**Development of a gene therapy strategy for Hutchinson-Gilford progeria syndrome**

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Hutchinson-Gilford Progeria Syndrome is a rare genetic disorder belonging to the laminopathies group, which includes diseases associated with mutations in the *LMNA* gene. In progeria, the mutation causes activation of an alternative splicing site in the transcript of *LMNA* gene and then the synthesis of a shortened form of lamin A called progerin, which leads to a disruption of a nuclear structure and nuclear envelope. An onset of initial symptoms occurs after the first year of life, ultimately leading to mortality around 13 years of age. Progeria is currently an incurable disease. That is why the development of gene therapy holds promise for enhancing the quality and duration of life of affected individuals, while also impeding the progression of symptoms.

This study aimed to select small interfering RNA that specifically recognizes progerin mRNA and downregulates protein synthesis and additionally are featured by increased stability in the blood.

In order to obtain an experimental model for convenient selection and evaluation of the efficiency of the designed oligonucleotides, it was necessary to prepare and characterise a new cellular model of progeria. It was decided to create a HeLa cells sublines with overexpression of GFP-progerin, GFP-lamin A fusion proteins and GFP alone. A new cellular model allowed an easy and quick analysis of the level and location of exogenous proteins. This model also allowed to obtain more material and significantly shortened the analysis time compared to commonly used primary cells from donors with progeria.

The gene therapy designed in this research relies on small interfering RNA, which selectively recognizes progerin mRNA, eliciting its degradation through RNA interference and downregulating protein synthesis. Several sequences were designed and tested in order to select the most effective and specific ones. It was confirmed that in contrast to progerin unmutated lamin A levels remained unaffected after oligonucleotide treatment.

The combination of the treatment with farnesylation inhibitor lonafarnib, an approved treatment for progeria, in combination with one of the selected oligonucleotides was investigated. The research results confirmed that both compounds can be used together, maintaining their effectiveness, and their combination has an additive effect.

In order to enhance stability in a blood serum, modified nucleotides were incorporated into the designed oligonucleotides. For one type of modification, increased stability

was confirmed along with maintaining the efficiency of the oligonucleotide to downregulate progerin level compared to the same sequence without modified nucleotides.

During the final stage, the most efficacious oligonucleotides were tested in fibroblasts derived from patients with progeria. A significant reduction in progerin level was noted after oligonucleotides application. A significant reduction in progerin level was found after oligonucleotides treatment, which was further enhanced with increased duration of the therapy and increased dose number. It was also demonstrated that tested oligonucleotides significantly reduced progerin level, without affecting the level of lamin A.

The developed therapeutic sequences can be used to create a genetic drug for Hutchinson-Gilford progeria. Double-stranded oligonucleotide sequences containing modified nucleotides can be conjugated with a carrier enabling their entry into cells. Oligonucleotides can also be used as single-stranded, which enables their independent uptake. Based on the developed sequences, an expression cassette can be created for the overexpression of micro RNA or small hairpin RNA.