SUMMARY

Nonsense mutations introduce premature termination codons (PTC) in the coding region of mRNA, leading to inappropriate termination of translation and, as a result, to the formation of non-functional proteins. It is estimated that the presence of PTC accounts for approximately 20% of all genetic diseases. Translational PTC readthrough induced by chemical compounds is a method, which potentially allows the restoration of functional protein expression and reduction of disease symptoms, without interfering with the patient's genome or transcriptome.

This work describes the investigation of the ability of selected aminoglycoside antibiotics (aminoglycosides; AAGs) to stimulate the translational readthrough of selected PTCs in genes, mutations in which are the cause of primary ciliary dyskinesia (PCD), inherited genetic disorder caused by the dysfunction of motile cilia and flagella. The effect of several AAGs on PTC readthrough has been studied in two experimental systems: *in vitro* and *ex vivo*, using constructs containing PTC sequences and its close nucleotide neighborhood. The efficiency of the PTC readthrough process in 17 mutations in five genes related to PCD pathogenesis was analyzed. The studies also included analysis of AAGs cytotoxicity on primary respiratory epithelial cells and their effect on the cilia formation and functioning.

PTC readthrough was observed in five mutations analyzed and its effciency differed with the AAGs type and concentration. In *in vitro* experiments its level varied between 1% and 28% of the translation from the corresponding wild-type constructs (without premature STOP codon). Under *ex vivo* conditions (transfected HEK293 cell line) the suppression of premature STOP codons was 3-5 times lower than corresponding values *in vitro*, despite using AAGs concentrations that were two orders of magnitude higher. In addition, the most effective AAGs- G418 was also the most toxic to the cells and in higher concentrations had a negative effect on cilia formation. The AAGs tested (gentamicin, paromomycin and amikacin) had no toxic effects on the epithelial respiratory cells.

The results obtained in this study allowed to identify four best-responding PTCs and select the most effective types of AAGs. At the same time, project indicated the need to look for other compounds that induce PTC readthrough, with significantly lower toxicity and higher permeability through the cell membrane.