**Heterogeneity of metastogenic neuroblastoma cells**

Christian Beltinger; University Hospital Ulm; Dept. of Pediatrics and Adolescent Medicine

**Background/State of the art.** Neuroblastoma (NB) often kills affected children by metastasis. While several molecules have been selected for analysis and were found to impact on NB metastasis in the preclinical (e.g., (Naftali et al., 2016; Xiang et al., 2015)) and, less often in the clinical setting (Cheung et al., 2014; Russell et al., 2004), no unbiased genome-wide investigations addressing NB metastasis have been performed in either setting. Thus, the molecules and pathways determining metastasis of NB remain largely unknown. Recently, a genome-wide loss-of-function screen using a CRISPR/Cas9 library has been performed in non-small-cell lung cancer cells, leading to the discovery of candidate metastasis-promoting genes in non-small-cell lung cancer (Chen et al., 2015).

**Preliminary work.** We showed that strong expression of CD57 in NB cells and their cells of origin increases metastogenicity and anchorage-independent growth, respectively (Schlitter et al., 2011). Furthermore, MYCN and survivin, poor prognostic factors in NB, cooperated to enhance anchorage-independent growth (Hipp et al., 2014; Saxena et al., 2013). Finally, in Ewing’s sarcoma, closely related to NB, CD57 also enhanced anchorage-independent growth (Wahl et al., 2010). Our data thus strongly suggests that NB cells are heterogeneous in their capacity to metastasize. Furthermore, we have performed preliminary work that will directly feed into the proposed project. This includes a genome-wide gain-of-function screen in NB cells using lentiviruses with catalytic inactive Cas9, targeted CRISPR-mediated gene knockouts in NB cells, an orthotopic (intraadrenal) NB model imaged by bioluminescence and MRI, 110 NB tissue samples linked to patient data and a clinically annotated expression data bank of more than 600 patient NB. Finally, we have procured privileged access to the TARGET databank of the US Children’s Oncology Group. This allows us to retrieve from 210 NB patients clinically annotated results and sequences from whole genome, whole exome, targeted and mRNA sequencing, as well as microarray expression and methylation data.

**Overall hypothesis.** We hypothesize that a genome-wide knock-out of genes that suppress metastasis will render some NB cells of non-metastatic human NB cells growing in mice to become metastatogenic. Deep sequencing of metastases will determine the genes that have been knocked-out, thus leading to metastasis. The data banks with human data will allow to determine the presence of these alterations, both on the genomic and mRNA levels, in patient tumors and to correlate them with metastatic disease.