Gene therapy is a very promising therapeutic approach that involves the introduction of nucleic acids into the patient's cells. It enables treatment of both, hereditary and multifactorial disorders. These therapeutic nucleic acids are delivered with vectors, among which viral vectors are the most effective in in vivo applications. Viral vectors can introduce their genetic material into the target cells (in other words, they can transduce target cells) and provide the expression of a beneficial gene. Recent clinical trials and approvals of first gene therapy drugs have proven efficiency and safety of adeno-associated viral (AAV) vectors as DNA carriers, but still one of the major obstacles in the majority of applications is the achievement of sufficient efficiency of gene delivery in the patients. As there is a continuous need for the development of new, more effective vectors for gene delivery, a deeper insight into AAVs biology and transduction mechanisms would be essential. Current findings show that there are many barriers for successful transduction of cells with AAV vectors including the availability of the appropriate receptors on the cell surface, intracellular modifications of the vector proteins or recognition of introduced genetic material as a foreign or damaged DNA. What is more, as the vectors are derived from viruses, they may elicit mild antiviral responses that will further decrease transduction efficiency.

The proposed study attempts to address one of the most important aspects regarding AAV vectors biology, which is the elucidation of their transduction mechanisms and identification of the cellular barriers influencing the successful transgene delivery and expression. The aim of this proposal is to identify signalling pathways activated in response to AAV vectors of different serotypes in cells permissive and refractory to AAV transduction and to investigate the consequences of these interactions in terms of antiviral response, reaction to foreign/damaged DNA, as well as transduction efficiency. To obtain reliable results, we will perform all the planned experiments on cardiomyocytes and fibroblasts obtained from human induced pluripotent stems cells. Such differentiated cells will closely resemble the cells that constitute the human heart muscle. Moreover, since the majority of previous studies investigating the AAVs biology were focused on AAV serotype 2, we aim to compare different AAV serotypes (AAV2, AAV6 and AAV9) and try to specify whether observed effects are serotype-dependent. The proposed study will involve tracking of all consecutive steps and barriers for successful transgene expression from AAV vectors. In terms of cytoplasmic response, we will focus on the assessment of modifications of viral capsid proteins, followed by the construction of mutant versions of vectors, that will be less prone to such alterations. Additionally, we will examine the role of antiviral response to AAV vectors in non-immune cells, as both cardiomyocytes and fibroblasts were shown to express numerous pattern recognition receptors, which play a significant role in the innate immunity. Next, we will analyse the nuclear translocation routes and the response of DNA repair machinery to AAV genetic material with concomitant identification of proteins interacting with vector DNA using mass spectrometry methods.

We believe that the comparison of normal cells exhibiting different permissivity to AAVs within the same tissue might facilitate identification of the most relevant factors limiting transduction efficiency. We are also convinced that the obtained results will fill several gaps crucial for a better understanding of AAV biology and may contribute to a design of new, improved, more efficient vectors, potentially reducing the costs of gene therapy with AAV vectors in the future.