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

Evaluation of Neddylation and Apoptosis-Related Gene Expression in Patients with Acute Myeloid Leukemia

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Introduction

Regulated cell death (RCD) is a process commonly dysregulated in patients (pts) with acute myeloid leukemia (AML), resulting in the survival of genetically unstable cells. Apoptosis, as the best-known form of RCD, interacts with neddylation, a process that contributes to tumorigenesis and apoptosis resistance.

Aims

This study was conducted to determine the expression of the selected genes involved in the neddylation pathway and to assess their clinical importance along with selected interacting proapoptotic genes. In addition, we correlated the expression levels of these genes with known prognostic factors in AML and determined their impact on the survival of AML pts.

Methods

The expression of selected proapoptotic genes *BIK, BAX, BAK, BCL2L11, BBC3* , *PMAIP1 CASP3, CASP7*, and neddylation-related genes: *CUL1, CUL2* , *CUL4A, CUL5, CUL7, CUL9, NEDD8* was determined in duplicates in bone marrow samples of newly diagnosed AML pts. Real-Time PCR was carried out using the TaqMan chemistry and the QuantStudio7 thermal cycler (Applied Biosystems-Thermo Fisher Scientific). Gene expression was normalized using reference genes selected by the Normirazor tool. The normalization was performed by $\Delta Ct = Ct$ (reference) - Ct (mRNA of interest). This approach results in higher values for higher mRNA expression. Differential expression analysis was performed using a Welch t-test. Kaplan-Meier method with the log-rank test was used to assess overall survival (OS).

Results

In a prospective study, we included 40 newly diagnosed AML pts (mean age 62; range 26-87) with a median (m) follow-up of 6.7 months (95% CI: 4.5-9.1). High-risk AML was present in 52.5%, intermediate-risk in 32.5%, and low-risk in 15% of pts, according to European LeukemiaNet (ELN) 2022 risk stratification. *NPM1* mutation was present in 23%, whereas *FLT3* ITD or TKD mutation in 25% of pts. Intensive treatment was introduced in 43% of pts, while 25% were treated with azacitidine+venetoclax. Non-intensive treatment with azacitidine in monotherapy was administered to the remaining pts.

BAK expression was detected in 70%, *BIK* in 98%, while expression of the other genes was found in all pts. In the high-risk group, we detected lower expression of both proapoptotic *BAX* (fold change - FC=0.7, p=0.01) and *BIK* gene (FC=0.4, p=0.01), as well as lower expression of *CUL9* (FC=0.7, p=0.03). Accordingly, in pts with *NPM1* mutation we revealed up-regulation of *BIK* (FC=6.1, p=0.001), and *CUL9* (FC=1.6, p=0.01). Whereas pts with *FLT3* mutation had lower expression of proapoptotic *BBC3* (FC=0.5, p=0.01), and neddylation-related *CUL1* (FC=0.6, p=0.01).

In cases of initial bone marrow blasts infiltration above median value (>49%) *BCL2L11 CUL5 , NEDD8* and *PMAIP1*were down-regulated (*p*=0.001-0.02), while *CUL7* (*p*=0.00) and *CASP3* (*p*=0.04) were upregulated. Patients with initial white blood cell count >20 G/l had lower expression of *BCL2L11* (*p*=0.005), and *CUL4A* (*p*=0.02), while higher expression of *CASP7* (*p*=0.04). The mOS in the study cohort was not reached. OS was longer in pts with higher (> -2.061, cutoff was determined with CutOff Finder) *BAX* expression (log-rank: *p*=0.03) [Figure 1]. The mOS was not reached in this group, whereas the mOS in pts with lower *BAX* expression was 1.3 months (95% CI: 0.6-1.3). In univariate Cox regression analysis, higher *BAX* expression (HR 0.31, 95%CI: 0.11-0.84, *p*=0.022), higher albumin level (HR 0.13, 95%CI: 0.04-0.41, *p*=0.0004), and intensive treatment (HR 0.18, 95%CI: 0.04-0.69, *p*=0.012) were factors influencing the outcome. In the multivariate model for OS, higher expression of *BAX* (HR 0.03, 95%CI: 0.003-0.33, *p*=0.004) retained its significant protective effect in the context of established prognostic factors [Table 1].

Conclusions

To our knowledge, we were the first to evaluate such a complex spectrum of neddylation and apoptosis-related genes in AML. We revealed dysregulation of these genes in AML. Our data indicate that upregulation of *BAX* at the diagnosis is associated with longer OS. Moreover, we reported significantly lower *BAX* expression in ELN high-risk group. Thus, indicating *BAX* may be a potential prognostic factor in AML. Our preliminary results did not prove significant differences in the effect of neddylation gene expression levels on the prognosis of AML patients. As studies are emerging on the potential of neddylation-targeted therapies, we are conducting further research to verify the effect of neddylation on AML.

Disclosures Krawiec: *Celgene/BMS:* Honoraria. **Strzalka:** *Celgene/BMS:* Honoraria. **Czemerska:** *Pfizer:* Honoraria; *Sandoz:* Honoraria; *Abbvie:* Honoraria; *Celgene/BMS:* Honoraria. **Wierzbowska:** *Servier:* Honoraria; *Pfizer:* Honoraria; *Gilead:* Honoraria; *Novartis:* Honoraria; *JazzPharmaceuticals/swixx:* Honoraria; *Celgene/BMS:* Honoraria; *Astellas:* Consultancy, Honoraria; *Abbvie:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees. **Pluta:** *Astellas:* Honoraria; *Celgene/BMS:* Honoraria; *Jazz Pharmaceuticals (Swixx):* Honoraria, Research Funding; *Pfizer:* Honoraria; *Abbvie:* Honoraria.

Figure 1. Kaplan–Meier analysis of overall survival (OS) according to the expression of *BAX* in AML patients.

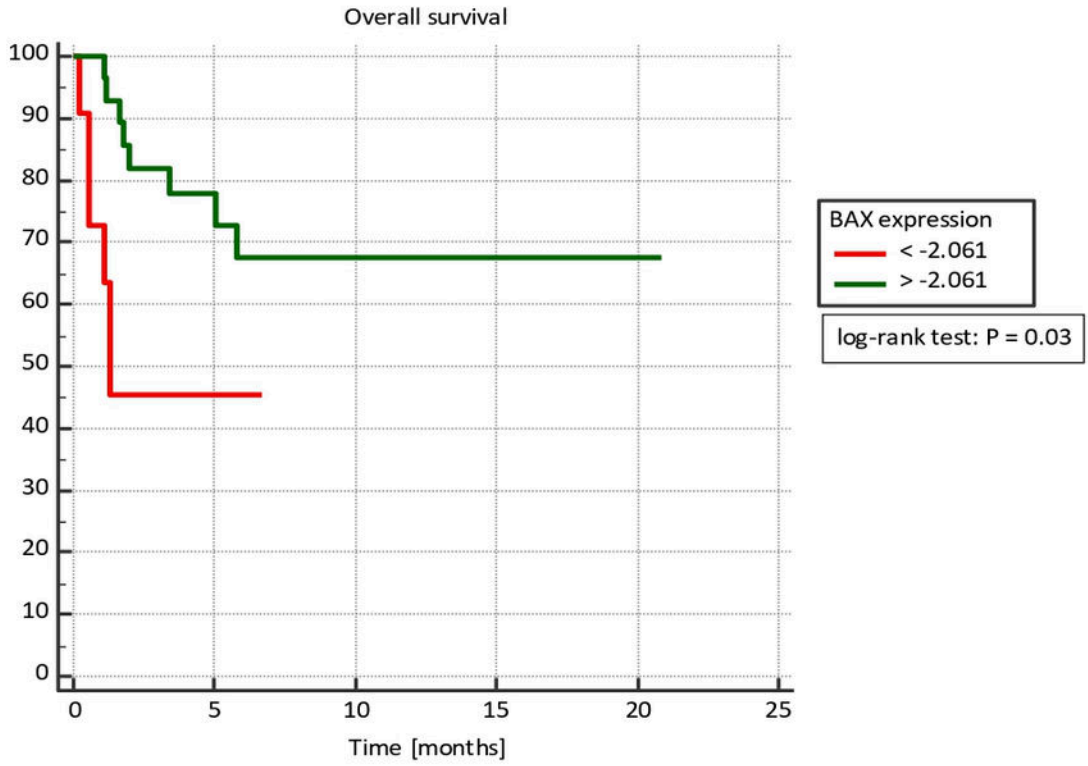

Table 1. Multivariate Cox regression model for overall survival (OS).

Variable	Coefficient	*p*-value	Hazard ratio (HR)	95% CI lower	95% CI higher
BAX expression	-3.474	0.004	0.031	0.003	0.33
albumin level	-7.335	0.001	0.001	0.0	0.046
Intensive treatment	-4.578	0.005	0.01	0.0	0.256
Azacitidine+Venetoclax	-2.462	0.049	0.085	0.007	0.985

Figure 1

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