Tick-borne encephalitis virus is an etiological agent of a very severe and acute viral disease of the central nervous system transmitted by ticks, known as tick-borne encephalitis. The data show that TBEV is present in at least 28 European countries, especially in Central and Eastern Europe, Scandinavia and parts of Asia. Based on the genetic analysis of strains isolated in different geographic regions as well as the E-glycoprotein sequences, three subtypes of TBE virus have been defined: European (TBEV-Eu), Far Eastern (TBEV-FE) and Siberian (TBEV-Sib). The incidence of TBE has increased over 400% times during the past 20 years in Europe, what makes TBE, after Lyme disease, the second most serious disease transmitted by ticks. Each year new outbreaks of the virus are detected in Finland, Denmark, Estonia, Lithuania, Sweden, Norway, Austria, Switzerland, Germany and Poland. Over the past 20 years, a total of about 200 000 clinical cases of TBE have been registered in Europe and Asia. However, it is known that these data are estimates and may constitute only 30% of all TBEV infections, as they relate only to registered = hospitalized cases of TBE. There are currently no licensed therapeutics for the treatment of TBEV infections.

The concept of the proposed research has arisen from the experience of Department of Recombinant Vaccines of IFB UG-MUG in the study of antiviral activity of designed and synthesized in Poland compounds against various viral pathogens. During last years, we have evaluated the exact mechanism of action for a few selected compounds, which showed the strongest antiviral activity. We have discovered that some of them act selectively on viral proteins. Moreover, the experimental data collected so far proved that some of tested compounds belonging to tunicamycin analogues are glycosylation inhibitors and are active against many viruses. What is important, the antiviral activity of few selected compounds against TBEV was confirmed in initial studies using virus-like particles (VLPs) in insect cells.

The first aim of the project is to gain the knowledge how inhibition of different steps of viral life cycle using tunicamycin analogues and mimetics exhibiting different mechanism of action can influence the production and infectivity of tick-borne encephalitis virus. We anticipate that studies using novel synthesized compounds, which are acting on different steps of TBEV life cycle, will lead to the development of effective methods for inhibiting viral replication. We hope that the data obtained during this project will be used for designing new therapies that can be beneficial for patients infected with TBEV. The proposed research aims at selecting specific, non-toxic potent antivirals against TBEV. In the project, we would like to focus particularly on the study of compounds that specifically block the activity of viral proteins but also to investigate the antiviral activity of compounds belonging to N-glycosylation inhibitors. The experience gained during last years will be employed for studying the effect of synthesized compounds on TBEV replication, synthesis of viral proteins, production, secretion and infectivity of progeny virus particles using a variety of methods of molecular biology, biochemistry and immunology. CPE inhibition assay and plaque reduction assay will be used during *in vitro* propagation of tick-borne encephalitis virus in cell culture. Modern research equipment, like mass spectrometers, confocal and electron microscopes, real-time PCR or flow cytometer will be used in our research to determine the effect of tested compounds on different steps of TBEV life cycle, growth kinetics of the virus and biochemical properties of viral glycoprotein. In order to study the biochemical properties of TBEV proteins several methods including Western blotting, immunoprecipitation and ELISA tests will be used. Moreover, recombinant mammalian cell lines with stable expression of TBEV proteins which can be used to study the antiviral activity of compounds on single viral protein will be constructed. The influence of antiviral compounds on TBEV particles formation will be also tested using VLPs system.

It has been proven, that over the last years, TBEV spreads to the new, previously unaffected areas. Furthermore, new outbreaks caused by new virus variants have been identified. Recent studies showed, that recombination between different TBEV strains may occur. The research hypothesis underlying the proposed project is the idea that new variants may be more pathogenic than the parental ones and with new features which allow them to bypass the host immune response. Due to this facts, the second main aim of the project is to search for co-infections in ticks and new variants of TBEV in environmental samples from different regions (Poland, Estonia, Sweden) which may improve the specificity of future vaccines against this pathogen. Therefore, in order to develop a specific method for the detection of co-infections in ticks as well as of new variants of tick-borne encephalitis virus, in the proposed project we will take advantage of the many years of experience in the molecular diagnosis of RNA viruses and the high quality of the molecular biology techniques available in our laboratory such as RT-PCR, RT-qPCR, dPCR, real-time PCR using specific TaqMan probes and sequencing. These research methods will be used to confirm TBEV infection or co-infections and to determine virus strains or species in the body of the tick. Moreover, we plan to study the *in vitro* recombination of different TBEV strains during co-infection in cell culture and to analyze the complete genomes by NGS method. We believe that the screening of TBEV in environmental samples from different regions will provide detailed information on endemic areas, new outbreaks and new viral strains. The collected data will be helpful in creating TBE risk maps. All collected data on the levels of viremia, genetic sequences and isolation areas will be valuable in creating future vaccines in order to maximize the effectiveness of immunization.