MicroRNA-based gene therapy for Huntington's disease : Silencing the villain

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ADDENDUM

ENGLISH SUMMARY

Huntington's disease (HD) is a devastating neurodegenerative disease caused by a single mutation, a CAG expansion, in the huntigntin (*HTT*) gene. The resultant mutant HTT protein has been shown to be the predominant toxic entity in the HD pathogenesis and therapeutic strategies that aim to lower the mutant HTT show a great promise. Micro (mi)RNAs are a class of small cellular RNAs that bind to target mRNAs based on the base-pair complementarity to mainly supress mRNA translation into a protein. Thus, miRNAs offer a therapeutic tool to lower the expression of disease genes such as *HTT*. For clinical applications, the expression cassette of therapeutic miRNAs can be delivered to the target tissue by the AAV vectors to achieve a long-term (in years) expression of miRNA precursors. These precursors are being further processed by the cellular RNA interference (RNAi) machinery to render mature a miRNA molecule that recognizes a specific sequence in the disease transcript and enables the reduction of toxic HTT protein, HTT lowering.

In **chapter 1**, we describe a current knowledge on the HD pathogenesis, HTT RNA-lowering treatment strategies, and HD cell and animal models enabling the preclinical development. We positioned the AAV-delivered-miRNA-based HTT gene therapy among currently investigated HTT lowering strategies. We provide a description of the efficacy outcome measures in the preclinical cell and animal HD models that present the proof-of-concept and proof-of-principle that are necessary to be addressed before the initiation of a clinical trial.

In **chapter 2,** we describe the pilot proof-of-concept study using the miRNA-based gene therapy approach. We have designed and tested *in vitro* and *in vivo* the efficacy of different miRNAs targeting various sequences in *HTT* exon 1, 50, and 67. Based on the generated results, we selected the miHTT construct targeting a sequence located one nucleotide upstream from the CAG expansion in exon 1 for further preclinical development. The miHTT construct showed the strongest efficacy *in vitro* and in the humanized HD 128/21 mouse model. Furthermore, as first in the HD field, we tested several pri-miRNA precursors to optimize the processing patterns of the selected miHTT to enhance the safety profile. We identified the human pre-miR-451 precursor as the only scaffold that does not produce the passenger strand both *in vitro and in vivo* thus, the use of this precursor offers a prevention of the unwanted off-target effects associated with the passenger strand.

To further establish the proof-of-mechanism, we addressed the efficacy of the miHTT-451 construct on supressing the downstream pathological HTT aggregation and neurodegeneration. In **chapter 3,** we injected the miHTT-451 construct delivered by AAV5 vectors bilaterally in the striatum of the lentiviral HD rat model to measure a therapeutic response. We showed strong reduction of HTT aggregation, which supressed the neuronal dysfunction at two months post injections. This study demonstrated a functional efficacy of the AAV5-miHTT-451 construct affecting downstream pathological events caused by

the mutant HTT protein. These results further supported the continuation of preclinical studies in large animals, which are beyond the scope of this thesis. Currently, the miHTT-451 construct is the product candidate AMT-130 in the development pipeline of uniQure, N.V. In October 2017, AMT-130 received the orphan drug designation in HD by the U.S. Food and Drug Administration. In January 2018, AMT-130 received an Orphan Medicinal Product Designation (OMPD) in HD from the European Medicines Agency, making it the first investigational AAV-gene therapy in HD to receive such designation. AMT-130 is expected to enter the clinical development in 2019.

The role of miRNAs in the cytoplasm is well established, however their functions in the nucleus are only recently being described. Lowering of HTT transcripts in the nucleus is intriguing because some level of HTT RNA toxicity in the nucleus has been reported. Moreover, extending the therapeutic effect of miRNAs to the nucleus could offer new applications for other indications in the future. In **chapter 4**, we designed miRNAs that specifically target a newly identified polymorphic sequence linked to the mutant HTT intron 22 with a deletion of four nucleotides, ΔACTT. This heterozygous polymorphism is largely characteristic for A1 haplogroup of HD patients and therefore it offers simultaneously an evaluation of allele-specific and nuclear HTT lowering. We showed a strong 90% HTT lowering of mutant protein in HD neuronal cultures heterozygous for ΔACTT and almost 0% lowering in ΔACTT-negative HD cultures, demonstrating the strongest allele-selective efficacy so far. Interestingly, these data challenge the traditional concept of mature miRNAs acting mainly in the cytoplasm. We propose the general concept of targeting nuclear disease-related genes by therapeutic miRNAs and we recommend further preclinical validation studies.

To enable the translation of HD gene therapy to the patients, it is important to identify informative pharmacokinetic/pharmacodynamic (PK/PD) measures, in easily obtainable biofluids such CSF or blood, which would correlate the expression of active AAV-miRNA vectors in the brain of treated HD patients with the efficacy and long-term effects. In **chapter 5,** we showed first indications that therapeutic miRNAs can be secreted within vesicles enriched for exosomes from human HD patient neuronal cultures. These findings are substantial and not only for the HD field. Having a therapy-specific PK/PD measure, which can be applicable to other CNS indications, will significantly impact the preclinical and clinical development of RNAi-based gene therapies currently facing these challanges. Further invistigations are ongoing beyond the scope of this thesis in order to provide better understandings on the mechanism of action and a possibility of the 'exosomal spread' of HTT silencing *in vivo*. Finally, in **chapter 6**, we summarize the current knowledge on HTT DNA and protein-lowering strategies, provide an overview of the safety preclinical measures for miRNA-based gene therapy and its future perspectives. To conclude, data presented in this thesis resulted in three first-author publications, one patent, and fuelled further preclinical and clinical development of AMT-130 gene therapy for HD.