

## **OPUS-26**

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### **Modulation of the N-glycosylation as a tool to improve the CAR-T immunotherapy efficacy**

Cancer immunotherapy has been of the most spectacularly developing branches in tumor treatment in the recent years. Among many modalities used as an immunotherapy, the development of chimeric antigen receptor (CAR) technology has brought a real milestone in this field. This technology enable genetic modification of T cells to re-direct their cytotoxic activity in a tumor antigen-specific manner recognized by CAR receptor. This recognition triggers the CAR-T cells to launch a powerful attack against the tumor cells. The CAR itself is made up of different parts, including an antibody-derived domain responsible for antigen recognition, internal activating domains from natural T cells, and co-stimulatory domains that influence the overall response of CAR T cells. However, the seemingly straightforward approach is complicated by the presence of a dense sugar-coating called glycocalyx on both tumor cells and CAR T cells. This coating is, among others, a result of a process called N-glycosylation, which involves the addition of sugar molecules to proteins. Dysregulation of this process in tumor cells can make them resistant to CAR T cell therapy. To overcome this challenge, several options were explored like genetic modifications of tumor cells and the use of a substance called 2-deoxy-d-glucose (2DG) to reverse abnormal N-glycosylation of tumor cells. This has proven effective in boosting the efficacy of CAR T cells against various cancers. Yet, there is much to learn about how changes in N-glycosylation affect the activity of T cells and CAR T cells against tumors. It's known that activating T cells through their natural T cell receptor leads to activation of intracellular in N-glycosylation process. However, it is not very well known if and how modulation of N-glycosylation in CAR-T cells impact CAR-T cells activity and especially their ability to recognize tumors.

Our preliminary data suggests that modulating global N-glycosylation in CAR-T cells using swainsonine or kifunesine enhances the effectiveness of CAR-T cells against tumor cells. This enhancement is observed in various aspects of CAR-T cells functionality, including CAR-T cell degranulation, cytokine production, and the ability to eliminate tumor cells.

We hypothesize that exposure of CAR T cells to specific N-glycosylation modulators induces changes, leading to a boost in CAR T cell activity. To delve deeper into this phenomenon, we plan to use advanced glycomic mass spectrometry to identify changes in N-glycan composition post CAR-T cell activation and in presence of global N-glycosylation inhibitors. We will investigate the enzymes responsible for these changes. We will then employ more targeted N-glycosylation modulators to assess their impact on CAR-T cell functionality. Additionally, we plan to explore if genetic manipulation of these enzymes will enhance the cytotoxic performance of CAR T cells.

The main goal of the project is to discover and describe changes in the N-glycosylation profile of CAR-T cells that occur in response to the recognition of antigens on cancer cells, especially those that improve the function of CAR-T cells after the application of global N-glycosylation inhibitors. Investigating this process will help us identify which of the enzymes involved in the N-glycosylation process can be targeted to enhance the recognition of cancer cells by CAR-T cells. In a broader perspective, our research aims to improve the effectiveness of CAR-T cell-based immunotherapy, particularly in solid tumors where optimal interactions between CAR-T lymphocytes and cancer cells appear to be crucial for the success of immunotherapy.