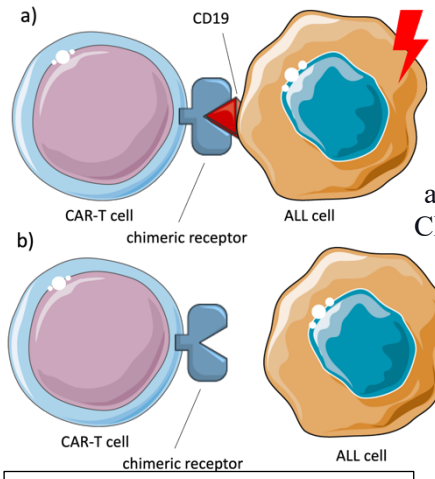
Mechanisms and consequences of CD19 loss in B-acute lymphoblastic leukaemia patients relapsing after CD19-CAR-T cell therapy: identification of novel therapeutic vulnerabilities

Acute B-cell Lymphoblastic Leukaemia (ALL) is a serious, potentially fatal disease caused by pathological, uncontrolled proliferation of B cells. Eventually, it leads to the degradation of bone marrow, leading to pancytopenia – a depletion of red and white blood cells, as well as platelets. The therapy is based mostly on chemotherapy followed by stem cells transplantation and induces complete remission in about 80% of cases. Unfortunately, about 60% of patients either don't respond to therapy or develop relapse, which results in a dismal prognosis. In recent years, a novel treatment strategy has been developed – Chimeric Antigen Receptor T-cells (CAR-Ts). It uses T cells, responsible for killing other, presumably hostile, cells. Their action is conditioned by the T-cell receptor (TCR), detecting specific surface proteins on potential targets. CAR-T technology modifies TCR to detect antigens on the surface of cancer cells. Importantly, the target for CAR-T cells must be present uniquely on malignant cells – otherwise, the therapy would damage healthy tissues and lead to severe side effects. Fortunately, ALL cells feature a suitable target, CD19 protein, expressed almost uniquely in B cells. It enabled the CAR-T therapy to greatly impact ALL treatment, inducing complete remission in 70%-90% of patients that have exhausted standard therapeutical measures. Unfortunately, despite initial success, 30%-60% of those patients eventually develop relapse. About half of those cases are caused by CAR-T target – CD19 protein loss. It is most commonly an effect of a mutation in the CD19 gene, but other mechanisms, such as alternative mRNA splicing leading to the production of damaged proteins, were also described. There is little known about the dynamics of the CD19 loss process. Are CD19- cells already present in some number before the treatment? How many mutations of *the CD19* gene are necessary to cause CD19 loss? If the occurrence of CD19- relapse is caused by an already present cellular subpopulation or determined by the presence of CD19 mutations, patients displaying such features would benefit little from anti-CD19 CAR-T therapy. Computational tool capable of detecting patients likely to develop CD19- relapse could be a valuable asset in selecting individuals for CAR-T treatment. **Therefore, we plan to assess the power of the CD19 mutation profile to predict and detect CD19- relapse by creating mathematical classifiers and evaluating their performance.** What is also important, CD19 protein is an essential modulator of signals required for B-cell proliferation and survival. We hypothesized that B cells without functional CD19 protein must rely on alternative cellular pathways to maintain their viability. Perturbing such pathways by additional drugs could lead to the death of CD19- relapse cells. We have validated our hypothesis in preliminary research, analyzing publicly available data of bone marrow sample transcriptome profiling (in single-cell resolution) for one ALL patient before CAR-T administration and after relapse development. By comparing the transcriptome of CD19+ and CD19- cells, we have detected multiple druggable cellular processes presumably sustaining CD19- cells survival. Moreover, we have found several distinct CD19- B cells populations, suggesting that there may be a variety of cellular mechanisms in use. **Thus, we also plan to validate and extend our findings by comparatively analyzing CD19+ and CD19- cells obtained from patients before CAR-T therapy and after relapse development.** We will enroll about 17 patients, collecting bone marrow samples before CAR-T infusion and after relapse. For each sample, we will perform deep targeted DNA sequencing of *CD19* to detect mutations, bulk RNA sequencing to evaluate the percentage of non-functional CD19 isoforms, and single-cell RNA sequencing (after dividing cells into CD19+ and CD19- groups). Using bioinformatical tools, we will build classifiers predicting and detecting CD19- relapse based on mutation profiles and evaluate their performance. Subsequently, we will comparatively analyze CD19+ and CD19- cells to validate our findings from the preliminary study and suggest additional drug candidates, potentially killing CD19- cells. Finally, we plan to test the effectiveness of selected drugs in CD19-knockout ALL cell lines experiments.



Overview of normal CAR-T function leading to cancer cell death **(a)** and cancer cell escape by loss of CD19 **(b)**