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ORAL ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Nucleophosmin (NPM1) Type D Genotype Is Associated with Distinct Outcome in Acute Myeloid Leukemia - an AIEOP-BFM and COG-SWOG Intergroup Collaboration

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Background: Nucleophosmin (NPM1) is a nucleo-cytoplasmic shuttling protein, predominantly located in the nucleolus that regulates a multiplicity of different biological processes. NPM1 localization in the cell is finely tuned by the nuclear export signal domain, where two tryptophan residues (Trp) are essential for the nucleolar localization. In acute myeloid leukemia (AML), several genomic subsets of *NPM1* frameshift mutations of 4 base pair insertions have been identified, with the type A variant (insertion of TCTG) being the most common, followed by type B (CATG) and D (CCTG). More recently, a series of different and rarer frameshifts have been found in adult and pediatric AML. We collected and studied the *NPM1* insertion genotypes in a large cohort of children with *de novo* AML enrolled in the AIEOP, BFM, ELAM02, NOPHO, DCOG, COG trials.
Methods: NPM1 mutations were sequenced in a group of 345 pediatric patients with *de novo* AML. 166 patients were enrolled in recent AIEOP, BFM, ELAM02, NOPHO, DCOG trials, and 179 in the CCG/COG AML trials (CCG-2961, AAML03P1, AAML0531 and AAML1031). A cohort of 75 patients of SWOG young adult (age 18-60) AML trial (S0106) were also included as validation cohort. The Kaplan-Meier method was used to estimate the probabilities of overall survival (OS) and event-free survival (EFS).
Results: In this cohort of 345 pediatric patients sequencing of exon 12 demonstrated multiple 4 bp insertion subtypes, with type A variant detected in 188 patients (55%), type B in 64 cases (19%) and type D mutation in 26 patients (7.5%). Other variants with the loss of the 2 Trp residues (namely A-like) were found in 42 patients (12%), whereas in 25 patients (7.5%) we identified mutations with the loss of 1 Trp residue (namely *non*-A-like). Comparison of disease characteristics demonstrated that NPM1 variants were prevalent in older patients (median age=13.3 years). WBC count was not significantly different among subgroups (Type A=30.3; *Alike*=25.2; B=16.8; D=41; *non-A-like*= 14.8x10 ^{3}/ul, p=0.14). FLT3-ITD prevalence was higher in Type A, B and D compared to the other Type *A-like* variants (42%, 44%, 46% vs. 26%); interestingly, FLT3-ITD was absent in *non-A-like* variants. Evaluation of clinical outcome based on NPM1 genotypes showed that although patients with type A, *A-Like*, *non-A-like* and type B had favorable outcomes, those with Type D had a significantly worse outcome with OS of 63% versus 86% for other genotypes (p=0.0053). Type D variant adverse outcome was confirmed in most of the trials and will be further examined considering different treatments. Each NPM1 variant mediated a different EFS, with most of early events

occurring within the D group (EFS 51.4%), and type A (EFS 68.8%) compared to the *non-A-like* NPM1, A like and B mutations (79.8%, 73%, and 75.5%, respectively) (p=ns). Validation data from adult SWOG trial (SWOG0106) demonstrated similar adverse outcome with EFS of 40% for type D vs. 61.4% for other NPM1 variants, indicating D as a peculiar NPM1 genotype /Figure A). Evaluation of overlapping mutations demonstrated that except for lack of FLT3-ITD in *non-A-like* genotype, there was similar distribution of high-risk mutations (FLT3-ITD, WT1, etc.) in those with different genotypes, thus not explaining the underlying biology responsible for the Type D adverse outcome. Even if the aminoacidic sequence of D and A variants is the same, the different prognosis suggests a further layer of regulation. We inquired whether Type D genotype might have a different NPM1 localization pattern compared to that in Type A variant. Immunofluorescence staining of primary AML cells from patients with Type D vs. Type A variant demonstrated that, in contrast to the canonical Type A variant with aberrant cytoplasmic localization, Type D variant maintains nuclear localization (Figure B). Further studies will provide the underlying biology of Type D being a silent mutation mediating a clinical outcome like NPM1 wild type AML.

Conclusion: This Eu/COG/SWOG collaborative study of over 400 pediatric and adult patients suggest a different functional biology behind NPM1 genotype where those with Type D variants have a significantly inferior outcome resembling feature of non-NPM1 mutated AML. Our data suggest that the specific genotype of NPM1 mutations can contribute to a more accurate risk-stratification, with D cases eventually shifting from standard to higher risk groups.

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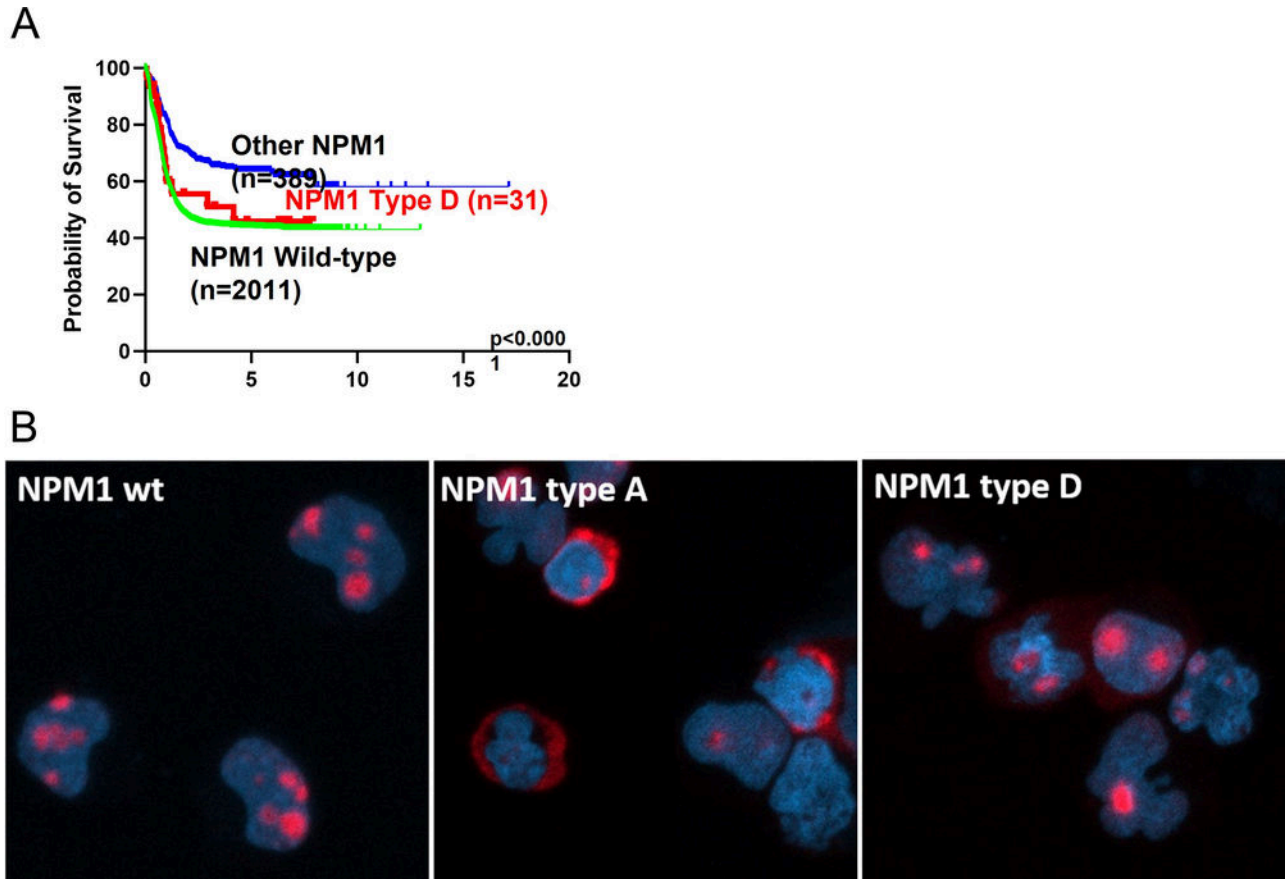

Figure 1

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