Potential SARS-Cov-2 antiviral strategy based on the inhibition of programmed ribosomal frameshifting
Principal Investigator: Prof dr hab Joanna Kufel, University of Warsaw

Coronaviruses are positive-sense single-stranded RNA viruses, meaning that their genome serves as a functional mRNA. Coronaviruses gained greater attention due to the severe acute respiratory syndrome (SARS) outbrake in 2002-2003 provoked by SARS-CoV-1. This epidemic has led to a significant progress in research into coronavirus biology and targeted antiviral strategies. The current global COVID-19 pandemic, caused by a very closely related coronavirus subtype, SARS-CoV-2, calls for the use of this knowledge to develop approaches to inhibit the propagation of this novel coronavirus.

The replication and multiplication of the virus requires tightly controlled expression of its genome. Several RNA viruses, including coronaviruses, use an uncanny strategy to ensure the most effective use of the coding potential of their small genome, i.e. ribosomal frameshifting. This programmed mechanism leads to the synthesis of different proteins from overlapping open reading frames in one mRNA molecule. Normally, the continuity of open reading frames is secured by the ribosome, the molecular machinery that carries out translation. However, specific sequence and structural elements present in virus mRNA are able to stall the ribosome, resulting in slippage and change of the reading frame. The -1 programmed ribosomal frameshifting is a key characteristic of the coronavirus genomes, where it is responsible for the production of the protein essential for viral replication and survival, RNA dependent RNA polymerase. Since inhibition of this frameshifting leads to reduction of viral infectivity and production, experimental approaches based on this concept represent potential effective antiviral strategies. In the case of SARS-CoV-1, two potential strategies for frameshifting inhibition have been proposed, both targeting RNA structures within the frameshifting region. The first one employed antisense oligonucleotides that reinforced the structure preventing ribosomes from entering frameshifting site, while the second was based on screening of small molecules interacting with a conserved frameshift-stimulating structure and resulted in identification of the novel ligand that disrupts this structure and dramatically inhibits frameshifting.

These promising observations are the basis of our proposal major objectives to implement and optimize both approaches for SARS-CoV-2. To this end, in the first place we plan to apply molecular modeling to design *in silico* effective blockers of viral ribosomal frameshifting based on already published data. These molecules, together with antisense oligonucleotides and small compound libraries, will be screened for CoV-2 antiframeshifting activities in human cell lines. Finally, appropriate toxicity assays for molecules with the best properties will be carried out to select non-hazardous candidates for further testing. Moreover, we want to answer more basic questions regarding coronavirus programmed ribosomal frameshifting, including the impact of translation initiation rate on frameshifting output. In addition, we will perform a highthroughput mutagenesis screen to assess the importance of sequence and structure of regulatory elements within viral mRNA on ribosomal frameshifting efficiency. This approach will assess evolutionary stability of regulatory sequences and will generate a detailed map of possible deleterious and enhancing mutations.

We believe that at least a few of compounds resulting from modeling and library screening will show promising inhibitory characteristics and will turn out as potent anti-SARS-CoV-2 agents. In addition, studies on the mechanism of action of these inhibitors will provide valuable information that will facilitate more effective anti-viral drug design. In particular, using antisense oligonucleotides represents a constructive approach, in which drug design relies only on viral genome sequence availability, which facilitates the process and reduces costs of its implementation. Generating efficient framework for antisense oligonucleotide-based inhibition of CoV-2 ribosomal frameshifting may enable application of the methodology to other viruses, most notably HIV or HCV. In turn, the map of changes in the CoV-2 regulatory region may be successively compared with in-fields samples of virus genomes, presenting a valuable tool as a proxy for the expected natural evolution and variability of this part of the SARS-CoV-2 genome.

This proposal addresses critical issues related to translation-mediated molecular mechanisms of SARS-CoV-2 that contribute to viral infectivity and propagation. It also includes the assessment of the prospect of utilization of identified inhibitors that may become the basis for new therapeutic strategies against COVID-19 before a vaccine becomes available.