The spread of pathogens resistant to antibiotics requires new treatments. With the dramatic increase of antibiotic resistance in *P. aeruginosa*, alternatives like bacterial viruses – bacteriophages are being re-evaluated. Phage therapy was used in the beginning of XX century, but was largely abandoned when antibiotics were discovered . Also, the development of molecular biology techniques has enabled the evolution of „traditional" phage therapy towards new opportunities, one of which is the use of phage-encoded proteins responsible for bacterial envelopes disruption. Endolysins as antibiotic alternative do not stimuli mechanisms of resistance. These enzymes, encoded by double-stranded DNA phages, are produced inside phage infected bacterial cell in the last stage of the phage lytic cycle, and are responsible for progeny release by the cleavage of peptidoglycan layer. In vitro and in vivo studies have shown that endolysin encoded by Gram-positive phages, used externally as recombinant proteins, cause lysis of cells in a few or ten seconds. In contrast to Gram-positive bacteria, endolysin activity against Gram-negative bacteria is considered to be low due to the presence of external membrane barrier (OM) and lack of enzymes to their target site (peptidoglycan).

The purpose of the project is to clarify whether the permeabilization of the bacterial cell membrane accompanying the physiological phenomena of pyroptosis promotes the lytic activity of recombinant phage endolysins against *Pseudomonas aeruginosa*. Pyroptosis is a natural immune response of eukaryotic cells to bacterial infection, leading to the secretion of the gasdermin protein, i.e. the N-terminal $GSDMD^{Nterm}$ domain, which forms pores in the cell membrane. Overcoming the barrier that is the outer membrane of gram-negative bacteria would allow the delivery of phage mural enzymes (endolysin) to the target site, ie peptidoglycan located in the periplasmic space. The scientific objective of the project will be to evaluate whether the natural immune response of human eukaryotic cells to bacterial infection, i.e. pyroptosis, can lead to effective eradication of bacteria when combined with exogenously applied phage endolysins.

The methodology planned in project: flow cytometry, ELISA, Western blot, RT PCR and microbiological cultivation techniques will allow us to assess whether the recombinant phage endolyses (lysozyme, amidase and endopeptidase) cause lysis of *Pseudomonas aeruginosa* in the conditions of LPS-mediated pyroptosis associated with i.a. cystic fibrosis.