ASSESSMENT OF APOPTOSIS-RELATED GENES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA – PRELIMINARY ANALYSIS

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INTRODUCTION

Apoptosis is the best-known form of regulated cell death (RCD), and its mechanisms are disrupted in patients with acute myeloid leukemia (AML). This results in the survival of genetically unstable cells, leading to the selection of progressively aggressive clones and disease progression [1]. Apoptosis is a target for new therapies in hematooncology, and is regulated by the BCL-2 family of pro- and anti-apoptotic proteins, the balance of which can significantly determine cell survival [2].

AIM

- This study was conducted to determine the clinical role of the expression of pro-apoptotic (*BIK, BIM, TP53, PUMA*, and *NOXA*) and anti-apoptotic (*MCL1, BCL-2, BCL-XL*) genes in AML patients [Figure 1].
- 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 patients.

METHOD

- We performed a prospective study in 50 newly diagnosed AML patients [Table 1, 2].
- The expression of apoptosis-related genes was determined in duplicates in samples from bone marrow (n=36) and blood (n=14). The material obtained from the blood contained a minimum of 20% blast cells.
- Real-Time PCR was carried out using the TaqMan chemistry and the QuantStudio™7 thermal cycler (Applied Biosystems-Thermo Fisher Scientific).
- Gene expression was normalized using reference genes selected by the Normirazor tool. The normalization was performed by ΔCt = *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).

Patients, n female, n (%) male, n (%)	50 23 (46) 27 (54)
Age mean (SD) median (IQR)	63 (±15) 66 (55-72)
Peripheral blood blasts %, median (IQR)	21 (5-63)
Bone marrow blasts %, median (IQR)	45 (29-68)
WBC G/l, median (IQR)	17 (2-80)
ANC G/l, median (IQR)	2 (0-6)
Hemoglobin g/dl, median (IQR)	9 (8-10)
Platelets G/l, median (IQR)	48 (24-93)
CRP mg/l, median (IQR)	22 (4-78)
LDH U/l, median (IQR)	438 (241-887)
Uric acid mg/dl, median (IQR)	6 (4-7)
Albumins g/dl, median (IQR)	4 (3-4)

SD – standard deviation, IQR – interquartile range, WBC – white blood count, ANC – absolute neutrophil count, CRP – C-reactive protein, LDH – lactate dehydrogenase

Table 1. Patients' characteristics.

RESULTS

- Among the genes tested, there was no expression detected of *BAK* in 26% of patients and *BIK* in 2%, while expression of the other genes was found in 100%.
- In older patients (>60 years) lower expression of *TP53* (fold change – FC=0.65, *P*=0.06) was detected.
- In patients with *NPM1* mutation upregulation of pro-apoptotic *BIK* (FC=4.2, *P*=0.0001) was found [Figure 2].
- *FLT3*-mutated patients had higher expression of *CASP3* (FC=2.24, *P*=0.026).
- Whereas, in *RUNX1* positive group, downregulation of proapoptotic *BIM* (FC=0.46, *P*=0.01) was revealed.
- No significant differences were detected in the expression of studied genes considering the presence of *IDH1* or *IDH2* mutation.

European Leukemia Net (ELN) 2022 risk stratification			
	Favorable	Intermediate	Adverse
% of patients	14%	40%	46%
Mutation status			
	NPM1	*FLT3-ITD or FLT3-TKD*	*IDH1 or IDH2*
% of patients	18%	24%	14%
Type of treatment			
	Intensive chemotherapy	Azacitidine+Venetoclax	Non-intensive
% of patients	36%	32%	32%

Table 2. Patients' characteristics.

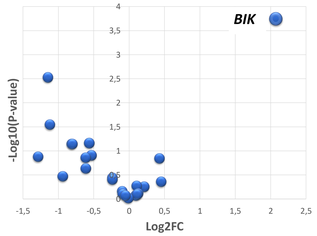


Figure 2. Volcano plot showing changes in genes expression depending on NPM1 mutation presence.

- Patients with confirmed *TP53* mutation had lower expression of pro-apoptotic *BIK* (FC=0.28, *P*=0.001) and *CASP7* (FC=0.34, *P*=0.001).
- In the favorable and intermediate-risk groups, higher expression of *TP53* (FC=1.39, *P*=0.05) was detected.
- Accordingly, median overall survival (mOS) was significantly longer in patients with higher *TP53* expression (log-rank: P=0.039) [Figure 3]. The mOS was not reached in this group. While mOS in patients with lower *TP53* expression was 12.9 months (95% CI: 1.8-14).

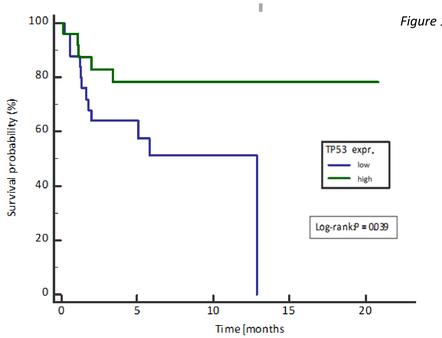


Figure 3. Kaplan–Meier analysis of overall survival (OS) according to the expression of TP53 in AML patients.

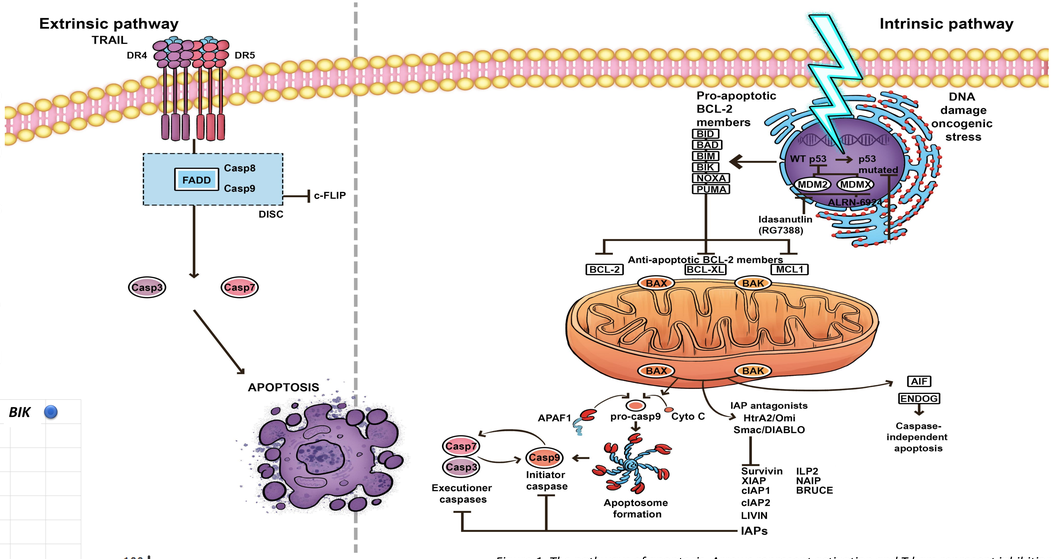


Figure 1. The pathways of apoptosis. Arrows represent activation and T bars represent inhibition.
Krawiec K et al. Targeting Apoptosis in AML: Where Do We Stand? Cancers (Basel). 2022 Oct 12;14(20):4995.

CONCLUSIONS

- Upregulation of *TP53* at the diagnosis is associated with longer OS. Moreover, higher *TP53* expression is found in ELN favorable and intermediate-risk groups.
- These results indicate that *TP53* expression level may be a potential prognostic factor in AML.
- Our preliminary results did not prove significant differences in the effect of *BIK, BIM, PUMA, NOXA, MCL1, BCL-2,* and *BCL-XL* gene expression levels on the prognosis of AML patients.
- However, a difference in the expression of these genes is highlighted depending on the 2022 ELN risk classification.
- As studies are emerging on the potential of apoptosis-targeted therapies, we are conducting further research to verify the impact of apoptosis gene expression on AML.

REFERENCES

1. **Garciaz S et al.** Targeting regulated cell death pathways in acute myeloid leukemia. *Cancer Drug Resist. 2023 Mar 15;6(1)*
2. **Krawiec K et al.** Targeting Apoptosis in AML: Where Do We Stand? Cancers (Basel). *2022 Oct 12;14(20)*

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