Changes in the sequence of DNA can have important consequences for the whole organism, from cancer to inherited diseases. Many of the inborn diseases are caused by the presence of small deleterious changes in the DNA, known as premature termination codons (PTC). PTCs interfere with the process of protein synthesis; as a consequence, the proteins are synthetized too short and do not function properly. Although many therapies have been designed to overcome the 'stop' signal that is given by PTC, the majority of methods require direct intervention into DNA of the patient and are thus burdened with risk. An alternative approach to the PTC problem is the use of certain chemicals to 'persuade' the protein synthetizing machinery to go over the PTC. This approach is known as the stimulation of PTC readthrough and has been successfully tested for some inherited diseases. Some substances are even tested in clinical trials.

Our group wants to examine the chances of using this approach in a rare inherited disease, called primary ciliary dyskinesia (PCD), which we are studying in our Department. PCD is caused by the defects of motile cilia, small cellular projections present on many cells of the body, including the cells that form lining of the respiratory airways and the tails of the sperm cells, which are a specialized form of cilia. Defects of motile cilia cause the hallmark symptom of PCD - recurrent respiratory infections, which with time lead to serious malfunction of lungs and even to lung transplantation. Other frequent symptoms involve male infertility (sperm cells do not move properly), and the reversed symmetry of the inner organs – with heart on the right side, liver on the left. With time, primarily due to the airway problems, patients' ability to work and their quality of life decrease, making PCD both a medical and social problem. The disease in one third of the PCD patients is caused by the presence of PTCs in genes responsible for cilia structure and function; therefore, it is important to test, whether the PTC readthrough approach can be used as a mode of treatment of the disease. However, many different factors influence this approach, and all of them have to be optimized before using the procedure in the patients is considered.

In our previous study we tested four most commonly used aminoglycoside antibiotics (AAG) for their potential to stimulate PTC readthrough in PTCs originating from the genes involved in the pathogenesis of PCD. Although promising, our study indicated the need for PTC readthrough-stimulating compounds with lower toxicity and better capacity to penetrate the cell membrane.

In this project, we plan to continue this line of research, testing several non-aminoglycoside compounds (NAAG, among others: PTC124, tylosin and negamycin, RTC13, RTC14, GJ071, GJ072) characterized by the reduced toxicity and lower mass (and thus better potential to enter the cell). We will investigate how these chemicals influence the viability and function of the respiratory epithelial cells, and the process of cilia formation. We will compare the efficacy of the NAAG compounds to stimulate PTC readthrough in PCD-related genes using two models: *in vitro* transcription and translation (TNT) and transfected epithelial cell lines. If possible (depending on the availability of patient's material), we will measure the amount of the transcripts in the cells from PCD patients carrying the PTC mutations examined and their susceptibility for degradation in NMD pathway- which protects the cell from the faulty transcripts.

So far, no work describing the use of PTC readthrough approach in PCD has been published. Defining the effect exerted by NAAGs on the respiratory epithelium will allow selecting compounds, which are least toxic to the epithelial cells and cilia; this is an essential preparatory step, when considering the future use of the PTC readthrough approach for the treatment of PCD**.** Comparison of the effectiveness of NAAGs with that of the previously tested AAGs will allow choosing the best combination of the compound and PTC. Identification of the mutations most susceptible to PTC readthrough will help selecting PCD patients who could potentially benefit from the application of PTC readthrough approach. The information regarding the susceptibility of particular mutations to PTC readthrough will also contribute to the general knowledge on the relationship between the DNA sequence context of PTCs, the nature of stimulating compounds and the resulting extent of PTC readthrough. This information will be of important for a variety of studies, from the basic research on the process of the PTC readthrough in the cell as well as the potential applications of this approach in inherited diseases caused by the presence of PTCs.