Expanded cytosine-adenine-guanine (CAG) trinucleotide repeats in the coding regions of certain genes are responsible for incurable neurodegenerative diseases called polyQ disorders (from expanded CAG motif which encodes glutamine). Currently, we can distinguish nine of these diseases, including, dentatorubral–pallidoluysian atrophy (DRPLA), Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), and six distinct spinocerebellar ataxias (SCAs). The symptoms are mainly related to the impairment of the nervous system due to the accumulation of the mutated protein in non-dividing neurons, which eventually leads to their degeneration. Recent human genetic data suggest that disturbances in DNA repair mechanisms are central to the pathogenesis of CAG repeat diseases.

Several approaches have been applied in potential therapies of polyQ disorders. One of them is based on degrading mutated mRNA. According to a new report, one of the promising strategy based on targeting mRNA had been halted in I/II and III phases because it failed to show higher efficacy than the placebo. Beside RNA targeting, the literature includes approaches in which mutated CAG repeat tract was removed at the DNA level by genome editing systems e.g. Clustered Regularly-Interspaced Short Palindromic Repeats-Cas9 (CRISPR-Cas9). Targeting DNA is more beneficial from a therapeutic point of view because the repaired sequence is pass to new cells during cell division.

CRISPR is a bacterial immune system that has been modified for genome engineering. This technology was named the breakthrough method in molecular biology and received the Nobel Prize in chemistry. It has been used to inactivate mutated genes, correct mutated sequences in various cellular and animal models, create new cellular models. In the context of therapy, ~30 studies are in progress, including the use of CRISPR-Cas technology, but no data is available for polyQ diseases.

In my research work, I use the CRISPR-Cas system to explore DNA repair mechanisms involved in the shortening of the CAG repeat tract. The task included in my proposal will be the continuation of the Etiuda 8 grant which I received a year ago. It was noticed that introduced double-strand breaks (DSBs) within CAG repeats lead to its expansions and contractions during DNA repair. Unfortunately, we still don't know which DNA repair mechanisms are responsible for shortening and how to manipulate them to shorten CAG repeats. Therefore, the aim of my project is to understand these mechanisms. The research will be carried out on the DRPLA cell model. For this purpose, I will conduct CRISPR interference screening that harnesses around 20.000 genes to check which DNA repair proteins are involved in CAG repeat contraction and expansion after the introduction of nuclease-induced DSBs. Moreover, I will compare the DNA repair outcome in neuronal and non-neuronal cells and I will edit both alleles with different length of CAG repeats in parallel. Modern analysis which will be used for this will allow examining the editing outcome on a longer fragment of the sequence. It may contribute to discovering interactions between alleles as well as take a deeper look into the editing outcome of repetitive sequences. As a model, I will use neuronal and non-neuronal cells differentiated from patient-derived cells suffering from DRPLA.

The results of this project may help to take a deeper look at DNA repair mechanisms in different cell types and may contribute to the development of potential gene therapy for all polyQ diseases.