Abstract SIOPEN 2015 : High frequency of subclonal *ALK* mutations in high risk neuroblastoma patients. A SIOPEN study.

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Introduction:
In neuroblastoma (NB), activating *ALK* receptor tyrosine kinase point mutations are detected in 8–10% at diagnosis using conventional sequencing. To determine the potential occurrence and the prognostic impact of *ALK* mutations in a series of high risk NB patients we studied *ALK* variation frequencies using targeted deep sequencing in samples of patients enrolled in the SIOPEN HR-NBL01 study.

Methods:
To date, a series of 326 diagnostic high risk NB samples enrolled in the HRNBL trial has been analyzed, focusing on the exons 23, 24 and 25 containing the F1174, F1245 and R1275 hotspots respectively. DNA was amplified via a two-step PCR approach, the second step consisting of addition of sample-specific barcodes for targeted resequencing in a single experiment. Amplicon sequencing (Illumina HiSeq2500) achieved an extremely high depth over the relevant hotspot (80,000X). The background base variability (error rate) in 32 control samples was 0.017%+/- 0.010 at the studied position. Given the mean coverage and error rate, a base frequency >0.06% is significantly different from background noise (Fisher's exact test).

Results:
At the F1174 hotspot, mutations were observed in 21/326 samples. For 11 cases, these mutations, observed with mutated allele fractions of >20%, occurred at a clonal

level, and in 10 additional cases they occurred at a sub-clonal level (range of mutated allele fractions: 0.207% - 4.195%). At the R1275 hotspot, mutations were observed in 26/326 samples, 12 at a clonal and 14 at a sub-clonal level. All clonal mutations were validated by Sanger sequencing. In one case, 2 sub-clonal *ALK* mutations (2.997% and 8.943%) were observed at the same position at the hotspot R1275 with another mutation observed at the hotspot F1174. Validation of all mutations observed at a sub-clonal level and analysis of other samples from collaborating groups is currently ongoing. Correlation between these findings and clinical and biological parameters will be established.

Discussion:

Our ongoing study documents a high frequency of clonal (7%) and sub-clonal *ALK* mutations (7.9%) in high risk NB patients. These findings are of utmost clinical importance given the potential role of ALK mutations in clonal evolution and relapse.