Prognostic value of expression of selected genes regulating the mitochondrial apoptosis pathway in patients with acute myeloid leukemia

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Introduction

- Genetic mutations and gene expression abnormalities are identified in >97% of patients with acute myeloid leukemia (AML), leading to evasion of regulated cell death (RCD), the best-known form of which is apoptosis [1-3].
- During oncogenesis, an overexpression of anti-apoptotic proteins - **BCL-2 family proteins** occur. They determine the survival and proliferation of abnormal haemopoietic cells [4].
- The BCL-2 family consists of three subfamilies: **pro-apoptotic BH3-only members** BIM, BID, PUMA, NOXA, HRK, BMF and BAD, **proapoptotic molecules** BAX and BAK and **anti-apoptotic BCL-2 family proteins** BCL-2, BCL-X, BCL-W, MCL-1, A1, and BCL-B [Fig 1].
- In response to an apoptotic stimulus the transcription of pro-apoptotic BH3-only members is upregulated, which bind anti-apoptotic members of the BCL-2 family and inhibit their activity [5].
- In AML **apoptosis is dysregulated** and there is **increased expression of apoptosis suppressor proteins** which **stimulate oncogenesis**.

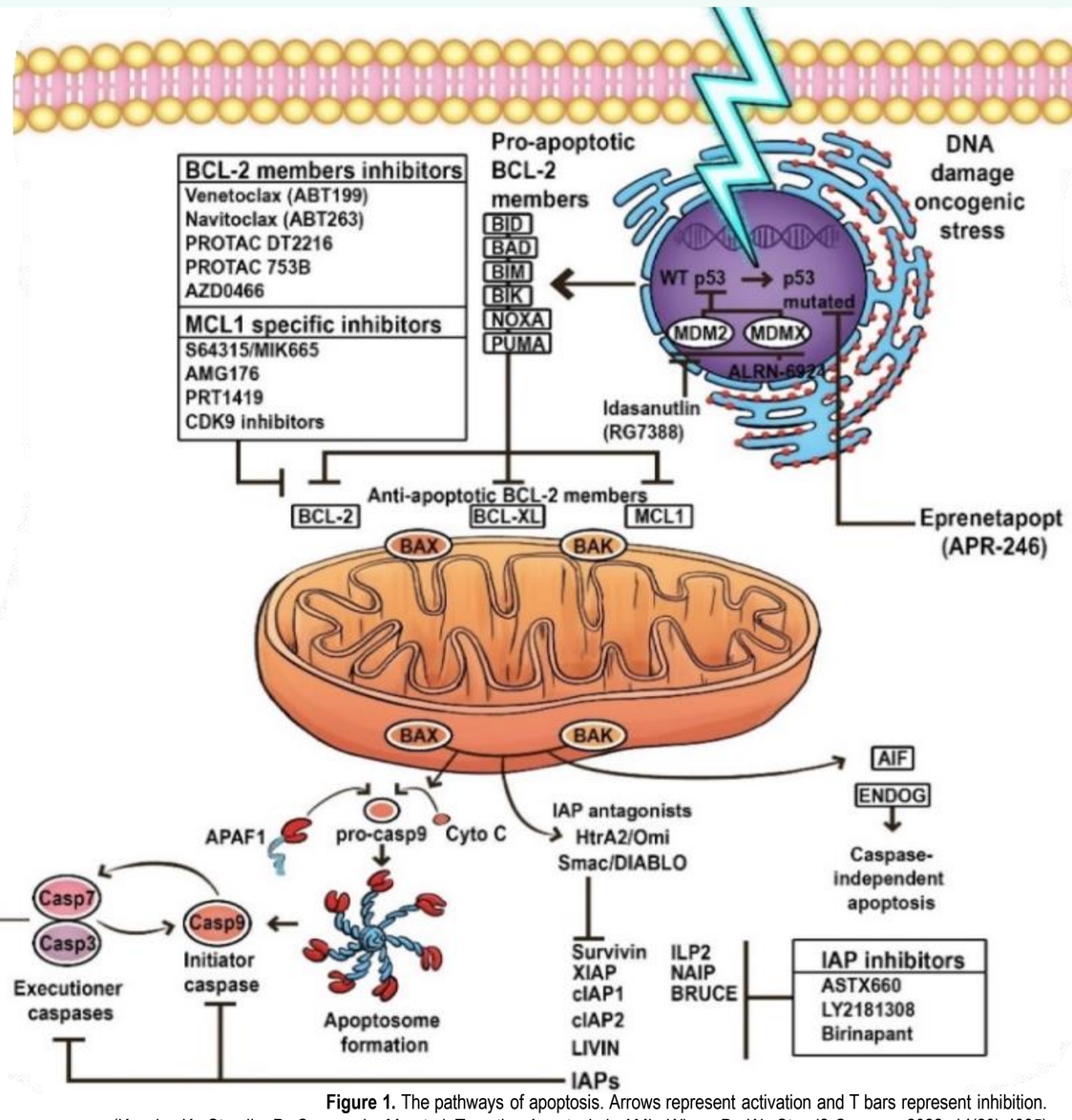

Figure 1. The pathways of apoptosis. Arrows represent activation and T bars represent inhibition. (Krawiec K., Strzałka P., Czemerska M., et al. Targeting Apoptosis in AML: Where Do We Stand? Cancers. 2022; 14(20):4995)

Hypothesis & Objectives

✓ Study hypothesis

Abnormalities in the expression of apoptosis and neddylation genes can contribute to the development and resistance of leukemic cells and affect prognosis.

✓ Primary objective

To evaluate the impact of ***BIM, BID, BIK, PUMA, NOXA, BAD, BAX, BAK, BCL2, BCLX, MCL1, NEDD8, SMAC/DIABLO, CASP3*** and ***CASP7, TP53, CUL-1-3, 4A-B, 5, 7, 9*** gene expression on the prognosis of patients with AML.

✓ Secondary objectives

1. Evaluation of differences in **prognosis** between patients with low and high expression of the respective apoptosis genes.
2. Evaluation of the usefulness of apoptosis gene expression determination **as new prognostic marker in AML**, which may optimise therapeutic management.
3. Correlation of apoptosis gene expression **with previously recognized prognostic factors**.
4. An attempt to select a group of patients who could **potentially benefit from apoptosis-based drugs**.

Material & Methods

1) Collection of the research population

- A prospective, single-centre study, including 86 patients with newly diagnosed AML, hospitalized in the Department of Hematology, Lodz.

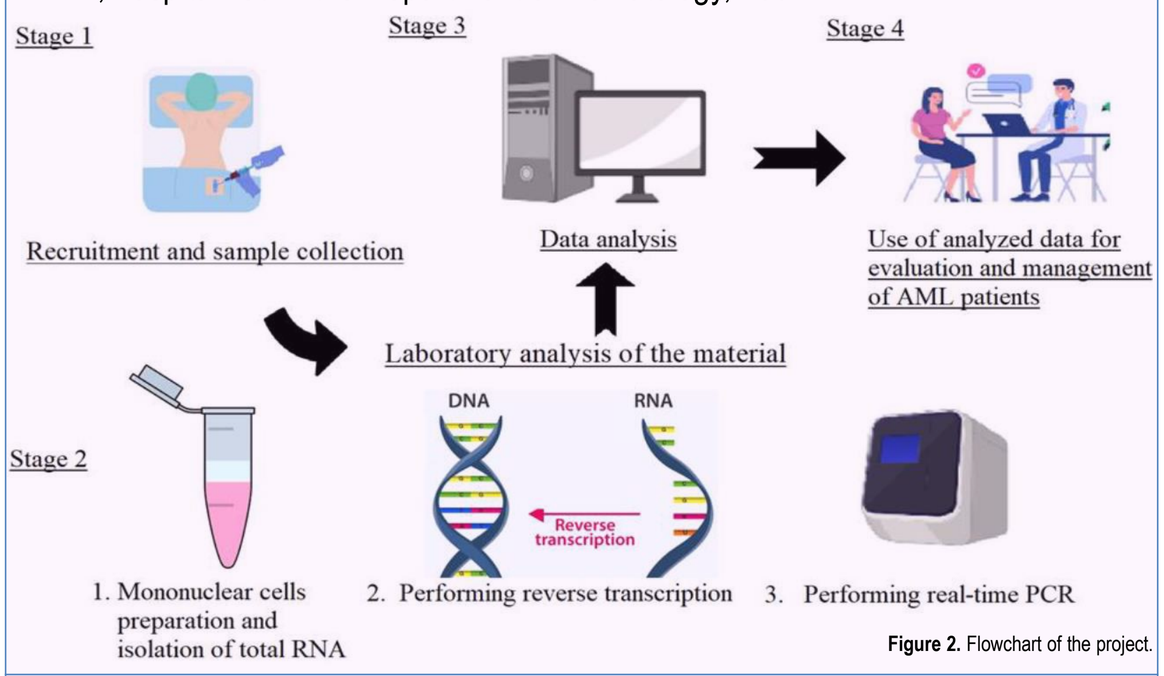

Figure 2. Flowchart of the project.

2) Laboratory tests

- The testing sample is an aspirate of approx. 10 ml bone marrow.
- Based on statistical analysis, I selected the reference genes *MRPL19* and *PPIA*.
- The genes I chose to analyze are *BIM, BID, BIK, PUMA, NOXA, BAD, BAX, BAK, BCL2, BCLX, MCL1, NEDD8, SMAC/DIABLO, CASP3, 7, TP53, CUL-1-5, 7, 9.*
- The expression of apoptosis and neddylation-related genes was determined in duplicates in samples from bone marrow using Real-Time PCR. The normalization was performed by ΔCt = Ct(reference) – Ct(mRNA of interest). The ΔΔCt method was used to calculate fold changes (FC) in mRNA expression.

3) Analysis of collected data

- Completion of patient recruitment and laboratory analysis is scheduled for 08.2024 after that, I will carry out statistical analysis of the obtained results and start work on the first original paper in the field of my research.
- So far, I have published a review article as part of my doctoral thesis. (Krawiec K., Strzałka P., Czemerska M., et al. Targeting Apoptosis in AML: Where Do We Stand? Cancers. 2022; 14(20):4995. doi: 10.3390/cancers14204995, IF: 6,575.)

1. Grove CS, Vassiliou GS. Acute myeloid leukaemia: a paradigm for the clonal evolution of cancer? Dis Model Mech. 2014;7(8):941-51. 2. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079-89. 3. Testa U, Riccioni R. Deregulation of apoptosis in acute myeloid leukemia. Haematologica. 2007;92(1):81-94.4. Fernald K, Kurokawa M. Evading apoptosis in cancer. Trends Cell Biol. 2013;23(12):620-33. 5. Nagata S. Apoptosis and Clearance of Apoptotic Cells. Annu Rev Immunol. 2018;36:489-517.

Results

- The median follow-up was 10.3 months (95% CI: 7.8-14.4). The median OS in the study cohort was 12.9 months (95% CI: 6.7-16.1).

- Our preliminary results showed a significant **upregulation of antiapoptotic genes** compared to **proapoptotic and neddylation genes** (FC=11.0, $p<0.001$, FC=2.7, $p<0.001$, respectively) in AML patients.

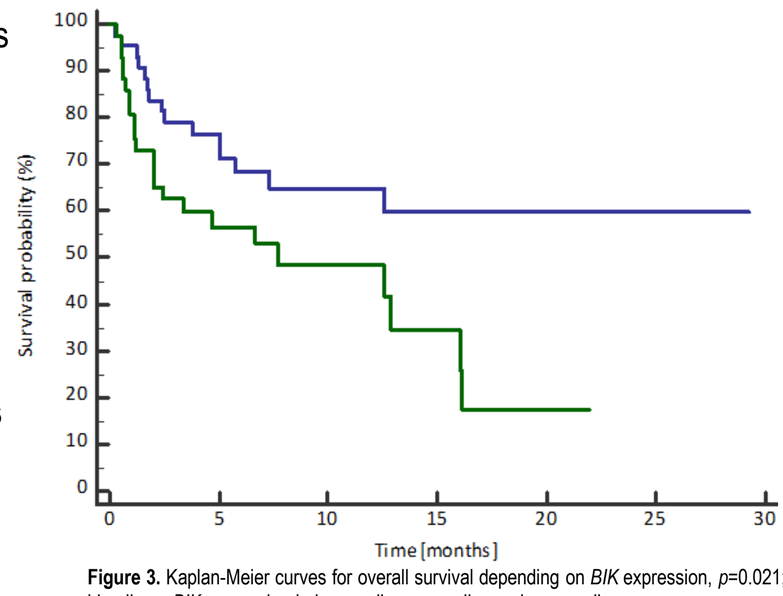

Figure 3. Kaplan-Meier curves for overall survival depending on *BIK* expression, $p=0.021$; blue line – *BIK* expression below median, green line – above median.

- In univariate Cox regression analysis, ***BIK* expression** (HR 1.2, 95%CI: 1.01-1.43, $p=0.041$), ***wtTP53* expression** (HR 0.61, 95%CI: 0.42-0.86, $p=0.005$), initial albumin level (HR 0.33, 95%CI: 0.17-0.62, $p=0.001$), age (HR 1.03, 95%CI: 1.00-1.06, $p=0.02$), and intensive treatment (HR 0.26, 95%CI: 0.12-0.57, $p=0.001$) were factors influencing the outcome.

- In the multivariate model for OS, **higher expression of *BIK*** (HR 1.27, 95%CI: 1.06-1.53, $p=0.011$) retained its significant negative impact, while ***wtTP53*** maintained the protective effect on OS (HR 0.56, 95%CI: 0.38-0.87, $p=0.008$) in the context of established prognostic factors.

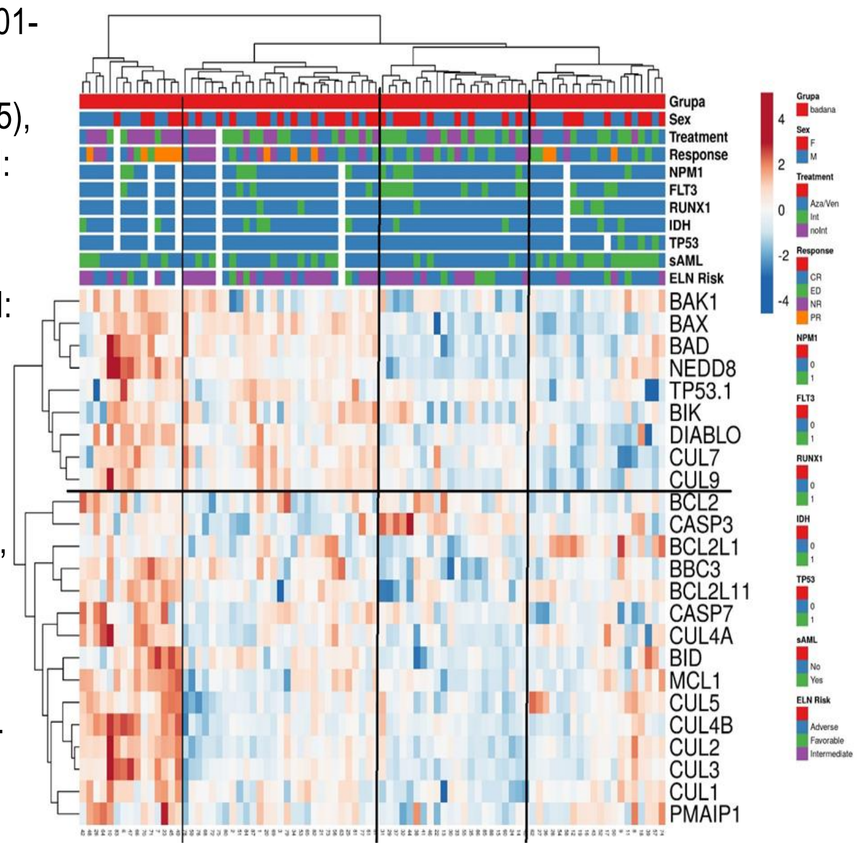

Figure 4. Heatmap of the studied mRNA expression with clustering according to clinical variables.

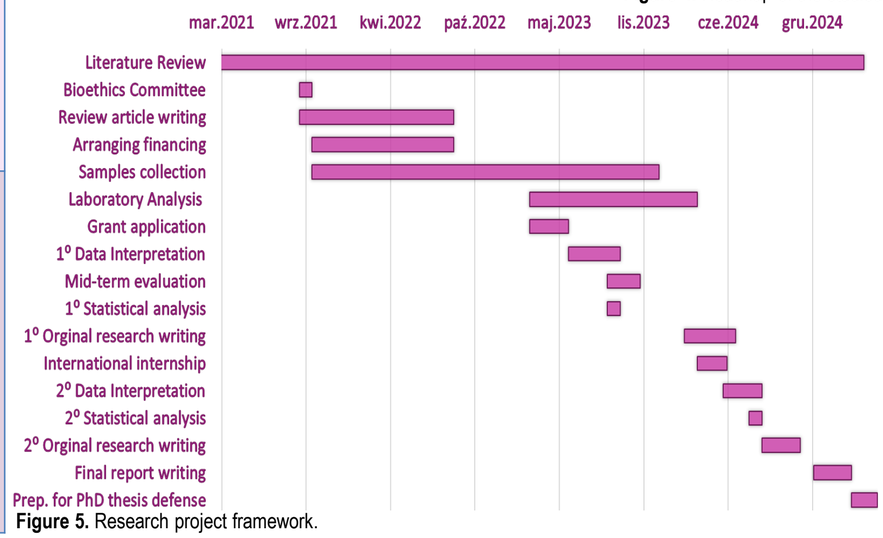

Figure 5. Research project framework.

CONCLUSIONS: Our study revealed dysregulation of apoptosis genes and indicated that both ***BIK* and *wtTP53* may be potential prognostic factors in AML.**
Our preliminary results did not prove significant differences in the effect of neddylation gene expression levels on the prognosis of AML patients, thus further research is needed.