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VCE Biology ¾
AOS 1 Revision [1.0]
SAC 4 Solutions

40 Marks. 5 Minutes Reading. 60 Minutes Writing.

Section A: SAC Questions (40 Marks)

CRISPR-Cas9 in Treating Transthyretin Amyloidosis

In an unprecedented clinical case study, CRISPR-Cas9 gene-editing technology was employed to target transthyretin amyloidosis, a debilitating genetic disorder. The trial involved a novel therapy, where patients received a one-time intravenous treatment designed to disrupt the mutated TTR gene within the liver, the site of the protein's synthesis.

The CRISPR-Cas9 system, delivered through lipid nanoparticles, was engineered to locate and cleave the specific DNA sequence harbouring the mutation responsible for the production of the defective TTR protein. This protein misfolds, and the accumulation of this misfolded protein as amyloid fibrils in tissues leads to systemic organ dysfunction and disease progression.

The trial's participants were closely monitored for efficacy and safety. Remarkably, results indicated a substantial reduction in serum TTR levels, correlating with decreased amyloid deposits. This reduction suggested that the treatment not only alleviated the clinical symptoms but also addressed the pathogenic root of the disease.

This therapeutic intervention represents a significant advance over traditional approaches, which have largely been confined to symptom management and do not modify the underlying genetic defect. Symptom management, it must be noted, has been found to be incredibly successful in providing patients with a relatively symptom-free life at a reduced cost. The success of the therapy in reducing the production of the mutant protein offers hope for a durable and potentially curative treatment.

The clinical trial also established the CRISPR-Cas9 system's safety profile, with most adverse events being mild and transient. Crucially, the study's longitudinal follow-up is assessing long-term outcomes, including any potential off-target effects and the stability of the gene edit over time.

The implications of this case study extend beyond transthyretin amyloidosis, setting a precedent for the use of in vivo gene editing in treating a range of genetic disorders. It underscores the therapeutic potential of CRISPR-Cas9 and opens new avenues for genetic research and medicine.

Moreover, the trial demonstrates the possibility of correcting genetic defects directly within the patient's body, a concept that shifts the paradigm from managing to curing inherited diseases. The success of this gene therapy heralds a new era in precision medicine, where treatments are tailored to the individual genetic makeup, offering a personalised approach to healthcare.

The case study is not only a testament to the power of genetic engineering but also emphasises the importance of ethical considerations in gene editing. With the capability to alter DNA comes the responsibility to ensure that such interventions are safe, ethical, and accessible to those in need.

In summary, the use of CRISPR-Cas9 in this clinical setting has shown that targeted gene editing can have profound effects on treating genetic diseases, potentially offering life-changing benefits to patients with conditions once deemed untreatable. As research progresses, it may pave the way for the approval and adoption of similar gene therapies for other genetic disorders, revolutionising the field of genetic medicine.

Question 1 (11 marks)

The primary cause of TTR is the result of a misfolded protein – this protein accumulating incorrectly is what leads to fibrosis. Scientists believe this to be the result of a mutation in the gene coding for TTR.

a. Explain how a mutation in the TTR gene could lead to changes in the structure of the TTR protein. (2 marks)

A mutation in the TTR gene could lead to changes in the structure of the TTR protein by:

Altering the amino acid sequence – A mutation may cause a change in the codon, leading to the substitution of a different amino acid during translation. This can affect protein folding.

Disrupting protein stability – The altered amino acid sequence can affect hydrogen bonds, disulfide bonds, or hydrophobic interactions, leading to misfolding of the TTR protein, which then accumulates abnormally and contributes to disease

The gene that codes for TTR can code for multiple proteins alongside the malfunctioning TTR.

b. Describe ONE reason why this may be the case, and explain how scientists can avoid affecting those proteins in editing for TTR. (2 marks)

The TTR gene can code for multiple proteins due to alternative splicing, where different exons are included or excluded during mRNA processing, leading to different protein isoforms.

To avoid affecting these proteins when editing for TTR, scientists can use CRISPR-Cas9 with a guide RNA specific to the malfunctioning TTR mutation, ensuring only the faulty sequence is targeted while leaving other isoforms intact.

The TTR protein will accumulate in tissues, causing fibrosis.

c. Name and describe the role of TWO organelles involved in exporting TTR from hepatocytes. (4 marks)

Endoplasmic Reticulum (ER) – The rough ER processes and folds the TTR protein after it is synthesized by ribosomes. It ensures proper folding and modifies the protein before it is transported to the Golgi apparatus.

Golgi Apparatus – The Golgi modifies, sorts, and packages the TTR protein into vesicles for secretion. These vesicles then transport the protein to the cell membrane, where it is exported from the hepatocyte into the bloodstream.

d. Explain, with reference to gene regulation, why the in vivo method of editing the TTR gene is successful in reducing serum TTR levels, as opposed to editing it in an embryo. (3 marks)

The in vivo method of editing the TTR gene is effective in reducing serum TTR levels because it specifically targets hepatocytes, where TTR is actively transcribed and produced. By disrupting the TTR gene in these cells, the production of misfolded TTR protein is reduced, leading to lower serum levels.

In contrast, editing the gene in an embryo would affect all cells, including non-hepatic cells where TTR is not normally expressed, making it less effective at directly reducing TTR production.

Additionally, gene regulation ensures that only hepatocytes actively transcribe the TTR gene, so in vivo editing specifically prevents TTR synthesis where it matters most.

Space for Personal Notes

Question 2 (9 marks)

There are a number of methods that scientists suggested in order to determine the success of gene editing. Frank suggested using PCR to increase the amount of protein and analyse its structure, whereas Josh was backing gel electrophoresis.

a. Explain why PCR cannot increase the amount of protein to analyse its structure. (1 mark)

PCR cannot increase the amount of protein because it amplifies DNA, not proteins, meaning it does not directly produce or replicate the TTR protein for structural analysis.

b. Describe the process of PCR. (3 marks)

The process of PCR involves three main stages:

Denaturation (94°C) – Hydrogen bonds between double strands of DNA are broken, separating the DNA into single strands.

Annealing (55°C) – Primers attach to the 3' ends of single-stranded DNA, providing a starting point for DNA synthesis.

Extension (72°C) – Taq polymerase adds complementary nucleotides in the 5' to 3' direction, joining the phosphate backbone via phosphodiester bonds to form a double-stranded DNA copy.

c. Explain how gel electrophoresis could be used to identify whether an edit has successfully been made or not. (3 marks)

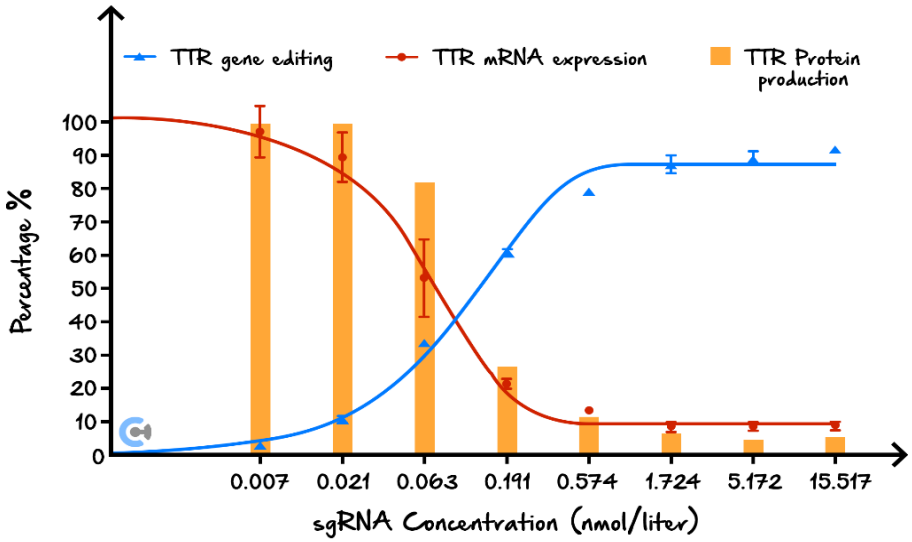
Gel electrophoresis can be used to identify whether a gene edit has been successfully made through the following steps:

DNA Fragment Separation: DNA samples, including edited and unedited sequences, are loaded into a gel and subjected to an electric field. Shorter fragments move faster and travel further than longer ones.

Comparison with Controls: The edited DNA is compared to a control (unedited DNA). A successful edit may result in a different band pattern due to changes in fragment size caused by insertions, deletions, or restriction enzyme sites.

Visualization: Staining with a DNA-binding dye (e.g., ethidium bromide) or fluorescent markers allows bands to be seen under UV light, confirming whether the expected edit has occurred.

The following were some of the results of this experiment.



In Vitro Evaluations of the Potency of NTLA-2001

Shown is the relationship between increasing concentrations of sgRNA and the consequent percentages of TTR editing, as well as TTR mRNA expression and TTR protein production in a single lot of primary human hepato-cytes. The primary indel patterns were a single-nucleotide deletion or insertion at the cut site, inducing a frameshift mutation (data not shown).

d. Describe the results and indicate what they mean. (2 marks)

As sgRNA concentration increases, TTR gene editing increases, leading to a decrease in TTR mRNA expression and TTR protein production. This indicates that successful gene editing disrupts TTR transcription and translation, effectively reducing TTR protein levels.

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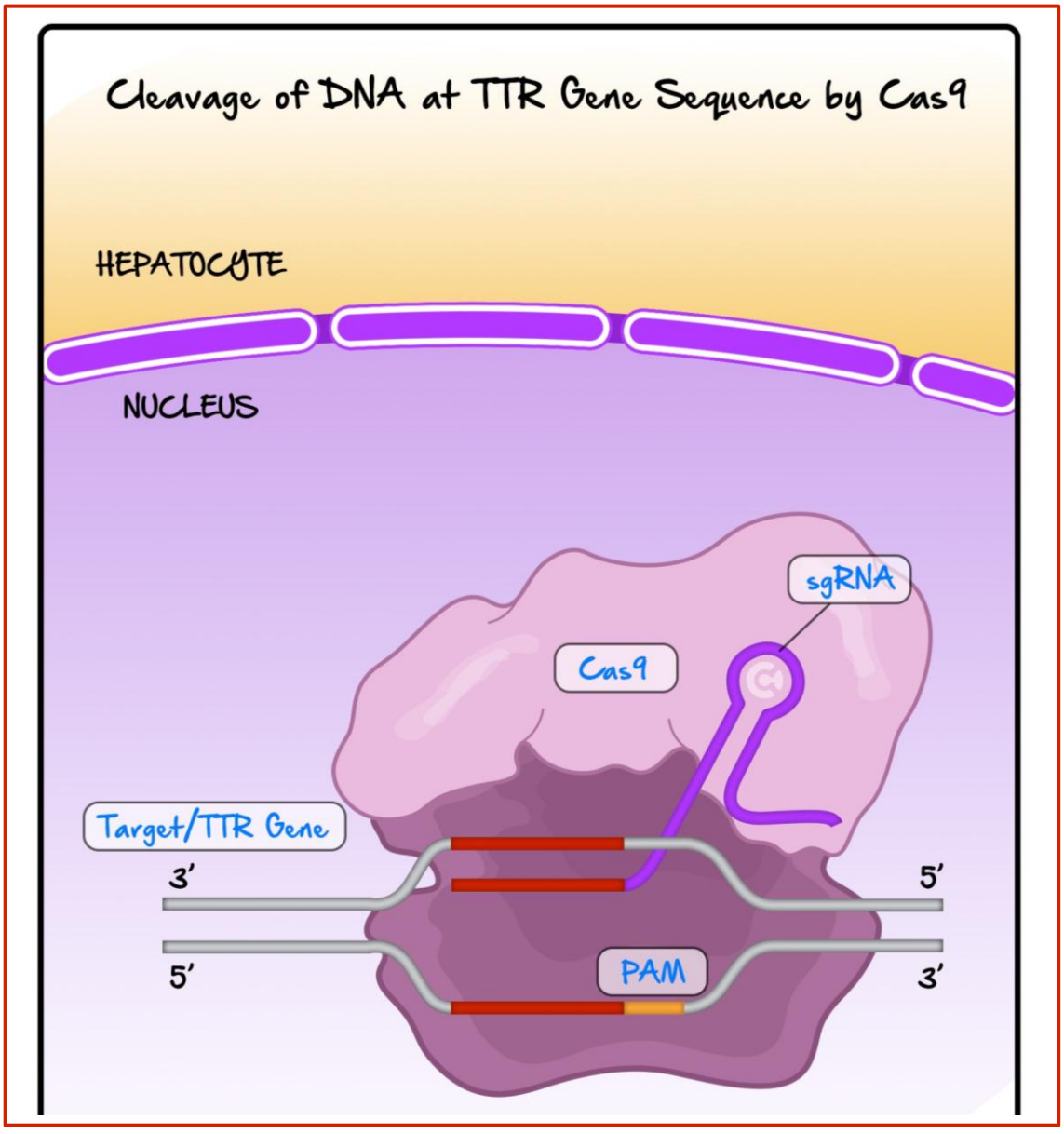
Question 3 (10 marks)

CRISPR-Cas9 in vivo editing is a revolutionary way to cure TTR.



a. Label this diagram. (4 marks)

b. Compare the function of the PAM in TTR editing compared to its function in bacteria. (2 marks)

In TTR editing, the protospacer adjacent motif (PAM) is essential for CRISPR-Cas9 to recognize and bind to the target DNA sequence, allowing precise gene editing. In bacteria, the PAM sequence helps the CRISPR system distinguish foreign viral DNA from the bacterium's own genome, ensuring only invading DNA is targeted for destruction.

c. Name the vector used to deliver the CRISPR-Cas9 complex to the patient in this case, and explain how it is specific to the liver cells. (2 marks)

The vector used to deliver the CRISPR – Cas9 complex in this case is lipid nanoparticles (LNPs). LNPs are specific to liver cells because they are taken up efficiently by hepatocytes due to their interaction with apolipoprotein E (ApoE), which facilitates receptor-mediated endocytosis in the liver.

d. Suggest two strategies to reduce the chance of off-target effects when editing using CRISPR-Cas9. (2 marks)

Designing a more specific guide RNA (gRNA) – Ensuring the gRNA sequence is highly specific to the target gene reduces the chance of binding to unintended sites.

Optimize sgRNA design – Selecting sgRNA sequences with minimal similarity to other genomic regions ensures that Cas9 binds only to the intended target site.

Question 4 (10 marks)

a. Analyse, with reference to justice and integrity, the ethics of editing symptomatic patients with CRISPR-Cas9. (4 marks)

Justice: Justice in bioethics refers to fair access to treatments. Editing symptomatic patients with CRISPR-Cas9 raises concerns about affordability and availability. If only wealthy individuals or certain countries can access the treatment, it may create health inequalities. Ensuring equitable distribution is essential.

Integrity: Integrity involves conducting gene editing responsibly and transparently. Scientists must ensure CRISPR-Cas9 is safe and effective before widespread use. Any risks, such as off-target effects, must be disclosed to patients, maintaining trust in medical research and practice.

b. Discuss the ethical implications of off-target effects in editing for TTR modification, with reference to the principles of respect. (2 marks)

Off-target effects in TTR modification raise ethical concerns related to respect for autonomy and respect for non-maleficence.

Respect for autonomy – Patients must be fully informed of potential off-target effects so they can make an informed decision about undergoing gene editing. Lack of transparency would undermine their right to choose.

Respect for non-maleficence – Off-target effects could cause unintended harm, contradicting the principle of "do no harm." Scientists must minimize these risks to ensure patient safety and ethical medical practice.

c. What ethical principle must be considered when performing trials for therapies such as CRISPR-Cas9 therapy? Discuss at least 2. (4 marks)

Two key ethical principles that must be considered when performing trials for CRISPR-Cas9 therapy are:

Beneficence – Researchers must ensure that the therapy provides a significant benefit to patients, outweighing potential risks. This includes rigorous testing to confirm safety and effectiveness before clinical use.

Justice – Access to the therapy should be fair and equitable, preventing discrimination based on socioeconomic status or geography. Trials must ensure that diverse populations are included so that the treatment benefits all affected individuals.

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