Lung cancer is the most common cancer worldwide and has been attributed as the leading cause of cancer-related deaths. Among all types of lung cancer, non-small cell lung cancer (NSCLC) represents approximately 85% of lung cancer cases with a lower than 15% 5-year survival. Because NSCLC is usually diagnosed at advanced stage of disease, it confers a poor prognosis, because there are not many available treatment options.

Many new personalized therapeutics, including tyrosine kinase inhibitors (TKIs), monoclonal antibodies, immunotherapy, and mammalian target of rapamycin inhibitors, were approved for NSCLC treatment in recent years. Combining this targeted drugs with the standard chemotherapeutics, increases the patient's quality of life and survival. However, **cancer cell acquire resistance to epidermal growth factor receptor (EGFR) TKIs (EGFR TKIs - gefitinib, afatinib, osimertinib)** In about **60% of NSCLC patients resistance to TKIs is developed,** and treatment regimen should be modified. However there are not many therapeutic options left in resistant NSCLC. Therefore, **overcoming the resistance to EGFR-TKIs in NSCLC is an unmet clinical need, which we address in the project.**

The project's main goal is to design novel Dual-Loaded Lipid Nanoparticles (DL-LNPs), a delivery carrier containing gefitinib, a first-generation EGFR-TKI and small interfering RNA (siRNA).

RNA-based therapeutics gained considerable interest in recent months as a new milestone in treating various diseases and vaccination. The most spectacular one was the development of vaccines against SARS-CoV-2 virus, which proved strong efficacy and safety profile.

In our project, the siRNA will be used to inhibit the expression of a certain gene, called *MET*, which is one of the factors responsible for the development of acquired resistance in NSCLC cells to EGFR-TKIs therapy. **The advantage of the project is that, instead of administering the two therapeutics - gefitinib and siRNA - separately, we will develop one carrier system co-delivering both molecules.**

The applicants think that simultaneous delivery of gefitinib and siRNA in a DL-LNP may delay the induction or overcome the resistance to EGFR-TKIs and enhance the efficacy of therapy overall. In this proposed study, the applicants will develop, manufacture and optimize the preparation process of double-loaded LNPs. Subsequently, DL-LNPs will be characterized, and their activity will be assessed in the NSCLC cell lines and in normal lung epithelial cells, which are healthy human cells. We will verify the influence of siRNA on the development of gefitinib resistance in NSCLC and the ability to re-sensitize cells to gefitinib.

The proposed strategy may open new doors in the field of drugs and genes delivery in combating drug resistance.

The presented project contributes to the development of RNA-based therapeutics and vaccines in Poland and worldwide. This is of great importance for a currently developed national strategy to implement RNA-based technology, where the advanced delivery systems are absolutely essential for therapeutic material protection and efficacious delivery, so crucial for proper therapeutic performance.
Above all, the presented project results may have great potential to significantly impact the field of drug delivery and improve the efficiency of therapy of lung and other types of cancer.