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Abstrakt: **„Development a preclinical therapeutic strategy for Huntington's disease and spinocerebellar ataxia type 3 using RNA silencing reagents targeting CAG repeats in *HTT* and *ATXN3* gene transcripts"**

Polyglutamine (polyQ) diseases belong to a group of 9 rare genetic diseases, i.e. Huntington's disease (HD), spinal-cerebellar ataxias (SCA) types 1, 2, 3, 6, 7, 17, dentatorubral-pallidoluysian atrophy (DRPLA) and spinal and bulbar muscular atrophy (SBMA). These diseases result from a special type of mutation involving the expansion of cytosine-adenine-guanine trinucleotide repeats (CAG) encoding the polyglutamine in the coding region of the causative genes. Patients with polyglutamine diseases most often have one allele with a normal number (around 5-35), while the other is a mutant allele with an increased number (>35) of CAG repeats. The incidence of polyQ diseases is in the range of about 1–10 cases per 100,000 people. HD (Huntington's disease, HD) and SCA3 (spinocerebellar ataxia type 3, SCA3) have the highest incidence rate worldwide and are the best described of all polyQ diseases.

A therapeutic strategy targeting the CAG tract in mRNA may prove to be an effective way to reduce the level of pathogenic protein in the case of HD and SCA3, as well as other polyQ diseases. I designed several different reagents, in vector and bivalent formats respectively, targeting CAG repeats in mRNA to test this therapeutic strategy *in vivo* Humanized HD ($Hu^{128Q/21Q}$) and SCA3 ($Ki^{150Q/21Q}$) mouse models with a mutant allele containing more than 100 CAG repeats and a normal allele containing 21 CAG repeats were used to simulate biallelic conditions in patients. AAV-PHP.eB vectors containing shRNA were administered by systemic injections into the orbital sinus as a non-invasive delivery of the therapeutic that passes through the blood-brain barrier (BBB). Bivalent particles with siRNA were administered intracerebrally.

The aim of work was to select reagents for further study by evaluating the safety by short-term post-operative observations and the efficiency of the designed shRNAs by conducting short-term experiments. This allowed to select A4(P10A) and A4(P10,11A) reagents characterized by the lowest side effects and the most effective reduction of mutant protein levels compared to other shRNAs. The selected shRNAs in AAV–PHP.eB were admitted for testing in long-term experiments, consisting of long-term post-operative observations, evaluation of behavioral

disorders, possible side effects, efficiency of lowering the levels of mutant and normal proteins, and the associated degree of allele-selectivity of the reagents, evaluation of the therapeutic effect at the cellular level (inclusions). It was observed that the AAV–PHP.eB vector containing shRNA transduced the brain and its specific regions effectively without peripheral tissue transduction. The efficiency of shRNA in lowering protein levels depended on the brain region transduction rate. The developed vector approach allowed for efficient transduction and delivery of CAG-targeting reagents to the brain over the BBB, which is particularly beneficial in treating polyQ and other CNS disorders. The second approach tested was bivalent molecules with siRNAs targeting the CAG sequence, which also achieved adequate transduction in the mouse brain, resulting in a reduction of the mutant protein; however, increasing side effects in the HD model. The bivalent reagents demonstrated therapeutic potential in the two models of the most common polyglutamine diseases; however, they require further study to minimize adverse effects.

In conclusion, the efficiency of a therapeutic approach targeting a sequence of CAG repeats in the mRNAs of mutant genes for HD and SCA3 is shown. The study shows that this approach can be applied to treating many polyQ diseases. Moreover, the shRNA reagents lower the mutant polyQ protein in the brain and are delivered by minimal invasive blood administration crossing the blood-brain barrier, which is of considerable clinical importance.