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# Anaplastic lymphoma kinase activity, a therapeutic target, suppresses neuroblastoma cell differentiation

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## Abstract

Neuroblastoma (NB) is the most common extracranial pediatric solid malignancy caused by the failed differentiation of precursor cells of the developing sympathetic nervous system. NB accounts for about 15% of childhood cancer-related deaths. Treatment failure and relapse are common in NB patients despite intensive chemotherapy and immunotherapy interventions, suggesting the need for new and effective treatment options. Common genetic aberrations associated with NB include MYCN amplification, chromosome 11q deletion, 1p deletion, 17q gain, 2p gain, and recurrent mutations in Anaplastic Lymphoma Kinase (ALK). While treatment of some categories of ALK-positive pediatric cancer patients such as non-Hodgkin lymphoma and inflammatory myofibroblastic tumour (IMT) with the first-generation ALK tyrosine kinase inhibitor (TKI), crizotinib, produced promising results, the outcome for ALK-positive NB patients was less encouraging, hence the need for more potent ALK TKIs for treatment of NB patients. This thesis aimed to further our understanding of ALK signalling and its role in NB differentiation and explore novel ALK TKIs in a neuroblastoma setting.

In the first study, we investigated the therapeutic efficacy of the second-generation ALK TKI, brigatinib, in an NB preclinical setting. Brigatinib was reported to be effective against ALK fusion-positive non-small cell lung tumours. We found that brigatinib potently inhibited both the activity of ALK full-length and growth of ALK-addicted NB cells in-vitro, in xenograft and Drosophila models. Compared to crizotinib, brigatinib inhibited the activities of different ALK-mutant alleles more effectively and potently inhibited crizotinib resistant ALK mutants in vitro.

In the second study, we characterized a novel ALK-I1171T mutant allele which we identified in a tumour from a 16 month old NB patient. We showed that ALK-I1171T is a gain-of-function mutation, which is resistant to crizotinib, but can be effectively inhibited by second- and third-generation ALK TKIs such as brigatinib, ceritinib and lorlatinib. Based on these results and the severe toxic side effect of the initially administered chemotherapy, ceritinib monotherapy was chosen for this child. After 7.5 months of ceritinib treatment, the primary tumour shrunk in size and was removed surgically. The patient showed complete metastatic remission and remains in remission at 58 months post-treatment.

In the third and last study, we investigated Disk large homologue 2 (DLG2), a gene reported to be uniquely upregulated in transient intermediary cells during Schwann cell precursor (SCP) differentiation to adrenal chromaffin cells. We found that DLG2, a gene located on the frequently deleted chromosome 11q in NB, is an NB tumour suppressor gene whose expression is lost in NB cell lines. Restoration of DLG2 expression inhibited NB cell growth and promoted NB cell differentiation. High expression of DLG2 in NB tumours is associated with good prognosis. Mechanistically we showed that oncogenic ALK maintains an undifferentiated NB cell phenotype by repressing DLG2 expression via the ERK1/2-SP1 signalling cascade.

In summary, these findings highlight the role of ALK in differentiation and therapeutic potential of targeting ALK in ALK-positive NB tumours.

Keywords: Neuroblastoma, ALK, 11q, DLG2, SP1, differentiation, crizotinib, ceritinib, brigatinib.

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av

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**Avhandlingen baseras på följande delarbeten**

I. **Joachim T. Siaw***, Haiying Wan*, Kathrin Pfeife, Victor M. Rivera, Jikui Guan, Ruth H. Palmer, Bengt Hallberg. (2016). Brigatinib, an anaplastic lymphoma kinase inhibitor, abrogates activity and growth in ALK-positive neuroblastoma cells, *Drosophila* and mice. *Oncotarget*, 7(20):29011-22. doi: 10.18632/oncotarget.8508.
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II. Jikui Guan*, Susanne Fransson*, **Joachim Tetteh Siaw***, Diana Treis*, Jimmy Van den Eynden, Damini Chand, Ganesh Umapathy, Kristina Ruuth, Petter Svenberg, Sandra Wessman, Alia Shamikh, Hans Jacobsson, Lena Gordon, Jakob Stenman, Pär-Johan Svensson, Magnus Hansson, Erik Larsson, Tommy Martinsson, Ruth H Palmer, Per Kogner, Bengt Hallberg. (2018). Clinical response of the novel activating ALK-I1171T mutation in neuroblastoma to the ALK inhibitor ceritinib. *Cold Spring Harb Mol Case Stud,* 4(4):a002550. doi: 10.1101/mcs.a002550.
(* Co-first author)

III. **Joachim Tetteh Siaw***, Niloufar Javanmardi*, Jimmy Van den Eynden*, Dan Emil Lind, Susanne Fransson, Angela Martinez-Monleon, Anna Djos, Rose-Marie Sjöberg, Malin Östensson, Helena Carén, Gunhild Trøen, Klaus Beiske, Ana P Berbegall, Rosa Noguera, Wei-Yun Lai, Per Kogner, Ruth H Palmer, Bengt Hallberg, Tommy Martinsson. (2020). 11q Deletion or ALK Activity Curbs DLG2 Expression to Maintain an Undifferentiated State in Neuroblastoma. *Cell Reports*, 32(12):108171. doi: 10.1016/j.celrep.2020.108171.
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