Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica

Edital Geral PRPGP/UFSM N° 065/2024

Seleção 20251/ 3ª janela – Nível Doutorado

Prova de conhecimentos gerais em Bioquímica e Biologia Molecular

Nome do candidato: ____

INSTRUÇÕES:

- A prova tem duração de duas horas (2h).
- É permitido consultar dicionário inglês/português (material físico).
- O caderno de questões e a folha de respostas definitiva devem ser entregues aos avaliadores ao final da prova.
- O caderno de questões e o gabarito final serão publicados na página do PPGBTox após a prova.
- As questões são objetivas e possuem apenas uma alternativa correta.
- Suas respostas devem ser embasadas no texto do caderno de questões e no seu conhecimento sobre o assunto.
- A folha de respostas definitiva encontra-se na próxima página e deve ser preenchida de maneira que seja possível identificar as suas respostas.

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FOLHA DE RESPOSTAS DEFINITIVA:

01	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
04	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
03	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
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06	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
07	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
08	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
09	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )
10	a) ( )	b) ( )	c) ( )	d) ( )	e) ( )

Assinatura do candidato: ____

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Caderno de Questões

Read the report below, adapted from the Science magazine website, and answer the questions that follow:

Gene-editing therapy made in just 6 months helps baby with life-threatening disease

Custom CRISPR paves the way for treating genetic disorders in tailormade ways

15 May 2025 By Jocelyn Kaiser

doi: 10.1126/science.zp4aln0

In a world first, researchers report easing the symptoms of a baby boy with a rare, life-threatening disease by giving him a version of the CRISPR gene editor tailored to a mutation he carries.

The boy, first treated in February at just 7 months old, still needs a special diet and medication; the experimental therapy alone isn't enough to prevent a dangerous buildup of ammonia in his blood caused by a faulty gene for a key liver enzyme. Still, the rapid development of a gene editor that appears to have repaired the defect in some of his liver cells is a landmark demonstration of a personalized approach that has tantalized rare disease researchers.

His treatment relies on a base editor, a variation on the better known CRISPR. With CRISPR, an enzyme fully cuts DNA at a specific site in the genome determined by a strand of guide RNA. In base editors, CRISPR's enzyme is altered so it only nicks one of DNA's double strands. A second enzyme then swaps out a DNA base, correcting a single "letter" misspelling. Base editors infused into a body to edit liver cells have shown success at treating adults with a high cholesterol disease and another genetic disorder.

Cardiologist Kiran Musunuru of the University of Pennsylvania Perelman School of Medicine, who helped develop one such treatment, and Rebecca Ahrens-Nicklas, a physician-scientist at Penn Medicine and the Children's Hospital of Philadelphia (CHOP) who treats metabolic diseases, wanted to move on to a bigger challenge. They set their sights on quickly designing base editors customized for young patients born with diseases called urea cycle disorders, who might avoid organ damage and other complications if treated very early in life. These conditions involve defects—often a single-base change—in genes encoding enzymes the liver needs to create harmless urea from the body's ammonia. Ammonia, produced by the breakdown of proteins, accumulates in the blood, where it can cause lethargy, coma, and brain damage.

The idea was to develop a pipeline to rapidly test various base-editing enzyme components and guide RNAs to find a combination that could effectively repair a patient's mutated gene. The Penn Medicine team and collaborators first practiced on mice with various mutations for a metabolic disorder called phenylketonuria. By streamlining steps such as inserting the disease gene into cultured cells, they reduced the time needed to make a custom base editor from 1 to 2 years to months and showed they could cure the mice.

In August 2024, they found an ideal human test case, a baby whose urea acid disorder would likely become so severe the potential benefits of treating him outweighed the risks. The infant, nicknamed KJ by his family, was diagnosed soon after birth with severe carbamoyl phosphate synthetase 1 (CPS1) deficiency, a disease that occurs in just one in 1 million births. Doctors immediately controlled KJ's ammonia levels with a low-protein diet and nitrogen-scavenging drugs, but he was likely going to need a risky liver transplant.

The CHOP/Penn Medicine team and collaborators raced to develop and test a base editor to correct one of KJ's broken copies of the CPS1 gene in just 6 months. After safety testing in lab animals, they got regulatory approval to infuse microscopic balls of fats, known as lipid nanoparticles, carrying messenger RNA encoding the editing tools into the bloodstream of the nearly 7-month-old.

Although the team didn't feel it could ethically do a risky liver biopsy to prove the base editor repaired KJ's cells, indirect evidence suggests it worked. After three doses, KJ can consume more protein and needs less medication to control his blood ammonia levels. The study's approval only allowed KJ to get three doses of the base editor, and he's clearly not cured. But his doctors hope he can avoid a liver transplant—and could in theory get more doses of the base editor as he grows. He is reaching developmental milestones and his father, Kyle Muldoon, said in a press call, "We're very, very happy with the results." He will soon go home from the hospital.

The treatment caused levels of certain liver enzymes to rise, signaling an immune response to the nanoparticles or their cargo, but they soon returned to normal. As with CRISPR, base editors have a theoretical risk of changing unintended DNA sequences—indeed, tests of KJ's base editor on cells revealed an off-target change, but not one likely to cause harm. "We're thrilled," Ahrens-Nicklas says. "This seems to be safe. … There's some early signs that this is going to benefit [KJ]." Musunuru adds, "Our hope is that this will be the start of something that many, many others around the world will pick up on."

Another promising approach relies on a CRISPR-like tool not to repair a gene, but to insert a whole working version at a specific site in the genome. A baby who received this treatment for a different urea cycle disorder, ornithine transcarbamylase (OTC), is now off all special diet and medication, a company reported in January and in recent scientific meeting presentations.

The gene insertion strategy is delivered with a virus, however, which carries its own risks. It can only be given once for now and is much more expensive to manufacture than base editors. Medical geneticist Cary Harding of Oregon Health & Science University, who is consulting on the trial for OTC, welcomes a diversity of strategies to treat these rare but devastating conditions. "At this stage, it's all experimental and all [approaches] deserve to be explored."

QUESTION 01.

Based on the article provided, which of the following statements best summarizes the central achievement and significance of the reported gene-editing therapy?

a) Researchers achieved a significant and lasting improvement in a baby's severe urea cycle disorder using an advanced form of CRISPR technology, leading to a near elimination of the need for dietary restrictions.

b) A novel base-editing therapy, customized and developed rapidly, showed promising results in a baby with a rare liver enzyme deficiency, reducing his need for medication and potentially avoiding a liver transplant.

c) The study primarily highlighted the potential of personalized RNA-based drugs for a wide range of inherited metabolic liver disorders in children, showcasing their less invasive nature compared to traditional treatments.

d) Scientists successfully achieved a nearly complete repair of a mutated gene in a substantial portion of a baby's liver cells through a cutting-edge viral gene therapy, leading to a substantial long-term benefit for his condition.

e) The primary focus of this groundbreaking research was to optimize gene-editing tool delivery systems for broader application across a multitude of rare genetic conditions, aiming for widespread accessibility rather than individual patient success.

QUESTION 02.

Baby KJ's case is presented as a landmark in gene therapy for inherited metabolic disorders. Given the life-threatening nature of his condition, which was characterized by the body's inability to effectively process a common byproduct of protein metabolism, which specific enzyme deficiency highlighted in the article, was the target of the gene-editing therapy?

a) Phenylketonuria (PKU), a disorder of phenylalanine metabolism, which can lead to severe neurological damage if untreated in infancy.

b) Ornithine transcarbamylase (OTC) deficiency, an X-linked urea cycle disorder that also causes hyperammonemia and severe symptoms in newborns.

c) Methylmalonic Acidemia (MMA), a disorder of organic acid metabolism that can lead to severe metabolic acidosis and neurological complications in early life.

d) Maple Syrup Urine Disease (MSUD), a disorder of branched-chain amino acid metabolism that causes toxic accumulation and severe neurological effects in infants.

e) Carbamoyl phosphate synthetase 1 (CPS1) deficiency, an autosomal recessive urea cycle disorder characterized by the liver's inability to convert ammonia into urea.

QUESTION 03.

Baby KJ's rare liver enzyme deficiency, as discussed in the article, leads to a critical buildup of a highly toxic substance in his bloodstream. This substance is a dangerous byproduct of protein metabolism that, when accumulated, can cause severe neurological damage and other life-threatening complications. What is this harmful substance?

a) Glucose

b) Urea

c) Amino Acids

d) Ammonia

e) Fatty Acids

QUESTION 04.

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins) technology has revolutionized genetic engineering by enabling precise genome editing. Considering the basic principles and diverse applications of the CRISPR-Cas system in biotechnology and medicine, which of the following statements is the most accurate?

a) The main characteristic of CRISPR-Cas is its ability to make random cuts in DNA to induce mutations, with its precision being a secondary concern, as the focus is on generating genetic variability.

b) Despite its precision, CRISPR-Cas technology still has a major limitation in the complete absence of "off-target" effects (edits at unintended locations), ensuring that all genetic modifications are always safe and predictable.

c) The targeting specificity of CRISPR-Cas is determined by a guide RNA sequence that pairs with the target DNA, while the Cas enzyme is responsible for making precise cuts in the double helix, paving the way for genetic insertions, deletions, or corrections.

d) Variations of the CRISPR system, such as base editors, are essentially tools for gene expression silencing, primarily acting to degrade mRNA, and are therefore not suitable for permanent corrections at the genomic DNA level, especially for single-point mutations.

e) The primary applications of CRISPR-Cas technology are restricted to basic laboratory research, with limited prospects for translation into human therapies or crop genetic improvement due to its complexity and high cost.

QUESTION 05.

Drawing upon the provided text and your broader understanding of molecular biology and genetic therapies, which of the following statements most accurately compares and contrasts "base editing" and "gene insertion" approaches, detailing their mechanisms, specific applications, and inherent challenges as presented in the article?

a) Base editing, a more precise variant of CRISPR, primarily corrects single-nucleotide "typos" in DNA by swapping out a base without full double-strand cuts, proving highly effective for liver-related disorders due to its delivery via lipid nanoparticles and potential for redosing; whereas gene insertion introduces an entire functional gene, often via viral vectors, which, despite offering a complete gene replacement, carries higher manufacturing costs, potential for immune response, and current limitations on redosing.

b) Both base editing and gene insertion fundamentally rely on the same CRISPR-Cas9 enzyme for precise double-strand DNA breaks, differing only in the type of guide RNA used, making them equally versatile for treating any genetic disorder regardless of the mutation type or target organ.

c) Gene insertion is inherently superior to base editing as it guarantees a permanent cure by fully replacing a defective gene in all affected cells, thereby eliminating the need for ongoing medication or dietary restrictions, as was unequivocally demonstrated in the case of baby KJ.

d) The primary advantage of base editing lies in its capacity to insert large, functional DNA sequences into specific genomic locations, making it ideal for complex genetic disorders, while gene insertion is specifically designed for the correction of single nucleotide point mutations.

e) Both base editing and gene insertion are currently considered risk-free, permanent curative therapies that do not elicit immune responses, have no off-target effects, and are easily and cheaply manufactured for global accessibility, as implied by their rapid development and initial success stories.

QUESTION 06.

The article discusses different gene therapy approaches, mentioning that RNA-based therapies are considered more suitable for neurological disorders, while base editors show promise for liver diseases. What is the most plausible inference about the reason for this specificity in therapy application, based on the information and general context of the text?

a) The specificity is due to neurological diseases being caused by different mutations than those affecting the liver, making RNA-based therapies more effective for the brain.

b) Lipid nanoparticles, used to deliver base editors, are readily absorbed by the liver, while RNA-based drugs are more efficient at crossing the blood-brain barrier to reach neural tissue.

c) Neurological diseases require double DNA cuts to be corrected, which is a characteristic of RNA drugs, not base editors.

d) The manufacturing of base editors is more expensive and complex for brain diseases, making RNA drugs a more viable option for these cases.

e) The specificity is arbitrary, and both technologies can be equally effective for neurological and liver disorders, depending only on the type of mutation.

QUESTION 07.

The text highlights "personalization" as a key aspect of new therapies. Which of the following options best describes the challenges and future potential of the personalized approach in gene therapy for rare diseases, considering the information provided?

a) The personalization of gene therapy is a fast and low-cost process, which will facilitate its immediate widespread adoption for all genetic diseases.

b) The main challenge of personalized therapy is the lack of specific genetic mutations to which it can be applied, limiting its use only to extremely rare diseases.

c) The ability to rapidly develop a customized therapy, as in 6 months for KJ, demonstrates the potential to treat rare diseases early, but it still faces regulatory, safety, and manufacturing cost challenges.

d) Personalized therapies like RNA-based drugs and base editing are mutually exclusive and compete with each other, making a diversified approach to treating rare diseases impossible.

e) Personalization completely eliminates the need for animal safety testing and regulatory approvals, as each treatment is unique to the patient.

QUESTION 08.

Baby KJ's severe metabolic disorder, characterized by the accumulation of a highly toxic substance, stems from a defect in the body's ability to properly process the breakdown products of which major macromolecule?

a) Carbohydrates

b) Lipids

c) Nucleic Acids

d) Proteins

e) Vitamins

QUESTION 09.

The text describes CPS1 deficiency as a condition where the liver cannot efficiently convert ammonia into urea. Considering the physiological and biochemical role of the urea cycle, as well as the toxicological implications of its malfunction, which of the following statements correctly describes the relationship between the enzymatic defect, toxicity, and the symptoms observed in patients like baby KJ?

a) Deficiency of the CPS1 enzyme results in the excessive conversion of proteins into urea, leading to renal overload and subsequent urea toxicity in the blood, manifested by lethargy and coma.

b) While ammonia is produced in various tissues and by intestinal metabolism, its systemic toxicity in cases of urea cycle deficiency is mitigated by rapid renal excretion, preventing significant long-term damage to vital organs beyond the liver.

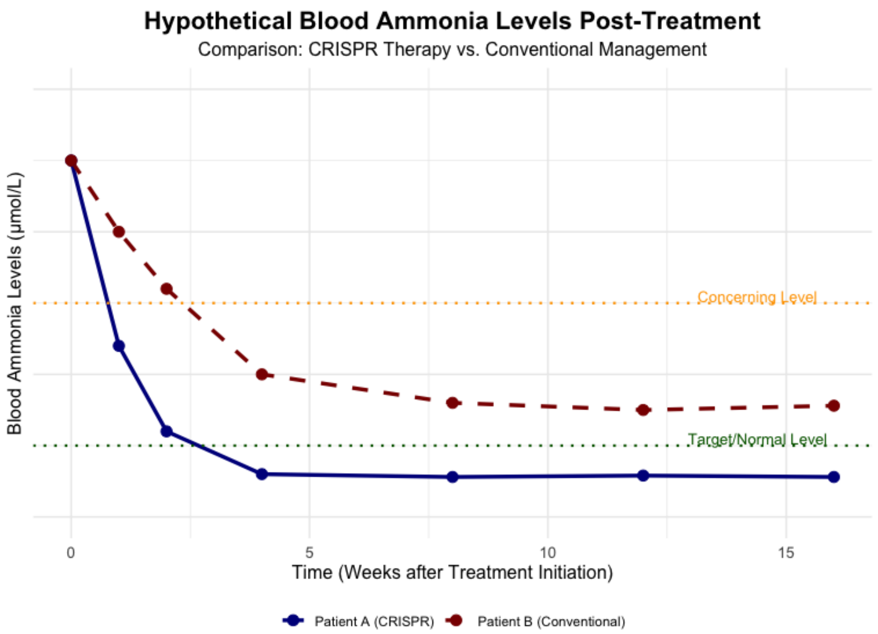
c) Failure of the CPS1 enzyme prevents the efficient conversion of ammonia, a highly neurotoxic substance produced by protein catabolism, leading to its accumulation in the blood and causing brain damage, lethargy, and coma, as observed in severe cases of urea cycle disorders.

d) Ammonia toxicity in patients with CPS1 deficiency is amplified because the urea cycle, essential for ammonia detoxification, is primarily active in brain cells, where ammonia is predominantly converted into urea.

e) Patients with CPS1 deficiency can metabolize ammonia through efficient alternative pathways in muscle, which prevents neurotoxicity and makes the disease asymptomatic in most cases.

QUESTION 10.

Urea cycle disorders lead to toxic ammonia accumulation in the blood, causing severe damage. New gene therapies aim to surpass conventional treatments. Observe the hypothetical graph representing blood ammonia levels of two patients (A and B) over time. Patient A received CRISPR-based gene therapy, while Patient B received conventional medical management. Both started with elevated ammonia. Based on the graph, which statement best compares the efficacy and impact of the two treatments?



a) Both treatments demonstrated similar efficacy in reducing ammonia levels, suggesting that CRISPR therapy offers no significant long-term advantages over conventional management.

b) Conventional treatment resulted in a faster and more complete reduction of ammonia, indicating its superiority over CRISPR therapy.

c) CRISPR-based gene therapy provided a more rapid, robust, and sustained control of ammonia levels, reflecting a potential correction of the underlying cause of the disorder.

d) Although CRISPR showed an initial improvement, conventional treatment ammonia levels eventually became lower and more stable over time.

e) Neither treatment was effective in reducing ammonia levels to safe thresholds, indicating the need for additional therapeutic approaches in both cases.