PRO-APOPTOTIC PARADOX: *BIK* OVEREXPRESSION WORSENS PROGNOSIS IN ACUTE MYELOID LEUKEMIA

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Introduction

- Molecular abnormalities in patients with acute myeloid leukemia (AML) affect the evasion of apoptosis, which is the best-known form of regulated cell death.
- An imbalance between pro-apoptotic modulators, which include *BIK*, and anti-apoptotic molecules contributes to the accumulation of abnormal hematopoietic cells, leading to the development of AML.
- Therefore, the apoptosis pathway is highlighted as important for the search for new targeted molecules (Figure 1).

Aim

- Evaluation of the expression and significance of genes regulating apoptosis in AML patients.

Methods

- A prospective study of 98 newly diagnosed AML patients (Table 1).
- The mRNA expression of pro-apoptotic *BIK, BBC3, PMAIP1, BAD, BAX, BAK1, BCL2L11, BID*, and anti-apoptotic *BCL2, BCL2L1, MCL1* genes was determined in duplicate in samples from bone marrow using real-time PCR.
- Normalization was performed by ΔCt = Ct(reference) – Ct(mRNA of interest). The ΔΔCt method was used to calculate fold changes (FC) in mRNA expression.

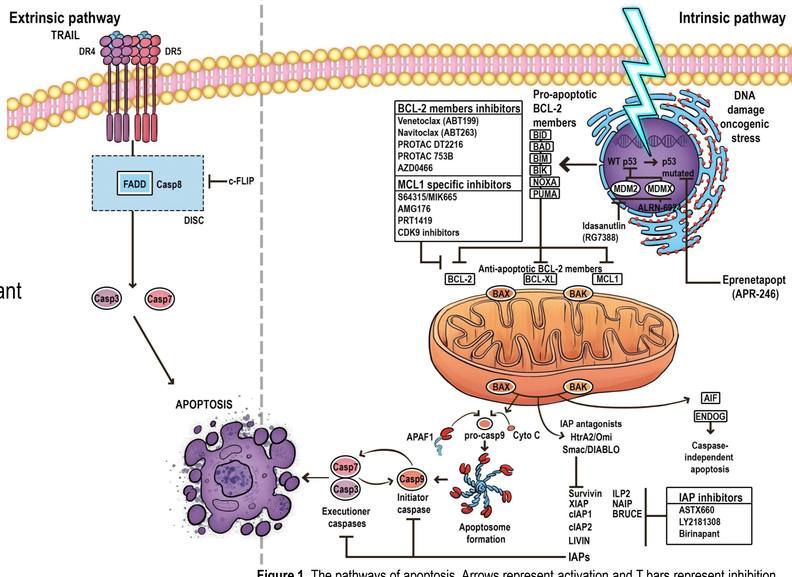


Figure 1. The pathways of apoptosis. Arrows represent activation and T bars represent inhibition. *Krawiec K et al. Targeting Apoptosis in AML: Where Do We Stand? Cancers(Basel). 2022 Oct12;14(20):4995.*

Results

- With a median follow-up of 22.5 months (IQR 17.5-26.6), median overall survival (OS) was 13 months (IQR 2.5-data censored) (Fig. 2A), and median event-free survival (EFS) was 5.9 months (IQR 1.7-16.8) (Fig. 2F).
- The **response to treatment** depending on the therapeutic group - Figure 3.

Table 1. Patients' characteristics.

Characteristics	Total
Patients, N	98
Sex, N (%)	F: 42 (43); M: 56 (57)
Age at diagnosis, median (IQR)	65 (55-73)
Patients >65 y, N (%)	54 (55%)
LAIP blasts, median (IQR)	47.5 (21-74.5)
BM blasts at diagnosis, median (IQR)	44.5 (24-68)
PB blasts at diagnosis, median (IQR)	17 (3-61)
Cytogenetic-molecular ELN2022 risk, N (%)	
favorable	11 (11.6)
intermediate	36 (37.9)
adverse	48 (50.5)
Treatment, N (%)	
intensive chemotherapy	38 (38.8)
azacitidine + venetoclax	27 (27.6)
non-intensive	33 (33.6)

LAIPs – leukemia-associated immunophenotypes, BM – bone marrow, PB – peripheral blood

Figure 3. Treatment response across therapeutic groups.

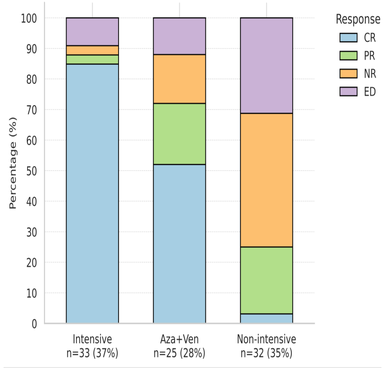


Figure 4. Spearman's correlation between apoptotic genes.

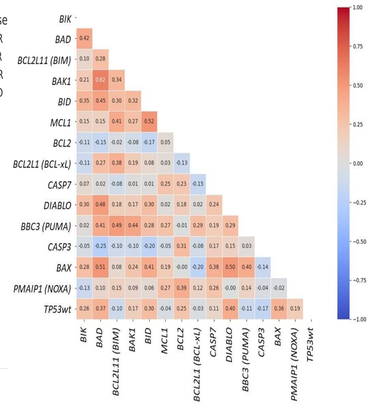
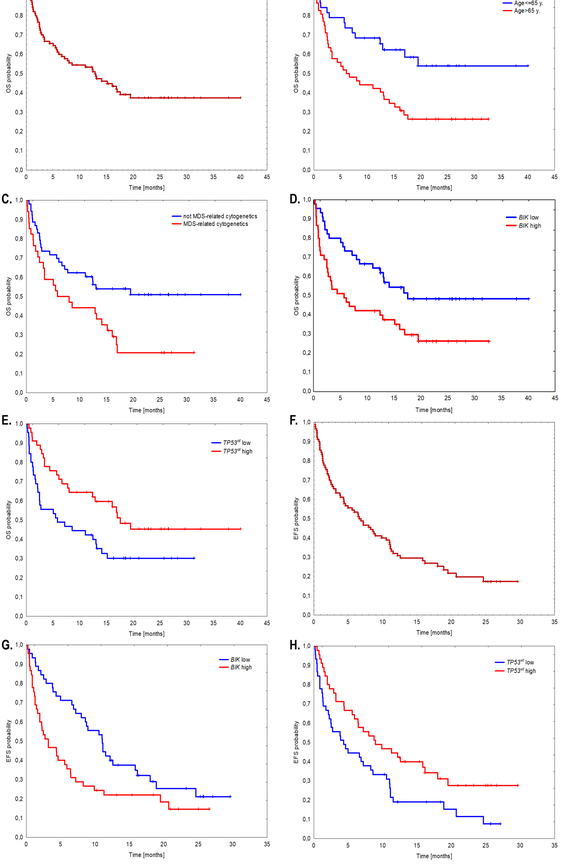


Figure 2. Overall survival (OS, A–E) as a whole group (A) and divided according to the age (B, p=0.006), MDS-related cytogenetics (C, p=0.015) or the expression level of *BIK* (D, p=0.015) and *TP53*^{wt} (E, p=0.025). Event-free survival (EFS, F–H) as a whole group (F) and divided according to the expression level of *BIK* (G, p=0.018) and *TP53*^{wt} (H, p=0.017).



- mRNA expression of *BCL2L11 (BIM), BID, PMAIP1 (NOXA), BAX, BCL2, BCL2L1 (BCL-xL), MCL1, SMAC/DIABLO, CASP3, TP53*^{wt} was detected in 100% of AML patients, while *BAK1* in 67,8% and *BAD, BIK, BBC3 (PUMA), CASP7* in 98,9%.
- **Upregulation** of **anti-apoptotic** genes compared to **pro-apoptotic** (FC=11.0, P<0.001) was detected.
- Kaplan-Meier survival analysis showed that **higher** expression (above median) of ***BIK*** at the time of AML diagnosis was associated with **worse prognosis** (p = 0.015 for OS and 0.018 for EFS, Figure 2D, 2G), while **higher *TP53*^{wt}** expression with a **better prognosis** (p = 0.025 for OS and 0.017 for EFS, Figure 2E, 2H).
- In both the univariate Cox regression analysis for OS (hazard ratio [HR]=1.9; 95% CI, 1.13-3.36; *P*=.016) and the multivariate model for OS* **higher *BIK*** expression had a significant **negative effect on OS** (HR=1.32; 95% CI, 1.13-1.54, *P*=.0004).
- **Higher *BIK*** expression also **negatively influenced EFS**, in both the univariate Cox regression analysis for EFS (HR=1.8; 95% CI, 1.11-2.86; *P*=.016) and the multivariate model for EFS* (HR=1.22; 95% CI, 1.07-1.39, *P*=.002).
- Moreover, **higher *BIK*** expression was an independent significant prognostic factor **negatively affecting** complete remission (**CR**) in multivariate logistic regression for CR* achievement (odds ratio [OR]=0.21, 95%CI 0.05-0.86, *P*=.03).
- ***BBC3 (PUMA)*** expression was **higher** in patients who achieved **CR/PR** (U Mann-Whitney test, p=0.024) after **azacitidine + venetoclax** (aza+ven), while ***BIK*** was significantly higher in patients who did not respond to aza+ven (U Mann-Whitney test, p=0.004).
- Correlation analysis of gene expression revealed a cluster of **positively correlated apoptotic genes** (Figure 4).

** adjusted for ELN 2022 classification, treatment approach, and baseline BM blast infiltration level*

Conclusions

- The study revealed **dysregulation of apoptosis pathway** gene expression **in AML**.
- Although *BIK* belongs to the pro-apoptotic genes, its high expression **negatively affects prognosis** and may contribute to **venetoclax resistance**.
- Further studies are needed to determine *BIK*'s role as a prognostic factor in AML.