Exploiting the weakness of lymphoma: spectroscopic identification of biomarkers for lymphoma metabolism and inducing its switch to enhance cell sensitivity to chemotherapy

Diffuse large B-cell lymphoma (DLBCL) is a **genetically and metabolically** heterogeneous group of aggressive malignancies. DLBCL is the most common form of non-Hodgkin's lymphoma in adults, and the standard first-line treatment is chemotherapy based on the R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone). Unfortunately, approximately 30-40% of patients relapse or fail to respond to treatment. **Drug resistance** in DLBCL is a complex phenomenon resulting from the interaction of various genetic, molecular and environmental factors. Cancer **metabolism** differs from healthy cells, which may contribute to the adaptation of DLBCL and their ability to acquire new molecular mechanisms that overcome standard treatment therapies. Growth abnormalities leading to an increased proliferation rate of DLBCL cells require significant energy needs and an increased demand for building blocks (i.e. nucleic acids, proteins and lipids). Consequently, the phenomenon of **metabolic plasticity** has become a well-known characteristic of cancer. The metabolism of nutrients in DLBCL can be altered depending on the prevailing conditions, allowing cells to survive in a constantly changing microenvironment. Therefore, an **important aspect is to investigate the ability of DLBCL cells to reprogram energy acquisition pathways and lipid metabolism.**

There may be many reasons for the resistance of DLBCL cells to treatment, e.g. genetic mutations and epigenetic mutations, microenvironment, alterations in signalling and metabolic pathways. A better understanding of DLBCL classification, enabling the systematic identification of molecular vulnerabilities and the development of targeted therapies may become a sure way to overcome drug resistance. Therefore, it was clinically essential to develop a classification system for DLBCL into OxPhos and non-OxPhos subtypes and highlight that **lymphoma cells depend on different survival mechanisms and exhibit specific metabolic pathways depending on conditions.** A better understanding of the impact of metabolic reprogramming on cell sensitisation to chemotherapy is a step towards developing new therapeutic strategies that can effectively break resistance and improve outcomes for DLBCL patients.

Meeting the project's objectives requires **sensitive and specific** molecular methods capable of identifying biochemical differences at the level of single cells (or even organelles). Such methods include **oscillatory spectroscopy**, i.e. Raman microscopy and infrared absorption spectroscopy, which can capture **molecular changes** resulting from, for example, pharmacological stimulation in a **non-destructive manner and without the need for cell labelling**. By examining the interaction of molecules with light, oscillatory spectroscopy can track biochemical changes (lipids, proteins, nucleic acids) occurring in individual cells. Thus, its application enables the identification of **biomarkers** that verify changes in cellular metabolism and the cell's response to drugs. Changing composition and degree of unsaturation of lipids, changes in protein conformation or changes in cytochrome content are just a few examples of markers that oscillatory spectroscopy techniques can determine.

The research hypothesis of the proposed project is that reprogramming the metabolism of DLBCL cells is able to sensitise drug-resistant cells to chemotherapy, thereby exposing a number of changes (chemical and/or morphological) detectable by oscillatory microscopy techniques. The project anticipates that spectral phenotyping of resistant and sensitive cells will allow the identification of cells capable of developing drug resistant DLBCL.

The project's main objective is to assess the impact of reprogramming oxidative phosphorylation-glycolysis metabolic pathways and blocking selected lipid metabolism pathways on the resistance of DLBCL cells to selected drugs used in the standard lymphoma treatment protocol. Previous results have shown that by using oscillatory spectroscopy techniques, it is possible to distinguish DLBCL cell lines exhibiting different energy acquisition mechanisms. In order to achieve this, a series of tasks will be carried out aimed at finding molecular, metabolic and morphological changes in DLBCL cell lines manifested in cells due to pharmacological stimulation using a multimodal approach based on Raman and fluorescence microscopy and infrared absorption spectroscopy, supported by reference biological assays.

The project combines basic research exploring the metabolic mechanisms of DLBCL cells with the development of new pathways to combat the drug resistance common in lymphoma. **As a result, new ways of identifying drug resistance in DLBCL cells and tracking the modulation of their metabolism will be developed, offering the possibility of finding new therapeutic targets and improving standard treatment regimens.**