Ewing's sarcoma is an aggressive pediatric bone and soft tissue cancer and second most common primary bone tumor. A chromosomal translocation t(11;22) results in expression of EWS-FLI1, a fusion protein present in 85% of cases and thus is an excellent therapeutic target. However, due to its nature as transcription factor, directly disrupting EWS-FLI1 activity is challenging. Alternative strategies include targeting EWS-FLI1 synthesis or its downstream genes, which are activated or repressed by its activity.

In my PhD project I aim to identify and rank EWS-FLI1 target genes essential for cancer cell survival potentially providing new therapeutic targets.

To this end, I plan to utilize CRISPR/Cas-based screening systems in Ewing's sarcoma cell lines with doxycycline inducible CRISPRi (interference) and CRISPRa (activation) systems. Selected single clones will be used for testing of two different sgRNA-libraries covering EWS-FLI1-repressed target genes for CRISPRa and EWS-FLI1-activated genes for CRISPRi. The target genes will be read out from EWS-FLI1 silencing studies and confirmed by EWS-FLI1 ChIP-seq data. These screens will be performed in vitro and in a xenograft mouse model. Potential hits will be validated and ranked by their effect on tumor viability if activated by CRISPRa or inhibited by CRISPRi, respectively.

Taken together, the establishment of inducible inhibition and induction systems will allow us to test EWS-FLI1 target gene libraries to find potential new therapy options.