Development and *in vivo* evaluation of CRISPR/Cas9-based therapies for Hutchinson-Gilford progeria syndrome

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Rational

Hutchinson-Gilford progeria syndrome is a rare autosomal dominant disease characterized by several aging-like alterations emerging at a very early age and leading to a premature death of the patient. The genetic basis of this syndrome resides in a point mutation in *LMNA* gene -encoding lamins A and C- which leads to the expression of an truncated lamin A variant known as *progerin* which accumulates in the nuclear envelope. Previous work from our group has allowed us to develop two different HGPS mouse models and demonstrate that the nuclear aberrations typical of HGPS and the progeroid phenotype are caused by the accumulation of the toxic prelamin A precursor, not by the loss of the normal protein. These animal models allowed us to test a variety of genetic and pharmacological approaches against progeria, including antisense oligonucleotide-based treatments aimed at blocking the aberrant splicing of the *LMNA* transcripts that gives rise to progerin production.

These approaches targeted the molecular roots of the syndrome and demonstrated a remarkable efficacy in our animal model. However, splicing-targeted therapies, such as those based on the use of antisense oligonucleotides are difficult to implement in humans due to potential off-target effects, toxicity and very high economic costs. The recent development of genomic edition systems provides promising alternatives, potentially capable of yielding a long-lasting or even permanent therapeutic solution. In this regard, the CRISPR/Cas9 genomic edition system has emerged as a potential tool for the correction of genetic alterations in an efficient and specific way, shedding some light on the treatment of genetic-based pathologies. This system involves a RNA-directed nuclease that generates double-stranded breaks in the DNA, stimulating non-homologous end-joining to produce disruption of a gene or homologous recombination to introduce specific changes in its nucleotide sequence.

Hypothesis

Our study is based on the idea that the development of CRISPR/Cas9-based therapeutic strategies against HGPS, aimed at modifying splicing signals located in the final portions of *LMNA* gene, could impair the production of progerin in HGPS patients leading to a specific and permanent therapeutic effect. Even though total *LMNA* correction in all the cells of the organism will not be feasible, we have already demonstrated that a mosaic HGPS mice model, in which coexist pogeroid and normal cells, has a completely normal phenotype. The results obtained with this mosaic mouse model indicate that a partial restoration of progerin accumulation could be sufficient for an important phenotype relief and support CRISPR/Cas9-based strategies would as a plausible treatment for HGPS children.

Objectives

1. To design and test in cell culture CRISPR/Cas9 nucleases capable of introducing changes in splicing sites of the final portion of the human and murine *LMNA* gene, yielding alleles incapable of driving progerin expression.
2. To test *in vivo* the activity of the nucleases selected in Objective 1, using our HGPS animal model (*Lmna*$^{G609G/G609G}$ mice, LAKI mice) and different *in vivo* gene delivery procedures, such as hydrodynamic gene delivery or *in vivo* transfection (Osorio et al., 2013).
3. To produce and validate viral vectors for efficient *in vivo* delivery of the CRISPR/Cas9 nucleases developed in Objectives 1 and 2.
4. To investigate the therapeutic potential of virally-delivered CRISPR/Cas9 anti-progerin nucleases in LAKI mice.

Total estimated budget: €_50.000_