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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 either 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 covering hotspots in exons 23-25, n=203) or both (n=499).

Results: High level genomic *ALK* amplifications were detected in 4.4% of cases (37/836); all but 2 showed *MYCN* amplification. As for *MYCN* amplification, *ALK*

amplification was more frequently observed in children aged<18 months at diagnosis (p=0.01). No correlation with the primary tumor site was observed.

ALK mutations were detected at a clonal level (>10% 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). Additionally, 3.7% of patients (22/586 by NGS) harbored ALK mutations at a subclonal level (MAF 0.5-10%) (F1174 n=11, R1275 n=6, both F1174 and R1275 or F1174 and F1245 n=3, other n=2). Although not statistically significant, *ALK* mutations were observed slightly more frequently in non-adrenal compared to adrenal primary tumors (p=0.08).

Whereas no statistically significant difference in survival was observed between patients with and without *ALK* mutations, patients with *ALK* amplification had a significantly poorer event free (EFS) and overall survival compared to those without *ALK* amplification (logrank, p<0.0001).

A multivariate analysis was performed to determine which parameters independently predicted EFS in this high risk population. Among 450 patients with complete datasets, a Cox proportional hazards procedure 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.

Conclusion: Taking into account amplifications, clonal and subclonal mutations, genetic alterations of *ALK* were observed in tumor samples of 17% of high-risk NB patients, of importance when considering ALK targeted therapies. Among the different genetic alterations, only *ALK* amplification predicted poorer survival.