

ALK in Neuroblastoma

Part 1 Background on the *ALK* Gene and Role in Neuroblastoma

- The *ALK* gene (which stands for “anaplastic lymphoma kinase”) encodes a protein called a tyrosine kinase receptor. This protein is activated (turned on) either by point mutations, amplification or chromosomal translocations, and has emerged as an important biomarker and therapeutic target in a subset of adult lung cancers ¹, as well as pediatric solid and liquid cancers ^{2,3}.
- *ALK* mutations were first discovered in both the hereditary (in the genetic make-up of the child’s DNA) and sporadic form (in tumor cells only) of neuroblastoma ⁴⁻⁷.
- The *ALK* receptor is typically expressed only in the developing nerve tissue and absent from normal tissues ⁸, providing an opportunity to develop targeted therapies.
- The implications of *ALK* mutations in neuroblastoma have been extensively studied in the laboratory using biochemistry to determine which mutations are “activating,” and human-derived neuroblastoma cell lines and mouse models to understand the impact of turning *ALK* off with different commercially available drugs ^{9,10}.
- Unlike cancers that have *ALK* fusions (non-small cell lung cancer, anaplastic large cell lymphoma, and inflammatory myofibroblastic tumors), *ALK* mutations in neuroblastoma have been shown to be intrinsically resistant to early-generation *ALK* inhibitors ^{9,10} but show exquisite sensitivity to the third generation *ALK* inhibitor, Lorlatinib ¹¹.
- Approximately 14% of children with high-risk neuroblastoma will have a tumor that has an *ALK* aberration (mutation or amplification) at diagnosis ⁹ (using low-resolution sequencing technology) with even higher rates reported at relapse ^{12,13}, and likely even higher rates at diagnosis with implementation of next generation sequencing ¹⁴.

Part 2 *ALK* alteration and impact on prognosis

- Two pivotal studies report on clinical correlates of somatic (in the tumor) *ALK* mutations in neuroblastoma and conclude that the presence of an *ALK* alteration (mutation or amplification) is a biomarker of inferior prognosis ^{9,14}.
- In a recent report describing patients with locoregional (non-metastatic) *ALK*-mutant neuroblastoma, there was a trend toward increased risk of incomplete surgical resection and tumor recurrence ¹⁵.
- Based on these findings, further work needs to be done to elucidate the risk conferred by *ALK* mutations in the context of modern chemoradioimmunotherapy.

Part 3 Spectrum of *ALK* alterations

- 85% of *ALK* mutations are accounted for by mutations at three hotspots (areas) of the gene: R1275 (43-49%), F1174 (30-35%), and F1245 (12%).
- Other mutations have been described as activating ⁹ and as more sequencing is done on tumor tissue, additional such mutations are identified, and their relevance is continuously being evaluated. When a previously unreported *ALK* variant is found, experts in the field should be contacted to determine if a patient is eligible for *ALK* inhibition therapy.

- ALK amplification occurs in 2-4% of neuroblastoma cases and predict for an even more inferior outcome ^{9,14}.
- ALK fusions are exceedingly rare in neuroblastoma.
- The presence of ALK expression by immunohistochemistry (IHC) does not mean that the tumor has an ALK alteration ⁸. Sequencing of tumor tissue (preferably using next-generation assays) should always be done to confirm the absence/presence of a neuroblastoma-specific ALK alteration.

Part 4 How do I know if my child's neuroblastoma has an ALK mutation or amplification?

- Tumor sequencing (at diagnosis and/or relapse) using next-generation sequencing assays; liquid biopsies (also referred to as Circulating tumor DNA), when a sample of blood can be collected to evaluate for the presence of any tumor DNA that is shed from the tumor ¹⁶⁻¹⁹.
- A test called Fluorescence In-Situ Hybridization (FISH) can be performed on the tumor biopsy to look for extra copies of the ALK gene (amplification).
- Important to know that an ALK alteration can be absent at diagnosis but can evolved over the course of therapy ^{12,13}. It is important to ask your doctor to look for the presence/absence of an ALK alteration from new tissue or from the blood if the neuroblastoma does not respond to standard treatment, or if you child suffers a relapse.

Part 5 Does my child's treatment change if his/her tumor has an ALK mutation/amplification?

- **ALK Inhibitors**
 - Targeted ALK inhibition holds significant promise for children whose tumors harbor an ALK alteration. ALK inhibitors are administered orally (either as a pill or liquid formulation depending on the drug).
 - **Crizotinib**, the first-in-class ALK inhibitor, has been FDA approved for cancers with ALK fusions: non-small cell lung cancer, relapsed/refractory pediatric and young adult anaplastic large cell lymphoma, and unresectable/recurrent pediatric and adult inflammatory myofibroblastic tumors. The latter two approvals came because of the Children's Oncology Group (COG) phase 1/2 study, ADVL0912 ²⁰.
 - The same COG study showed discouraging results when crizotinib was evaluated in patients with relapsed/refractory ALK+ neuroblastoma ²¹, as predicted by the early laboratory studies. Crizotinib can be combined with chemotherapy to enhance its activity ^{22,23} which served as the rationale for integration of crizotinib into standard front-line chemotherapy for newly diagnosed patients with high-risk NB harboring an ALK mutation (ANBL1531, accrual started in April 2018). This is the first pediatric solid tumor Phase 3 trial to integrate a patient-specific molecularly targeted therapy.
 - Common side effects of crizotinib include gastrointestinal symptoms (abdominal pain, nausea, vomiting, diarrhea), visual disturbance, headache, musculoskeletal pain, mouth sores, fatigue, fever, cough and pruritis (itching). Laboratory abnormalities most commonly include decreased neutrophils and rise in creatinine (kidney) though it does not impair kidney function. All side effects are reversible when the drug is stopped.
 - **Lorlatinib**, a third-generation ALK inhibitor with superior activity against all neuroblastoma hot spot mutations and robust penetration through the blood brain barrier, has shown unprecedented anti-tumor preclinical activity in mouse models of neuroblastoma ¹¹ as well as in Phase 1 testing though the New Approaches to

Neuroblastoma Therapy (NANT) Consortium²⁴. In light of these data, a major amendment was approved to replace crizotinib with lorlatinib upfront in COG study ANBL1531 (activated April 2022), and to incorporate lorlatinib into the ongoing European Phase 3 trial.

- The NANT study continues expansion to refine our understanding of lorlatinib in the relapse setting.
- Common side effects of lorlatinib in children include increased appetite/weight gain and a rise in cholesterol levels; less common side effects in children include cognitive impairment (difficulty with memory and word finding), peripheral neuropathy (nerve pain tingling), and peripheral edema (swelling of hands and/or feet). All side effects are reversible when the drug is stopped.

Part 6 Are there any open clinical trials for patients with *ALK*-mutant neuroblastoma?

- Newly diagnosed
 - ANBL1531; COG *upfront Phase 3 trial* (<https://clinicaltrials.gov/ct2/show/NCT03107988>)
- Relapse
 - Lorlatinib monotherapy or combination with chemotherapy; NANT Phase 2 study (<https://clinicaltrials.gov/ct2/show/record/NCT03107988>)

References:

1. Kwak EL, Bang YJ, Camidge DR, et al: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *The New England journal of medicine* 363:1693-703, 2010
2. Mosse YP: Anaplastic Lymphoma Kinase as a Cancer Target in Pediatric Malignancies. *Clin Cancer Res*, 2015
3. Pearson ADJ, Barry E, Mosse YP, et al: Second Paediatric Strategy Forum for anaplastic lymphoma kinase (ALK) inhibition in paediatric malignancies: ACCELERATE in collaboration with the European Medicines Agency with the participation of the Food and Drug Administration. *Eur J Cancer* 157:198-213, 2021
4. Chen Y, Takita J, Choi YL, et al: Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* 455:971-4, 2008
5. George RE, Sanda T, Hanna M, et al: Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* 455:975-8, 2008
6. Janoueix-Lerosey I, Lequin D, Brugieres L, et al: Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* 455:967-70, 2008
7. Mosse YP, Laudenslager M, Longo L, et al: Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* 455:930-5, 2008
8. Carpenter EL, Haglund EA, Mace EM, et al: Antibody targeting of anaplastic lymphoma kinase induces cytotoxicity of human neuroblastoma. *Oncogene* 31:4859-67, 2012
9. Bresler SC, Weiser DA, Huwe PJ, et al: ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. *Cancer Cell* 26:682-94, 2014
10. Bresler SC, Wood AC, Haglund EA, et al: Differential inhibitor sensitivity of anaplastic lymphoma kinase variants found in neuroblastoma. *Sci Transl Med* 3:108ra114, 2011
11. Infarinato NR, Park JH, Krytska K, et al: The ALK/ROS1 inhibitor PF-06463922 overcomes primary resistance to crizotinib in ALK-driven neuroblastoma. *Cancer Discov*, 2015
12. Padovan-Merhar OM, Raman P, Ostrovnya I, et al: Enrichment of Targetable Mutations in the Relapsed Neuroblastoma Genome. *PLoS Genet* 12:e1006501, 2016
13. Schleiermacher G, Javanmardi N, Bernard V, et al: Emergence of New ALK Mutations at Relapse of Neuroblastoma. *J Clin Oncol* 32:2727-34, 2014
14. Bellini A, Potschger U, Bernard V, et al: Frequency and Prognostic Impact of ALK Amplifications and Mutations in the European Neuroblastoma Study Group (SIOPEN) High-Risk Neuroblastoma Trial (HR-NBL1). *J Clin Oncol*:JCO2100086, 2021
15. O'Donohue T, Gulati N, Mauguen A, et al: Differential Impact of ALK Mutations in Neuroblastoma. *JCO Precis Oncol* 5, 2021
16. Van Paemel R, Vandeputte C, Raman L, et al: The feasibility of using liquid biopsies as a complementary assay for copy number aberration profiling in routinely collected paediatric cancer patient samples. *Eur J Cancer* 160:12-23, 2022
17. Peneder P, Stutz AM, Surdez D, et al: Multimodal analysis of cell-free DNA whole-genome sequencing for pediatric cancers with low mutational burden. *Nat Commun* 12:3230, 2021
18. Yagyu S, Iehara T, Tanaka S, et al: Serum-Based Quantification of MYCN Gene Amplification in Young Patients with Neuroblastoma: Potential Utility as a Surrogate Biomarker for Neuroblastoma. *PLoS One* 11:e0161039, 2016
19. Chicard M, Boyault S, Colmet Daage L, et al: Genomic Copy Number Profiling Using Circulating Free Tumor DNA Highlights Heterogeneity in Neuroblastoma. *Clin Cancer Res*, 2016
20. Mosse YP, Voss SD, Lim MS, et al: Targeting ALK With Crizotinib in Pediatric Anaplastic Large Cell Lymphoma and Inflammatory Myofibroblastic Tumor: A Children's Oncology Group Study. *J Clin Oncol* 35:3215-3221, 2017

21. Foster JH, Voss SD, Hall DC, et al: Activity of Crizotinib in Patients with ALK-Aberrant Relapsed/Refractory Neuroblastoma: A Children's Oncology Group Study (ADVL0912). Clin Cancer Res 27:3543-3548, 2021
22. Greengard E, Mosse YP, Liu X, et al: Safety, tolerability and pharmacokinetics of crizotinib in combination with cytotoxic chemotherapy for pediatric patients with refractory solid tumors or anaplastic large cell lymphoma (ALCL): a Children's Oncology Group phase 1 consortium study (ADVL1212). Cancer Chemother Pharmacol 86:829-840, 2020
23. Krytska K, Ryles HT, Sano R, et al: Crizotinib Synergizes with Chemotherapy in Preclinical Models of Neuroblastoma. Clin Cancer Res, 2015
24. Goldsmith KC, Kayser K, Groshen SG, et al: Phase I trial of lorlatinib in patients with ALK-driven refractory or relapsed neuroblastoma: A New Approaches to Neuroblastoma Consortium study. Journal of Clinical Oncology 38:10504-10504, 2020

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