ONLINE FIRST

This is a provisional PDF only. Copyedited and fully formatted version will be made available soon.

Acta Haematologica Polonica

Evaluation of polymorphism in BCL-2, PD-1, and PD-L1 genes in myelodysplastic neoplasms

Authors: Bartłomiej Kuszczak, Piotr Łacina, Marta Dratwa-Kuźmin, Katarzyna Bogunia-Kubik, Tomasz Wróbel, Justyna Rybka

DOI: 10.5603/ahp.104373

Article type: Original research article

Submitted: 2025-01-06

Accepted: 2025-03-26

Published online: 2025-03-31

ORIGINAL RESEARCH ARTICLE

DOI: 10.5603/ahp.104373

Evaluation of polymorphism in BCL-2, PD-1, and PD-L1 genes in myelodysplastic neoplasms

Gene polymorphism in BCL-2, PD-1, and PD-L1 in MDS

Bartłomiej Kuszczak[1](https://orcid.org/0000-0002-8036-073X), Piotr Łacina[2](https://orcid.org/0000-0002-9659-9403), Marta Dratwa-Kuzmin[2](https://orcid.org/0000-0003-4077-7538), Katarzyna Bogunia-Kubik[2] (https://orcid.org/0000-0001-9744-0376), Tomasz Wróbel[1](https://orcid.org/0000-0002-6612-3535), Justyna Rybka[1](https://orcid.org/0000-0001-7965-3589)

[1]Department and Clinic of Hematology, Cellular Therapies and Internal Medicine of Wroclaw Medical University, Wroclaw, Poland

[2]Laboratory of Clinical Immunogenetics and Pharmacogenetics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Address for correspondence:

Bartłomiej Kuszczak, Department and Clinic of Hematology, Cellular Therapies and Internal Medicine of Wroclaw Medical University, Pasteura 4,

50–367 Wroclaw, Poland;

e-mail: bartkuszczak@gmail.com

Received: 06.01.2025 **Accepted:** 26.03.2025 **Early publication:** 31.03.2025

Abstract

B-cell CLL/lymphoma 2 (BCL-2) family proteins regulate apoptosis, while programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) act as negative regulators of

the immune system. An association has been found between abnormalities in BCL-2 and PD-1/PD-L1 proteins and susceptibility to myelodysplastic neoplasms. The aim of this study was to assess polymorphisms in genes encoding BCL-2, PD-1, and PD-L1 and their impact on the clinical course of myelodysplastic neoplasms (MDS), treatment effectiveness and correlation with other prognostic factors in MDS. The study included 50 individuals with a median age of 70 who had been diagnosed with MDS. Genotyping for *BCL2* (rs1564483, rs2279115), *CD274* (rs2297136, rs4143815), and *PDCD1* (rs10204525, rs2227981) was performed using LightSNiP assays. Real-time PCR reactions were conducted on a LightCycler 480 II device. Mann-Whitney U test and Fisher's exact test were employed for analysis. Kaplan-Meier curves and the log-rank test were used for survival analysis. The analysis revealed that the *BCL2* rs1564483 *G* allele is associated with better overall survival compared to patients with the *BCL2* rs1564483 *AA* genotype ($p = 0.037$), and is more common in patients who achieve complete remission (CR) or partial remission (PR) after first-line treatment (regardless of the therapy used) ($p = 0.021$).

Key words: *PD-1/PD-L*, *BCL-2*, MDS, polymorphism

Acta Hematol Pol

Introduction

The immune system plays a key role in eliminating cancer cells, and thus influences individual susceptibility to proliferative processes. Abnormalities in gene expression, gene polymorphisms, and abnormalities in key signaling pathways play an essential role in the pathogenesis of all cancers, including hematological neoplasms.

Proteins belonging to the BCL-2 (B-cell CLL/lymphoma 2) family are responsible for regulating apoptosis, or programmed cell death. Deregulation within the BCL-2 family proteins facilitates the survival of pathological cells and leads to their abnormal proliferation [1]. High expression of *BCL2* is found in various types of cancer i.e. lung cancer, breast cancer, prostate cancer, and esophagal cancer, as well as in lymphoproliferative disorders such as chronic lymphocytic leukemia and B-cell non-Hodgkin's lymphomas [2]. Over the past two decades, proteins belonging to the BCL-2 family have been better understood and their individual functions identified. In the BCL-2 family, it has become possible to differentiate anti-apoptotic proteins (such as BCL-2, MCL1, BCL-XL, BFL1/A1, BCL-W, and BCL-2L10), which have three BCL-2 homologous (BH) domains and a transmembrane domain

(TM). Pro-apoptotic proteins are categorized into those with a single BH3 domain (such as BID, BIM, PUMA, BAD, BIK, HRK, NOXA, and BMF) and those with multiple BH domains (such as BAX, BOK, and BAK) [3].

As *BCL2* expression is much higher in cancer clone cells than in normal cells, the action of BCL-2 inhibitors will have little effect on the function of the normal cell. Overcoming resistance by inhibiting anti-apoptotic proteins of the BCL-2 family represents a new therapeutic target for cancer treatment [4]. Polymorphism of the gene encoding the BCL-2 protein influences increased susceptibility, and is a prognostic factor in the course of many cancers. In the available literature, three significant polymorphisms of the *BCL2* gene have been described: rs2279115, rs1801018 and rs1564483. Two large meta-analyses have demonstrated that the rs2279115 polymorphism is linked to a greater risk of developing cancer in Asian populations [5, 6].

Programmed cell death protein 1 (PD-1) and PD-L1 (programmed death ligand 1) are negative immune system regulators. They are expressed on CD4+, CD8+ lymphocytes, monocytes, NK/T cells, and B lymphocytes. PD-1 inhibits the T-cell-dependent immune response and the production of effector cytokines. The PD-1/PD-L1 axis helps maintain immune tolerance, offering specific protection against autoimmune processes [7]. The genes encoding PD-1/PD-L1 are known to be polymorphic. The best-studied PD-1 polymorphisms include: rs36084323, rs11568821, rs2227981, rs2227982 and rs7421861. Hashemi et al. showed that the presence of the rs2227981 and rs11568821 polymorphisms can reduce the risk of certain cancers, but that the rs7421861 polymorphism significantly increases this risk [8, 9].

Myelodysplastic neoplasms (MDS) comprise a heterogeneous group of hematopoietic malignancies, characterized by ineffective hematopoiesis, dysplastic changes in the bone marrow, and an increased risk of transformation to acute myeloid leukemia. More than 86% of patients with MDS are over the age of 60, and the median age at diagnosis is 76 years [10, 11]. Disorders responsible for the pathogenesis of MDS originate at the level of bone marrow progenitor cells; however, the exact immunological and molecular mechanisms that lead to the development of MDS remain unknown and are the subject of many research projects. One contributory factor to the development of MDS is genetic instability. Between 80% and 90% of MDS patients have at least one confirmed somatic molecular mutation [12, 13]. The polymorphism of genes encoding BCL-2, PD-1/PD-L1 and their impact on the clinical course of MDS remain unknown.

The aim of this study was to evaluate polymorphisms in the genes encoding BCL-2 (BCL2), PD-1 (PDCD1), and PD-L1 (CD274), and their impact on the clinical course of MDS, treatment efficacy, and correlation with other prognostic factors in MDS, such as the Revised International Prognostic Scoring System (IPSS-R), cytogenetic changes, and response to treatment used.

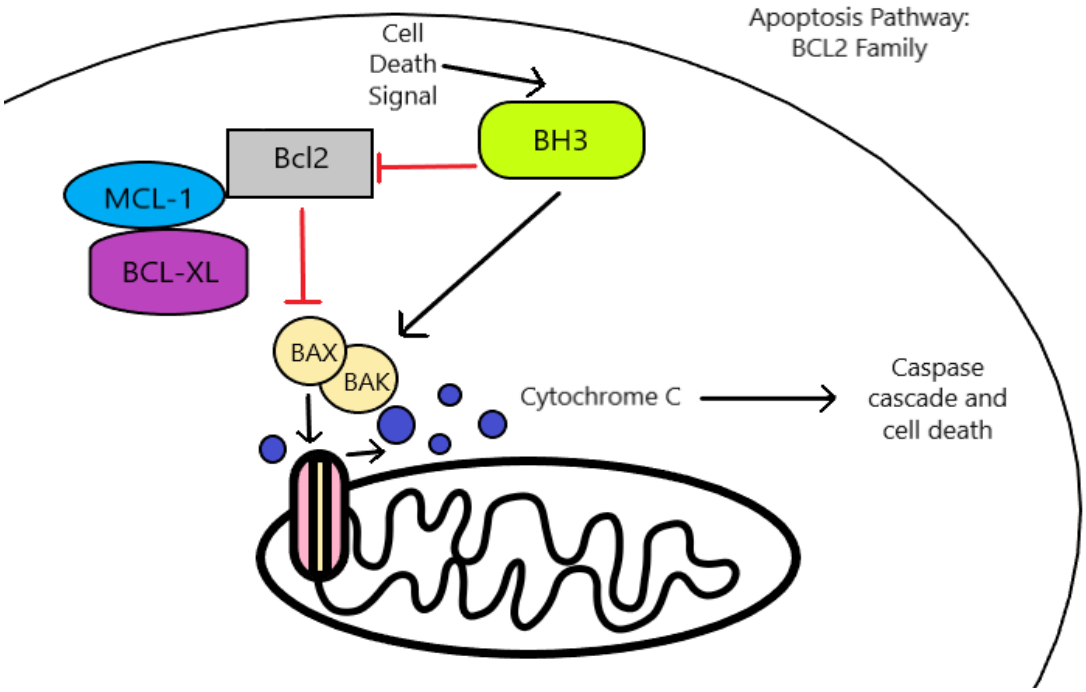

Figure 1. Cellular pathways of BCL-2 family

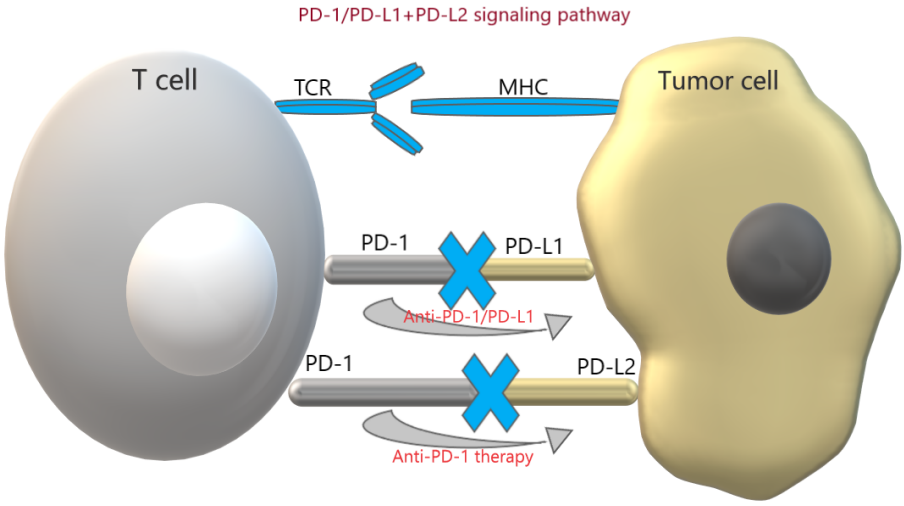

Figure 2. PD-1/PD-1L signaling pathway

Material and methods

Peripheral blood was collected from 50 patients diagnosed with MDS during routine diagnostic tests. The Bioethics Committee at Wroclaw Medical University granted consent for the study. The research was carried out under a grant from the National Science Center (Poland) at Wroclaw Medical University with the number MINI.C140.21.001.

The response assessment was based on the International Working Group 2023 criteria. Due to the limited study population, patients who achieved CR bilineage, CR unilineage, CR with limited count recovery, and CR with partial hematological recovery, were classified into a single CR group [14].

The following polymorphisms for *BCL2* (rs1564483 C>T, rs2279115 G>T), *CD274* (rs2297136 G>T, rs4143815 G>C), and *PDCD1* (rs10204525 C>T, rs2227981 A>G) were determined in our MDS cohort as well as in a healthy control group. DNA was extracted using a NucleoSpin Blood kit (MACHEREY-NAGEL, Dueren, Germany) according to the manufacturer's instructions. DNA purity and concentration were then investigated on a DeNovix DS-11 spectrophotometer (DeNovix, Wilmington, DE, USA). Extracted DNA was subsequently stored at –20°C until further use. LightSNiP assays (TIB MOLBIOL, Berlin, Germany) were used for genotyping for *BCL2*, *CD274*, and *PDCD1* polymorphisms. Real-time PCR reactions were performed on a LightCycler 480 II device (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions.

Statistical analysis was performed using a Real Statistics Resource Pack for Microsoft Excel 2013 (version 15.0.5023.1000, Microsoft, Redmond, WA, USA), GraphPad Prism (GraphPad Software, La Jolla, CA, version 8.0.1) and RStudio (Posit PBC, Boston, MA, USA, version 2024.12.0). A Mann-Whitney U test was used for the analysis of associations with age, IPSS-R, blasts, hemoglobin, white blood cell (WBC) count, neutrophils, lymphocytes, monocytes, platelets, bilirubin, ALAT, ASPAT, GGTP, alkaline phosphatase, total protein, creatinine, uric acid, urea, C-reactive protein (CRP), sodium, potassium, and lactate dehydrogenase (LDH). A Fisher's exact test was used to test for associations with sex, infection, presence of the del5q mutation, Ogata score, and response to treatment [15]. Kaplan-Meier curves and the log-rank

test were used to analyze survival. The hazard ratio was calculated using GraphPad Prism and the Mantel Haenszel method. Median survival and follow-up were calculated using the ggsurvfit package and survfit function in R; furthermore, the Schemper and Smith method was used to estimate median follow-up. For all results, *p*-values <0.05 were considered as statistically significant.

Results

The study included 50 patients (30 men and 20 women) diagnosed with MDS. The median age was 70 years (range: 45–91). The diagnosis of MDS was based on the revised 2022 WHO criteria [16]. According to the IPSS-R classification, 17 patients had low or very low risk, 15 had intermediate risk, 10 had high risk, and eight patients had very high risk. First-line treatment in the analyzed population included azacitidine in 31 patients, luspatercept in two patients, and lenalidomide in one patient [17]. Two study participants had leukocytosis above >10 $\times 10^3/\mu l$, specifically 12.23 $\times$ $10^3/\mu l$ and 32.71 $\times$ $10^3/\mu l$. Both these patients had an infection at the time of diagnosis, accompanied by elevated CRP levels >80 mg/l. Additionally, the patient with the higher leukocytosis was receiving corticosteroids. They did not meet the diagnostic criteria for any myeloproliferative neoplasms or myelodysplastic/myeloproliferative neoplasms, and their bone marrow findings were consistent with MDS. Therefore, they were included in the study population. 16 patients were not eligible for MDS therapy. The control group consisted of 20 healthy subjects (13 women and seven men) with a median age of 69.5 years. The patient's clinical data is set out in Table I.

Distribution of *BCL2, PDCD1,* and *CD274* polymorphic variants in MDS patients and control group

There was no statistically significant difference between the polymorphisms in MDS patients and controls. However, it was observed that the *CD274* rs4143815 *C* allele was more common in MDS patients than in healthy controls ($p = 0.057$).

BCL2 gene polymorphisms in MDS patients

The rs1564483 *C* allele in the BCL-2 gene was found to be significantly more common in patients who achieved CR or PR after first-line treatment, regardless of the therapy used ($p = 0.021$) (Fig. 3). We also found that the presence of the *BCL2* rs1564483 *C* allele in patients with MDS was associated with longer overall survival compared to patients with the *BCL2*

rs1564483 *TT* genotype (p = 0.037) (Fig. 4). Median follow-up time was 16.1 months. Median survival of patients with genotype *TT* was 30.7 months and was not reached for patients with genotype *CT*/*CC*. Hazard ratio was 6.22 (95% CI: 1.11–34.75).

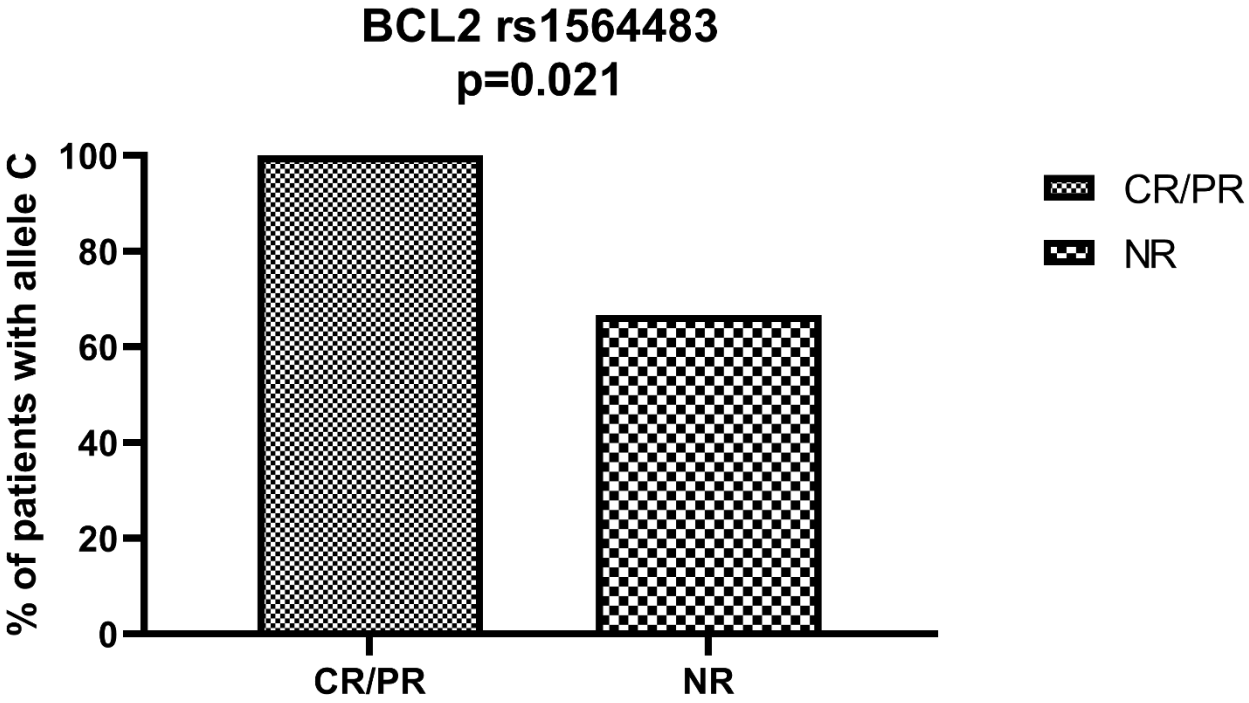

Figure 3. Association between *BCL2* rs1564483 *C* allele and response rate. Allele *BCL2* rs1564483 *C* was more frequent in patients with complete/partial response (CR/PR) than in patients who did not respond to first-line treatment (NR)

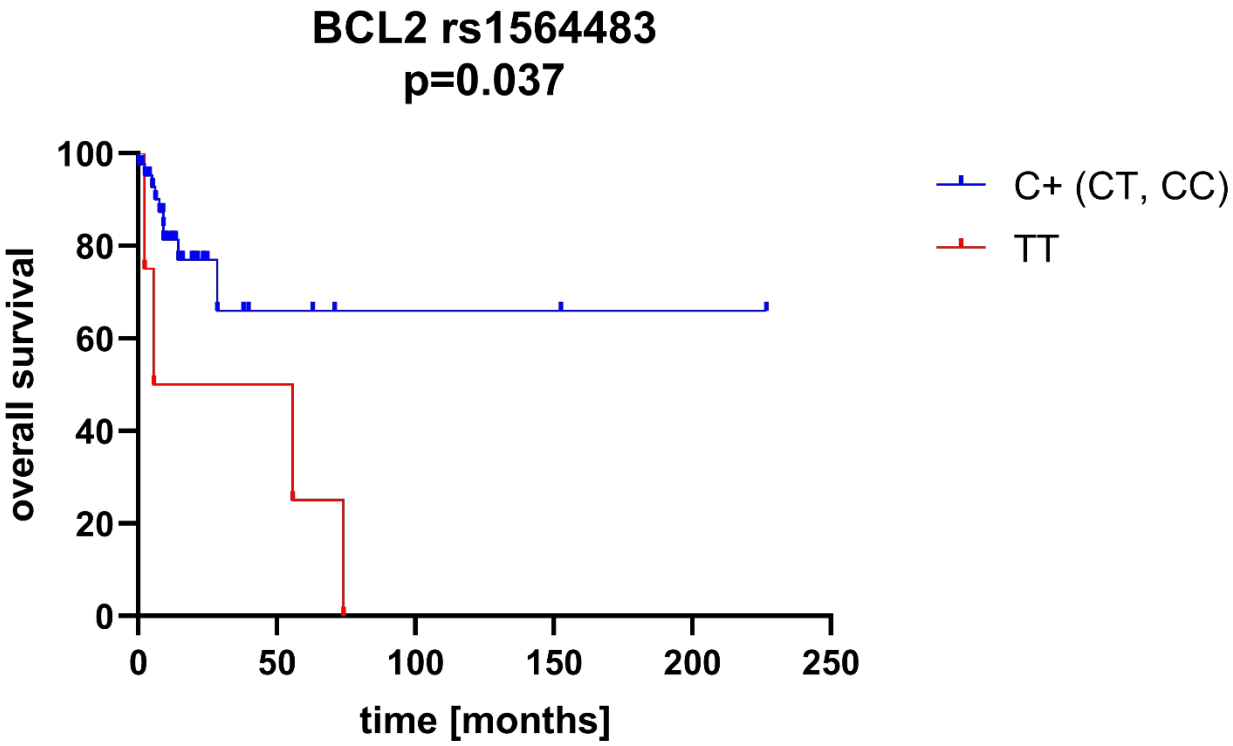

Figure 4. Overall survival of patients with different *BCL2* rs1564483 genotypes. Patients with allele *C* were characterized by longer overall survival

Regarding clinical parameters, the BCL2 rs2279115 genotype (GT and GG) showed a correlation with lower neutrophil counts compared to the BCL2 rs2279115 TT genotype (p = 0.017) (Fig. 5). Additionally, the BCL2 rs2279115 G genotype was linked to lower platelet counts than the BCL2 rs2279115 TT genotype (p = 0.027) (Figure 6). This assessment was conducted at the time of diagnosis.

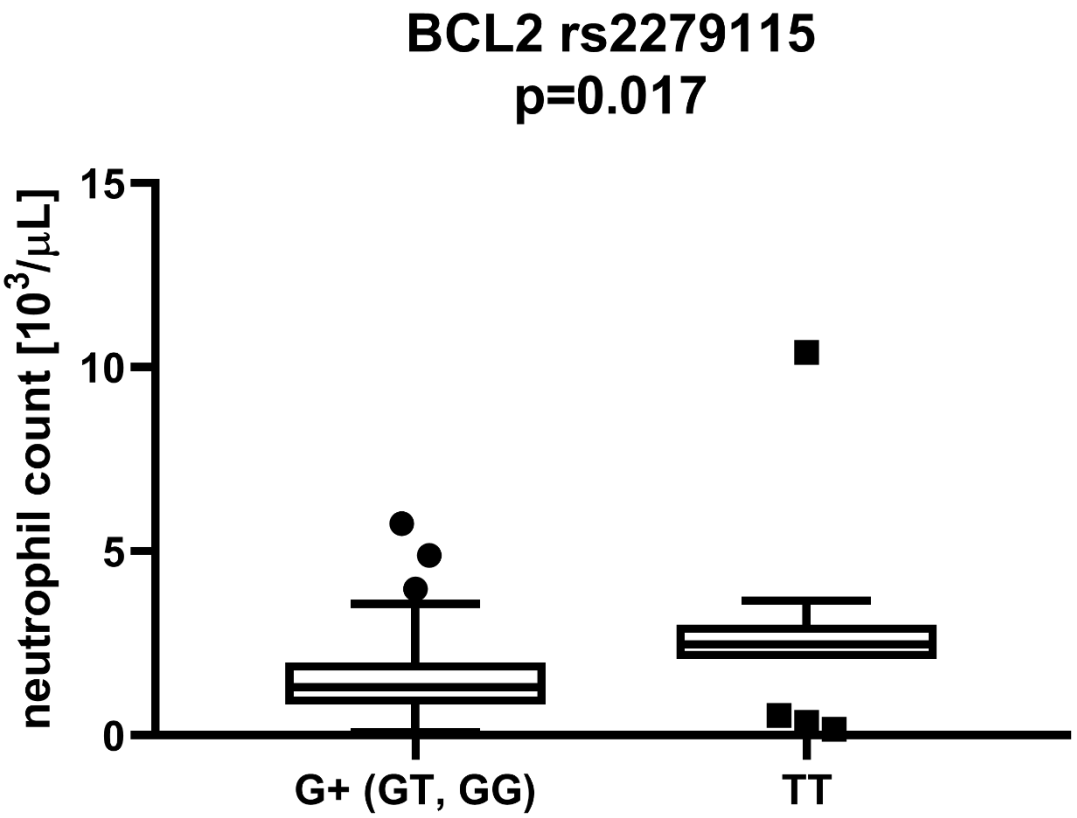

Figure 5. Association between presence of *BCL2* rs2279115 *G* and neutrophil count. Patients with allele *G* were characterized by lower neutrophil count

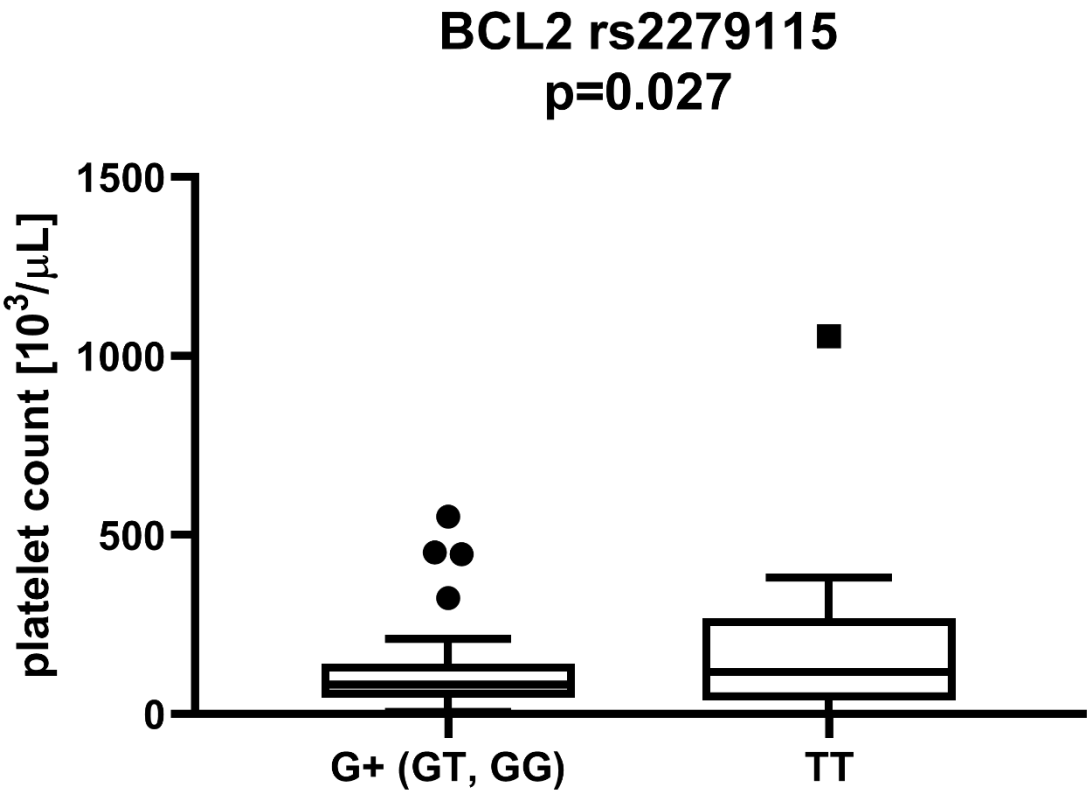

Figure 6. Association between presence of *BCL2* rs2279115 *G* and platelet count. Patients with allele *G* were characterized by lower platelet count

***PDCD1* and *CD274* gene polymorphisms in MDS patients**

Patients with higher leukocyte counts were more likely to have the *CD274* rs4143815 *C* allele than the *CD274* rs4143815 *GG* genotype ($p = 0.032$) (Fig.7).

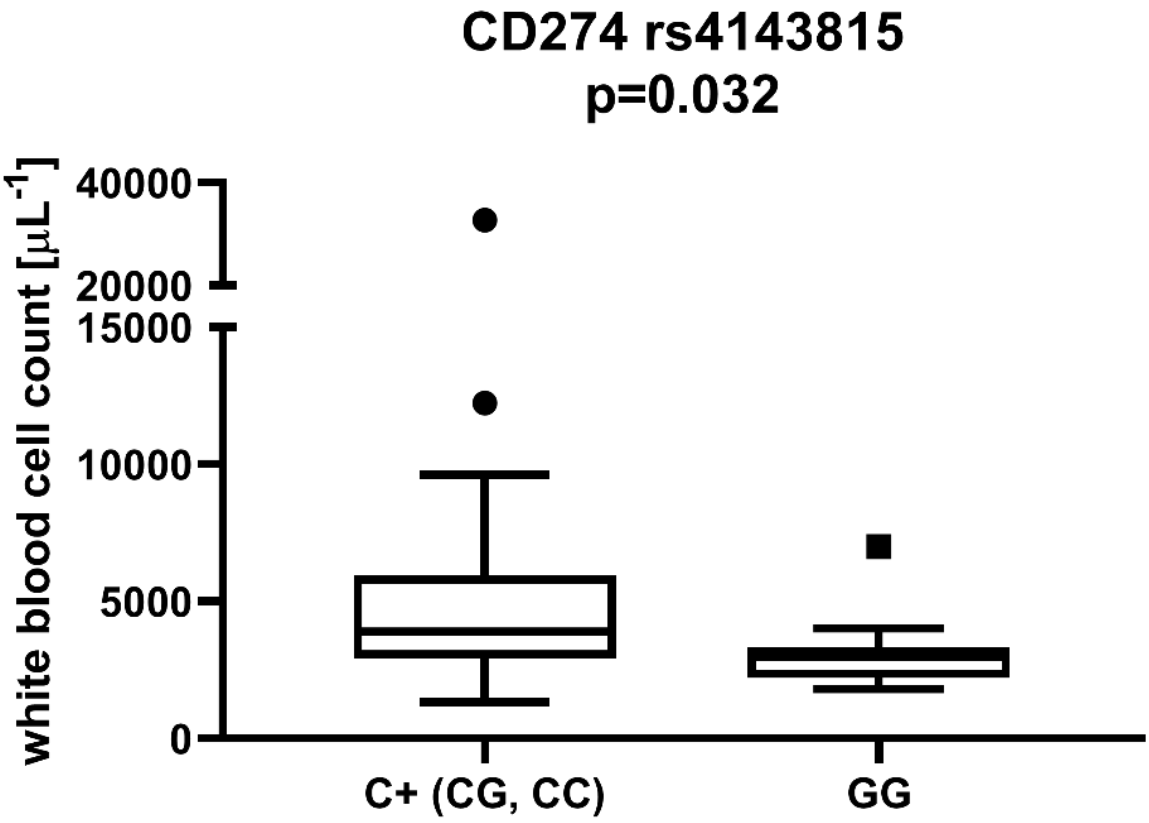

Figure 7. Association between presence of *CD274* rs4143815 *C* and leukocyte count. Patients with allele *C* were characterized by higher white blood count

In contrast, patients with the *CD274* rs4143815 *C* allele (*CG* and *CC* genotypes) showed higher neutrophil count compared to *CD274* rs4143815 *GG* (p = 0.046) (Figure 8). *CD274* rs4143815 *G* allele was associated with fewer monocytes than *CD274* rs4143815 *CC* (p = 0.048).

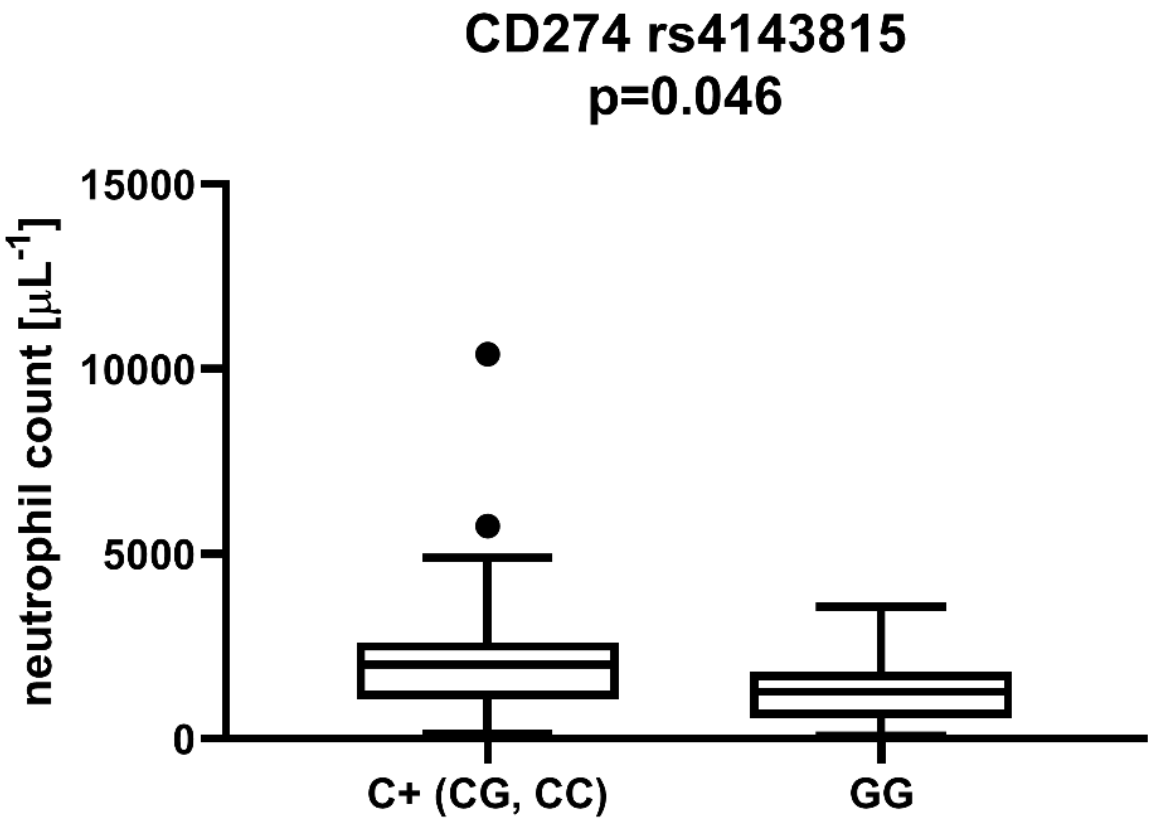

Figure 8. Association between presence of *CD274* rs4143815 *C* and neutrophil count. Patients with allele *C* were characterized by higher neutrophil count

There were no statistically significant (p >0.05) correlations between the polymorphisms studied and any of the following parameters: IPSS-R, blast cell percentage, hemoglobin, age, presence of infection, or Ogata score. Table II presents the relationship between BCL-2, PD-1, and PD-L1 gene polymorphisms and other variables.

Discussion

Over the past decade, our understanding of the pathogenesis of myelodysplastic neoplasms has expanded significantly. This has been facilitated by the rapid development of new diagnostic methods associated with advanced genetic testing technology. Recognizing new genetic variants of MDS in conjunction with well-established clinical factors such as age,

number of cytopenias, karyotype, and number of blasts allows for a personalized evaluation of patients with MDS. However, the effectiveness of available therapies for patients with MDS remains limited. Only around 40–50% of MDS patients respond to treatment with the hypomethylating drug azacitidine, and just 10–20% of patients reach CR [18]. The prognosis for patients following the failure of azacitidine treatment is poor, with a median survival of six months [19]. Researchers are seeking new prognostic factors, knowledge of which will enable the classification of patients into specific risk groups: those less responsive to therapy and those more rapidly progressing to acute myeloid leukemia (AML). Cytogenetic aberrations such as — 5q/5q, –7/7q, +8, 20q-, or 17p–, along with molecular abnormalities in the UTX, SF3B1, TP53, and RUNX1 genes, as well as epigenetic changes in DNMT3A, TET2, IDH1/2, or ASXL1, play a significant role in the pathogenesis of MDS [12, 20]. The role of abnormalities in the genes encoding the BCL-2 (BCL2), PD-1 (PDCD1), and PD-L1 (CD274) proteins in the etiology of MDS remains unclear.

Two opposing processes play a role in the pathogenesis of MDS. On the one hand, there is uncontrolled proliferation of blast cells, while on the other hand there is activation of apoptosis. The number of CD34+ cells undergoing apoptosis is higher in lower-risk MDS patients who have transformed to AML [21]. Increased expression of BCL-2 may be one of the causal factors in this process [22]. Vidal et al. conducted a retrospective analysis involving 70 patients with MDS who underwent hypomethylating treatment. They found that patients who did not respond to therapy exhibited significantly higher expression of BCL-2L10, another member of the BCL-2 family, compared to those who responded to treatment. Patients with elevated BCL-2L10 expression had a lower CR rate and a shorter median survival than the group with lower BCL-2L10 expression (9 months vs. 15.6 months) [23].

In the present study, we have demonstrated that the BCL2 rs1564483 C allele correlated with a higher response rate, regardless of the therapy used. Patients with the BCL2 rs1564483 C allele also experienced longer overall survival than those with the BCL2 rs1564483 TT genotype. BCL2 rs1564483 is an SNP located within a predicted miRNA-binding site in the 3' UTR region. Potential miRNAs that may bind to this site include miR-513c, miR-149, and miR-296-3p [24]. A previous study indicated that miR-296-3p is a likely candidate for binding at the rs1564483 site [25]. However, miR-149 also seems interesting in this context, as it has been shown to affect BCL-2 expression and drug sensitivity in AML cells [26]. To the best of our knowledge, rs1564483 has never been studied in MDS or any other hematological disorders. Studies on various tumors, including breast cancer, esophageal cancer, and

laryngeal squamous cell carcinoma, have shown no association between the SNP and the risk of disease [25, 27]. In contrast to our study, Yang et al. reported that allele rs1564483 *T* was associated with better survival in patients with lung cancer [28], while Jouneghani et al. showed that rs1564483 *CT* heterozygotes were more likely to develop gastric cancer [25]. These discrepancies may suggest tissue-specific expression of miRNAs potentially influenced by rs1564483.

Nevertheless, our results indicate that BCL2 rs1564483 could serve as a potential predictive marker for treatment efficacy in MDS patients, possibly paving the way for personalized therapy in the future.

In addition to the results previously discussed, we also found that the BCL2 rs2279115 G allele was more common in MDS patients with lower neutrophil counts, which may directly increase the risk of infection in this population. These findings may indicate the influence of polymorphisms within the BCL2 gene on sensitivity to the treatment administered, as well as the risk of complications during therapy. Interestingly, previous studies have linked allele G with reduced BCL2 expression, and allele T with increased expression [29–31]. Rs2279115 is situated in an inhibitory P2 promoter of the BCL2 gene, and increased activity of P2 is linked to reduced activity of the primary P1 promoter, which results in lower BCL2 expression [32]. Allele rs2279115 T and genotype TT have been identified as risk factors or markers for shorter survival in various cancers [33]. However, many other studies have indicated that allele rs2279115 G and genotype GG are risk factors [34]. Given the association of allele G with reduced BCL2 expression, these observations might be explained by the fact that, in addition to BCL-2's role as an oncogene, it has also been observed to function as a tumor suppressor [35].

A relationship between PD-1 and PD-L1 expression and disease progression has been described in various cancers [22]. However, their role in MDS has not been clearly defined. A combination of AZA and durvalumab, an anti-PD-L1 antibody, was investigated in high-risk MDS (HR-MDS) and AML as part of a randomized, open-label study. The HR-MDS cohort was divided into two arms, each consisting of 42 patients, with no significant differences in ORR or median OS between them. [36]. In MDS patients, increased PD-1 expression was confirmed on effector cells and Treg lymphocytes, while PD-L1 expression was confirmed on CD34+ myeloblasts, but only in patients with an increased blast cell count >5%. PD-L1 has also been shown to correlate with high IPSS-R [37]. In our current study, we did not find any association with the PD-1 (PDCD1) polymorphism; however, the PD-L1 (CD274) genotype

rs4143815 GG was found to be associated only with low neutrophil and white blood cell counts. Rs4143815 is located within a potential miRNA binding site in the 3' UTR region [24]. Earlier studies have identified miR-570 as the miRNA most likely affected by this SNP [38, 39], and allele rs4143815 *G* has been linked to lower PD-L1 expression [39, 40]. Interestingly, many previous studies found allele *G* to be a risk factor in various cancers or to negatively affect survival [38, 41], which appears to align with our results.

The presence of TP53 mutations can coincide with increased PD-L1 expression. Therefore, abnormalities in the PD-1/PD-L1 pathway are likely one of the mechanisms contributing to the poorer prognosis of patients with TP53 mutations [42]. According to the available literature, patients with high-risk MDS exhibit higher CD274 expression compared to those who have transformed to AML [42, 43]. In the group of patients we analyzed, only one patient with MDS had a TP53 mutation. This patient presented the following genotypes: BCL2 rs1564483 GA, BCL2 rs2279115 CA, CD274 rs2297136 GG, CD274 rs4143815 GG, PDCD1 rs10204525 GG, and PDCD1 rs2227981 CC. Compared to the entire study group, these genotypes are either the most common within a given polymorphism (BCL2 rs2279115 CA, PDCD1 rs10204525 GG, PDCD1 rs2227981 CC), or the second most common (BCL2 rs1564483 GA, CD274 rs2297136 GG, CD274 rs4143815 GG). The CD274 rs4143815 C allele, which was associated with higher leukocyte levels in most patients, was not present in the patient with the TP53 mutation. This patient underwent an allotransplantation procedure involving stem cells from an unrelated donor. Analyzing differences in the frequency of each genotype in relation to sex, we showed that the *CD274* rs2297136 *G* allele was more common in men than in women, and correlated with a lower monocyte count.

In conclusion, single nucleotide polymorphisms in the genes encoding BCL-2, PD-1, and PD-L1 can vary among individual MDS patients. Our study suggests that some of these could be potentially useful for evaluating patients with MDS. However, the findings we have presented here need confirmation in a larger population of MDS patients.

Table I. Clinical data of patients

Characteristic	
Age range, median [years]	45–91, 70
Hemoglobin, median [g/dl]	8.70 (5.0–12.2)
Leukocytes × 10^3/µl, median	3.31 (1.32–32.71)
Neutrophils × 10^3/ µl, median	1.59 (0.17–10.40)
Platelets × 10^3/ µl, median	103 (1.0–306)
Monocytes × 10^3/ µl, median	0,29 (0.4–2.74)
WHO 2022 classification	**Number of patients**
MDS-LB	30
MDS-5q	1
MDS-IB1	8
MDS-IB2	10
MDS-TP53	1
IPSS-R risk group	**Number of patients**
Low and very low	17
Intermediate	15
High	10
Very high	8
First line treatment	**Number of patients**
Azacitidine	31
Luspatercept	2
Lenalidomide	1
No treatment	16
Response after 1st line treatment	**Number of patients**
CRi	15
PRi	5
NR	14

CRi — complete remission with incomplete hematological recovery; IB1 — increased blasts type 1; IB2 — increased blasts type 2; IPSS-R — Revised International Prognostic Scoring System; LB — low blasts; MDS — myelodysplastic neoplasm; MDS-5q — myelodysplastic

neoplasm with isolated 5q deletion; MDS-IB1 — myelodysplastic neoplasm with increased blasts type 1; MDS-IB2 — myelodysplastic neoplasm with increased blasts type 2; MDS-LB — myelodysplastic neoplasm with low blasts; MDS-TP53 — myelodysplastic neoplasm with TP53 mutation; NR — no response; PRi — partial remission with incomplete hematological recovery; TP53 — tumor protein p53; WHO — World Health Organization

Table II. Relationship between BCL-2, PD-1, and PD-L1 gene polymorphisms and other variables

	BCL-2							**CD274**						**PDCD1**				
	rs1564483				rs2279115			rs2297136			rs4143815			rs102045 25		rs222798 1		
	G	G	A	A	C	C	A	G	G	A	G	C	C	GG	AG	C	T	T
	G	A	A	G	C	A	A	G	A	A	G	C	G			C	T	C
WBC	X	X	X	X	X	X	X	X	X	X	↑	↓	↓	X	X	X	X	X
ANC	X	X	X	X	↑	↑	↓	X	X	X	↑	↓	↓	X	X	X	X	X
AMC	X	X	X	X	X	X	X	X	X	X	↑	↓	↑	X	X	X	X	X
PLT	X	X	X	X	↑	↑	↓	X	X	X	X	X	X	X	X	X	X	X
GGTP	X	X	X	X	X	X	X	X	X	X	↓	↑	↑	X	X	X	X	X
Urea	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	↑	↓	↓
1st line CR	↓	↓	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
OS	↓	↓	↑	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
IPSS-R	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BLAST%	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HGB	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LYMP	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ALAT	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ASPAT	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Creatinine	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Uric acid	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CRP	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Na^+	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LDH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Age	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyt.mut	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mol.mut	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ogata score	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

X — no correlation, ↓ — negative correlation, ↑ — positive correlation

ALAT — alanine aminotransferase; AMC — absolute monocyte count; ANC — absolute neutrophil count; ASPAT — aspartate aminotransferase; BLAST% — percentage of blast cells; CRP — C-reactive protein; CRi — complete remission with incomplete hematological recovery; Cyt. mut. — cytogenetic mutation; FA — alkaline phosphatase; GGTP — gamma-glutamyl transpeptidase; HGB — hemoglobin; IB1 — increased blasts type 1; IB2 — increased blasts type 2; IPSS-R — Revised International Prognostic Scoring System; LDH — lactate dehydrogenase; LB — low blasts; Lymph% — percentage of lymphocytes; MDS — myelodysplastic neoplasm; MDS-5q — myelodysplastic neoplasm with isolated 5q deletion; MDS-IB1 — myelodysplastic neoplasm with increased blasts type 1; MDS-IB2 — myelodysplastic neoplasm with increased blasts type 2; MDS-LB — myelodysplastic neoplasm with low blasts; MDS-TP53 — myelodysplastic neoplasm with TP53 mutation; Mol. mut. — molecular mutation; Na^+ — sodium; NR — no response; OS — overall survival; PLT — platelets; PRi — partial remission with incomplete hematological recovery; Score — composite prognostic score; TP53 — tumor protein p53; WBC — white blood cells; WHO — World Health Organization

Article information and declarations

Data availability statement

The data that supports the findings of this study is available from the corresponding author, Bartłomiej Kuszczak, upon reasonable request.

Funding statement: This research was carried out under a grant from the National Science Center (Poland) at Wroclaw Medical University with the number MINI.C140.21.001.

Conflict of interest

The authors declare no conflict of interest.

Ethics statement

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was obtained from the ethics committee at Wroclaw Medical University.

Patient consent statement

Written informed consent was obtained from each study participant.

Authors' contributions

BK — data collection, analysis and interpretation; study conception and design; manuscript writing; final approval.
PŁ — experiments; data analysis and interpretation; figure preparation; final approval.
MD-K — experiments; final approval.
KB-K — provision of key reagents; data analysis and interpretation; study supervision; final approval.
TW — study supervision; final approval.
JR — data analysis and interpretation; study conception and supervision; final approval.

References

1. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol. 2020; 17(7): 395–417, doi: 10.1038/s41571-020-0341-y, indexed in Pubmed: 32203277.
2. Campbell KJ, Tait SWG. Targeting BCL-2 regulated apoptosis in cancer. Open Biol. 2018; 8(5), doi: 10.1098/rsob.180002, indexed in Pubmed: 29769323.
3. Kuszczak B, Wróbel T, Wicherska-Pawłowska K, et al. The Role of BCL-2 and PD-1/PD-L1 Pathway in Pathogenesis of Myelodysplastic Syndromes. Int J Mol Sci. 2023; 24(5), doi: 10.3390/ijms24054708, indexed in Pubmed: 36902139.

4. Buja LM. The cell theory and cellular pathology: Discovery, refinements and applications fundamental to advances in biology and medicine. Exp Mol Pathol. 2021; 121: 104660, doi: 10.1016/j.yexmp.2021.104660, indexed in Pubmed: 34116021.

5. Peng X, Shi J, Sun W, et al. Genetic polymorphisms of IL-6 promoter in cancer susceptibility and prognosis: a meta-analysis. Oncotarget. 2018; 9(15): 12351–12364, doi: 10.18632/oncotarget.24033, indexed in Pubmed: 29552316.

6. Zhang X, Weng W, Xu W, et al. Role of Bcl-2 -938 C>A polymorphism in susceptibility and prognosis of cancer: a meta-analysis. Sci Rep. 2014; 4: 7241, doi: 10.1038/srep07241, indexed in Pubmed: 25430556.

7. Okazaki T, Maeda A, Nishimura H, et al. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci U S A. 2001; 98(24): 13866–13871, doi: 10.1073/pnas.231486598, indexed in Pubmed: 11698646.

8. Salmaninejad A, Khoramshahi V, Azani A, et al. PD-1 and cancer: molecular mechanisms and polymorphisms. Immunogenetics. 2018; 70(2): 73–86, doi: 10.1007/s00251-017-1015-5, indexed in Pubmed: 28642997.

9. Hashemi M, Karami S, Sarabandi S, et al. Association between and Polymorphisms and the Risk of Cancer: A Meta-Analysis of Case-Control Studies. Cancers (Basel). 2019; 11(8), doi: 10.3390/cancers11081150, indexed in Pubmed: 31405171.

10. Mądry K, Machowicz R, Waszczuk-Gajda A, et al. Demographic, Hematologic, and Clinical Features of Myelodysplastic Syndrome Patients: Results from the First Polish Myelodysplastic Syndrome Registry. Acta Haematol. 2015; 134(2): 125–134, doi: 10.1159/000375149, indexed in Pubmed: 25925777.

11. Zeidan AM, Shallis RM, Wang R, et al. Epidemiology of myelodysplastic syndromes: Why characterizing the beast is a prerequisite to taming it. Blood Rev. 2019; 34: 1–15, doi: 10.1016/j.blre.2018.09.001, indexed in Pubmed: 30314642.

12. Papaemmanuil E, Gerstung M, Malcovati L, et al. Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013; 122(22): 3616–27; quiz 3699, doi: 10.1182/blood-2013-08-518886, indexed in Pubmed: 24030381.

13. Mądry K, Drozd-Sokołowska J, Lis K, et al. Diagnosis of myelodysplastic syndromes in Poland: Polish Adult Leukemia Group (PALG) 2021 recommendations. Acta Haematologica Polonica. 2022; 53(1): 3–18, doi: 10.5603/ahp.a2022.0001.

14. Zeidan AM, Platzbecker U, Bewersdorf JP, et al. Consensus proposal for revised International Working Group 2023 response criteria for higher-risk myelodysplastic syndromes. Blood. 2023; 141(17): 2047–2061, doi: 10.1182/blood.2022018604, indexed in Pubmed: 36724453.

15. Majcherek M, Kiernicka-Parulska J, Mierzwa A, et al. The diagnostic and prognostic significance of flow cytometric bone marrow assessment in myelodysplastic syndromes according to the European LeukemiaNet recommendations in single-centre real-life experience. Scand J Immunol. 2021; 94(2): e13028, doi: 10.1111/sji.13028, indexed in Pubmed: 33577137.

16. Zhang Y, Wu J, Qin T, et al. Comparison of the revised 4th (2016) and 5th (2022) editions of the World Health Organization classification of myelodysplastic neoplasms. Leukemia. 2022; 36(12): 2875–2882, doi: 10.1038/s41375-022-01718-7, indexed in Pubmed: 36224330.

17. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012; 120(12): 2454–2465, doi: 10.1182/blood-2012-03-420489, indexed in Pubmed: 22740453.

18. Visconte V, Tiu RV, Rogers HJ. Pathogenesis of myelodysplastic syndromes: an overview of molecular and non-molecular aspects of the disease. Blood Res. 2014; 49(4): 216–227, doi: 10.5045/br.2014.49.4.216, indexed in Pubmed: 25548754.

19. Mądry K, Budziszewska B, Lis K, et al. Treatment recommendations developed by MDS experts of the Polish Adult Leukemia Group (PALG) for management of myelodysplastic syndromes (MDSs) and other MDS-related conditions in Poland for 2021. Acta Haematologica Polonica. 2022; 53(2): 75–93, doi: 10.5603/ahp.a2022.0009.

20. Calvi LM, Li AJ, Becker MW. What is the role of the microenvironment in MDS? Best Pract Res Clin Haematol. 2019; 32(4): 101113, doi: 10.1016/j.beha.2019.101113, indexed in Pubmed: 31779976.

21. Boudard D, Vasselon C, Berthéas MF, et al. Expression and prognostic significance of Bcl-2 family proteins in myelodysplastic syndromes. Am J Hematol. 2002; 70(2): 115–125, doi: 10.1002/ajh.10108, indexed in Pubmed: 12111784.

22. Mittelman M, Oster HS, Hoffman M, et al. The lower risk MDS patient at risk of rapid progression. Leuk Res. 2010; 34(12): 1551–1555, doi: 10.1016/j.leukres.2010.05.023, indexed in Pubmed: 20573398.

23. Vidal V, Ginet C, Karsenti J, et al. BCL2L10 Quantification Is a Predictive Factor of Response to Azacitidine in Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML). Blood. 2014; 124(21): 3261–3261, doi: 10.1182/blood.v124.21.3261.3261.

24. Xu Z, Taylor JA. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res. 2009; 37(Web Server issue): W600–W605, doi: 10.1093/nar/gkp290, indexed in Pubmed: 19417063.

25. Azadeh Jouneghani M, Keshavarzi F, Haghnazari N, et al. The Investigation of the Association Between the 3'-UTR rs1564483 Polymorphism and miR-296-3p in the Development of Breast and Gastric Cancers. Clin Med Insights Oncol. 2023; 17: 11795549231207835, doi: 10.1177/11795549231207835, indexed in Pubmed: 37928451.

26. Chen Xi, Song Y, Tian Y, et al. miR-149-3p Enhances Drug Sensitivity of AML Cells by Inhibiting Warburg Effect Through PI3K/AKT Pathway. Cell Biochem Biophys. 2024; 82(4): 3287–3296, doi: 10.1007/s12013-024-01412-8, indexed in Pubmed: 39154128.

27. Pan W, Yang J, Wei J, et al. Functional BCL-2 regulatory genetic variants contribute to susceptibility of esophageal squamous cell carcinoma. Sci Rep. 2015; 5: 11833, doi: 10.1038/srep11833, indexed in Pubmed: 26132559.

28. Yang X, Gao F, Ma F, et al. Association of the functional BCL-2 rs2279115 genetic variant and small cell lung cancer. Tumour Biol. 2016; 37(2): 1693–1698, doi: 10.1007/s13277-015-3934-9, indexed in Pubmed: 26311051.

29. Renner W, Langsenlehner U, Krenn-Pilko S, et al. BCL2 genotypes and prostate cancer survival. Strahlenther Onkol. 2017; 193(6): 466–471, doi: 10.1007/s00066-017-1126-9, indexed in Pubmed: 28396899.

30. Kaderi MA, Norberg M, Murray F, et al. The BCL-2 promoter (-938C>A) polymorphism does not predict clinical outcome in chronic lymphocytic leukemia. Leukemia. 2008; 22(2): 339–343, doi: 10.1038/sj.leu.2405042, indexed in Pubmed: 18046447.

31. Bhushann Meka P, Jarjapu S, Vishwakarma SK, et al. Influence of BCL2-938 C>A promoter polymorphism and BCL2 gene expression on the progression of breast cancer. Tumour Biol. 2016; 37(5): 6905–6912, doi: 10.1007/s13277-015-4554-0, indexed in Pubmed: 26662799.

32. Seto M, Jaeger U, Hockett RD, et al. Alternative promoters and exons, somatic mutation and deregulation of the Bcl-2-Ig fusion gene in lymphoma. EMBO J. 1988; 7(1): 123–131, doi: 10.1002/j.1460-2075.1988.tb02791.x, indexed in Pubmed: 2834197.

33. Masago K, Togashi Y, Fujita S, et al. Effect of the BCL2 gene polymorphism on survival in advanced-stage non-small cell lung cancer patients who received chemotherapy. Oncology. 2013; 84(4): 214–218, doi: 10.1159/000342854, indexed in Pubmed: 23364242.

34. Hirata H, Hinoda Y, Nakajima K, et al. The bcl2 -938CC genotype has poor prognosis and lower survival in renal cancer. J Urol. 2009; 182(2): 721–727, doi: 10.1016/j.juro.2009.03.081, indexed in Pubmed: 19539330.

35. Hirata H, Hinoda Y, Nakajima K, et al. The bcl2 -938CC genotype has poor prognosis and lower survival in renal cancer. J Urol. 2009; 182(2): 721–727, doi: 10.1016/j.juro.2009.03.081, indexed in Pubmed: 19539330.

36. Bieliński K, Puła B, Bołkun Ł. Checkpoint inhibitors as potential therapeutics in acute myeloid leukemia. Acta Haematol Pol. 2024; 55(6): 343–353, doi: 10.5603/ahp.102572.

37. Kondo A, Yamashita T, Tamura H, et al. Interferon-gamma and tumor necrosis factor-alpha induce an immunoinhibitory molecule, B7-H1, via nuclear factor-kappaB

activation in blasts in myelodysplastic syndromes. Blood. 2010; 116(7): 1124–1131, doi: 10.1182/blood-2009-12-255125, indexed in Pubmed: 20472834.

38. Wang W, Li F, Mao Y, et al. A miR-570 binding site polymorphism in the B7-H1 gene is associated with the risk of gastric adenocarcinoma. Hum Genet. 2013; 132(6): 641–648, doi: 10.1007/s00439-013-1275-6, indexed in Pubmed: 23430453.

39. Lee SY, Jung DK, Choi JE, et al. Functional polymorphisms in PD-L1 gene are associated with the prognosis of patients with early stage non-small cell lung cancer. Gene. 2017; 599: 28–35, doi: 10.1016/j.gene.2016.11.007, indexed in Pubmed: 27838455.

40. Ohhara Y, Tomaru U, Kinoshita I, et al. Polymorphisms of the PD-L1 gene 3'-untranslated region are associated with the expression of PD-L1 in non-small cell lung cancer. Genes Chromosomes Cancer. 2024; 63(1): e23216, doi: 10.1002/gcc.23216, indexed in Pubmed: 38169142.

41. Tan D, Sheng Li, Yi QH. Correlation of PD-1/PD-L1 polymorphisms and expressions with clinicopathologic features and prognosis of ovarian cancer. Cancer Biomark. 2018; 21(2): 287–297, doi: 10.3233/CBM-170357, indexed in Pubmed: 29171986.

42. Sallman DA, McLemore AF, Aldrich AL, et al. TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. Blood. 2020; 136(24): 2812–2823, doi: 10.1182/blood.2020006158, indexed in Pubmed: 32730593.

43. Tcvetkov N, Morozova E, Epifanovskaya O, et al. Profile of Checkpoint Molecules Expression on Bone Marrow Cell Populations in Patients with High-Risk Myelodysplastic Syndrome. Blood. 2020; 136(Supplement 1): 43–44, doi: 10.1182/blood-2020-141997.