Precision double strike against non-small cell lung cancer. Insights into multidirectional therapies and the landscape of mutational variation and drug resistance.

Lung cancer remains a formidable challenge in the medical field due to its high mortality rate and the limited success of current treatment options, particularly in non-small cell lung cancer (NSCLC). Despite advancements such as targeted therapies and immunotherapy, many patients experience relapse as cancer cells develop resistance to treatment. Several factors contribute to the limited efficacy of these treatments, including significant intra-tumoral heterogeneity, frequent genetic mutations in key components of receptor tyrosine kinase signaling pathways (such as EGFR, ALK, HER2), mTOR pathway, oxidative stress response, cell cycle progression and proliferation, which is associated with the emergence of alternative kinase signaling pathways and the development of cellular resistance. The lack of validated predictive biomarkers of treatment response and the failure to identify novel gene mutations also affect the efficacy of therapies. Therefore, in order to fight NSCLC more effectively, it is necessary to develop new, more specific compounds that target not only one target, but also its downstream molecular targets.

The goals of the project are to design novel multi-target EGFR kinase and tubulin (EGTUB) inhibitors and to characterize novel molecular targets associated with treatment resistance in NSCLC. Additionally, using bioinformatics analysis, we plan to develop more innovative directional therapeutic approaches, such as combination therapies with known kinase inhibitors.

Interdisciplinary project encompasses *in silico* drug design, synthesis, and extensive biological testing on various models, including NSCLC cell lines, patient-derived cell lines, and 3D spheroid and organoid models. This research aims to characterize the mechanism of action of novel inhibitors and their therapeutic response. Furthermore, the project includes toxicological studies and *in vivo* efficacy testing in a *Danio rerio* model. In addition, detailed genetic characterization of cellular models will enable an understanding of gene expression profiles and genetic changes that may affect response to therapy. These efforts may result in the identification of potential genes - predictive biomarkers that can determine the greatest benefit of therapy, while genes associated with survival and disease progression may serve as prognostic biomarkers to help monitor treatment efficacy and predict clinical outcomes. Ultimately, this comprehensive approach will create a map of crucial molecular targets, facilitating the selection of kinase inhibitors for combination therapy with our EGTUB compounds.

The proposed project aims to bring new light to the fight against lung cancer by combining modern research and therapeutic approaches, which could contribute to the development of more effective treatments for this deadly cancer.