Tick-borne encephalitis virus (TBEV) is an increasingly **serious public health concern, especially in Poland and other parts of Europe and Asia where the number of reported cases has been rising steadily over recent years**. This neurotropic flavivirus is transmitted mainly by ticks and can cause severe, sometimes fatal, neurological infections. Currently, **no antiviral drugs exist to treat TBEV infection, which makes preventive vaccination the most effective strategy to protect vulnerable populations.** Despite the availability of traditional inactivated vaccines, there remain challenges such as incomplete coverage and complex immunization schedules. Therefore, developing novel and improved vaccine platforms is essential to enhance protection and control the spread of this pathogen.

The TBEV particle is composed mainly of structural proteins prM (precursor membrane) and E (envelope), which form the viral surface and are critical targets for neutralizing antibodies. Modern vaccine approaches focus on mimicking the native virus structure to stimulate robust and specific immune responses. Virus-like particles (VLPs), which resemble the virus but lack genetic material, can safely present viral antigens in their native conformation. Similarly, synthetic messenger RNA (mRNA) vaccines encoding prM and E proteins have shown great promise due to their ability to induce strong humoral and cellular immunity. **The primary objective of this project is to evaluate the immunogenicity profile of two advanced TBEV antigen forms: VLPs displaying the E glycoprotein in a covalently linked dimeric conformation and synthetic mRNA encoding prME sequence. Particular emphasis will be placed on assessing the quality, magnitude, and nature of immune responses elicited by these antigens. In the second step, the impact of combining these components in a heterologous prime-boost vaccination regimen will be investigated, as such approaches have shown enhanced and broader immunogenicity in the context of other viral infections**. The introduced dimeric mutation in the E protein is expected to stabilize the mature conformation of the antigen, which may improve its immunogenic and protective potential.

The research is divided into three interconnected stages. **First, plasmid constructs encoding both wild-type and mutated forms of the TBEV structural proteins prM and E will be engineered to enable the production of synthetic mRNA**. The mRNA will be synthesized in vitro, capped, and tested for expression efficiency in human and murine cell lines relevant to vaccine development and immune response. **Second, the project will focus on producing and purifying the designed antigens** in two forms: mRNA and mutated VLPs produced in mammalian cells. The use of Expi293F suspension cells allows for scalable production of complex glycosylated proteins mimicking the native virus structure. Purification techniques such as ultracentrifugation and chromatography will ensure high-quality vaccine candidates suitable for animal studies**. In the final stage, immunogenicity of the proposed antigens will be tested in mice, using advanced immunological assays to measure antibody responses, T-cell activation, and virus neutralization**. Heterologous prime-boost schedule combining mRNA and VLPs, will be evaluated to optimize the immune response. State-of-the-art lipid nanoparticle formulations will be employed for mRNA delivery, while established adjuvants will enhance the VLP-based antigen efficacy.

Successful completion of this project is expected to generate valuable insights into the design and performance of next-generation vaccines against TBEV. By incorporating the latest vaccine technologies - mRNA and VLPs **- this research aims to investigate the immune response induced by these antigens and assess the impact of their use in a heterologous prime-boost vaccination regimen on the type of the immune response, which is particularly important in the context of effective TBEV control.** The combined use of mRNA and VLP-based antigens is anticipated to activate both humoral and cellular immunity, leading to a more balanced and robust response than that achieved with homologous regimens. **The results of this project will not only inform future development of an effective and safe TBEV vaccine but may also contribute to advancing vaccination strategies against other similar flaviviruses.**