The role of sirtuin family histone deacetylases as biomarkers, predictive factors and potential therapeutic targets in patients with acute myeloid leukemia.

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INTRODUCTION

- The biology of the leukemic clone in Acute Myeloid Leukemia (AML) determines resistance to chemotherapy or an increased risk of relapse.
- Enzymatic proteins of the sirtuin family are NAD+-dependent histone deacetylases with pleiotropic effects on metabolism, ageing processes and cell survival [1].

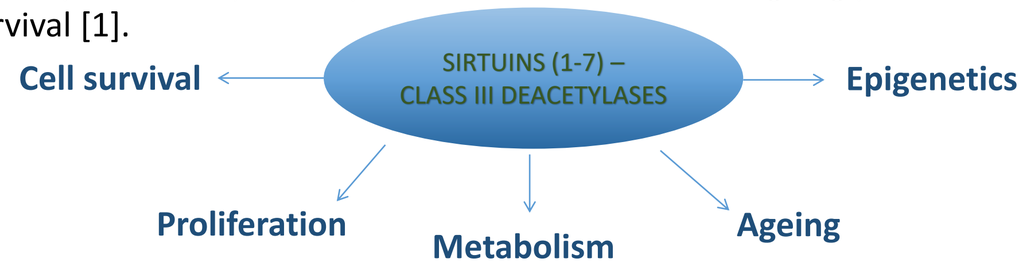


Figure 1. Selected biological effects of sirtuins activity.

- Another epigenetic mechanism for controlling gene expression, which is related to the action of sirtuins, is microRNA-based regulation (miRNAs).
- For example, miR-34a can indirectly increase TP53 levels by inhibiting negative regulators of TP53, such as SIRT1, in colorectal cancer; however, more research is needed in AML [10, 11].

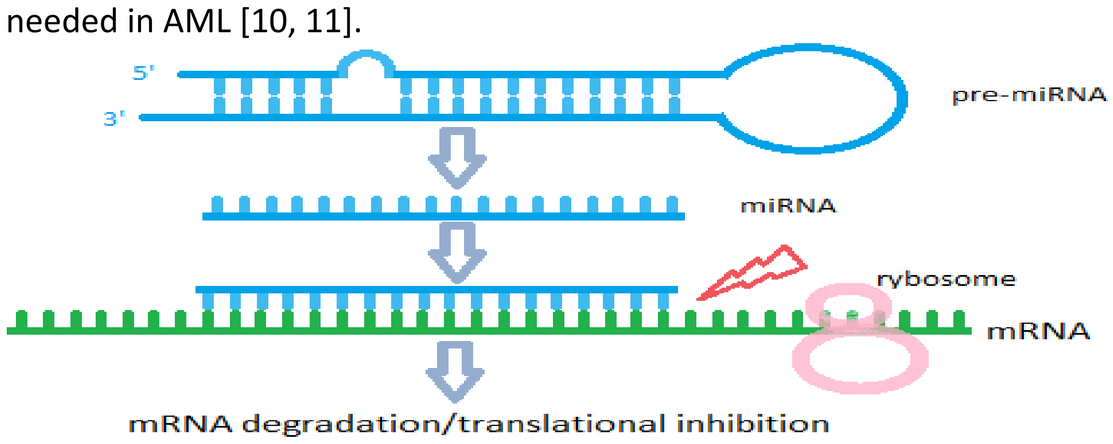


Figure 2. A simplified scheme of miRNA processing and their basic functions.

- **SIRT1** expression is higher in patients classified as intermediate or high cytogenetic-molecular risk and this protein may increase leukemic stem cells (LSCs) survival through its effect on reducing **TP53** activity [2].
- **SIRT2** is found to be involved in the proliferation and survival of AML blasts [3].
- **SIRT3** may affect leukemia cells resistance to conventional chemotherapy [4].
- **SIRT4** shows low expression in blastic cells; its role in AML is still unclear [5,6].
- **SIRT5** inhibitors acted to suppress the proliferation and colony formation of leukemic cells [7].
- There was described the occurrence of increased **SIRT6** activation during therapy with hypomethylating drugs [8].
- On the other hand, it has been shown that the last of the sirtuins, **SIRT7**, may suppress tumors derived from bone marrow progenitor cells [9].

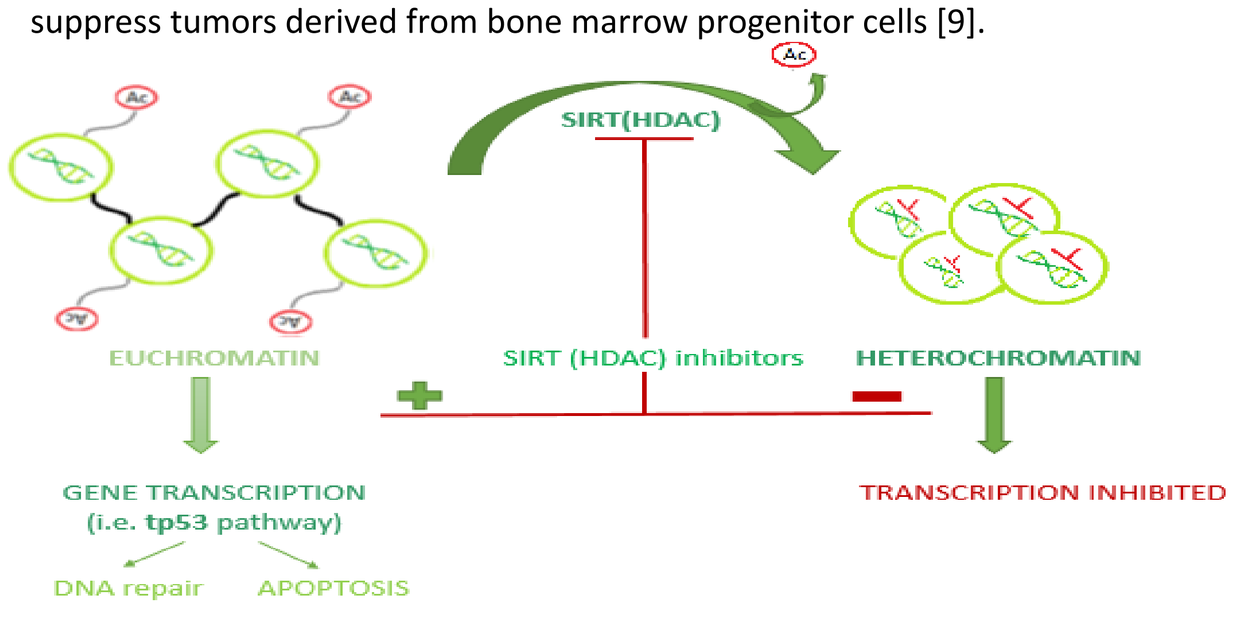


Figure 3. Simplified scheme of deacetylation process and potential SIRT inhibitors impact.
HDAC – Histone deacetylases.

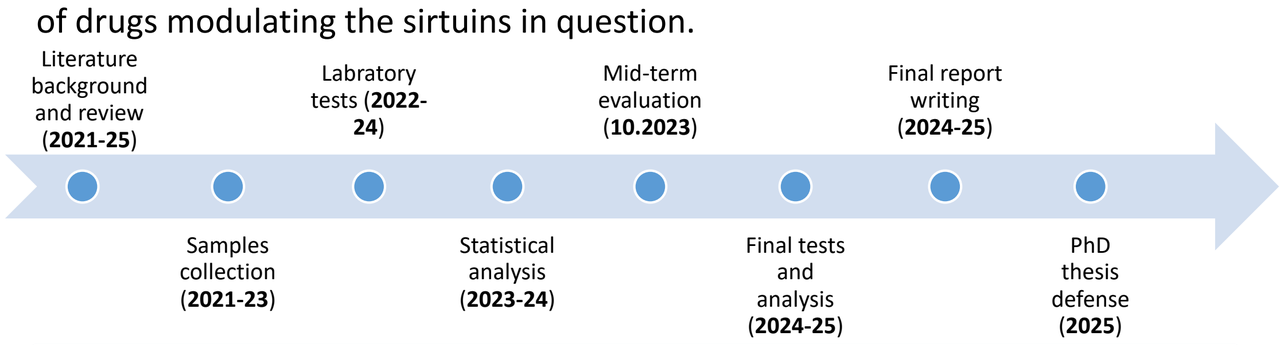
Hypothesis & Aims

Research hypothesis: The gene expression level of sirtuins is important in the biology of AML clone and affects the course of the disease and patient prognosis.

Primary objective: To comprehensively evaluate the impact of individual SIRT on AML blasts and patients prognosis by examining the expression of *SIRT(1-7)* mRNAs and the expression of sirtuin-dependent genes, as well as to describe their relationship with selected miRNAs.

Secondary objectives:

1. To evaluate mRNA expression of *SIRT1-7* in bone marrow samples of patients with newly diagnosed AML.
2. To correlate gene expression levels of individual sirtuins with baseline LSC levels routinely assessed at diagnosis of AML by bone marrow immunophenotyping.
3. Correlation of gene expression levels of individual sirtuins with baseline cytogenetic-molecular risk and other known prognostic factors.
4. To examine the relationship between sirtuins and treatment outcomes.
5. To assess the prognostic significance of *SIRT* and miRNA expression based on response to therapy, relapse rate, overall survival (OS), event-free survival (EFS).
6. Correlation of *SIRT* and *TP53* expression and selected miRNAs molecule levels.
7. Attempt to identify a group of patients who can potentially benefit from the use of drugs modulating the sirtuins in question.



Materials & Methods

- A prospective, single-center study is being conducted.
- Considering retrospective epidemiological data, the expected number of patients meeting the inclusion criteria is ~80. As of 20/04/2023, diagnostic material from 60 patients has been banked. ~15 samples were undiagnostic.

Inclusion criteria	Exclusion criteria
• Newly diagnosed AML according to WHO 2016. • Age above 18 years old. • Informed, voluntary, written consent.	• Presence of other active malignancies. • Pregnancy. • Acute promyelocytic leukemia (APL).

Laboratory procedures and tests:

- Basic tests are performed: myelogram, immunophenotyping, cytogenetic testing, and molecular testing of AML-specific gene mutations, including NGS.
- Additionally, mRNA expression of ***SIRT1-7***, ***TP53***, and **14 miRNA** is assessed.
- I collect test samples of bone marrow aspirate in a volume of 10 ml.
- The cellular layer is harvested and cells are prepared and frozen at -80 °C.
- This is followed by the isolation of total RNA, including the miRNA fraction.
- Mononuclear cells were isolated from 40 selected samples (March 2023).
- The expression is tested using real-time PCR and compared to reference genes.

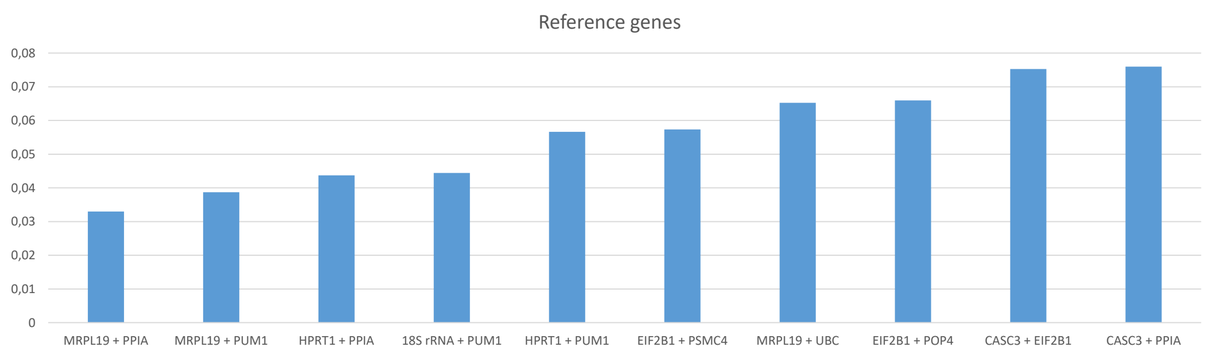


Figure 4. The best pairs of reference genes sequentially from the left.

Data analysis:

- Comparison of ΔCt between the expression of *SIRT* and reference genes.
- Division into groups according to the expression levels of the genes studied.
- Statistical analysis of the results, correlation with the levels of miRNAs or *TP53*.
- Evaluation of the expression of the studied genes in relation to the clinical data, cytogenetic-molecular risk groups and search for potential prognostic factors.
- Conclusion summary and publication of results.

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