The reasons for choosing the research topic: In recent years, we have witnessed significant progress in the treatment of aggressive hematological malignancies, such as diffuse large B-cell lymphoma and acute lymphoblastic leukemia. The treatment of these diseases has been one of the greatest challenges for oncologists so far. CD19-targeting immunotherapy using genetically modified with chimeric antigen receptors (CARs) cytotoxic cells – T lymphocytes or NK cells, has been particularly successful. Up to 40-80% of patients treated with this immunotherapy achieve complete response, which was unattainable until recently. From 2021, this therapy is also reimbursed in Poland for patients with acute lymphoblastic leukemia. However, despite such promising results, many reports indicate that a significant percentage of patients experience resistance following CD19 CAR-T therapy. One of the most commonly reported resistance mechanisms is the CD19 loss on the surface of the tumor cells. Despite the intensive research, the causes and mechanisms of cancer resistance to immunotherapy are still poorly understood and caused among others by the limited access to material from patients. One solution that will help to overcome this problem and support basic research in this field would be the usage of the CD19 CAR-T resistance model of malignant cells. As part of the preliminary research for this project, we created a models of a lymphoma tumor cell line resistant to anti-CD19 CAR-T cells. In these models, we observed many changes, including a decrease in CD19 expression on the surface of tumor cells, also reported in patients after CAR-T anti-CD19 therapy. In addition, to obtain the above models, we used CAR constructs containing the 4-1BB or CD28 co-stimulatory domain, identical to those used in clinical products. The use of such models within current proposal will enable us to conduct extensive research on the development of resistance mechanisms in hematological cancers, including a direct comparison of these mechanisms depending on the structure of the therapeutic CAR.

Aims of the project: The aim of this proposal is to characterize and compare the *in vitro* models of lymphoma and B-ALL after long-term pressure of CD19 CAR-T cells, with the structure of clinical CARs incorporating 4-1BB or CD28 co-stimulatory domains. We will study the biology of CD19 antigen loss and identify the unique phenotypic changes accompanying resistance development taking into account the differences in the structure of the therapeutic CAR constructs.

Implementation of the project: Within the project, we have planned the implementation of three research tasks. In Task 1, we will characterize the CD19 on the level of surface epitopes and total protein in tumor cells after prolonged exposures to CD19 CAR-T cells. Additionally, we will broadly phenotype the surfaceome of our studied models in the context of screening for novel hits accompanying the tumor immune escape phenotype. In Task 2, we will perform the transcriptomic analysis to define the genetic-related factors of CD19 loss in long-term effectors' pressure models, as well as to look for novel transcriptomic hits. Moreover, in all the above analyses, we will include the comparison of CAR structure, CAR-4-1BB and CAR-CD28, used for model generation. Finally, in Task 3, we will verify newly identified hits in primary samples from lymphoma- and B-ALL-resistant patients.

Expected results: We believe, that the results of this project will provide new insight into the antitumor immune response of CAR-T cells used in therapeutic products and will further contribute to the improvement of clinical immunotherapies efficacy. Undoubtedly, the success of our project would have a chance to influence the significant development of medicine and public health.