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Genetic alterations of *ALK* in high-risk neuroblastoma patients. A SIOPEN study.

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Background: In neuroblastoma (NB), the ALK receptor tyrosine kinase can be constitutively activated through genomic amplification or activating point mutations. We studied *ALK* genetic alterations in high-risk NB patients to determine their frequency and prognostic impact.

Methods: Diagnostic NB samples from 1039 patients enrolled in the SIOPEN-HR-NBL1 trial were studied to determine the *ALK* amplification status (copy number analysis; n=337), the *ALK* mutational profile (Sanger and/or NGS including deep sequencing, n=203) or both (n=499).
The sensitivity of ALK mutated/ALK amplified or ALK wildtype NB cell lines ((CLB-GA (R1275Q), CLB-GE (F1174V; ALK-A), SKNBE-2C (ALK wt)) to simultaneous or

consecutive combinations of ALK TKIs (crizotinib/lorlatinib) and/or chemotherapy (Etoposide and Doxorubicin) was then tested.

Results: Genomic *ALK* amplifications were detected in 4.4% of cases (37/836); all but 2 showed *MYCN* amplification. *ALK* mutations were detected at a clonal level (>20% mutated allele fraction, MAF) in 9.8% of cases (69/702) (F1174 n=25, R1275 n=32, both F1174 and R1275 n=1, F1245 n=6, others n=5) and at a subclonal level (MAF 0.5-20%) in 3.7% of patients (22/586) (F1174 n=11, R1275 n=6, both F1174 and R1275 or F1174 and F1245 n=3, other n=2).

A significantly poorer OS and EFS was observed in cases with clonal *ALK* mutations, versus all others (3-years OS 47% +/-6.4% versus 65% +/-2%, logrank, p< 0.0001) and in those with *ALK* amplifications, versus all others (3-years OS 31% +/-8.5% versus 66% +/- 1.9%; logrank, p<0.0001).

A Cox proportional hazards procedure (450 patients with complete clinical/biological datasets) retained stage 4 disease (as opposed to non-stage 4) and *ALK* amplification as factors with a higher hazard of relapse/progression (hazard 2.3 and 2.2, respectively), whereas *ALK* mutation, *MYCN* amplification and age>18 months were not retained.

The consecutive treatment of Doxorubicin followed by Lorlatinib had a synergistic effect in *ALK* mutated/amplified NB cell lines.

Conclusion: Genetic alterations of *ALK* (clonal mutations, amplifications) in high-risk NB patients are associated with poorer survival. Further preclinical data are required to determine optimal treatment modalities for integration of TKI in upfront treatment strategies of HR NB patients with *ALK* alterations.