Quiz-2 **BT-637 Genome Editing and Engineering** **Date: 11/11/2024**

Q1. Write the full form of: **1.5 marks**

Ans: a) GVHD - Graft-Versus-Host Disease

b) ESC - Embryonic Stem Cell

c) iPSC - Induced Pluripotent Stem Cell

d) scFv - Single-chain Variable Fragment

e) CAR - Chimeric Antigen Receptor

f) HPFH - Hereditary Persistence of Fetal Hemoglobin

Q 2. **1.5 marks**

a) Write down the guide RNA sequence that binds to the **MC1R** gene, and the complementary DNA sequence that it binds to. Label the 5' and 3' ends of both strands.

5’-------CCCACAGCCATCCCCCAGCTGGGTCAGCCTCCCTAAGA----------3’

3’------GGGTGTCGGTAGGGGGTCGACCCAGTCGGAGGGATTCT---------5’

Ans: a) Guide RNA Sequence and Complementary DNA Sequence:

Guide RNA: 5'-CCCACAGCCAUCCCCCAGCU-3'

Complementary DNA: 3'-GGGTGTCGGTAGGGGGTCGA-5'

b) Compare the sequences in the above answer you just made to Question 2. How did the sequence of the gene change due to CRISPR-Cas9? Where was this change made (to the RNA, to one DNA strand, or to both DNA strands)?

Ans: Comparison of Gene Sequence Change Due to CRISPR-Cas9:

Using CRISPR-Cas9 causes a double-strand break (DSB) at the target sequence. This change affects both DNA strands, leading to possible insertions or deletions (indels) upon repair by non-homologous end joining (NHEJ), which can disrupt gene function.

c) How might this change inactivate, or “knock out,” a gene?

Ans: Indels introduced by NHEJ can lead to frameshift mutations, creating premature stop codons and disrupting protein synthesis. This "knock-out" inactivates the gene by producing a non-functional or truncated protein.

Q3. Consider the following statements on genome editing- **1 mark**

1. CRISPR-Cas9 is one of the techniques of genome editing.
2. CRISPR is an enzyme used to edit the DNA
3. Cas9 is used to determine the DNA segment to be altered.

Which of the above statements is/are true?

A) 1 only B) 1 and 2 only C) 1 and 3 only D) All of the above

Q4. Fill in the blanks: **2 marks**

a) Cas12 is used for targeting DNA molecules, while Cas13 is specific for RNA molecules.

b) iPSCs are derived from somatic cells that are reprogrammed to a pluripotent state.

c) Second-generation CAR T cells contain one co-stimulatory domain, while third-generation CAR T cells contain two co-stimulatory domains.

d) Sickle cell anemia results from a mutation in the HBB gene, which alters hemoglobin structure.

Q5. If a gene is flanked by two loxP sites in the same orientation and Cre recombinase is introduced, what is the likely outcome? How would this outcome change if the loxP sites were in opposite orientations? **1 mark**

Ans: Same Orientation of loxP Sites: Cre recombinase will delete the gene flanked by the two loxP sites, resulting in excision.

Opposite Orientation of loxP Sites: Cre recombinase will invert the gene between the loxP sites, resulting in an inverted sequence within the genome.

Q6. In a cytotoxicity experiment, you test second- and third-generation CAR T cells against target cells. If second-generation CAR T cells kill 60% of target cells and third-generation cells kill 80%, how many target cells remain from an initial population of 1,000 after exposure to each CAR T cell type? **1 mark**

Ans: Initial Target Cell Population: 1,000

Second-generation CAR T cells (60% killed):

60% of 1,000 = 600 cells killed, leaving 400 target cells.

Third-generation CAR T cells (80% killed):

80% of 1,000 = 800 cells killed, leaving 200 target cells.

Q7. CAR T cells often face limitations in persistence and efficacy in solid tumours. Describe two specific modifications to CAR T cell constructs that could enhance their ability to infiltrate and persist within solid tumor environments. Discuss the potential drawbacks of each modification. **2 marks**

Ans: Incorporation of Chemokine Receptors: Adding chemokine receptors (e.g., CCR2) helps CAR T cells follow chemokine gradients into the tumor microenvironment.

Drawback: This may increase off-target effects if the chemokine receptors lead to accumulation in non-tumor tissues expressing the same chemokines.

Addition of Costimulatory Domains or Cytokine Secretion: Adding an IL-15 or IL-7 signaling domain can improve CAR T cell survival and persistence.

Drawback: This could lead to overstimulation and exhaustion of CAR T cells or increased cytokine release syndrome (CRS), posing safety concerns.

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