Children's Neuroblastoma Cancer Foundation

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) Consortium [24]. 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/eight 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 their 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)

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If you have any questions regarding this chapter, please contact us at: info@cncfhope.org