Name: _____ Date: _____

In this exam sheet, there are 18 questions.
Each answer yields up to 2 points according to its correctness and completeness.

1. What are the Reactive Oxygen Species? Name at least three of them.

2. As an ionizing particle goes through water it produces a certain number of radiolytic species. Recall the definition of the *G* value to find a simple formula that returns the mean number of ROS of a given type produced by *N* identical ionizing particles with an initial kinetic energy of *E* eV when they are stopped in water.

3. What is the approximate duration of the cell cycle for proliferating human cells? If a single initial cell could proliferate without limit, how many cells would you find after one year?

4. Explain what stroma, parenchima and epithelium are.

5. What is a codon? How are they related to the aminoacids?

6. What are the ribosomes?

7. What is the difference between *direct* and *indirect* damage caused by radiation in cells?

8. What is catalase? Why is it important?

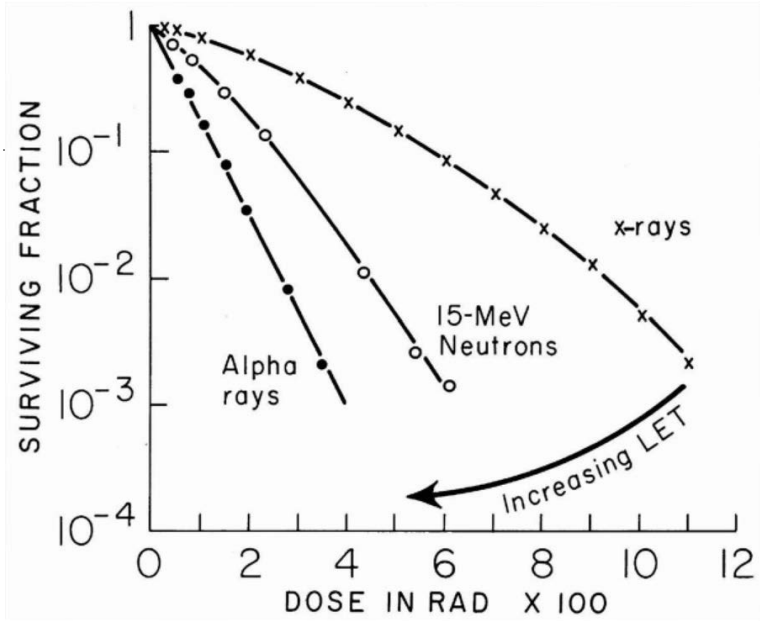
9. What is clonogenic death?

10. In the Poisson model of survival of irradiated cells, a cell dies if a single sensitive target is hit. In this model, the probability of NOT being hit is $S(D) = e^{-D/D_0}$, and this is also the expression of the survival probability in the Poisson model. How is this survival probability represented in a logarithmic plot?

11. The linear-quadratic model is an empirical description of the survival probability *S(D)*. What is its range of validity? How does the shape of the survival curve change when the α/β ratio is large?

12. Describe the multitarget model, derive its mathematical expression, and compare it to the Poisson model of the surviving fraction.

13. Consider the following figure and use it to compute the RBE of 15-MeV neutrons at the surviving fraction level of 10^{-2}.

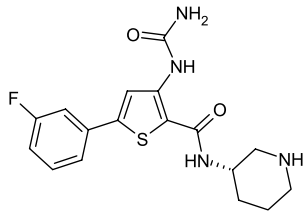


14. What is the NTCP? How is it usually modeled?

15. The U-251MG cell line (one of the cell lines of the brain tumor *glioblastoma multiforme*) has the following LQ parameters: $\alpha = 0.36$ Gy^{-1} and $\beta = 0.06$ Gy^{-2}. When we irradiate these cells in a fractionated treatment with a series of 2 Gy doses, what is the effective D_0?
(Hint: the effective D_0 is defined in the Poisson model description of the surviving fraction: $S(D) = e^{-D/D_0}$)

16. What is the expression for the biologically effective dose (BED) and how is it corrected for cell proliferation?

17. AZD7762 is a novel drug that is administered in combination with DNA-damaging agents, to enhance the efficacy of both conventional chemotherapy and radiotherapy and increase patient response rates in a variety of settings.



AZD7762

It works by abrogating the S and G2 checkpoints. How does this explain its radiosensitizing activity?

18. List the 5 R's of radiobiology, along with a short description of their meaning.

Answers

1. When irradiated, water molecules can be broken into ions by the action of the ionizing particles. The resulting ions can combine with neighboring water molecules or other ions and the whole process produces several types of radiochemical species which are strongly reactive and are collectively called *Reactive Oxygen Species* (ROS). ROS are also synthesized endogenously, by the cellular metabolism.
Common ROS are O_2^-, H_2O_2, OH^-, OH, H_3O^+, e_{acq}^-.

2. According to the definition, G is the mean number of copies of a given ROS produced by the ionizing particle for every 100 eV of its initial energy, so that in this case the mean number of ROS copies is

$$n = G \times E(\text{eV})/100$$

and finally, the total number of copies is, on average

$$Nn = N \times G \times E(\text{eV})/100$$

3. The duration of the cell cycle in human cells is approximately one day. Since there are 365 days in one year, if the cells could really proliferate without limit, there would eventually be $2^{365} \approx 10^{110}$ cells at the end of one year (2^1 at the end of day 1, 2^2 at the end of day 2, 2^3 at the end of day 3, ... , 2^{365} at the end of day 365; since $2 \approx 10^{0.3}$, this means $2^{365} \approx (10^{0.3})^{365} \approx 10^{110}$).

4. The stroma is that part of tissue or organ that has connective and structural role (like connective tissue, blood vessels, nerves, etc.). The parenchima is that part of tissue that performs the function of the tissue or organ. Finally, the epithelium is a tissue that lines cavities and surfaces of blood vessels and organs.

5. A codon is a triplet of consecutive nucleotides in the DNA molecule that can encode either an aminoacid or an instruction that corresponds to the start or stop of the genetic sequence that defines a whole gene.

6. The ribosomes are complex molecular machines that are built of two main parts, the *small unit* and the *large unit*. These two pieces assemble around a messenger RNA (mRNA) molecule, and use the information that it carries (a sequence of codons), to pick aminoacids in the cytoplasm and synthesize a protein.

7. An ionizing particle can break chemical bonds in the DNA molecule when it passes in its vicinity (or when a photon is absorbed close to the molecule), and this is the direct damage. It can also produce ROS in the cytoplasm, and the damage to DNA can be caused by these ROS as they drift close to it (indirect damage).

8. Catalase is an enzyme that converts oxygen peroxide (H_2O_2) into water and molecular oxygen. This is an important defense mechanism against DNA damage, because H_2O_2 is a ROS.

9. Clonogenic death occurs when a cell is unable to form clones, i.e., to proliferate.

10. Since

$$\ln S(D) = -D/D_0$$

the survival probability is represented by a straight line in a logarithmic plot.

11. The linear-quadratic model is valid for an absorbed dose lower than about 6 Gy. The alpha coefficient is related to the linear part, while the beta coefficient is related to the quadratic part. When the alpha coefficient is large with respect to beta, the survival probability is represented by a curve close to a straight line in the logarithmic plot.

12. In the Poisson model, a cell dies if a single sensitive target is hit; in this model, the probability of NOT being hit is $S(D) = e^{-D/D_0}$. In the multitarget model we assume that a cell dies only when multiple targets are all hit. So, if the Poisson probability of not hitting a given target is e^{-D/D_0}, then the probability of hitting the same target *at least once* is $1 - e^{-D/D_0}$, and therefore the probability of hitting *all* of the *n* targets at least once is $(1 - e^{-D/D_0})^n$, and finally, the probability of NOT hitting all of them at least once is

$$S(D) = 1 - (1 - e^{-D/D_0})^n$$

13. The RBE, i.e., the ratio between the doses D_X (photons) and D (neutrons) that correspond to $S(D) = 10^{-2}$ is approximately 2.2.

14. The NTCP is the normal tissue complication probability. One often uses the Lyman description, which is given by the expression

$$NTCP = \frac{1}{2\pi}\int_{-\infty}^{u} e^{-t^2/2}dt$$

with

$$u = \frac{D - TD_{50}}{m \cdot TD_{50}}$$

15. The surviving fraction in the LQ model is described by the expression

$$S(D) = e^{-(\alpha D + \beta D^2)}$$

In the present case $\alpha D = 0.72$; $\beta D^2 = 0.24$, and therefore $\ln S(2\text{ Gy}) = -0.96 = -\frac{2\text{ Gy}}{D_0}$. Thus, $D_0 \approx 2.08$ Gy.

16. The survival probability with *n* doses *D* is $[S(D)]^n$, and the corresponding biological effect is

$$\begin{aligned} E &= -\ln[S(D)]^n = -n \ln S(D) \\ &= n(\alpha D + \beta D^2) \\ &= \alpha(nD)\left(1 + \frac{D}{\alpha/\beta}\right) \end{aligned}$$

The biologically effective dose is defined as follows

$$\text{BED} = \frac{E}{\alpha} = (nD)\left(1 + \frac{D}{\alpha/\beta}\right)$$

After a "kickoff time" T_k, tumor cells start proliferating again, therefore the tumor population after treatment changes by the total factor

$$N(T)/N_0 = [S(D)]^n 2^{(T-T_k)/T_p}$$

where T_p is the tumor cells' duplication time. Taking logarithms, one finds

$$n \ln[S(D)] + \frac{T - T_k}{T_p/\ln 2} = -\alpha n D\left(1 + \frac{D}{\alpha/\beta}\right) + \frac{T - T_k}{T_p/\ln 2}$$

and finally

$$\begin{aligned} \text{BED}(D, n, T) &= (nD)\left(1 + \frac{D}{\alpha/\beta}\right) - \frac{T - T_k}{\alpha T_p/\ln 2} \\ &= \text{BED}(D, n) - \frac{T - T_k}{\alpha T_p/\ln 2} \end{aligned}$$

17. When DNA is damaged a proliferating cell stops at checkpoints to repair DNA and the cell cycle restarts only when the repair is complete. By abrogating checkpoints in combination with DNA-damaging agents, it is thus possible to kill proliferating cells.

18. The 5 R's of radiobiology are:

- *Repair*: repair of sublethal damage must be accounted for, because it affects the tolerance of healthy tissue to radiotherapy (allowing cells to repair we can continue a treatment that should otherwise be interrupted), and because tumor cells often have a reduced ability to repair damage, e.g., when they have a mutated P53 gene
- *Redistribution of cells within the cell cycle*: Proliferating cells have different radiosensitivities, in particular cells in the S phase are *less* sensitive to radiation.

After a session, more of the cells in the S phase survive, and waiting for a redistribution of cells in different phases helps in killing them.

- *Repopulation*: Repopulation takes place both in healthy and in diseased tissues. At least some tumors display accelerated repopulation after 4-5 weeks into treatment. This means that this repopulation must be countered in long treatments.
- *Reoxygenation*: Many tumor tissues are hypoxic, and this protects tumor cells from radiation because of the Oxygen Effect. Therefore, one useful strategy consists in helping oxygen diffuse through tissues. Reoxygenation can be achieved by killing cells closer to blood vessels, so that oxygen penetrates more deeply into the tumor tissue.
- *Radiosensitivity*: Radiosensitivity differs in different cell types, and this factor must be included in therapeutic strategies. Radiosensitivity can also be enhanced in tumor cells with proper sensitizing chemicals.