Efficacy and safety of viral and non-viral vectors for the delivery of CRISPR/Cas9 to the retina

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ABSTRACT

CRISPR/Cas9 is now a reference in terms of gene editing. This system, which can modify the sequence of a gene in a precise, rapid and efficient manner, is revolutionizing the possibilities of gene therapy and there is an urgent need to ensure its safety. Currently, viral vectors, particularly adeno-associated viruses (AAV), are the most efficient for transferring CRISPR/Cas9 *in vivo*. However, AAV can induce immune reactions, leading to inflammation and toxicity. But this has not been extensively studied in the eye. This is a concern for gene therapy application as this would potentially limit the efficacy of the treatment. Thus, the transient transfer of CRISPR/Cas9 as Cas9 protein complexed with its guide RNA (gRNA) and encapsulated in a lipid non-viral vector could limit the risks of immune reactions.
In this context, my project aims to **compare the efficacy and safety of CRISPR/Cas9 using AAV versus lipid vectors *in vivo*, in mice photoreceptors**.

To do so, after sub-retinal injection in wt mice of the CRISPR/Cas9 system by AAV or lipids, I will collect the retina and the vitreous. I will then extract the DNA and the RNA.
Efficacy will be measured by NGS sequencing of the retinal DNA to quantify the indels generated by CRISPR/Cas9.
Immune response will be monitored by **measuring the expression of innate immune markers on the vitreous and retinal RNA by using the 1-step RT-qPCR system (Catalog number A6020)**. Innate immune markers will include Iba1, a microglial marker; H2-Eb1 and H2-Ab1, which are expressed in APCs; and CyBB, a macrophage marker. Measuring the RNA level will allow rapid and sensitive detection and relative quantification of the different markers' expression using small amounts of starting materials, as mouse retina and vitreous are small tissue and fluids.

The study presented here in mouse models is a prerequisite for developing safe and efficient gene therapy in humans. Monitoring the immune response against AAV versus non-viral vectors to transfer CRISPR/Cas9 has the potential to change current research and clinical paradigms in gene therapy for currently untreatable retinal diseases.