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ELEVENTH ANNUAL MEETING

EVALUATION OF SIRTUINS AND *TP53* GENE EXPRESSION AND THEIR IMPACT ON PROGNOSIS IN ACUTE MYELOID LEUKEMIA – PRELIMINARY ANALYSIS

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INTRODUCTION

Sirtuins (SIRTs) are a family of class III histone deacetylases with 7 representatives (SIRT1-7) that affect numerous cellular processes such as metabolism, proliferation and aging [1]. They influence gene expression or can modulate pathways related to cell survival, for example, by downregulating the suppressor protein p53 [2]. However, they are known for their dual action on tumorigenesis, as the anti-oncogenic effects of some sirtuins have also been described [3]. There are reports that in cancer, SIRTs regulate processes such as epithelial-mesenchymal transition, invasion and metastasis [4]. Nevertheless, in hematologic malignancies such as acute myeloid leukemia (AML), the role of SIRTs and their interactions with proteins such as p53 are not yet fully understood.

AIM

To evaluate *SIRTs* and *TP53* gene expression in patients with newly diagnosed AML and search for their prognostic relevance and potential relationship.

METHOD

- A prospective study involving 50 adult patients newly diagnosed with AML was conducted [Table 1].
- Mononuclear cells were isolated from bone marrow (n=36) or peripheral blood samples (n=14).
- The expression of *SIRT1-7* and *TP53* was examined in duplicates by real-time PCR (QuantStudio™7, Applied Biosystems-Thermo Fisher Scientific).
- *MRPL19* and *PPIA* were selected as reference genes using GeNorm, BestKeeper and NormFinder. The mRNA levels were normalized using the formula ΔCt = Ct(reference) — Ct(particular mRNA).
- Group comparisons were performed using Welch's t-test. Overall survival (OS) was assessed by Kaplan-Meier method with the log-rank test. Pearson correlation evaluated the relationship between individual gene expression.

Table 1. Patients' characteristics.

Patients, n	**50**
- female, n (%)	**- 23 (46)**
- male, n (%)	**- 27 (54)**
Age	
- median (IQR)	- 66 (55-72)
Treatment approach, n (%)	
- intensive	- 18 (36)
- azacitidine+venetoclax	- 16 (32)
- non-intensive	- 16 (32)
Cytogenetic-molecular risk (ELN2022), n (%)	
- favorable	- 7 (14)
- intermediate	- 20 (40)
- adverse	- 23 (46)
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)

n – number, SD – standard deviation, IQR – interquartile range, ELN2022 – European LeukemiaNet 2022, WBC – white blood count, ANC – absolute neutrophil count, CRP – C-reactive protein, LDH – lactate dehydrogenase.

RESULTS

- *SIRT4* expression was detected in 90% of patients, *SIRT5* in 96%, while expression of the other genes studied was found in all patients. All of the genes studied showed underexpression relative to the reference genes.
- *SIRT1* presented lower expression in patients with *TP53* [Figure 1] or *RUNX1* mutation (fold change – FC=0.64, *P*=0.03, FC=0.61, *P*=0.03, respectively) in this preliminary analysis.

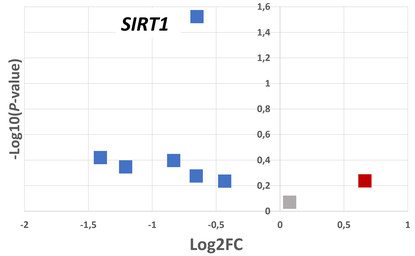


*Figure 1. Volcano plot showing changes in genes expression depending on **TP53** mutation presence.*

- *TP53* expression was upregulated in younger patients (<60 years) (FC=1.54, *P*=0.05).
- There were significant positive correlations between the expression of individual SIRTs, with the strongest expression between SIRT2 and SIRT7 (r=0.81, *P*=0.00) [Figure 2].

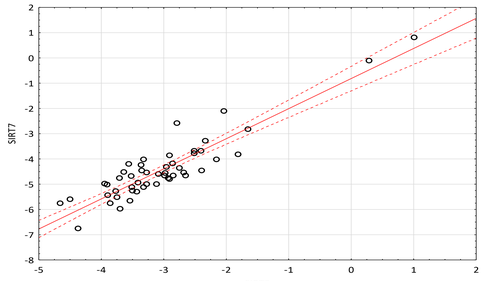


Fig 2. Pearson's correlation between SIRT2 and SIRT7 expressions levels, r=0.81, P=0.00.

- OS was the longest in patients receiving intensive chemotherapy, followed by the azacitidine+venetoclax group, and non-intensive treatment (median OS not reached vs 12.9 months (95% CI: 3.4-12.9) vs 2.0 months (95% CI: 1.3-5.0), *P*=0.009) [Figure 3].

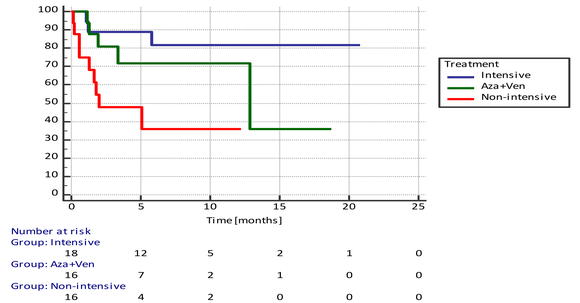


Fig 3. Kaplan–Meier analysis of overall survival (OS) depending on treatment approach. Log-rank P=0.009.

- In the univariate Cox proportional hazard model, lower *TP53* expression (below median) (HR= 2.92, 95% CI: 1.01–8.46, *P*=0.05), WBC level (HR= 1.01, 95% CI: 1.00–1.02, *P*=0.01), LDH (HR= 1.00, 95% CI: 1.00–1.01, *P*=0.01), uric acid (HR= 1.23, 95% CI: 1.02–1.47, *P*=0.03), albumins (HR= 0.20, 95% CI: 0.07–0.51, *P*=0.00), and intensive treatment (HR= 0.16, 95% CI: 0.04–0.63, *P*=0.01) were variables significantly affecting OS.
- In the multivariate Cox proportional hazard model, none of the genes had a prognostic effect on OS, while albumins, LDH, and intensive treatment significantly impacted OS [Table 2].

Table 2. Multivariate Cox proportional-hazard regression for OS in AML patients.

	Coefficient	P	HR	95% CI	
				lower	upper
Albumins	-5.21	**0.00**	**0.01**	0.00	0.08
LDH	0.01	**0.01**	**1.00**	1.00	1.01
Intensive treatment	-2.80	**0.00**	**0.01**	0.00	0.12
WBC	0.01	0.32	1.01	0.99	1.02
Uric acid	-0.05	0.64	0.95	0.76	1.19
TP53 expression (<median)	-0.10	0.82	0.81	0.14	4.75

HR – hazard ratio, CI – confidence interval, LDH – lactate dehydrogenase, WBC – white blood count.

CONCLUSIONS

- **These preliminary results showed that *SIRTs* expression in AML appears to be downregulated and has no clear impact on prognosis or treatment outcomes.**
- **The expression of individual *SIRTs* is closely correlated with each other, but the analysis did not show their direct relationship with *TP53* expression.**
- **Evaluation of the *SIRT1* role in patients with high risk mutations requires confirmation in larger studies.**
- **Further research on *SIRTs* is needed to assess their impact on AML.**

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