Reverting the *PAH*:c.1222C>T mutation via Cas9 guided adenine base editor on Phenylketonuria mice models

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INTRODUCTION

Phenylketonuria stands as the most common disorder stemming from a genetic glitch in amino acid metabolism, rooted in a mutation within the ***PAH*** gene with an occurrence in Europe of approximately one case per 10,000 live births. Consequently, there is a buildup of **phenylalanine** in the bloodstream, reaching toxic levels in the brain. Left untreated, the condition leads to progressive cognitive decline, autism, seizures, and motor impairments. Besides, while **dietary limitation** of phenylalanine remains the primary treatment, ongoing research promises emerging therapeutic avenues for phenylketonuria. Moreover, above all *PAH* variants, the most prevalent is **c.1222C>T** (RefSeq: NM_000277.3). This two and a half years project presents a **single administration** of a construct that includes a **guided adenine base editor** to revert this mutation. The on-target effect of the system should be enough to have consequently the synthesis of the completely functional protein hence normalising the phenylalanine blood levels. If the treatment is applied **as soon as possible**, this disease should be stopped, and the most severe phenotypes should be evaded.

HYPOTHESIS & OBJECTIVES

Since this variant causes a **missense** mutation, I hypothesise that if the mutation is **reverted**, the **phenotype** should be **restored**.

For that, a follow-up of sub-objectives has been planned:

- Mice **colony** establishment.
- Build the **RNP** that will do the reversion.
- **LNP** identification and optimization.
- Genome edition corroboration via **Sanger.**
- Functional analysis via **transcriptome** and phenylalanine in blood.
- Monitor the **body weight** of the mice.
- Results **divulgation.**

METHODS

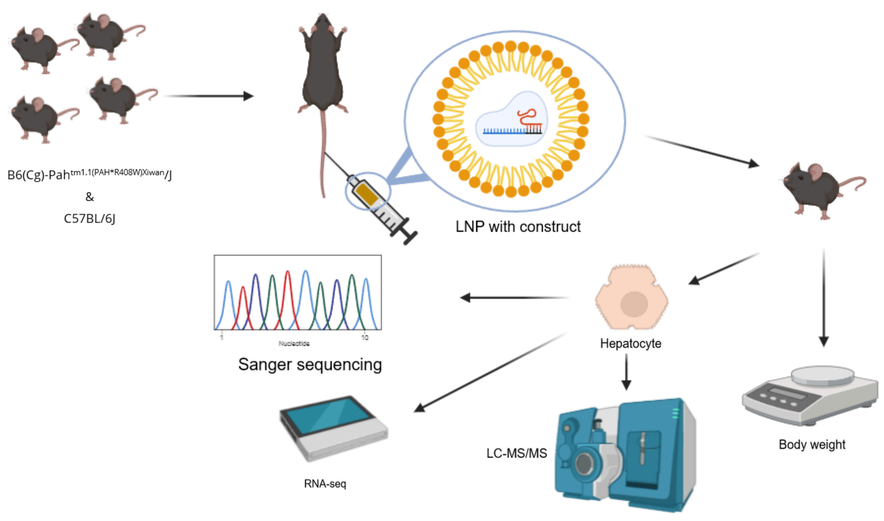

Figure 2: the visual representation of the follow-up of experiments this project will have.

ADVANCED THERAPY MEDICINAL PRODUCT

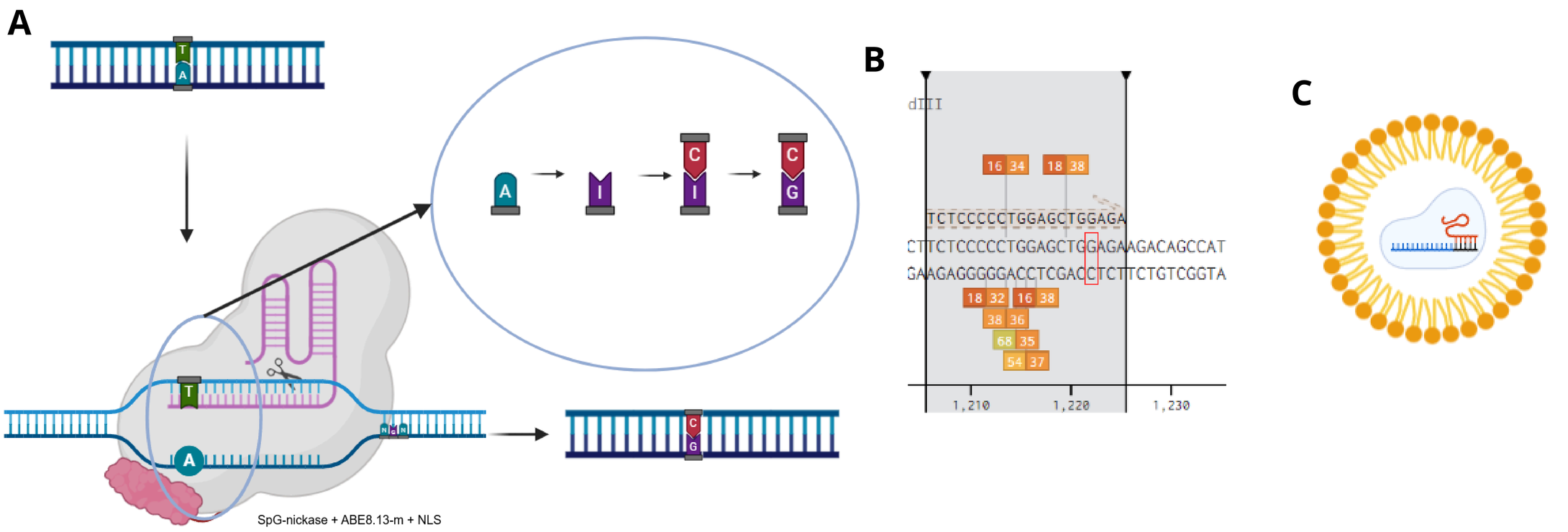

Figure 1. Advanced therapy medicinal product design and action: (A) Diagram of the action of the SpG nickase with an NGN PAM bonded to the ABE8.13-m. The action of the ABE8.13-m, which will make the change from an adenine to an inosine that will be paired with guanine, is going to be directed by the SpG with an NGN PAM. (B) Image obtained by the Benchling programme of one possible guide for the SpG that has AGA as PAM at 5', designed with the *PAH* gene sequence (RefSeq: NM_000277.3). The red square indicates, in the wild-type sequence, where the mutation occurs. (C) Non-viral vector which is a lipid nanoparticle (LNP).

EXPECTED RESULTS

- Reversion in a high percentage of hepatocytes restoring **>20%** of PAH activity.
- Molecular reversion of the mutation from the pair **A:T to G:C** in the **1222** position of the *PAH* gene.
- **No alteration** of the **transcriptome.**
- After the treatment, **phenylalanine** blood levels are expected to be **normalised.**
- **Normal growth** and body weight of the mice.

IMPACT & CONTRIBUTION

This project aims to correct the most common mutation in Phenylketonuria (*PAH*:c.1222C>T) using **lipid nanoparticles** (LNPs) containing **ABE8.13-m** and an **RNP SpG nickase**. Successful results could lead to testing in complex animal models and clinical trials, potentially revolutionizing treatment.
Publishing results will expand knowledge in gene therapy and base editing. The project's benefits extend to other diseases, as its mutation-reversing mechanism can achieve the **wild-type phenotype**. **Early detection and treatment** can prevent severe symptoms and halt disease progression.

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