

# Mechanistic Implications and Characterization of Anaplastic Lymphoma Kinase (ALK) mutations in Neuroblastoma

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## Akademisk avhandling

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**Abstract**

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that was first reported as a fusion partner of nucleophosmin in Anaplastic large cell lymphoma in 1994. ALK is involved in myriad of cancers including neuroblastoma which is the most common extracranial solid tumor affecting young children. It arises in the neural crest cells of sympathetic nervous system origin and is responsible for 12% of all childhood cancer deaths. Several point mutations in ALK have been described in both familial and sporadic neuroblastoma.

With the aim to understand the role of ALK in neuroblastoma further, we investigated the point mutations in ALK reported in patients. Using cell culture based methods and *Drosophila* as a model organism; we first characterized these mutations under three broad categories: 1) Ligand independent mutations that were constitutively active, 2) Kinase dead mutation and 3) Ligand dependent mutations that behaved as inducible wild type. Further, to understand the activation mechanism of ALK, we constructed mutations that could potentially alter ALK's conformation based on the available crystal structure. From the data generated, we were able to provide a new perspective to the activation of full length ALK receptor. This was more in line with activation mechanism of insulin receptor and different from that suggested for ALK fusion protein. From a clinical point of view, all the mutations in the study were blocked to different degrees using the ALK inhibitor, crizotinib. Lastly, we identified potential downstream targets of ALK using phosphoproteomics. From the various targets identified, we focused on STAT3 and confirmed its role as a mediator in ALK initiated MYCN transcription. We showed that STAT3 inhibition led to reduction of MYCN levels and thereby identifying it as a potential therapeutic target in neuroblastoma. Overall, our study highlights clinical relevance of ALK mutations in neuroblastoma and from a basic biology viewpoint; it reveals important mechanistic insight into receptor activation.

**Keywords** neuroblastoma, ALK, crizotinib, receptor tyrosine kinase, STAT3, MYCN

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