**Heterogeneity of metastogenic neuroblastoma cells**

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**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.

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**Intra-individual tumor heterogeneity of NSCLC: Longitudinal in vivo analysis of clonal evolution and selection of chemo-resistant clones**

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**Background/State of the art.** Non-small cell lung cancer (NSCLC) is among the tumors with the largest number of acquired mutations per tumor (Alexandrov et al. 2013). In accordance, not only inter-individual tumor heterogeneity of these tumors poses a major challenge for treatment concepts, but also the intra-individual
heterogeneity, which contributes to the development of resistance and treatment failure (Vogelstein et al. 2013). Thus, a better understanding of the intra-individual tumor heterogeneity is a prerequisite for new therapeutic approaches that might be able to delay or even prevent the development of chemo-resistance via a “precise” combination therapy.

**Preliminary work.** The supervisors have a longstanding track record in studying tumor heterogeneity by applying novel state of the art molecular genetic techniques. For example, L. Bullinger’s group was among the first to use next-generation sequencing to decipher the genomic heterogeneity of several tumor entities such as e.g. acute myeloid leukemia (AML) (Dolnik et al. 2012), acute lymphoblastic leukemia (ALL) (Mar et al. 2014), and multiple myeloma (Kortüm et al. 2014). Similarly, R. Marienfeld has been involved in the genomic characterization of hairy cell leukemia (Lennerz et al. 2012) and primary mediastinal B cell lymphoma (Nagel et al. 2014). Furthermore, there is also a tremendous expertise to link genomic information with clinical data in order to come up with clinically relevant implications (Gaidzik et al. 2013). With regard to the intended study of lung cancer, L. Bullinger has significantly contributed to build up the LuCa bioregistry, which already contains samples from over 300 clinically well annotated lung cancer cases (including follow-up samples from individual cases, which will enable “minimal residual disease” detection and/or monitoring of the occurrence of resistant clones). In parallel, in close collaboration L. Bullinger and R. Marienfeld have established a targeted resequencing panel for the “routine”-analysis of solid tumor samples, including protocols that work with little DNA that can be obtained from FFPE conserved tumor biopsies. In addition, in-house data analysis pipelines for both targeted resequencing and whole exome sequencing have been established and are running well. Finally, functional analyses based on CRISPR/Cas9 technology have been set up successfully in leukemia and solid tumor derived cell line models and can be applied to lung cancer cell line models as well as primary lung cancer derived cell models (*in vitro* culture of primary cells and/or xenograft models).

**Overall hypothesis.** We will study the intra-individual heterogeneity within selected patients with adenocarcinoma using next generation sequencing (NGS) technology.