Investigating the impact of *Nr4a1* on immune synapse in lymphoma

Introduction:

Aggressive lymphomas represent the most common type of lymphoid malignancies with a five-year survival rate of 60%. Despite effective initial treatment, one-third of all patients will experience a relapse, warranting more research to discover novel therapeutic strategies. We recently detected a significant reduction of nuclear receptor NR4A1 expression in aggressive lymphoma patients that correlated with poor cancer-specific survival. We observed that loss of *Nr4a1* leads to a marked acceleration of lymphomagenesis in vivo, concomitant with increased expression of immune checkpoints. Immuno-competent, but not immune-deficient, mice transplanted with Nr4a1-deficient lymphoma cells exhibited rapid lymphoma development, reduced survival, and upregulation of immune checkpoints.

Hypotheses:

These data indicate that Nr4a1 and the tumor immune response form a functional axis of checkpoint-mediated immune evasion in aggressive lymphomas, which we aim to investigate comprehensively in this project.

Methods:

To achieve these aims, we will use banked tissues of human aggressive lymphoma, murine lymphoma cell models, and viable cryopreserved patient-derived DLBCL cell suspensions – all characterized for low and high *NR4A1* expression levels to allow co-culture functional modeling with syngenic murine or autologous human CD8+ T cells, respectively.

First, we will perform co-culture experiments using murine lymphoma cells (with or without *Nr4a1* loss) and CD8+ T cells to test anti-lymphoma immune responses under blockade of several immune checkpoint axes alone or in combination. Furthermore, Nr4a1 agonists as well as specific inhibitor(s) of a certain pathway(s) knowing to impact on checkpoint molecules will be added to these experiments. Immune cell-mediated lymphoma cell lysis and T cell activation will be determined upon treatment to determine anti-lymphoma immune responses.

Second, the treatment/s resulting in a high lymphoma cell lysis and T cell activation in the murine models will be validated in co-cultures using our human setting cryopreserved patient-derived DLBCL cell suspensions and autologous CD8+ T cells as a translational approach.

Third, the most potent treatment (highest immune cell-mediated lymphoma cell killing and/or T cell action) will be tested using organotypic slice cultures with fresh obtained lymphoma tissue from DLBCL patients to mimic *in vivo* conditions. Anti-lymphoma immune response will be evaluated by staining for T cell activation marker and set in relation to the NR4A1 expression status.

Expected results & impact:

With these complementary approaches, we aim to identify novel mechanisms of NR4A1-mediated lymphomagenesis via immune checkpoint regulation and to establish new biomarkers to aid treatment decisions with checkpoint inhibitors.