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

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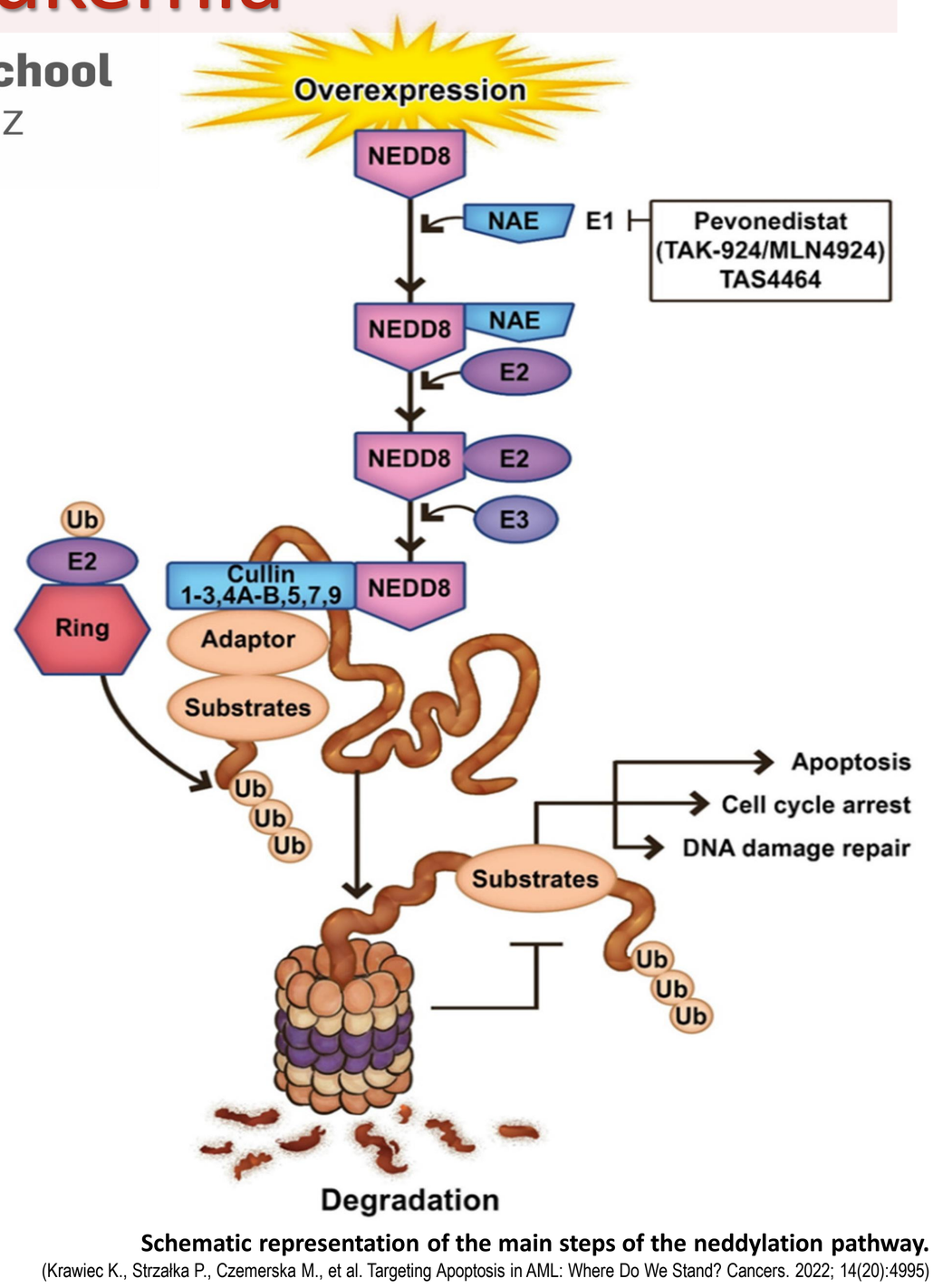
Introduction

- Genetic mutations and gene expression disorders are identified in **>97%** of patients with acute myeloid leukemia (AML), leading to unleashed proliferation and evasion of regulated cell death (**RCD**) [1].
- The most prominent form of RCD is apoptosis which eliminates unnecessary cells in the course of following cellular stress [2, 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**.
- Further research in the field of AML biology can contribute to identify **new prognostic markers and finding new therapeutic targets**.



Schematic representation of the main steps of the neddylation pathway.
(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

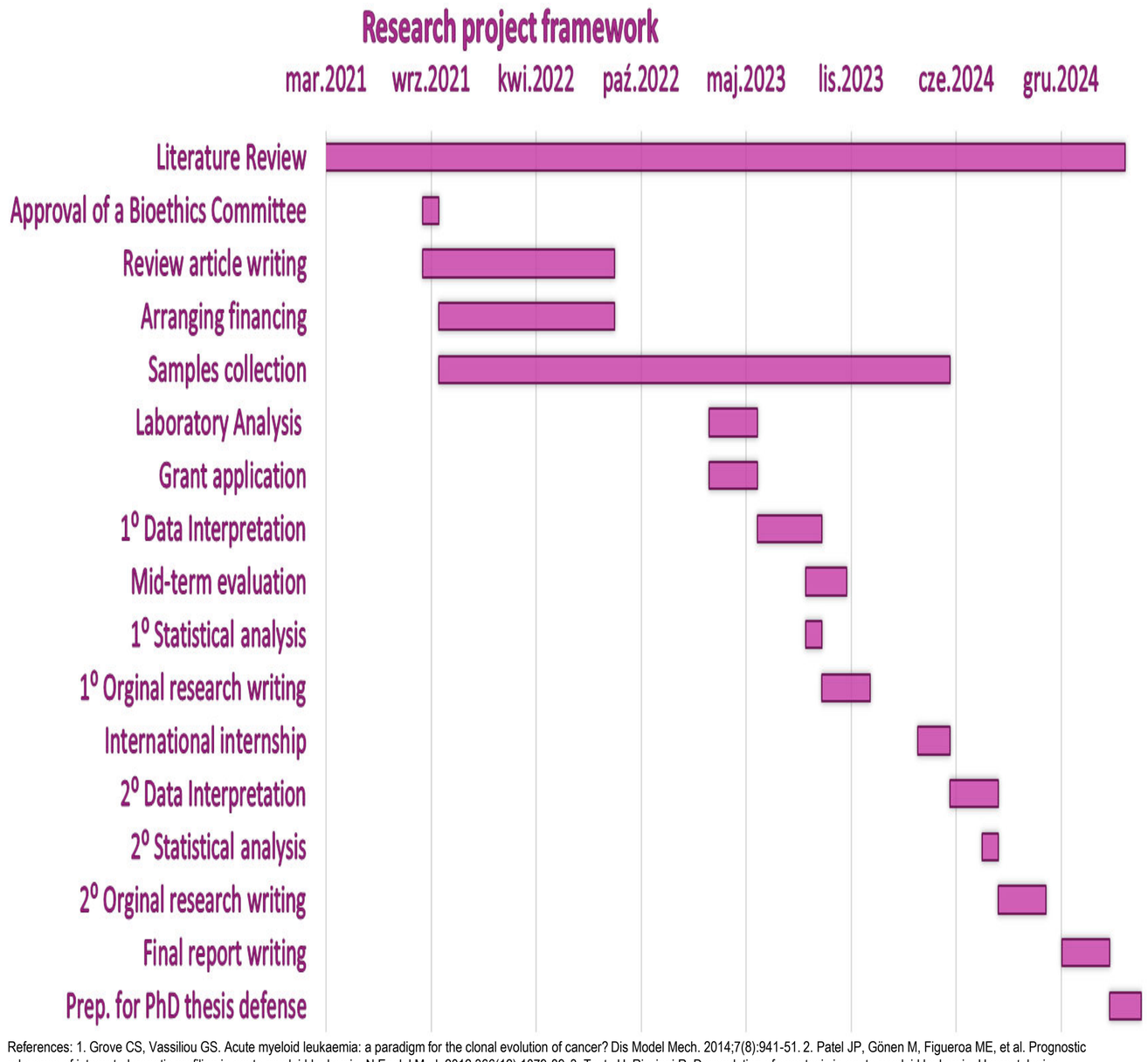
Abnormalities in the expression of genes related to the mitochondrial apoptosis pathway have an important role in the biology of AML and affect prognosis.

✓ Primary objective

Evaluation of the impact of disorders in the expression of selected genes regulating the mitochondrial apoptosis pathway: ***BIM***, ***BID***, ***BIK***, ***PUMA***, ***NOXA***, ***BAD***, ***BAX***, ***BAK***, ***BCL2***, ***BCLX***, ***MCL1***, ***NEDD8***, ***SMAC/DIABLO***, ***CASP3*** and ***CASP7***, ***TP53***, CULLIN family: ***CUL-1-3, 4A-B, 5, 7, 9*** on the prognosis of AML patients.

✓ Secondary objectives

1. Evaluation of differences in **overall survival (OS)** and **disease-free survival (DFS)** 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 and methods

1) Collection of the research population

❖ A prospective, single-centre study. The analysis includes patients hospitalized in the Department of Haematology, Lodz.

Inclusion criteria	Exclusion criteria
1. Newly diagnosed AML according to WHO 2016.	**1.** Pregnancy
2. Age between 18-85 years old.	**2.** Acute promyelocytic leukemia
3. Providing voluntary, informed consent.	**3.** Other active malignancy.

❖ Currently (as of April 2023), 60 patients are included in the study, including 40 patients who underwent intensive treatment for AML at a later stage.

2) Laboratory tests

❖ The testing sample is an aspirate of approx. 10 ml bone marrow.
❖ I have currently isolated and banked mononuclear cells from 60 patients.
❖ The study will be conducted in two tranches.
❖ I started the analysis of the first tranche of material on 03.2023. I included material collected from 40 patients who have undergone intensive treatment for AML.
❖ The rest of the material remains banked until further laboratory tests are made.
❖ 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 isolation of genetic material in the form of total RNA was performed in 03.2023.
❖ The material is currently subjected to molecular analysis, using reverse-transcription polymerase chain reaction (RT-PCR).
❖ In further analysis, the variation in gene expression levels will be compared with reference genes.

3) Analysis of collected data

❖ Laboratory analysis of the first tranche of material will be conducted until 06.2023, 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.
❖ The prognosis evaluation will focus on the assessment of response to treatment.
❖ We will analyse the significance of the expression of selected apoptosis genes as potential prognostic factors and correlate the results with parameters such as cytogenetic-molecular risk group assessment and tumour size markers.
❖ 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.)

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