

## **Title: Overcoming CAR-T cell exhaustion via CRISPR- based enhancer modification**

For decades, the mainstay of cancer treatment was surgery, chemotherapy, and radiotherapy. That changed with the introduction of immunotherapy - therapy that uses and boosts a patient's immune system to attack tumor cells - which has rapidly become what many call the "fifth pillar" of cancer treatment. CAR-T cell therapy is a type of treatment where the patient's T cells (obtained from blood) are changed using genomic engineering tools in the laboratory to attack cancer cells. In this process, a special receptor called chimeric antigen receptor (CAR) that binds to a specific protein on the patient's cancer cells is attached to the T cells. Since 2017, the Food and Drug Administration (FDA) has approved six CAR-T therapies and although they have been successful in the treatment of some blood cancers, durable remission following CAR-T cell therapy is not guaranteed. One of the reasons for the therapy failure is connected to CAR-T cell exhaustion, the condition where CAR-T cells become dysfunctional and lose their ability to kill cancer cells. Since CAR-T cells are unable to destroy target cells when this phenomenon occurs, developing anti-exhaustion strategies is critical for treatment success.

Enhancers are short regions of DNA that form a platform for the assembly of various proteins to promote gene transcription. Studies showed that enhancers constitute a promising target for genome manipulation in search of effective treatment for genetic disorders. A previous study unraveled multiple state-specific candidate genes and related enhancers in terminally exhausted T cells. Targeting those enhancers provides an attractive starting point for improving adoptive T cell products, such as CAR-T cells.

The purpose of this project is to overcome CAR-T cell exhaustion by targeting enhancers of genes associated with exhaustion. Using a genomic engineering tool (CRISPR/Cas9) we will study the effect of enhancers inhibition on three genes overexpressed in exhausted T cells: RBPJ, HAVCR2, and RUNX2. Then, we plan to check the influence of our manipulations on the CAR-T cells' phenotype and transcriptome.

Our results will not only increase our understanding of the mechanisms of interactions of selected genes and their enhancers but also have the translational potential to improve the effectiveness of CAR-T cell therapy.