 2005. PubMed link
    b. Jain RK. Antiangiogenic therapy for cancer: Current and emerging concepts, Oncology, 19(4 Suppl 3):7-16, 2005. PubMed link

2. D The DAHANCA trial of nimorazole reported that this 5-nitroimadazole hypoxic cell radiosensitizer can be delivered without serious, dose-limiting side effects. Because nimorazole has its NO_2 group at the 5 rather than the 2 position on the imidazole ring, it is a less efficient radiosensitizer than either misonidazole or etanidazole. Loco-regional failure and disease-specific mortality were more frequent in patients assigned to the radiation plus placebo arm of the trial than for those patients given radiation plus nimorazole. Thus, it was the recommendation of the authors of this study that nimorazole **should** be used routinely in the treatment of these types of head and neck cancer.
    a. 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 link
    b. Overgaard J, Eriksen JG, Nordsmark M *et al.* Danish Head and Neck Cancer Study Group.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, 6:757-764, 2005. PubMed link

3. E The critical factor in determining whether a new agent will be clinically valuable when combined with radiation is whether it produces a therapeutic gain, that is, it increases tumor toxicity or reduces normal tissue toxicity without a commensurate increase or decrease, respectively, in the other tissue. Synergy with radiation will not produce a therapeutic gain if it occurs equally in both tumor and critical normal tissues. A therapeutic gain will not be produced unless the proliferation in tumors and critical normal tissues show significant differences that result in the modulator producing a selective increase in the radiation response of the tumor. A therapeutic gain cannot be achieved unless the vasculature in tumors and critical normal tissues show differences that result in the modulator producing a selective increase in the radiation response of the tumor. The cytotoxicity of most biological response modulators is minimal and manageable; their efficacy as cancer treatments result primarily from their ability to modulate radiation sensitivity. Minimal normal tissue toxicity alone does not necessarily lead to a therapeutic gain; in fact, a therapeutic gain can be obtained despite significant toxicity in normal tissue, provided the relative cytotoxic effect is greater in the tumor.

4. E Overgaard has published a meta-analysis using data obtained from over 10,000 patients in 86 randomized trials who received radiotherapy and either oxygen or nitroimidazoles as hypoxic cell radiosensitizers, compared to radiotherapy alone. His findings were that these attempts at modification of tumor hypoxia significantly improved the effect of radiotherapy, with an odds ratio of 0.77 for loco-regional tumor control and an associated significant survival benefit (with an odds ratio of 0.87).
    a. Overgaard J. Hypoxic radiosensitization: Adored and ignored, J Clin Oncol, 10;25(26):4066-4074, 2007. PubMed link

5. B dFdCDP formed in cells treated with gemcitabine interferes with ribonucleotide reductase, causing depletion of deoxynucleotide triphosphates necessary for DNA synthesis. This is thought to be a mechanism leading to radiosensitization.
    a. Shewach DS, Lawrence TS. Antimetabolite radiosensitizers, J Clin Oncol, 25:4043-4050, 2007. PubMed link

6. D The use of an hypoxic cell radiosensitizer, such as nimorazole, would not be expected to affect the response of normal tissues to radiotherapy since normal tissues generally do not possess regions of hypoxia. A change in fraction size may affect both the incidence and the severity of the radiation response in normal tissues, particularly late-responding tissues. A step down in field size would spare at least some normal tissues the full treatment dose. A gap in treatment may lessen the severity of the response in acutely-responding normal tissue, as repopulation of surviving cells during the gap would compensate to some extent for the damage caused by the radiation. Administration of amifostine, a radioprotector, also may protect normal tissues.

7. E Cisplatin has a substantial amount of laboratory- and clinical-based evidence supporting its action as a radiosensitizing chemotherapeutic in tumor cellsCurcumin has been shown to reduce DNA damage-induced foci formation by certain DSB repair genes, although further studies are needed. Amifostine is thought to act as a radioprotector although its true clinical efficacy has been questioned recently and thus is controversial. Neomycin and ciprofloxacin are not thought to have radiosensitizing effects.

8. B The hypoxic environment of many tumors treated with radiotherapy is overcome with conventional radiotherapy by allowing reoxygenation to occur between the many doses of treatment. By using fewer fractions, reoxygenation does not occur as readily during SBRT, and therefore hypoxic cell radiosensitizers could be advantageous (20832663). Answer “a” is true, but SBRT is defined by 1-5 fractions so this answer does not apply well to the question. SBRT is thought to cause acute damage to endothelial cells of the tumor vasculature (1275052) with or without a hypoxic cell radiosensitizer. Cancer stem cells (CSCs) have been hypothesized to reside in hypoxic regions (19249645), but hypoxic cell radiosensitizer targeting of CSCs has not been tested thoroughly. Answer “e” is not correct.

9. A Radiation-induced late normal tissue toxicity is increasingly being appreciated as a phenomenon of ongoing changes in tissue after radiation but prior to the manifestation of toxicity. These events include ongoing mitotic cell death and perpetually active cytokine cascades that can lead to vascular damage, tissue hypoxia, and excessive extracellular matrix deposition. Radiation mitigators aim to interrupt these cascades or intervene to prevent the perpetuation of damage and thus reduce the expression of toxicity. Alternatively, radiation mitigators can be agents delivered during or shortly after exposure to repopulate a critical cell compartment such as the mucosa or bone marrow. In this instance, the mitigator is used to prevent acute toxicity. For radiologic terrorism and space research, much of the focus of mitigators has been in the field of developing chemopreventatives to reduce carcinogenesis of total body exposures (20413641). Most correct answer is “a”. Answer “b” is a radioprotector; “c” is treatment; “d” could be either a protector or mitigator; and “e” could be a radiosensitizer or radioprotector.

10. A The NF-κB pathway mediates transcriptional upregulation of (i) anti-apoptotic genes; (ii) cytokines and growth factors that induce proliferation and survival of HP and other stem cells; and (iii) potent ROS-scavenging antioxidant proteins, such as MnSOD. Mice with a genetic defect in NF-κB signaling displayed heightened GI radiosensitivity. Pharmacological activators of NF-κB, like flagellin, where found that exploited the natural mechanisms by

which the innate immune system responds to microbial infections. Various pathogen-associated molecular patterns (PAMPs) are recognized by host cells due to their specific interaction with Toll-like receptors (TLRs), which leads to activation of NF-κB (22479357 and 18403709).

11. B Existing in highly proteotoxic environments, tumor cells are subjected to chronic and acute hypoxia, increased levels of DNA damage, high levels of reactive oxygen species, and protein complex imbalances due to aneuploidy. Survival under these conditions is enabled by the aid of efficient cellular stress response machinery, such as heat shock protein 90. Hsp90 inhibition imparts radiosensitization through multiple mechanisms via downregulation of multiple HSP90 clientele that function in: (1) reassortment of cancer cells into G2-M; (2) downregulation of radioresistance signal transduction pathways like the PI3K-AKT-mTOR pathway; and (3) downregulation of the DNA checkpoint and repair pathways such as the ATR-Chk1 pathway (23863691). Only "b" is correct. Hsp90 inhibition does not result in answer "a", "c" or"d" and furthermore, S-phase is the most radioresistant phase of the cell cycle (answer "c").

12. E All are correct so "e" is the most correct answer (17510418, 16982770, 1616630 and 17460771).

13. A Answer "a" is the most correct answer (24027197 and 24027196). Successful treatment by genotoxic modalities including radiotherapy is commonly hampered by treatment resistance in advanced cancers. Two new studies reveal that androgen receptor signaling transcriptionally upregulates a large subset of DNA repair genes, thereby enhancing the repair capacity and promoting radioresistance of prostate cancer. These results provide a mechanistic rationale for a combined treatment by ionizing radiation and androgen deprivation therapy. All the other answers do not apply to ADT + radiation synergy. ADT can actually induce the PI3K-AKT-mTOR pathway (21575859).

14. C A radiation protector is an agent that reduces the injury caused by ionizing radiation, often through a chemical process such as free radical scavenging. These agents must be present at the time of or very shortly after irradiation (10-3 seconds after irradiation) to exert their effects. Radiation mitigators reduce the damage from radiation by inhibiting or altering processes initiated after irradiation but before expression of injury. Radiation sensitizers enhance the cell killing from irradiation. Anti-fibrotics would be considered a treatment of radiation injury that has already been established. PubMed link

15. E Amifostine is a sulfhydryl compound that scavenges free radicals. It also concentrated more rapidly by normal tissues compared to tumor. Although amifostine may cause hypotension, there is no evidence that this contributes to its selectivity. Amifostine has no known impact on hemoglobin. PubMed link

16. A Palifermin is a truncated human recombinant keratinocyte growth factor that stimulates proliferation of mucosal cells of the gastrointestinal tract to proliferate and thus to decrease the severity. It has been shown to reduce the duration and severity of mucositis after transplantion for hematologic malignancies. PubMed link

Hyperthermia

1. E Radiofrequency ablation is accomplished by inserting a RF probe into or near a tumor mass, and then heating it to temperatures that produce frank tissue necrosis. RF ablation is typically used singly, not simultaneously with radiation therapy.

2. C The greatest heat radiosensitization is produced when the heat is delivered as close to the time of irradiation as possible, since a likely mechanism for the sensitizing effect is heat denaturation of the proteins (enzymes) associated with the repair of radiation damage.
    a. Moyer HR, Delman KA. The role of hyperthermia in optimizing tumor response to regional therapy, Int J Hyperthermia, 24:251-261, 2008. PubMed link

3. E The thermal enhancement ratio (TER) is defined as the radiation dose to produce an effect in cells or tissues irradiated at normal physiologic temperature divided by the dose of radiation for cells or tissues irradiated at elevated temperature to produce the same effect. There are large differences in the sensitivity of cells to heat depending on their position in the cell cycle ("age response"), with S phase cells being most sensitive. This is the opposite of radiation's age response, in which S phase cells exhibit the greatest resistance. This "complementarity" of toxicities of heat and radiation forms part of the basis for combining the two modalities. A second justification for combining radiation and heat is that heat enhances radiation injury by denaturing proteins/enzymes needed for the repair of radiation damage; heat does not create additional DNA damage in and of itself. Thermotolerance is an acquired resistance to heat, and is thought to be mediated by so-called heat shock proteins, cellular chaperones that help stabilize structures damaged by heating (membranes, proteins, cytoskeleton, etc.). The time course for the appearance, maintenance and eventual disappearance of heat shock proteins in cells undergoing hyperthermia mirrors the time course for the development and decay of thermotolerance. The development of thermotolerance is not a genetic change and therefore is **not** heritable in the progeny of previously-heated cells. Step-up heating may be useful clinically only if it can be used to protect normal tissues selectively; the procedure involves a pre-heating at mild hyperthermic temperatures so as to induce thermotolerance, followed by high temperature heating sufficient to produce cytotoxicity. Step-down heating has also been attempted for the purposes of sensitizing tumors to hyperthermia. In this case, a tumor is pre-heated at a very high temperature – which temporarily inhibits the development of thermotolerance – followed by heating at a somewhat lower, but still cytotoxic temperature.

4. B Tissues maintained under conditions of low pH tend to be sensitive to heat. G_2 cells are quite radiosensitive, but somewhat more resistant to heat killing, comparatively speaking. It is the chronically hypoxic cells in tumors (that typically exist in acidic microenvironments) that tend to be more sensitive to heat than acutely hypoxic cells. In laboratory rodents, hyperthermia usually results in increased blood flow in normal tissues and decreased blood flow in most tumors, not vice versa. Because the vasculature in normal tissues is generally more "mature" and responsive to external stimuli than tumor vasculature, it can more readily respond to elevated temperatures by dilating and increasing blood flow so as to carry away excess heat and restore normal physiologic temperature. The amount of cytotoxicity produced by a hyperthermic treatment at 43ºC for 10 minutes would be less, not more, than that produced by 46ºC for 5 minutes. This would be predicted from the thermal dose calculation (applicable for heat exposures at 43ºC and above) $t_2/t_1 = 2^{T1-T2}$, where t_1 and t_2 are the exposure times at temperatures T1 and T2 to produce equal biological effects. Thus, if T1 is 46 ºC and T2 is 43ºC, then the treatment at the lower temperature would need to be 8 times as long as at the higher temperature to produce the same amount of cell killing.

5. D Hyperthermia using gold nanoshells results in preferential sensitization of tumor stem cells. PubMed link

Radiation Carcinogenesis

1. C Individuals treated as infants with radiation therapy for an enlarged thymus were found to have an increased incidence of thyroid cancer.
    a. Boice JD. Radiation-induced thyroid cancer -- What's new?, J Natl Cancer Inst, 97:703-705, 2005. PubMed link

2. A Because different tissues have different sensitivities with respect to radiation carcinogenesis, for risk estimation and radiation protection purposes, tissues are assigned "weighting factors" (W_T) that correct the absorbed dose a tissue receives for biological equivalence. For example, the breast is assigned a $W_T = 0.12$, whereas bladder and gonads have W_T's = 0.05, and brain and kidney, 0.01.

3. C Thyroid cancer was the most common cancer observed among children who lived in the Chernobyl area at the time of, and subsequent to, the accidental radiation release., This was a result of the high level of environmental contamination with radioactive iodine which homed to the thyroid.

4. D In the Childhood Cancer Survivor Study, there was no evidence of an increase in pancreatic cancer, however increased incidences of skin cancer, sarcoma, meningioma and thyroid cancer were observed in childhood cancer survivors who received radiotherapy as part of their treatment.
    a. Armstrong GT, Stovall M, Robison LL. Long-term effects of radiation exposure among adult survivors of childhood cancer: Results from the childhood cancer survivor study, Radiat Res, 174:840-850, 2010. PubMed link
    b. Sadetzki S, Mandelzweig L. Childhood exposure to external ionising radiation and solid cancer risk, Br J Cancer, 7;100(7):1021-1025, 2009. Review. PubMed link

5. D Using a low dose rate risk estimate for the working population of 0.04 radiation-induced fatal cancers per Sv, and assuming a linear extrapolation of the risk estimate to 0.25 Sv, it would be anticipated that this person would have a 1% excess risk for the development of a cancer resulting from his/her activities as a radiation oncologist.
    a. 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, 100:428-436, 2008. PubMed link
    b. Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2 (2006) National Research Council, National Academies Press, 2006.
    c. Charles MW. LNT -- An apparent rather than a real controversy? J Radiol Prot 26:325-329, 2006. PubMed link
    d. 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 link
    e. Wakeford R, Little MP. Risk coefficients for childhood cancer after intrauterine irradiation: A review, Int J Radiat Biol, 79:293-309, 2003. PubMed link
    f. Preston DL, Ron E, Tokuoka S, Funamoto S, Nishi N, Soda M, Mabuchi K, Kodama K. Solid cancer incidence in atomic bomb survivors: 1958-1998, Radiat Res, 168:1-64, 2007. PubMed link

6. D Using the risk estimate of 0.05/Sv for a general population exposed to X-rays from CT scanning, it would be anticipated that (10^7 people) x (0.01 Sv per person) x (0.05 radiation-induced fatal cancer deaths) = 5,000 excess cancer deaths.

7. B Radiation-induced leukemias have a medium latent period of 3-7 years, whereas solid tumors do not appear for at least 10 years following irradiation, if not several decades later.
    a. Finch SC. Radiation-induced leukemia: Lessons from history, Best Pract Res Clin Haematol 20(1):109-118, 2007. Review. PubMed link
    b. 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 link

8. D The EPA has estimated that approximately 20,000 of the annual 160,000 lung cancer deaths in the U.S. each year are due to exposure to indoor radon through the production of α-particles resulting from the decay of radon to α-emitting daughter products.
    a. http://epa.gov/radon/healthrisks.html

9. B Treatment of ankylosing spondylitis, which at one time involved radiation therapy, has been associated with an increased incidence of leukemia.

10. D Correct answer is "d". Several studies have called into question the widely held view that the DNA-damage response is integral to the actions of p53 as a tumor suppressor (16957739, 14729946, and 10082576). The possibility that the acute DNA damage response may be dispensable for p53-mediated tumor suppression has intriguing implications. First, although DNA damage is very efficient at triggering p53-dependent cell death, as evident from the widespread apoptosis of radiosensitive tissues following irradiation, it is highly inefficient at generating tumor cells: mice lacking p53 are immune to much of the pathological cell death induced by irradiation yet still take months to develop rare, clonal tumors. Thus, only a tiny number of damaged cells that p53 kills would ever have evolved into tumors had they survived. Widespread activation of p53 following DNA damage is therefore, at best, an unwieldy tool with which to cull a few potential tumor cells. In contrast, because p19ARF is induced only in those rare preneoplastic cells that, as a consequence of DNA damage, acquire oncogenic mutations, its expression is highly specific to those few cells set on a neoplastic trajectory. Second, the p53-dependent DNA-damage response mediates much of the life-threatening pathology and side effects that accompany radiation exposure and chemotherapy. Restoring p53 function only at a later time abrogates all of the pathology yet, by focusing p53 activity only on those incipient tumor cells expressing p19ARF, preserves much of the tumor suppression. Such observations suggest that transient pharmacological inhibition of p53 during, or shortly after, acute genotoxic injury may be helpful in ameliorating the pathology, without compromising subsequent tumor suppression. Perhaps, most perversely, it is even possible that the linkage between p53 and DNA damage actually drives erosion of the efficacy of p53 as a tumor suppressor by imposing a lifelong selective pressure to inactivate p53 in cells sustaining such damage. Once lost, p53 is then unavailable to act as a tumor suppressor.

11. C Cancer is a stochastic effect of radiation. Its severity is not determined but the probability to occur is determined by radiation dose. Different organs have dissimilar radiation induced cancer risks. For internally deposited radioisotopes, the sites of deposition are additional factors of organ specific cancer risk.

12. A Regardless of the receipt of radiotherapy, breast cancer patients are at a higher risk of second malignancies, including a higher risk of contralateral breast, gynecologic, and thyroid cancer. Receipt of radiation further increases risk, particularly in the esophagus, lung, and soft tissues. However, the estimated percentage of second malignancies (excluding non-melanomatous skin cancers) attributable to radiation therapy is only 3.4-9%. As many patients are concerned about secondary malignancy risk, it is important to counsel them that the risk of getting a future non-radiation-related cancer is much higher than a radiation-related one. Furthermore, the risk of cancer recurrence without radiation treatment is often much higher than the risk of a second, radiation-attributable cancer. However, for

younger women treated with breast cancer radiotherapy, the risk of a future second cancer can be nearly double that of age-matched controls.

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Berrington de Gonzalez et al. Br J Cancer (2010) PMID: 19935795

Heritable Effects

1. C The current estimate for the development of a hereditary disorder in the children of an irradiated person is 0.002/Sv.
    a. Schull WJ. The children of atomic bomb survivors: A synopsis, J Radiol Prot, 23: 369-384, 2003. PubMed link

2. B The GSD or genetically significant dose, which represents the average dose to the gonads weighted to reflect the child-bearing potential of the people that comprise that population, is estimated at 0.3 mSv for radiation exposures from “man-made” radiation (imaging procedures, commercial nuclear power, air travel, weapons testing fallout, ect).

3. E A dose of 0.83Gy will cause a significant drop in the sperm count that may result in oligospermia and infertility for about a year following the irradiation. After a period of about six months following irradiation, the more differentiated members of the spermatogenic series that were susceptible to mutation will have all matured and been lost. Based on studies with laboratory rodents, this period of time should also be adequate to permit a return to the baseline population risk for mutations in offspring. In males, a dose >6Gy to the testes may cause permanent disability. Also, a dose of 0.83 Gy would be too low to cause a hormonal dysfunction.

Embryonic Effects

1. D Prenatal irradiation puts individuals at a dose-dependent, increased risk for the development of a radiation-induced cancer at some time later in life. The woman should not be advised to discontinue treatment until reaching term, as the scattered dose to her fetus is likely small. Her personal risk in delaying therapy, while her cancer continues to progress, would effectively present a much greater concern. In addition to carcinogenesis, the fetus would also be at (an even higher) risk for radiation-induced congenital abnormalities, because irradiation took place during the first trimester of pregnancy when most of the organs are undergoing active development. The scattered dose to the fetus would certainly not be large enough to result in death and miscarriage or stillbirth, however it is likely greater than 0.01 cGy.

2. C The thyroid of a developing fetus will incorporate radioactive iodine from about the 10^{th} week of gestation onward. The Society of Nuclear Medicine recommends that females of child-bearing age and capability should have a pregnancy test within 72 hours prior to I-131 treatment. (PMID: 20300595

3. E The dose to the breasts associated with a screening mammogram is on the order of 10 mSv, with the scattered dose to the ovaries being only a small fraction of this dose. The estimated risk for a mutation being produced in the child of an irradiated individual is only about 0.2% per Sv, so the probability that this woman's future children would inherit a radiation-induced mutation is very small. For this low a dose, no hormonal effects would be expected and no ova should be killed. It would be incorrect to tell the woman that her ovaries received **no** dose since there would always be some amount of scattered radiation, although the total dose received would be extremely low. The dose to her ovaries would be far lower than the estimated 1-2 Sv assumed to be the approximate "genetic doubling dose" for humans. The doubling dose is the dose that doubles the spontaneous incidence of mutations among offspring of irradiated parents.

4. B Temporary growth inhibition would most likely be observed if a developing mouse was irradiated during the organogenesis period of gestation. Mice irradiated during this gestational stage tend to have low birth weights due to cell depletion, however they usually catch up in size during infancy. Hall chapter 12: Effects of radiation on the embryo and fetus.

5. B The organs that are actively undergoing development, (i.e., those that have high rates of cell division and ongoing differentiation), at the time of irradiation are the most susceptible to radiation injury during gestation.

6. C A dose of 0.1 Gy to an embryo or fetus at the 10 day to 25 week period of gestation (organogenesis: 10 days – 6 weeks; fetal period: 6 weeks - 9 months) is generally accepted as the minimum dose above which a physician should discuss with a pregnant patient the risk of radiation-induced birth defects (including possible congenital abnormalities and mental retardation), and possible actions to be taken, including therapeutic abortion.

1. D Historically, the annual dose equivalent received from medical diagnostic tests in the US is quoted as approximately 0.4-0.5 mSv per year, which constitutes about 15% of average yearly radiation exposure. This is in comparison to the 3 mSv received from natural background radiation sources (including radon), and the 0.1 mSv from other sources. However, as a result of the large increase in the use of CT scanning, for which the doses are higher than for most other diagnostic tests, the average annual dose equivalent resulting from use of medical X-rays may now be as high as 3 mSv (or closer to 50% of the total average annual dose). Also, background radiation exposure generally increases with increasing altitude since there would be less atmosphere to attenuate the cosmic rays from space.
    a. 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 link

2. C It is estimated that an average of 0.3 mSv to the gonads are received each year resulting from use of diagnostic X-rays, although this value may now be somewhat greater due to the increased use of CT scanning. In contrast, the human genetic doubling dose is estimated at 1-2 Gy. Thus the ratio of these values is closest to 3,000.

3. C An order for a diagnostic X-ray examination may only be based upon medical need and not for the purpose of limiting legal liability for the radiologist. Using the current estimate, that the average annual effective dose equivalent associated with diagnostic radiology is 3 mSv, calculations suggest that (3×10^{-3} Sv)(5×10^{-2} radiation-induced fatal cancers/Sv)(3×10^{8} people) = 45,000 fatal, radiation-induced cancers would be produced per year from imaging procedures. This would constitute about 8% of all cancer deaths each year in the
U.S. This risk estimate is based on the currently accepted, linear, no threshold model of radiation carcinogenesis. There is reason to believe that this number may be an over-estimate since the majority of people receiving these medical exposures tend to be older adults who are less susceptible to radiation carcinogenesis than young people. Nevertheless, even accounting for age differences in sensitivity to radiation carcinogenesis, the risk estimate for radiation-induced cancers still would suggest that more than 1% of fatal cancers are induced by medical radiation. However, not all scientists agree that use of the linear, no threshold model is appropriate in the case of such small radiation doses, especially given the amount of extrapolation necessary, and therefore that these risk estimates are probably over-estimates. Nevertheless, how much of an over-estimate remains to be seen.

4. A According to NCRP guidelines, a member of the public may receive a maximum of 1 mSv per year resulting from exposure to radioactive waste materials. Background radiation and the radiation exposure resulting from medical exposures, that are performed to either diagnose or treat disease, do not count towards this annual limit.
    a. NCRP Report 116. Limitation of Exposure to Ionizing Radiation, 1993. PubMed link
    b. ICRP (1991). *1990 Recommendations of the International Commission on Radiological Protection. ICRP Publication 60*. *Ann of the* ICRP 21, 1-3 Pergamon Press, Oxford.

5. C Radiation-induced mental retardation resulting from in utero irradiation is a deterministic effect that has a threshold dose below which the effect is not observed. It should be noted that some forms of mental retardation can occur from mutation-induction in eggs or sperm. However, some forms of mental retardation induced by mutations in eggs or sperm would be stochastic. In contrast, cancer

(breast and leukemia) and inherited genetic disorders (phenylketonuria and galactosemia) are stochastic effects, characterized by a no dose threshold and endpoints that are “all or nothing”. ”.

6. C The term stochastic is used to describe an effect of radiation that occurs by chance. Cancer induction and radiation induced hereditary effects are examples of stochastic effects. Deterministic effects depend on dose, dose rate, and dose fraction and have a threshold below which the effect does not occur.

Molecular Techniques

1. C An exonuclease is an enzyme that hydrolyzes the phosphodiester bonds of DNA to cleave nucleotides sequentially from the end of a polynucleotide chain.

2. C RNA polymerase is an enzyme that transcribes a copy of a DNA template into RNA. This would likely not serve as a useful reporter gene since it does not produce a product that can be detected easily.

3. B An antibody would be useful to screen an expression library, which synthesizes the protein encoded by each gene in the library. If nucleotide sequences are not available as probes for library screening (eg. sequence is not known), antibodies could be used for screening, if available. To do this one must create an expression library (ie. a library that not only contains the DNA fragments of interest but one that can actually manufacture the protein coded by the fragment) so that it may be detected by the antibody. This requires that the cDNA fragment within the vector be inserted downstream of a bacterial promoter, which will cause the inserted fragment to be expressed.

4. D The basic steps of a polymerase chain reaction (PCR) are denaturing, annealing, and extending. The 95°C denatures the DNA, a temperature of approximately 57°C permits binding of primers to the DNA (depending on the primer sequence and length, the temperature may vary around this range), and then -extension at 72°C which is the optimal temperature for synthesis of DNA by Taq polymerase.

5. B Subtractive hybridization is a technique that compares amounts of mRNA in different samples. All the other assays are used to analyze genomic alterations. Single nucleotide polymorphisms are ancestral genetic variations that occur when a single nucleotide in a genome is altered. Variations in the DNA sequences of humans can affect how humans develop diseases and respond to pathogens, radiation, chemicals, drugs, etc. This research is generally performed by comparing regions of the genome between matched cohorts with and without a disease or reaction. The increased interest in SNPs has been reflected by the development of a diverse range of SNP genotyping methods, including the single-strand conformation polymorphism (SSCP) assay, TaqMan assay, invader assay and the use of molecular beacons. TaqMan is based on PCR and is limited to applications that involve a small number of SNPs, since optimal probes and PCR reaction conditions must be designed for each SNP. Molecular beacons make use of a specially engineered probe. If the probe encounters a complementary sequence, it undergoes a conformational change, which allows the molecule to fluoresce. Alternatively, if the probe encounters a target sequence with as little as one non-complementary nucleotide, the molecular beacon will remain in its original state and no fluorescence will be observed. The invader assay utilizes a specific endonuclease that catalyzes structure-specific cleavage. This cleavage is highly sensitive to mismatches and can be used to interrogate SNPs with a high degree of specificity. Single strand conformation polymorphism (SSCP) involves the electrophoretic separation of single-stranded nucleic acids, based on subtle differences in sequence (often a single base pair) which results in a different secondary structure and a measurable difference in mobility through a gel. The mobility of double-stranded DNA in gel electrophoresis is dependent on strand size and length, but is relatively independent of the particular nucleotide sequence. The mobility of single strands, however, is noticeably affected by very small changes in sequence, possibly one changed nucleotide out of several hundred. Small changes are detectable because of the relatively unstable nature of single-stranded DNA; in the absence of a complementary strand, the single strand may experience intrastrand base pairing, resulting in loops and folds that give the single strand a unique 3D structure,

regardless of its length. A single nucleotide change could dramatically affect the strand's mobility through a gel by altering the intrastrand base pairing and its resulting 3D conformation

a. Abravaya K, Huff J, Marshall R, Merchant B, Mullen C, Schneider G, and Robinson J. Molecular beacons as diagnostic tools: Technology and applications, Clin Chem Lab Med, 41: 468-474, 2003. PubMed link
b. McGuigan FE, Ralson SH. Single nucleotide polymorphism detection: Alleic discrimination using TaqMan, Psychiatr Genet, 12: 133-136, 2002. PubMed link
c. Olivier M. The Invader assay for SNP genotyping, Mutat Res, 573:103-110, 2005. PubMed link
d. Orita M, Iwaha H, Kanazawa H, Hayashi K, and Sekiya T. Detection of polymorphism of human DNA by gel electrophoresis as single-strand polymorphism conformation, PNAS 66:2766-2770, 1989. PubMed link

6. A Exons can generally be identified by their lack of stop codons, since only a single one appears per mature mRNA strand. Exons are coding regions of a gene and introns are intervening sequences whose function is unknown. It is estimated that up to 99% of DNA is intronic, non-coding DNA. The primary transcript (RNA) is the exact copy of the entire gene, including introns as well as exons. The difference between the primary transcript and DNA is the base difference of thymine (DNA)→ uracil (RNA). The process of splicing removes the introns from the RNA and joins the exons together to create the messenger RNA (mRNA).The mRNA contains the coding sequence (CDS), which is translated into a string of amino acids based on the three-letter mRNA genetic code. CDS starts with the start codon, AUG (methionine). The mRNA also includes an untranslated region on each end, the 5'UTR and 3'UTR. The 3'UTR sequence starts with one of three stop codons (UAG, UAA, or UGA) that end the process of translation.

7. E A DNA ligase rejoins simple strand breaks. A DNA polymerase performs the resynthesis step during nucleotide excision repair. DNA ligase IV plays an important role in the **final** step of non-homologous end joining repair of DNA double strand breaks. During nucleotide excision repair, DNA endonuclease recognizes a particular type of damage and produces single strand cuts on either side of the damaged nucleotide to remove it. An AP endonuclease recognizes and removes a damaged base from DNA as an initial step in base excision repair.

8. E A Northern blot, in which RNA is subjected to gel electrophoresis and screened with a probe for a particular RNA transcript, would best be used to study the expression of a particular gene. A Western blot is used to detect the presence of a particular protein, using an antibody to detect it. The electrophoretic mobility gel shift assay, or EMSA, is used to map transcription factor binding sites in the regulatory portions of genes, and is based on the reduced electrophoretic mobility of a DNA-protein complex compared to unbound DNA. For a Southern blot, DNA is cut with a restriction enzyme and then separated with gel electrophoresis. The DNA fragments are transferred onto a membrane and probed for a particular DNA sequence. DNAase I footprinting is used to identify a protein binding site in DNA.

9. D Fluorescent *in situ* hybridization or FISH involves the use of a fluorescently-labeled probe for a particular gene in order to identify the location of that gene on a chromosome. Promoter bashing is used to identify that portion of a promoter where a transcription factor binds. The Enzyme-Linked ImmunoSorbent Assay, or ELISA, is used to detect the presence of an antibody or an antigen in a sample. A two-hybrid screen is used to characterize protein-protein interactions. A restriction fragment length polymorphism (RFLP) results when the location cut by restriction enzymes varies between individuals, due to insertions, deletions or transversions.

a. Braselmann H, Kulka U, Baumgartner A, et al. SKY and FISH analysis of radiation-induced chromosome aberrations: A comparison of whole and partial genome analysis, Mutat Res, 578:124-133, 2005. PubMed link
b. 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 link

10. B Sequencing of a cDNA can be used to predict the amino acid sequence of the protein encoded by the original gene, since this represents the expressed portion of a gene. The cDNA is synthesized from the mature, processed mRNA, and therefore contains only the information from the DNA's exons. A functional complementation assay involves the transfer of a gene to a mutant cell in order to determine whether doing so restores the normal phenotype. A cDNA library is created from mature mRNAs, not whole genomic DNA. A unique oligonucleotide probe for a particular gene cannot be backwards engineered from the amino acid sequence of the protein encoded by that gene due to the redundancy in the genetic code (i.e., a particular amino acid can be designated by more than one triplet codon).

11. B The comet assay is a method of single cell electrophoresis which allows the DNA of a cell to be separated based on the weight of the DNA. Cells with many breaks will have smaller fragments of DNA and thus the fragments will move faster from the original location of the nucleus. Under neutral conditions, DNA remains bound to its complimentary strand, and double strand breaks are measured. Under alkaline conditions, the complementary strands dissociate, thus, single strand breaks are measured. A northern blot is used to assess RNA and a western blot is used to evaluate proteins. Thus, neither a northern nor western blot would play a role in the direct assessment of DNA double strand break repair. Polymerase chain reaction (PCR) is used to amplify specific segments of DNA but is not generally used to evaluated DNA double strand breaks or their repair.

12. A Single-Cell RNA-Seq is a powerful technique that allows transcriptional profiling of tens of thousands of individual cells which helps to understand not only what genes are expressed, but also how this might differ within a heterogeneous sample. Cellular subpopulations can be identified and interrogated on an individual basis with some limitations. By comparison, more conventional bulk RNA-Seq and microarrays can only provide the expression profile as an average for all cells in a sample and therefore critical differences between different cellular subsets will be lost. A typical workflow for scRNA-seq involves isolation of a single cell, reverse transcription and barcoding, amplification, sequencing library preparation, sequencing and bioinformatic data analysis. ChIP-seq, on the other hand, is a method that uses chromatin immunoprecipitation combined with DNA sequencing in order to identify binding sites of DNA-associated proteins, i.e. most often transcription factors. Steps in the protocol involve cross-linking proteins to DNA, chromatin fragmentation, immunoprecipitation, DNA recovery and purification, sequencing. Although, RNA might also be cross-linked to the protein, it won't be reversed transcript or sequenced.

Stuart T, Satija R. Integrative single-cell analysis. Nat Rev Genet. 2019 May;20(5):257-272. doi: 10.1038/s41576-019-0093-7. PMID: 30696980.

13. D TCRseq (also called immunosequencing) is a high-throughput next generation sequencing technology that allows the identification of every T cell clone in a sample, be it whole blood, PBMC or tumor infiltrating lymphocytes or other tissues. It is based on sequencing a subsection of the T cell receptor (TCR). TCRs are heterodimers on T cells consisting of a and b chains or in some rare cases of g and d chains. ab TCRs recognize and bind peptides presented within the MHC molecule. During lymphocyte development random recombination events amongst the VDJ gene segments that take place in the thymus yield the immensely diverse repertoire of ab T cells (about 10^{18} different abTCRs)

which is then further shaped through negative and positive clonal selection. In most cases, TCR sequencing tend to focus on the hypervariable complementary determining region 3 (CDR3) which is a short region spanning the VD and DJ junction on the TCR b subunit that comes in direct contact with the MHC-bound peptide and the region with the most variability, i.e. unique to each TCR. VDJ recombination is done at the DNA level and does not involve RNA splicing. Most commercial TCRseq platforms are in fact based on DNA, although mRNA can also be used.

Matos TR, de Rie MA, Teunissen MBM. Research Techniques Made Simple: High-Throughput Sequencing of the T-Cell Receptor. J Invest Dermatol. 2017 Jun;137(6):e131-e138. doi: 10.1016/j.jid.2017.04.001. PMID: 28532760; PMCID: PMC5912324.

Woodsworth DJ, Castellarin M, Holt RA. Sequence analysis of T-cell repertoires in health and disease. Genome Med. 2013;5(10):98. Published 2013 Oct 30. doi:10.1186/gm502

14. D Whole exome sequencing (WES), is a genomic technique for sequencing all of the protein-coding regions of genes in a genome (known as the exome, about 1.5% of all DNA). The term exon was derived from “EXpressed regiON,” since these are the regions that get translated, or expressed as proteins, as opposed to the intron, or “INTRagenic regiON” which is not represented in the final protein. It generally involves two steps: selection of the protein coding region on the DNA prior to sequencing using any high-throughput DNA sequencing technology. Exome sequencing is a good choice for scientists today who are looking for rare mutations, especially when used as a complement to studies of common variation like GWAS. GWAS (whole genome association study) captures a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait. They typically focus on associations between single-nucleotide polymorphisms (SNPs) and traits like major human diseases. The entire genome is being probed, in contrast to methods that specifically test a small number of pre-specified genetic regions.

Frayling TM. Genome-wide association studies: the good, the bad and the ugly. Clin Med (Lond). 2014;14(4):428-431. doi:10.7861/clinmedicine.14-4-428

Petersen BS, Fredrich B, Hoeppner MP, Ellinghaus D, Franke A. Opportunities and challenges of whole-genome and -exome sequencing. BMC Genet. 2017 Feb 14;18(1):14. doi: 10.1186/s12863-017-0479-5. PMID: 28193154; PMCID: PMC5307692.

https://www.broadinstitute.org/blog/what-exome-sequencing
https://en.wikipedia.org/wiki/Genome-wide_association_study

15. B Mass cytometry (or CyTOF -cytometry by time of flight) is a technique that combines two experimental platforms: flow cytometry and elemental mass spectrometry. Cells are stained with a cocktail of antibodies or probes coupled with unique stable, heavy-metal isotopes. Cells are passed in a single-cell suspension into a nebulizer, prior to entering the mass cytometer. Cells travel through an argon plasma, in which covalent bonds are broken to produce free atoms, which become charged in the process. The resulting ion cloud is passed through a quadrupole, enriched for heavy-metal reporter ions, and separated by their mass-to-charge ratio in a time-of- flight mass spectrometer. The ion counts are converted to electrical signals and ultimately into a data matrix in which every column represents a distinct isotope measured and each row represents a single mass scan of the detector. Essentially, it is a high-throughput single-cell analysis platforms similar to flow cytometry but at increased resolution and parameterization and used to identify different cell subsets in a mixture of cells and/or physiological endpoints such as cell signaling or activation status.

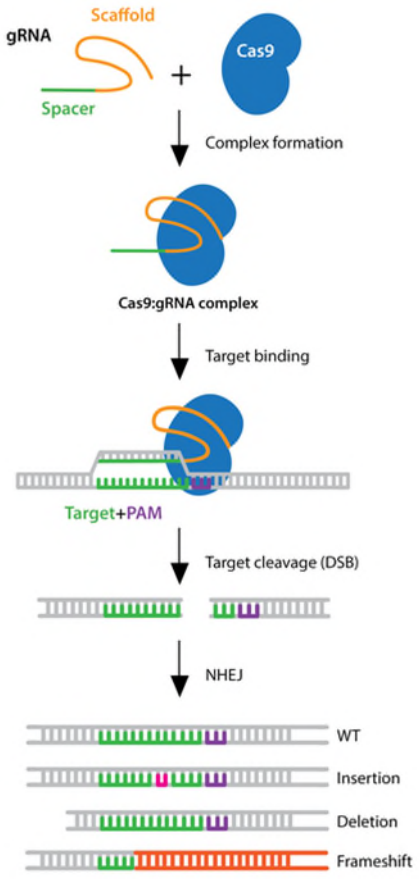
Spitzer MH, Nolan GP. Mass Cytometry: Single Cells, Many Features. Cell. 2016;165(4):780-791. doi:10.1016/j.cell.2016.04.019

16. B CRISPR Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are used as gene editing tools so that single base-pair changes can be made in a large genome. Luciferase is a reporter gene. GWS is genome-wide sequencing. RT-PCR (real time-PCR) is a method used to quantitate mRNA. ChIP, chromatic immunoprecipitation allows for detection of sequences bound to DNA, most often promoter sequences.

17. A Promoter bashing experiments involve the deletion of regions around the start of transcription of a gene in the promoter itself to identify sequences that are required for transcription under certain cicrumstances. (Hall and Giaccia, Fig. 17.22 has an example). Proteins bound to promoters are identified in electrophoretic mobility shift assays (EMSA). Double strand breaks are detected by several means: comet assay, gamma-H2Ax antibody binding, pulsed field gel electrophoresis and others. Most often sequence similarity is used to identify miRNA binding regions in mRNA. Many methods quantitate gene expression: Northwestern blots, microarray mRNA assays, RT-PCR, protein expression assays and more.

18. A The CRISPR reaction needs the guide RNA and the cas9 (CRISPR associated protein 9) in order to take place. The other ingredients in the answer are used in other reactions, but none of them are needed for CRISPR.

19. D The Clustered Regularly Interspased Short Palindromic Repeats (CRISPR)/Cas9 system is a versatile system to modulate target gene expression. It is composed of 2 elements: the guide RNA (gRNA) and Cas9 protein. The gRNA is an RNA molecule that directs the Cas9 protein to a specific area of the protein-encoding genome by complementary base paring. Cas9 is an endonuclease of bacterial origin that cuts double-stranded DNA at the location directed by the gRNA. This dsDNA break induces the host DNA damage response in an attempt to repair the cut. While some percentage of cuts will be repaired by error-free homologous recombination (HR), the majority of cuts are repaired by error prone non-homologous end joining (NHEJ) often resulting in small insertions and deletions (aka “indels”). These can cause the protein coding sequence of the gene to be altered in such a way as to disrupt protein expression and/or function. The net effect is a reduction in functional protein expression. Note that if the dsDNA cut is repaired perfectly, it re-creates the target sequence for the CRISPR/Cas9 system to cut yet again. It will do so until the target is repaired imperfectly and is no longer recognized by the gRNA.



From: https://www.addgene.org/guides/crispr/

20. A CRISPR/Cas9-mediated gene knockout is a secondary response to imperfect DNA damage repair. The CRISPR/Cas9 system is composed of 2 elements: the guide RNA (gRNA) and the Cas9 protein. The gRNA is an RNA molecule that directs the Cas9 protein to a specific area of the protein-encoding genome by complementary base paring. Cas9 is bacterial DNA endonuclease that cuts double-stranded DNA at the location directed by the gRNA. Once Cas9 cuts at the designated location, the host cell DNA damage response attempts repair. This primarily occurs through imperfect non-homologous end joining (NHEJ), which results in small insertions, deletions, and frameshifts that result in disrupted protein expression. The bulk of the DNA for the target gene remains in the genome, but is not expressed. mRNA degradation is the primary mechanism of gene suppression by RNA interference technology.

    Of note, subsequent applications of the CRISPR/Cas9 system have been developed to increase (CRISPR activation, or CRISPRa) or decrease (CRISPR interference, or CRISPRi) the transcription of specific genes. Increased gene transcription is accomplished by expressing a fusion protein of an endonuclease defective Cas9 (dCas9, which localized to gRNA-directed DNA but does not cut DNA) fused with a protein that recruits the transcriptional machinery to induce transcription. In this case the gRNA is designed to target the Cas9-transcription activator fusion protein to the promoter of the target gene. Conversely, suppression of gene transcription is accomplished in a similar way, but the dCas9 protein is fused with a transcriptional repressor protein. Subsequent generations of the CRISPR/Cas9 system have been developed to specifically alter epigenetic modifications at specific sites in the genome.

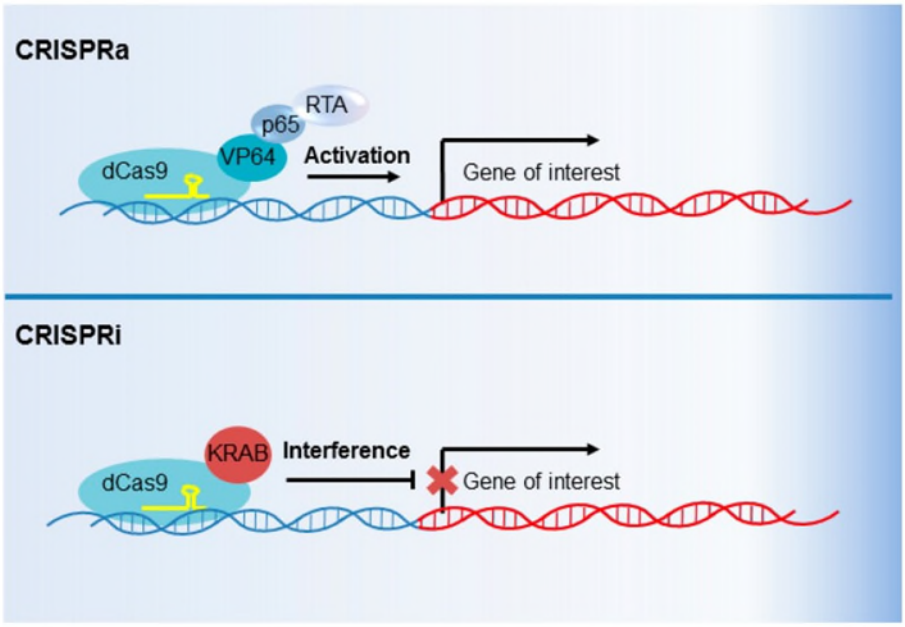


Image from: https://europepmc.org/article/pmc/pmc5850362

References:

https://www.addgene.org/guides/crispr/

https://ocg.cancer.gov/e-newsletter-issue/issue-14/crispri-and-crispra-new-functional-genomics-tools-provide

21. C SNPs are changes at a single locus with a minor allele frequency of greater than 1% in at least 1 population. There are approximately 10 million SNPs in the human genome. The basis of SNP research in cancer is purely correlative; the existence of a SNP does not indicate any particular functional consequence of that SNP, but rather only an association with a tested outcome (disease likelihood, chemo response, disease-free survival, overall survival, etc.). SNPs that are recurrently associated with a phenotype may be associated with the underlying cause of the phenotype by linkage disequilibrium and not by a true functional outcome of the SNP itself. Thus, answer D is incorrect. If present in a protein coding region, SNPs may be silent or result in an alteration in protein sequence. The biased manner of most research examining SNPs (i.e., the "candidate gene approach") means that often only protein encoding regions of specific genes are examined, however the vast majority of SNPs occur in noncoding regions of DNA.

Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2410167/

Molecular Imaging

1. B 18-Fluorodeoxyglucose is the most commonly used metabolic radiotracer for PET scanning at present.

2. C 18-Fluorine labeled thymidine has been used to image DNA synthesis in humans in vivo.
   a. Salskov A, Tammisetti VS, Grierson J, Vesselle H. Semin Nucl Med. 2007 Nov;37(6):429-39. PubMed link

3. C The prostate-specific membrane antigen (PSMA) is a promising, well-characterized biomarker of prostate cancer and is associated with tumor aggressiveness. Histologic studies have associated high PSMA expression with metastasis, androgen independence, and progression. PSMA imaging by SPECT using the agent 111In-Capromab Pendetide (ProstaScint™), approved by the Food and Drug Administration, demonstrated poor performance due to several factors, including the inherent limitations of intact antibody-mediated imaging (poor tumor penetration and slow blood-pool clearance), the relatively coarse resolution of SPECT, and the fact that the 7E11-C5.3 antibody on which Indium-111 Capromab Pendetide is based binds to an intracellular epitope of PSMA. Radiolabeled antibodies with their poor tumor penetration and slow blood-pool clearance tend to have lower tumor-to-background ratios (23590171). The correct answer is "c". The other answers are incorrect and are not true of 111Incapromab pendetide as this agent has poor tumor penetration, slow blood-pool clearance, lower tumor-to-background ratios for low volume disease and is a SPECT imaging agent. Answer "c" is correct. The other answers incorrect describe 111Incapromab pendetide.

4. E The prostate-specific membrane antigen (PSMA) is a promising, well-characterized biomarker of prostate cancer and is associated with tumor aggressiveness. Histologic studies have associated high PSMA expression with metastasis, androgen independence, and progression. Low molecular weight molecules have inherent advantages over antibodies, such as rapid tumor uptake and clearance from nontarget sites. Many low-molecular-weight inhibitors of PSMA are in development including DCFBC. 18F-DCFBC PET/CT may be superior molecular imaging of prostate cancer versus 111In-Capromab Pendetide for several reasons. As a druglike molecule, it should have rapid and high tumor penetration along with rapid blood-pool clearance, compared with radiolabeled antibodies, allowing for higher tumor-to-background ratios. It targets a more accessible, external binding domain of PSMA, rather than an intracellular domain. Additionally, PET allows for higher resolution and is highly amenable to quantification in contrast to SPECT, and the relatively long (110 min) physical half-life of 18F enables regional clinical distribution (23590171). Answer "e" is correct. The other answers describe characteristics of an imaging agent that are not ideal for a highly efficacious molecular imaging agent.

5. A, B, C, and D