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# The role of neuronal apoptosis inhibitory protein (NAIP) in acute myeloid leukemia patients

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**Abstract**

Acute myeloid leukemia (AML) is a heterogeneous, highly malignant neoplasm. Apoptosis is a complex process executed by caspases and suppressed by the inhibitor of apoptosis (IAP) family. Neuronal apoptosis inhibitory protein (NAIP), IAP's member, may play an exceptional role in the mechanisms of tumors' resistance to chemotherapy. The aims of the study were to assess the expression of NAIP in leukemic blasts of AML patients using flow cytometry and to evaluate its influence on disease outcome. NAIP expression was found in 106 out of 108 patients. A higher complete response rate was associated with a low expression of NAIP, age < 60 yo, and white blood cell count < 20 G/L ($p = 0.009$, $p = 0.033$, and $p = 0.076$, respectively) in univariate analyses and a low NAIP expression and age < 60 yo ($p = 0.025$ and $p = 0.013$, respectively) in multivariate analyses. Longer overall survival (OS) in the univariate analysis was influenced by a low NAIP expression, age < 60 yo, and intensive chemotherapy ($p = 0.033$, $p < 0.001$, and $p < 0.001$, respectively). In the intensively treated group, better OS was observed in patients with age < 60 yo, *de novo* AML, and a low NAIP expression ($p = 0.03$, $p = 0.024$, and $p = 0.07$, respectively). In multivariate analysis, longer OS was associated with age < 60 yo ($p = 0.009$) and *de novo* AML ($p = 0.007$). In conclusion, we suggest that NAIP might play an adverse role in response to chemotherapy.

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**Keywords:**
acute myeloid leukemia, apoptosis, NAIP

## Introduction

Acute myeloid leukemia (AML) is an infrequent (1.3%), highly malignant neoplasm responsible for a large number of cancer-related deaths [1, 2]. In the USA and other highly developed countries, the incidence has been near stable over the last years and is about 4 cases per 100,000 citizens per year [1, 2, 3]. The median age at diagnosis is 67 years [1–3]. AML is a heterogeneous and complex disease in which genomic and proteomic alterations and the interactions between them result in various apoptosis abnormalities [1–3].

Apoptosis, programmed cell death, is a highly regulated cellular signaling pathway important for normal development that is needed for the correct functioning of diverse processes such as embryogenesis and tissue homeostasis, as well as tumorigenesis in multicellular organisms [4]. The apoptotic signaling cascades can be initiated by various extracellular (extrinsic pathway) or intracellular (intrinsic pathway) stimuli. Apoptosis is executed by a family of cysteine proteases known as caspases, which are produced in cells as inactive zymogens and become active proteases subsequent to proteolysis [5–8]. Once active, caspases cleave a limited number of substrates that result in the destruction of the cells and their eventual disposal by phagocytic cells.

The inhibitors of apoptosis (IAP) are a family of proteins comprising intrinsic negative regulators of the caspase cascade; these are the only known endogenous proteins that interfere with the activity of both initiator (caspases 8 and 9) and effector enzymes (caspases 3, 6, and 7) [9–11]. Eight human IAP family members have been recognized: neuronal apoptosis inhibitory protein (NAIP), X-linked inhibitors of apoptosis protein (XIAP), cellular inhibitors of apoptosis protein 1 (cIAP1), cellular inhibitors of apoptosis protein 2 (cIAP2), survivin, baculoviral IAP repeat-containing ubiquitin-conjugating enzyme (BRUCE/apollon), livin (ML-IAP, KIAP) and IAP-like protein 2 (ILP-2) [10]. Various IAP family members have been shown to influence the outcome of AML, either alone or in combination [12–15]. It has been demonstrated that overexpression of IAP family members in AML leukemic cells correlates with a low complete remission (CR) rate and shorter overall survival (OS) [16–18]. Especially, a high survivin protein expression is associated with shorter OS [16]. Studies on XIAP have shown that its overexpression was associated with a lower CR rate and worse OS as compared to the in patients with absent or low XIAP expression [17,18]. Various levels of IAP family member expression have been described in AML patients. Simultaneously, a high expression of 1, 2, 3, and 4 proteins has been found to be strongly correlated with CR rates 71%, 60%, 57%, and 33%, respectively [16]. Additionally, absent or a low expression of all examined proteins (XIAP, cIAP1, cIAP2, and survivin) was associated with 100% CR rate [16]. These results demonstrate that apoptosis resistance is very complex in AML patients.

NAIP is comprised of three 3 zinc-binding baculovirus IAP repeat (BIR) domains and, uniquely among IAPs, a nucleotide-binding and oligomerization (NOD) domain and a leucine-rich repeat (LRR) domain [10, 19]. NAIP has emerged as an important regulator of innate immune signaling [20]. Overexpression of this protein has been described in inflammatory disorders such as neurodegenerative disorder spinal muscular atrophy (SMA) [21]. NAIP is also responsible for the intracellular recognition of flagellin, the main structural component of the bacterial flagellum. It takes part in the formation of the inflammasome, multiprotein oligomer responsible for the activation of inflammatory responses. This process activates procaspese-1 cleavage and the extracellular secretion of proinflammatory interleukins: IL-1 and IL-18

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[22]. NAIP also has the same antiapoptotic function as other IAPs. Apoptosis inhibition takes place via blockade of caspases 3, 7, and 9 [20, 21]. All these processes suggest that NAIP possesses unique activity, which might be due to highly variable region of the chromosome (5q13) that encodes NAIP.

The overexpression of NAIP has been described in inflammatory diseases, motor neurons in SMA, multiple sclerosis, Alzheimer's and Parkinson's diseases. Moreover, the presence of NAIP has been noted in some malignancies, including hematological ones: lymphomas, myelodysplastic syndromes (MDS), and AML cells [23–26]. The highest expression was noticed in AML refractory to chemotherapy [25].

Studies on NAIP expression suggest that it may play an exceptional role in neoplasms, including leukemia; however, little information exists about its exact role in AML.

Hence, the present study examines the expression of NAIP protein and its influence on treatment outcome and survival of AML patients.

## Materials and methods

### Patients

The study involved 108 patients with newly diagnosed AML who were hospitalized between January 2008 and December 2012 in the Department of Hematology, Medical University of Lodz. The characteristics of the patients are summarized in table I. The study was approved by the Ethics Committee of the Medical University of Lodz. Written informed consent was obtained from all the patients.

The diagnosis was based on the World Health Organization classification of hematopoietic and lymphoid tissues [3]. Median age of the patients was 61 years (ranged from 18 to 87 years).

Forty-nine patients received intensive induction chemotherapy according to the Polish Adult Leukemia Group (PALG) protocols [27, 28]. Fourteen patients received DA daunorubicin and cytarabine (DA) induction treatment, while 35 received daunorubicin, cytarabine, and cladribine (DAC). Fifty-nine patients received non-intensive treatment, 23 patients were treated with low-dose cytarabine (LD-AraC), 8 with azacitidine (AZA), and 22 with hydroxyurea (HU). Six patients underwent best supportive care (BSC) only.

Response to the treatment was defined in accordance with the revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia [3, 24]. Briefly, complete response (CR) was defined as the presence of less than 5% of bone marrow (BM) blasts with a neutrophil count higher than 1.0 G/L, a platelet count higher than 100 G/L, and no extramedullary disease. Partial remission (PR) was established with either 5–25% BM blasts, a 50% or a higher decrease in BM blasts, or BM blasts < 5% but with the presence of Auer rods. No response (NR) was established for the patients who did not fulfill any of the abovementioned criteria. Early death (ED) was defined as a death from any cause within 4 weeks after the induction therapy and was considered as treatment failure. Disease-free survival (DFS) was calculated from the first day of CR until documentation of a relapse [3]. OS was calculated from the time of diagnosis until death [3].

### Laboratory tests

#### *Blood and BM sampling*

Venous blood or BM samples were collected at the time of diagnosis into pyrogen-free ethylenediaminetetraacetic acid (EDTA) tubes. Immunophenotyping of leukemic cells was performed routinely in the whole peripheral blood or BM using flow cytometry: the "lysed-not washed" method. A routine panel of monoclonal antibodies HLA-DR, CD34, CD45, CD13, CD15, CD33, and CD117 conjugated with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) was applied (all BD Pharmingen, San Diego, CA, USA).

#### *Assessment of expression of NAIP protein*

Peripheral blood or BM mononuclear cells were isolated from EDTA samples by centrifugation on a Histopaque-1077 (Sigma Diagnostics, St Louis, MO, USA) density gradient for 30 minutes at 3600 rpm. The interphase region containing mononuclear cells was collected and washed, once in Hanks' Balanced Salt Solution (Biomed, Lublin, Poland) and twice in phosphate-buffered saline (PBS; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Then, the samples were fixed in 1% paraformaldehyde (PFA) (15 minutes at 4°C) and 90% ethanol for 30 minutes at -20°C for the flow cytometry analysis.

#### *Flow cytometry cell preparation*

The frozen cells were washed in PBS, centrifuged (5 minutes, 1100 rpm), and incubated in 0.01% saponin for 1 minute. The cells were then washed in PBS and centrifuged (5 minutes, 1100 rpm). The incubation with primary antibody (Ab) at a dilution of 1:50 of anti-NAIP (AF 829, polyclonal rabbit anti-human Ab IgG; R&D

***Table I. Patient characteristics***

| **Number of patients** | *n* = 108 |
|---|---|
| **Median age (range), years** | 61 (18–87) |
| **Sex:** n **(%)** | |
| Male | 47 (44) |
| Female | 61 (56) |
| **Median WBC, range, G/L** | 15.6 (0.67–408) |
| **Median LDH, range, U/L** | 399 (119–2936) |
| **Median BM blasts, range, %** | 57 (20–91.5) |
| **AML,** n **(%)** | |
| *De novo* | 67 (61) |
| Secondary | 41 (39) |
| **Cytogenetic risk subgroups, according to SWOG,** n **(%)** | |
| Good | 7 (6) |
| Intermediate | 57 (53) |
| Poor | 19 (18) |
| Unknown | 25 (23) |
| **Treatment,** n **(%)** | |
| Intensive | 49 (45) |
| DA | 14 (13) |
| DAC | 35 (32) |
| Nonintensive | 59 (55) |
| LD-AraC | 23 (21.5) |
| Azacitidine | 8 (7.5) |
| HU | 22 (20.5) |
| BSC | 6 (5.5) |

*WBC – white blood cells; LDH – lactate dehydrogenase; BM – bone marrow; AML – acute myeloid leukemia; SWOG – Southwest Oncology Group; DA – daunorubicin and cytarabine; DAC – daunorubicin, cytarabine, and cladribine; LD-AraC – low-dose cytarabine; HU – hydroxyurea; BSC – best supportive care*

System, Minneapolis, MN, USA) was performed at 4°C overnight. On the following day, the samples were washed in PBS, centrifuged (5 minutes, 1100 rpm), and incubated for 120 minutes with secondary FITC-conjugated Abs at a dilution of 1:20. Afterwards, the samples were washed in PBS, centrifuged (5 minutes, 1100 rpm) before being resuspended in 400 µL PBS, and subjected to the flow cytometry analysis. At the same time, samples with isotype controls were also prepared (normal rabbit IgG control, 1:100 dilution; R&D System, Minneapolis, MN, USA). All preparations have been previously described in detail [16, 30].

#### *Flow cytometry analysis*

All fluorescence measurements were performed by the use of flow cytometry (FACScan; Becton-Dickinson, San Jose, CA, USA). An acquisition gate was established based on forward scatter (FSC) and side scatter (SSC) that included mononuclear cells according to the previous immunophenotype. Cell fluorescence was measured using standard emission filters: FL1 (green, $\lambda$ 515–545 nm) and FL2 (orange, $\lambda$ 564–606 nm). For each analysis, 10,000 events were acquired and analyzed using CellQuestPro software (Becton Dickinson, San Jose, CA, USA). Expression of NAIP was presented as a percentage of NAIP-positive cells in the whole population of leukemic blasts. All the flow cytometry measurements were performed on 10,000 cells per sample. The population of NAIP-positive cells was identified after gating based on appropriate isotype controls. For data quantitation, FACS DIVA software was used. “High” and “low” expression were established according to the median of NAIP-positive cells estimated in the whole group of patients. Examples of flow cytometry images are presented in Figure 1.

### Statistical analysis

The statistical analysis was performed with Statistica 12.0 (Tulusa, OK, USA) software. Correlations between the variables were assessed using the Spearman rank test. OS was estimated using the Kaplan–Meier method. The chi-square and logistic regression tests were applied to investigate the dependence between individual factors and CR rate. The log-rank test was used to evaluate the univariate effects of particular variables on OS. For the multivariate analysis of factors affecting OS, the Cox proportional hazard regression model was applied. Comparisons and correlations between the examined parameters were considered as significant when $p$ was < 0.05.

## Results

### Intracellular flow cytometry protein expression

NAIP protein expression was found in 106 of 108 (98%) newly diagnosed AML patients. Median expression was 11.9% (range 1–79.6%).

### NAIP expression and treatment outcome

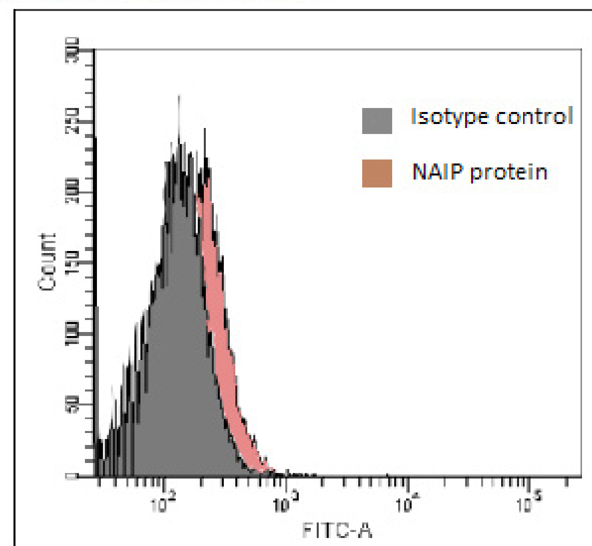
#### *Response to the treatment*

Thirty-three (67%) of 49 intensively treated patients achieved CR. The univariate analysis found age below 60 yo, white blood cell (WBC) count < 20 G/L and a low expression of NAIP protein to be associated with a higher probability of CR achievement ($p$ = 0.033, $p$ = 0.076, and $p$ = 0.009, respectively, Table II).

The multivariate analysis found age below 60 yo and low NAIP protein expression to be the only factors associated with a higher probability of CR achievement ($p$ = 0.013, and $p$ = 0.025, respectively, Table III).

#### *Overall survival*

Median time of OS for the whole cohort of patients was 4.7 months (range 0.03–120.6 months). The probability of 5-year survival in our cohort was 15.5%. In the univariate log-rank analysis, better OS was influenced by age less than 60 yo, intensive therapy and a low expression of NAIP protein ($p$ < 0.001, $p$ < 0.001, and $p$ = 0.033, respectively, Table IV). In multivariate analysis, intensive treatment was the only significant factor associated with longer OS ($p$ <



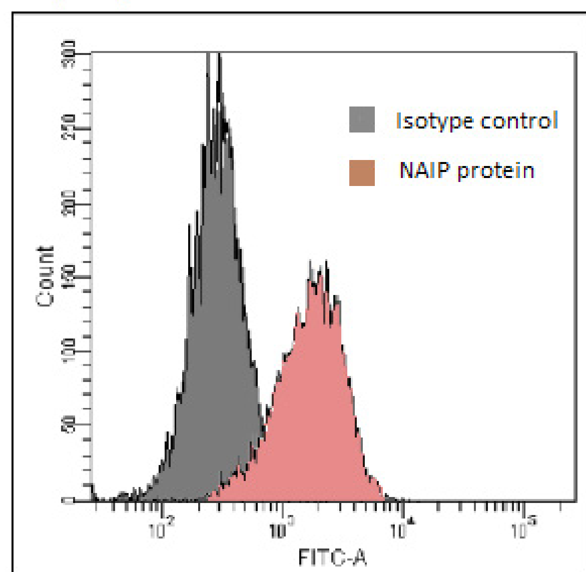




*Fig. 1. Expression of NAIP in flow cytometry*

***Table II. Univariate analysis (chi-square test) of the factors associated with CR rate in intensively treated patients***

| Factor | *n* | CR (%) | *p*-value |
|---|---|---|---|
| **Age, years** | | | |
| <60 | 40 | 31 (77) | 0.033 |
| >60 | 8 | 2 (25) | |
| **Risk group by karyotype** | | | |
| Good, intermediate, unknown | 41 | 29 (71) | 0.51 |
| Poor | 7 | 4 (57) | |
| **AML** | | | |
| *De novo* | 29 | 20 (69) | 0.88 |
| *vs.* Secondary | 19 | 13 (68) | |
| **Blasts in BM (%)** | | | |
| <50 | 18 | 12 (67) | 0.98 |
| >50 | 30 | 21 (70) | |
| **WBC (G/L)** | | | |
| <20 | 32 | 23 (72) | 0.076 |
| >20 | 16 | 10 (62) | |
| **LDH (IU/L)** | | | |
| <UNL | 14 | 8 (57) | 0.22 |
| >UNL | 27 | 20 (74) | |
| **NAIP** | | | |
| <Me | 27 | 23 (85) | 0.009 |
| >Me | 21 | 10 (47) | |

*CR – complete remission; AML – acute myeloid leukemia; BM – bone marrow; WBC – white blood cell; LDH – lactate dehydrogenase; UNL– upper normal limit; NAIP – neuronal apoptosis inhibitor protein*

***Table III. Multivariate analysis (logistic regression) of the factors associated with CR rate***

| Factor | CR (95% CI) | *p*-value |
|---|---|---|
| Age < 60 yo *vs.* Age > 60 yo | 0.29 (0.12–0.77) | 0.013 |
| WBC < 30 G/L *vs.* WBC > 30 G/L | 0.80 (0.38–1.68) | 0.56 |
| NAIP < Me *vs.* NAIP > Me | 0.42 (0.19–0.89) | 0.025 |

*CR – complete remission; WBC – white blood cell; NAIP – neuronal apoptosis inhibitor protein*

0.001, Table V). The analysis of the intensively treated group alone showed that better OS was influenced by age < 60 yo and *de novo* AML ($p$ = 0.03 and $p$ = 0.024, respectively, Table IV) and longer survival tended to be observed in patients with a low expression of NAIP ($p$ = 0.07, Table IV). Additionally, in multivariate analysis, age below 60 yo and *de novo* AML were important for longer OS ($p$ = 0.009 and $p$ = 0.007, respectively, Table V).

### *Disease-free survival*

Median time of DFS for the whole cohort of patients was 15.5 months (range 1.8–119 months). The probability of a 5-year DFS in the intensively treated cohort was 55%. Age < 60 yo was the only factor that influenced longer DFS ($p$ = 0.016).

### Relationship between the expression of NAIP protein and standard AML risk factors

Expression of NAIP protein in leukemic blasts did not differ significantly in the risk groups divided according to age (< 60 yo *vs.* ≥ 60 yo), karyotype (good, intermediate, and unknown vs. poor), WBC count (≤ 20 G/L *vs.* >20 G/L), percentage of leukemic blasts in BM (≤50% *vs.* >50%), and lactate dehydrogenase (LDH) (≤UNL, i.e., upper normal limit, *vs.* >ULN).

## Discussion

Induction of apoptosis is a crucial process in AML treatment. Identifying the factors responsible for cell death inhibition may influence the future potential targets for the treatment. NAIP suppressed apoptosis by inhibiting initiation of apoptosome formation, which blocked the proteolysis of main effector capspases-9, -3, and -7. A literature review revealed limited data regarding NAIP expression in AML. Tamm et al. studied the expression of IAP family (NAIP, XIAP, cIAP1, cIAP2) mRNA in a panel of 60 different human tumor cell lines using a real-time reverse transcriptase polymerase chain reaction (RT-PCR) [15]. They found that NAIP was not detectable in AML cell lines (HL60 and RPMI-8226), unlike XIAP, cIAP1, and cIAP2 [15].
Yamamoto et al. using RT-PCR approach described overexpression of NAIP mRNA in BM of all examined patients with MDS ($n$ = 13) and *de novo* AML ($n$ = 10) as compared to healthy BM ($n$ = 13) [25]. The expression of mRNA of NAIP was almost equal to that of MDS and AML cells.
Nakagawa et al. studied NAIP mRNA expression in BM samples from 13 healthy individuals, 9 patients with AML, 7 with acute lymphoblastic leukemia (ALL) and 8 with acute mixed linage leukemia (AMLL) by quantitative RT-PCR. They found that NAIP expression was present in all examined cases and was higher in AML, ALL, and AMLL than in healthy BM samples [26].

*Table IV. Factors associated with the probability of 5-year survival (univariate analysis: log-rank test)*

| Probability of five-year survival | | | | |
|---|---|---|---|---|
| | Whole group | | Intensively treated group | |
| | | *p*-value | | *p*-value |
| **Age**<br>≤60<br>*vs.*<br>>60 | 31% (20.9%; 47.1%)<br><br>1.8% (0.3%; 12.2%) | <0.0001 | 39% (26.6%; 57.2%)<br><br>0% (NA; NA) | 0.03 |
| **AML**<br>*De novo*<br>*vs.*<br>Secondary | 20.9% (13.1%; 33.3)<br><br>8.1% (2.7%; 24.0%) | 0.36 | 48.3% (33.1%; 70.4%)<br><br>11.8% (3.2%; 43.2%) | 0.024 |
| **Cytogenetic risk group (SWOG)**<br>Low, intermediate<br>*vs.*<br>High | 16.9% (10.6%; 26.7%)<br><br>10.5% (2.8%; 39.0%) | 0.23 | 33.3% (21.7%; 51.1%)<br><br>28.6% (8.9%; 92.2%) | 0.53 |
| **Therapy**<br>Intensive<br>*vs.*<br>Low dose, BSC | 32.7% (21.8%; 48.8%)<br><br>0.02% (0.2%; 11.8%) | <0.0001 | | |
| **WBC**<br>≤30 G/L<br>*vs.*<br>>30 G/L | 15.2% (8.6%; 26.8%)<br><br>16.7% (8.5%; 32.8%) | 0.38 | 28.1% (16.2%; 48.9%)<br><br>41.2% (23.3%; 72.7%) | 0.43 |
| **BM blast**<br>≤50%<br>*vs.*<br>>50% | 11.6% (5.1%; 26.5%)<br><br>18.5% (11.1%; 30.8%) | 0.47 | 21.1% (8.8%; 50.3%)<br><br>40.0% (25.8%; 62.0%) | 0.1 |
| **LDH**<br>Normal limit<br>*vs.*<br>>Upper normal limit | 14.3% (5.0%; 40.7%)<br><br>16.7% (11.6%; 30.7%) | 0.56 | 21.4% (7.9%; 58.4%)<br><br>44.4% (23.7%; 67.8%) | 0.19 |
| **NAIP**<br>≤Me<br>*vs.*<br>>Me | 23.5% (14.3%; 38.6%)<br><br>8.8% (3.8%; 20.3%) | 0.033 | 42.9% (27.9%; 65.7%)<br><br>19.0% (7.9%; 46.0%) | 0.07 |

*NA – not applicable; AML – acute myeloid leukemia; SWOG – Southwest Oncology Group; BSC – best supportive care; WBC – white blood cells; BM – bone marrow; LDH – lactate dehydrogenase; NAIP – neuronal apoptosis inhibitor protein*

*Table V. Multivariate analysis (Cox model) of the factors associated with the decreased probability of OS*

| Factor | Whole group | | Intensively treated group | |
|---|---|---|---|---|
| | HR (95% CI) | *p*-value | HR (95% CI) | *p*-value |
| Age < 60 years | 1.138 (0.545; 2.379) | 0.730 | 0.290 (0.114; 0.734) | 0.009 |
| AML *de novo* | 0.997 (0.621; 1.601) | 0.991 | 0.355 (0.167; 0.752) | 0.007 |
| Therapy – intensive | 0.233 (0.106; 0.515) | 0.0003 | | |
| NAIP ≤ Me | 0.956 (0.598; 1.530) | 0.852123 | 0.688 (0.327; 1.449) | 0.326 |

*OS – overall survival; AML – acute myeloid leukemia; NAIP – neuronal apoptosis inhibitor protein*

The present study is the first to assess the expression of NAIP protein in AML cells using cytometry. It identified NAIP expression in 98% of AML patients with a range 1–79.6%. The presence of NAIP mRNA and protein expression in leukemic cells suggests that it may be an important factor for escape from cell death.

Although various IAP family members have been shown to have an impact on CR achievement and survival in AML patients [15, 16], no data have been presented regarding the influence of NAIP protein on treatment outcome and survival; however, the univariate and multivariate analyses in the present study indicate that a low expression of NAIP was associated with a higher CR rate in AML (85% vs. 47%; $p = 0.009$ and $p = 0.025$, respectively). Moreover, a low NAIP protein expression was associated with longer survival in the whole group and a trend was observed toward better OS in intensively treated patients ($p = 0.033$ and $p = 0.07$, respectively). The influence of NAIP expression on AML outcome suggests that it modulates leukemic cell survival.

Choi et al. measured mRNA NAIP expression in 117 breast cancers and 10 normal breast tissues using RT-PCR [31]. NAIP was present in all breast samples. It was overexpressed in the cancer tissue as compared to healthy breast tissue, and particularly a high expression was observed in patients with unfavorable risk factors [31]. The authors did not find any correlation between NAIP expression and 3-year relapse-free survival. However, it should be mentioned that this is quite a short period of time in breast cancer patients, and the authors underline that longer observation in larger patients' group is necessary to determine the role of NAIP in breast cancer. Nevertheless, these findings regarding the significant role of NAIP

are supported by a previous study suggesting that NAIP might be responsible for the resistance of MCF-7 cell line to bleomycin treatment and that NAIP was shown to be downregulated by tumor suppressor p53 [32].

Chiu et al. showed using RT-PCR and Western blot approaches that NAIP mRNA and protein were expressed in prostatic cancer cells [32]. Moreover, the authors observed that increased NAIP expression significantly lowered the response to androgen therapy in an *in vitro* and *in vivo* mouse model [32]. It also corresponded with increased DNA-binding activity of NF-kappaB, an NAIP up-regulator, and resulted in treatment resistance [32]. In addition, a Spanish group studied NAIP expression in 95 patient samples of prostatic cancer and 35 cases of benign prostatic hyperplasia as well as 10 normal prostatic cells using immunohistochemistry. NAIP protein was present only in samples with prostatic neoplasm, and this protein expression was in more than 60% of cells [33]. Normal prostatic cells displayed a lack of NAIP protein expression; unfortunately, NAIP expression was not correlated with clinical and pathological parameters [34]. Nevertheless, studies on prostate cancer suggest the NAIP may play a role in carcinogenesis.

Additionally, the expression of IAP family members (XIAP, cIAP1, and NAIP) was found to increase with carcinoma stage in a hamster model of squamous-cell carcinogenesis. Simultaneous RT-PCR assessment of p53 mutation and IAP methylation suggests that IAP expression might be modulated by both genetic (mutant p53) and epigenetic mechanisms [35]. This observation suggests that combined treatment may be a future therapeutic option.

Our results are convergent with those in breast and prostatic cancer. Higher expression of NAIP is an adverse prognostic factor in treatment outcome and survival of AML patients.

Currently, based on the knowledge about IAP's family, 2 types of mechanisms are under investigation in phase I and II of clinical trials in the cancer patients. One is based on proapoptotic Smac small molecule, which binds IAP's members and promotes activation of caspases leading to apoptosis [35]. The second mechanism uses pan-IAP inhibitor. The blockade of IAP's members induces cleavage of caspases 3 and 8 and activates apoptosis [36]. We may expect the development of the targeting treatment focusing on IAP's pathway in near future.

## Conclusions

NAIP may play an important role in the response to chemotherapy and development of refractory disease. However, further studies are necessary to establish its exact role in the treatment of AML patients.

### *Authors' contributions/Wkład autorów*

The first and last version of the manuscript was written by AP, AW and TR . All authors collected patient data and cooperated on the final shape of the manuscript.

### *Conflict of interest/Konflikt interesu*

The authors have no conflicts of interest that are directly relevant to the content of this paper.

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### *Etyka/Ethics*

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, EU Directive 2010/63/EU for animal experiments, and Uniform Requirements for Manuscripts submitted to biomedical journals.

## References
## Piśmiennictwo

[1] www.seer.cancer.gov.

[2] Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424–47.

[3] Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009;114:937–51.

[4] Gupta S. A decision between life and death during TNF-alpha-induced signaling. J Clin Immunol 2002;22:185–94.

[5] Nicholson DW. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 1999;6:1028–42.

[6] Zhivotovsky B, Orrenius S. Carcinogenesis and apoptosis: paradigms and paradoxes. Carcinogenesis 2006;27:1939–45.

[7] Zhivotovsky B, Orrenius S. Cell death mechanisms: cross-talk and role in disease. Exp Cell Res 2010;316:1374–83.

[8] Hassab AH, Nafea DA, Swelem RS, Ghazal BM. Study of CD 25 expression on leukemic cells: a prognostic factor in acute myeloid leukemia. Acta Hematol Pol 2018; 49:20–7.

[9] Liston P, Fong WG, Kelly NL, et al. Identification of XAF1 as an antagonist of XIAP anti-caspase activity. Nat Cell Biol 2001;3:128–33.

[10] Liston P, Fong WG, Korneluk RG. The inhibitors of apoptosis: there is more to life than $Bcl_2$. Oncogene 2003;22:8568–80.

[11] Cohen GM. Caspases: the executioners of apoptosis. Biochem J 1997;326:1–16.

[12] Wrzesień-Kuś A, Smolewski P, Sobczak-Pluta A, Wierzbowska A, Robak T. The inhibitor of apoptosis protein family and its antagonists in acute leukemias. Apoptosis 2004;9:705–15.

[13] Smolewski P, Robak T. Inhibitors of apoptosis proteins (IAPs) as potential molecular targets for therapy of hematological malignancies. Curr Mol Med 2011;11:633–49.

[14] Fulda S. Inhibitor of apoptosis (IAP) proteins in hematological malignancies: molecular mechanisms and therapeutic opportunities. Leukemia 2014;28:1414–22.

[15] Tamm I, Kornblau SM, Segall H, et al. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. Clin Cancer Res 2000;6:1796–803.

[16] Pluta A, Wierzbowska A, Cebula-Obrzut B, et al. Prognostic value of inhibitor of apoptosis protein family expression in patients with acute myeloid leukemia. Leuk Lymphoma 2015;56: 2529–35.

[17] Sung KW, Choi J, Hwang YK, et al. Overexpression of X-linked inhibitor of apoptosis protein (XIAP) is an independent unfavorable prognostic factor in childhood de novo acute myeloid leukemia. J Korean Med Sci 2009;24: 605–13.

[18] Tamm I, Richter S, Scholz F, et al. XIAP expression correlates with monocytic differentiation in adult de novo AML: impact on prognosis. Hematol J 2004;5: 489–95.

[19] Liston P, Roy N, Tamai K, et al. Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature 1996;379:349–53.

[20] Maier JK, Lahoua Z, Gendron NH, et al. The neuronal apoptosis inhibitory protein is a direct inhibitor of caspases 3 and 7. J Neurosci 2002;22:2035–43.

[21] Davoodi J, Ghahremani MH, Es-Haghi A, Mohammad-Gholi A, Mackenzie A. Neuronal apoptosis inhibitory protein, NAIP, is an inhibitor of procaspase-9. Int J Biochem Cell Biol.2010;42:958–64.

[22] Maltez VI, Miao EA. NAIP inflammasomes give the NOD to bacterial ligands. Trends Immunol. 2014;3:503–4.

[23] Kesari A, Misra UK, Kalita J, et al. Study of survival of motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP) gene deletions in SMA patients. J Neurol 2005;252:667–71.

[24] Yamamoto K, Abe S, Nakagawa Y, et al. Expression of IAP family proteins in myelodysplastic syndromes transforming to overt leukemia. Leuk Res. 2004;28:1203–11.

[25] Nakagawa Y, Hasegawa M, Kurata M, et al. Expression of IAP-family proteins in adult acute mixed lineage leukemia (AMLL). Am J Hematol 2005;78:173–80.

[26] Holowiecki J, Grosicki S, Giebel S, et al. Cladribine, but not fludarabine, added to daunorubicin and cytarabine during induction prolongs survival of patients with acute myeloid leukemia: a multicenter, randomized phase III study. J Clin Oncol 2012;30:2441–48.

[27] Pluta A, Robak T, Wrzesien-Kus A et al. Addition of cladribine to the standard induction treatment improves outcomes in a subset of elderly acute myeloid leukemia patients. Results of a randomized Polish Adult Leukemia Group (PALG) phase II trial. Am J Hematol 2017;92:359–66.

[28] Cheson BD, Bennett JM, Kopecky KJ, et al. International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 2013;21:4642–9. Erratum in: J Clin Oncol 2004;22:576.

[29] Pluta A, Wrzesień-Kuś A, Cebula-Obrzut B, et al. Influence of high expression of Smac/DIABLO protein on the clinical outcome in acute myeloid leukemia patients. Leuk Res 2010;34:1308–13.

[30] Choi J, Hwang YK, Choi YJ, et al. Neuronal apoptosis inhibitory protein is overexpressed in patients with unfavorable prognostic factors in breast cancer. J Korean Med Sci 2007;22:S17–23.

[31] Yang L, Zhao W, Wei P, Zuo W, Zhu S. Tumor suppressor p53 induces miR-15a processing to inhibit neuronal apoptosis inhibitory protein (NAIP) in the apoptotic response DNA damage in breast cancer cell. Am J Transl Res 2017;9:683–91.

[32] Chiu HH, Yong TM, Wang J, et al. Induction of neuronal apoptosis inhibitory protein expression in response to androgen deprivation in prostate cancer. Cancer Lett 2010;292:176–85.

[33] Rodríguez-Berriguete G, Fraile B, de Bethencourt FR, et al. Role of IAPs in prostate cancer progression: immunohistochemical study in normal and pathological (benign hyperplastic, prostatic intraepithelial neoplasia and cancer) human prostate. BMC Cancer 2010;10:18.

[34] Hsue SS, Wang WC, Chen YK, Lin LM. Expression of inhibitors of apoptosis family protein in 7,12-dimethylbenz[a]anthracene-induced hamster buccal-pouch squamous-cell carcinogenesis is associated with mutant p53 accumulation and epigenetic changes. Int J Exp Pathol 2008;89:309–20.

[35] Boddu P, Carter BZ, Verstovsek S, Pemmaraju N. SMAC mimetics as potential cancer therapeutics in myeloid malignancies. Br J Haematol 2019;185:219–31.

[36] Infante JR, Dees EC, Olszanski AJ, et al. Phase I dose-escalation study of LCL161, an oral inhibitor of apoptosis proteins inhibitor, in patients with advanced solid tumors. J Clin Oncol 2014;32:3103–10.