 doi:10.20944/preprints202207.0033.v1

Article

A Circulating Serum Mirna-Based Model to Predict Early Mortality in Multiple Myeloma Patients Treated with Bortezomib-Based Regimens

Anna Puła[1,2,†], Paweł Robak[2,3,†], Dariusz Jarych[4,†], Damian Mikulski[2,5], Małgorzata Misiewicz[1,6], Izabela Drozdz[7], Wojciech Fendler[5], Janusz Szemraj[8] and Tadeusz Robak[1,6*]

[1]Department of Hematology, Medical University of Lodz, 93-510 Lodz, Poland; robaktad@csk.umed.lodz.pl (T.R.);anna.pula@ume.lodz.pl (A.P.); malgorzata.misiewicz@umed.lodz.pl (M.M.)

[2] Department of Hematooncology, Copernicus Memorial Hospital, 93-510 Lodz, Poland;

[3]Department of Experimental Hematology, Medical University of Lodz, 93-510 Lodz, Poland; pawel.robak@umed.lodz.pl (P.R.);

[4]Laboratory of Virology, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland; djarych@cbm.pan.pl (D.J.);

[5]Department of Biostatistics and Translational Medicine, Medical University of Lodz, 92-215 Lodz, Poland; damian.mikulski@stud.umed.lodz.pl(D.M.); wojciech_fendler@dfci.harvard.edu (W.F.);

[6] Department of General Hematology, Copernicus Memorial Hospital, 93-510 Lodz, Poland.

[7]Department of Clinical Genetics, Medical University of Lodz, 92-213 Lodz, Poland; izabela.drozdz@umed.lodz.pl (I.D.);

[8]Department of Medical Biochemistry, Medical University of Lodz, 92-215 Lodz, Poland; janusz.szemraj@umed.lodz.pl (J.S.);

[*]Author to whom correspondence should be addressed.

[†] These authors contributed equally.

Abstract: Multiple myeloma (MM) is a hematological malignancy characterized by the clonal proliferation of plasma cells in the bone marrow (BM) microenvironment. Despite the progress made in treatment, some MM patients still die within the first year of diagnosis. Numerous studies investigating microRNA (miRNA) expression patterns suggest they may be good prognostic markers. The primary aim of this study was to analyze the expression of selected miRNAs in the serum of MM patients subsequently treated with bortezomib-based regimens and determine their potential to predict early mortality. The study was conducted in 69 prospectively-recruited patients with newly-diagnosed MM admitted to the Department of Hematology of the Copernicus Memorial Hospital, Lodz (Poland) between 2017 and 2021. Among them, 17 patients experienced death within 12 months of diagnosis. The expression of 31 selected miRNAs was determined using miRCURY LNA miRNA Custom PCR Panel. The obtained clinical data included patient characteristics on diagnosis, treatment regimen, response to treatment, and follow-up. Differential expression analysis found two miRNAs to be significantly downregulated in the early mortality group: hsa-miR-328-3p (fold change- FC: 0.72, p=0.0342) and hsa-miR-409-3p (FC: 0.49, p=0.0357). Univariate and multivariate logistic regression analyses were performed to assess the early mortality rate. The final model consisted of hsa-miR-409-3p, hsa-miR-328-3p, age and R-ISS 3. It yielded an area under the curve (AUC) of 0.863 (95%CI: 0.761-0.965) with 88.2% sensitivity and 77.5% specificity. Further external validation of our model is necessary to confirm its clinical value.

Keywords: multiple myeloma; early mortality; blood plasma; circulating miRNA; hematological malignancies; molecular biomarker; multiparametric model; prognosissurvival

 doi:10.20944/preprints202207.0033.v1

1. Introduction

Multiple Myeloma (MM) is a malignant, heterogenous disease characterized by the clonal expansion of antibody-producing plasma cells, mostly with their origin in the bone marrow. In addition, symptomatic MM is associated with hypercalcemia, renal impairment, anemia, lytic destruction of bones (the 'CRAB' criteria).[1] MM is the second most common hematological malignancy, accounting for about 10% of hematological cancers, with an annual incidence of 4.5-6 cases per 100,000 people [2] and a standardized rate of 6.1 per 100,000 inhabitants for male patients and 3.8 for female patients. In the US, it was estimated that there were 34,920 new MM cases and 12,410 deaths in 2021.[3] The advent of novel agents, such as immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs) and monoclonal antibodies (MoAbs), have markedly improved patient outcome.[4,5] However, MM is still an incurable disease with a median overall survival (OS) of approximately five years.[6]

Despite significant improvements in MM treatment modalities, a subset of patients still experiences abbreviated responses and short survival.[5,7] Treatment is still burdened by high patient mortality early in the course of disease, which is often attributed to combined effects of active disease and comorbid factors.[8,9] Early mortality (EM) is often defined as death within two to twelve months of diagnosis.[10–13] In some studies, cut-offs of two or six months are also used.[8,9,14–18] It is estimated that 4% to 25% of MM patients die within one year of diagnosis, depending on the clinical trial.[19–25]

To maximize treatment outcomes, it is important that patients receive optimal treatment depending on the disease characteristics and patient status. An increasing number of studies underline the role of miRNAs in the pathogenesis of MM and their potential role in its diagnosis and prognosis.[26,27] It was previously found that the expression of several micro RNAs (miRNAs), such as miR-15a and miR-16, is markedly decreased in MM patients while miR-21 and miR-221 are strongly increased. In addition, several miRNAs, are associated with drug resistance, and the miRNA expression patterns can be used as prognostic markers. Papanota et al propose a miRNA signature to facilitate MM bone disease diagnosis and provided evidence of the prognostic role of let-7b-5p and miR-335-5p as non-invasive prognostic biomarkers in MM.[28] However, no study has yet fully examined the types of miRNA present in MM patients experiencing early death. Identifying the miRNAs which are linked to early mortality may distinguish very-high-risk patients and improve proper treatment selection. Therefore, the present study analyzes selected serum miRNA expression patterns in the serum of newly diagnosed MM patients subsequently treated with bortezomib-based regimens.

2. Materials and Methods

2.1. Patients and treatment.

The study was performed in previously-untreated MM patients who were qualified for treatment with bortezomib-based regimens based on the decision of the medical board. Sets of clinical and laboratory data were obtained: patient characteristics on diagnosis, treatment regimen, response to treatment and follow-up. For inclusion in the study, the patients needed to have newly-diagnosed, untreated multiple myeloma and measurable disease confirmed by the presence of monoclonal protein in blood or Bence-Jones proteinuria. Patients were treated at the Copernicus Memorial Hospital, Lodz, Poland in years 2017-2021. Responses to treatment, progression free survival (PFS) and overall survival (OS) were assessed according the International Myeloma Working Group (IMWG) criteria.[29,30] Early mortality was defined as death within one year of diagnosis. The study and experimental protocol were conducted according to good clinical and

laboratory practice rules and the principles of the Declaration of Helsinki. All procedures were approved by the local ethical committee (The Ethical Committee of the Medical University of Lodz, No RNN/103/16/KE). All patients included in the study gave written informed consent for all examinations and procedures.

2.2. Isolation of miRNA

Venous blood samples were collected from previously-untreated MM patients in serum separating tubes. The samples were processed within two hours of collection by centrifugation at 2400× g for 10 min, as described previously.[31] Serum samples were stored at −80 ◦C. Isolation of cell-free total RNA, including miRNA, was performed from serum using the miRNeasy Serum/Plasma Advanced Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Briefly, 200 µl of serum was mixed with Buffer RPL to ensure a complete lysis and to release and stabilize RNA from plasma proteins and extracellular vesicles. To allow for normalization of sample-to-sample variation in RNA isolation and to control the quality of RNA isolation, before purification, each serum sample was spiked with 22 nt synthetic miRNAs added to the RPL Buffer, using the RNA Spike-In Kit, For RT (Qiagen), according to the manufacturer's protocol. Briefly, each sample was spiked with UniSp2 (2 fmol), UniSp4 (0.02 fmol), UniSp5 (0.00002 fmol), each at 100-fold reductions in concentration. The sample was then mixed with Buffer RPP to precipitate proteins and inhibitors and then centrifuged. Isopropanol was added to the supernatant to provide the appropriate conditions for RNA molecules (>18 nucleotides) to bind to the silica membrane. The sample was then applied to the RNeasy UCP MinElute spin column, where RNA binds to the membrane and other contaminants are washed away in subsequent wash steps. In the final step, total RNA (>18 nucleotides) was eluted using 20 µl of RNase-free water. RNA quality was determined with Agilent High Sensitivity RNA ScreenTape using 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA). Directly after the isolation, RNA was subjected to reverse transcription.

2.3. Reverse transcriptase reaction

cDNA was synthesized from the obtained mRNA by reverse transcription using the miRCURY LNA Reverse Transcription Kit (Qiagen), according to the guidelines provided by the manufacturer. Mature miRNAs were polyadenylated by poly(A) polymerase and reverse transcribed into cDNA using oligo-dT primers. Polyadenylation and reverse transcription were performed in parallel in the same tube. The oligo-dT primers have a 30-degenerate anchor that allows amplification of mature miRNA in the real-time PCR step. 2 µL of the isolate was added to the reaction tube containing 2 µl of 5× miRCURY RT Reaction Buffer, 1 µl of 10x miRCURY RT Enzyme Mix and filled with RNase-free water to make up a final volume of 10 µl reaction mix. Two synthetic miRNAs: UniSp6 (0.075 fmol) and cel-miR-39-3p (0.001 fmol) were used as a positive control for cDNA synthesis. The reaction took place at 42 °C for 60 min, followed by inactivation at 95 °C for 5 min using a TProfessional thermal cycler (Biometra, Analytik Jena, Jena, Germany). The cDNA was stored at -20 °C until further use.

2.4. Determination of microRNA expression

Before the miRNA expression analysis, the RNA samples were subjected to quality control using the miRCURY LNA miRNA QC PCR Panel (Qiagen), according to the manufacturer's protocol. The Panel contains 12 predefined assays for miRNAs that are expressed in a wide range of sample types used for evaluating samples in a biological context (data not shown). Two of the miRNAs in the panel (miR-451 and miR-23a) were used to evaluate the level of hemolysis in serum samples. If the ΔCq (miR-23a – miR-451) is close to or higher than 7, there was an increased risk that the samples are affected by

 doi:10.20944/preprints202207.0033.v1

hemolysis, and these samples were rejected from the further analysis (in case of high levels of hemolysis, miRNAs from red blood cells will make a significant contribution to the overall miRNA expression).

The expression of miRNAs was determined in 69 patients using miRCURY LNA miRNA Custom PCR Panel (Qiagen). The selection of miRNAs included in this study was performed based on our previous analysis of 752 miRNAs and was based on the p value from the univariate logistic regression analysis for overall survival.[31] The miRNA expression was performed for 31 miRNAs in duplicate. The list of selected miRNAs can be found in Supplementary Table 1.

2.5.Real-Time PCR (qPCR)

A premix of 3 µL of cDNA template (diluted 1:30), 5 µl of 2X miRCURY LNA SYBR Green PCR Master Mix and filled with RNase-free water to the final volume of 10 µl, was aliquoted into the PCR plate. Real-time PCR was performed on a LightCycler480 II Real-Time PCR System (Roche, Pleasanton, CA, USA). The reaction was performed at 95 °C for 2 min, followed by 55 amplification cycles at 95 °C for 10 s and 56 °C for 1 min. Fluorescence signal (SYBR Green I assays) was measured after each cycle. Melting curve analysis was performed after each qPCR to assess the specificity of the amplification (data not shown). Absolute quantification of miRNA was determined using the LightCycler® 480 Software, Version 1.5. Relative quantification of miRNA was determined by comparative Ct method.

2.6. Statistical Analysis

The data normalization was performed by using the mean expression value of three miRNAs in a given sample (miR-23b-3p, miR-151a-5p and miR-152-3p) as in our previous study.[31] The formula used to calculate the normalized Ct values was: Normalized ΔCt = mean Ct of miR-23b-3p, miR-151a-5p and miR-152-3p—Ct miRNA.

Study group description and intragroup association were conducted based on assumption testing using the Chi2 test. The normality of the distribution of continuous variables was verified with the Shapiro-Wilk test. The difference between two groups for continuous variables was evaluated using a two-sided independent Student's t-test when the data was normally distributed, and with the Mann-Whitney U-test when it was not, or if the variable was ordinal.

For a more comprehensive analysis, a logistic regression model was generated. The early mortality was used as outcome and the clinical features together with miRNAs expression as predictors. Student's t-test was used to preselect variables for the development of the classification model. miRNAs with FDR < 0.2 were chosen as candidate variables in this step. Both a univariate model for each of the selected miRNA and a multivariate model that included all selected predictors were estimated. Forward stepwise and backward stepwise selection approaches were used to restrict the model. The lowest AIC (The Akaike information criterion) value was chosen as the determinant of the best final model. Receiver operating characteristics (ROC) and area under the curve (AUC) analysis were performed to determine the predictive power of the model and its ability to accurately predict early mortality. The goodness of fit of the model was tested with the Hosmer-Lemeshow statistic, where high p values indicate a good fit.

All statistical analyses were conducted using Statistica Version 13.1 (TIBCO, Palo Alto, CA, USA) and R programming language (version 4.1.3). The OmicSelector R package was used partly used in the analysis.[55] P values lower than 0.05 were considered statisti-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0033.v1

cally significant. As all the analyses were preplanned, no correction for multiple comparisons was applied.

3. Results

The study was performed in 69 previously-untreated MM patients with a mean age at diagnosis of 64.84 ± 10.97 including 34 men and 35 women. Among them, 17 patients experienced death within 12 months of diagnosis. Demographic, clinical and laboratory characteristics of the 69 MM patients enrolled for the study can be found in Table 1.

Table 1. Patient characteristics. LCD—light chain disease; R-ISS – Revised International Staging System. # - Pearson's Chi-squared test, *- Fisher's Exact Test

Characteristic (n=69)		**No. (%)**			
		All	Early mortality	Control	P-value
Age, years, mean (SD)		64.84 (10.97)	72.61 (7.81)	62.3 (10.7)	**0.0005**
Sex					
	Male, n (%)	34 (49)	12 (17.4)	22 (31.9)	
	Female, n (%)	35 (51)	5 (7.2)	30 (43.5)	**0.04**#
R-ISS					
	Stage I, n (%)	3 (2.9)	1 (1.8)	2 (3.5)	
	Stage II, n (%)	34 (50.7)	9 (15.8)	25 (43.9)	
	Stage III, n (%)	20 (29)	7 (12.3)	13 (22.8)	0.8*
Type					
	IgA kappa, n (%)	10 (14.5)	2 (2.9)	8 (11.6)	
	IgA lambda, n (%)	5 (7.2)	0 (0)	5 (7.2)	
	IgG kappa, n (%)	28 (40.6)	7 (10.1)	21 (30.4)	
	IgG lambda, n (%)	13 (18.8)	5 (7.2)	8 (11.6)	
	LCD kappa, n (%)	6 (8.7)	2 (2.9)	4 (5.8)	
	LCD lambda, n (%)	7 (10.2)	1 (1.4)	6 (8.7)	0.68*
Cytogenetics					
	del17p, n (%)	6 (8.7)	2 (3.4)	4 (6.8)	1.0*
	t(4;14), n (%)	3 (4.3)	0 (0)	3 (5.2)	0.55*
	t(14;16) , n (%)	2 (2.9)	0 (0)	2 (3.4)	1.0*
	t(14;20) , n (%)	0 (0)	0 (0)	0 (0)	-
	t(11;14), n (%)	5 (7.2)	3 (5.6)	2 (3.7)	0.15*
	del1p, n (%)	5 (7.2)	2 (3.6)	3 (5.4)	0.62*
	amp1q, n (%)	25 (36.2)	7 (12.3)	18 (31.6)	0.99#
	del13q, n (%)	8 (11.6)	3 (10)	5 (16.7)	1.0*
Anemia at diagnosis, n (%)		30 (43.5)	8 (11.6)	22 (31.9)	0.73#
Bone disease, n (%)		50 (72.5)	13 (19.7)	37 (56.1)	0.74*
Creatinine > 2 mg/dl at diagnosis, n (%)		13 (18.8)	4 (5.8)	9 (13)	0.72*
Hypercalcemia at diagnosis, n (%)		23 (33.3)	7 (10.1)	16 (23.2)	0.43#

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 July 2022 doi:10.20944/preprints202207.0033.v1

Patients with early mortality were significantly older (72.61 vs. 62.3 years, p=0.0005) and were more frequently men (p=0.04). No differences in R-ISS distribution or CRAB symptoms were found between the groups. Similarly, no significant trends in percentage of myeloma infiltration in the bone marrow were noted with regard to laboratory results, including LDH, serum M protein, albumin, CRP and uric acid.

In differential expression analysis, two miRNAs were significantly downregulated in the early mortality group- hsa-miR-328-3p (fold change- FC: 0.72, p=0.0342) and hsa-miR-409-3p (FC: 0.49, p=0.0357). A volcano plot of miRNAs with significant differences in expression is shown in Figure 1. The results of differential expression analysis for all miRNAs can be found in Supplementary Table 2.

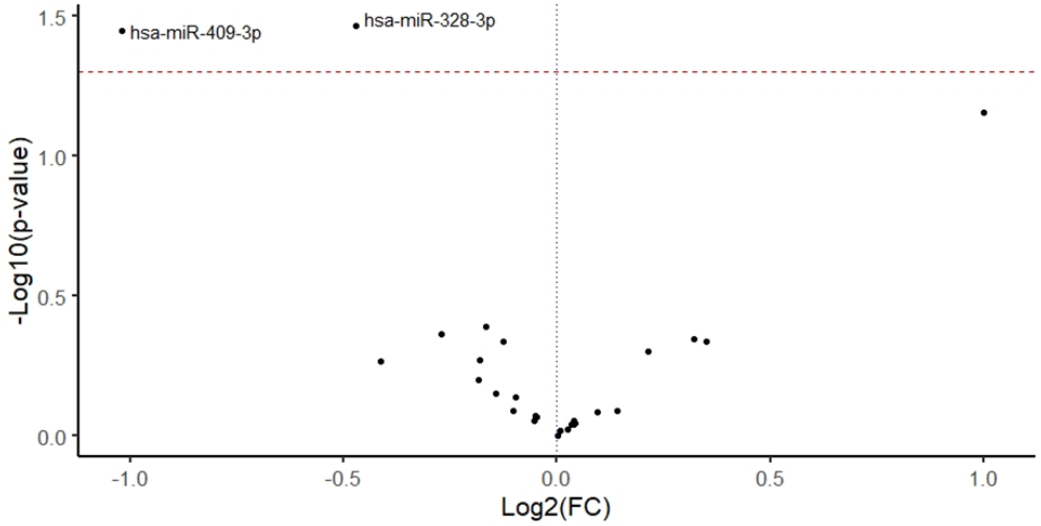

Figure 1. Volcano plot of all 28 miRNAs. Significant miRNAs have been labeled.

Univariate logistic regression analyses were performed to assess the early mortality occurrence. Similarly, hsa-miR-328-3p (OR 0.44, 95%CI: 0.20-0.97, p=0.0415) and hsa-miR-409-3p (OR 0.69, 95%CI: 0.48-0.98, p=0.0400) were acting protectively in relation to the occurrence of early mortality. The results of univariate logistic regression analyses for all miRNAs can be found in Supplementary Table 3.

A multivariate logistic regression analysis was also performed. A forward stepwise and backward stepwise selection approach were used to restrict the model to the most predictive miRNAs together with clinical features. Forward stepwise selection yielded a final model with the lowest AIC value. In the multivariate logistic regression analysis, the two miRNAs retained their significance against the combined established clinical prognostic factors. The final model (Table 2) consisted of hsa-miR-409-3p (OR 0.61, 95%CI: 0.37-0.99, p=0.0480), hsa-miR-328-3p (OR 0.33, 95%CI: 0.13-0.87, p=0.0254), age (OR 1.13, 95%CI: 1.03-1.23, p=0.0096) and R-ISS 3 (OR 2.91, 95%CI: 0.63-13.47, p=0.1723). The combination of hsa-miR-409-3p, hsa-miR-328-3p, age at diagnosis and R-ISS 3 could discriminate patients with high risk of early mortality.

Table 2. Final multivariate regression model for predicting early mortality in MM patients.

Variable	OR	95%CI	P

 doi:10.20944/preprints202207.0033.v1

		lower	upper	
hsa-miR-409-3p	0.607	0.370	0.996	0.0480
hsa-miR-328-3p	0.331	0.126	0.873	0.0254
Age	1.128	1.030	1.235	0.0096
R-ISS 3	2.908	0.628	13.467	0.1723

Area under the receiver operating characteristics (ROC) analysis for the model yielded an area under the curve (AUC) of 0.863 (95%CI: 0.761-0.965). At the optimal cut-off value of 0.28, the sensitivity and specificity reached 88.2% and 77.5%, respectively. The ROC curve is shown in Figure 2.

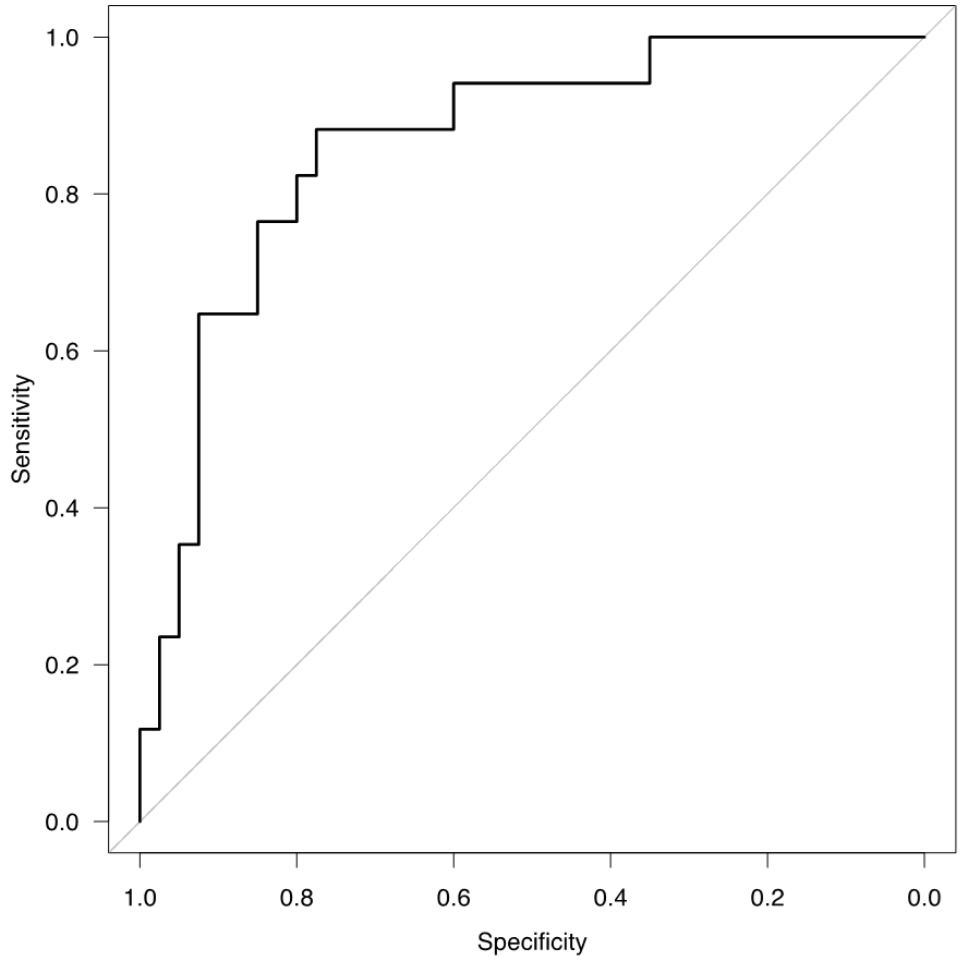

Figure 2. ROC curve of the final model for assessing factors associated with early mortality in MM patients.

4. Discussion

Our findings from this cohort study demonstrate that a combination of certain miRNA levels together with selected clinical factors may predict the occurrence of early mortality in newly-diagnosed MM patients. This is the first study to evaluate the potential of miRNA expression as a predictor of early death.

Despite spectacular advancements in therapy, early mortality remains a significant problem in MM patient care. Clinical trials have found between 4% and 25% of MM patients to die within one year of diagnosis.[19–25] Despite this, data on early mortality (EM) outside the context of clinical trials remain extremely scarce.

Fortunately, the mortality rate during the first year after diagnosis has reduced considerably over the years.[13] The most common causes of early death are reportedly infections, progression of MM, cardiovascular diseases and renal failure.[9,32] The phenomenon of EM is often attributed to combined effects of active disease and comorbid factors.[33] However, various clinical features such as beta 2-microglobulin, serum lactate dehydrogenase level, performance status, age at diagnosis, ISS, history of cardiovascular diseases or diabetes mellitus, presence of bone disease, diminished renal function have been associated with EM by various studies. [8,32,34]

To date only two approaches have been accepted in the prognosis of MM: the International Staging System (ISS) which combines albumin and β2-microglobulin levels, and the presence of chromosomal abnormalities detected via FISH.[6,35] However, it is not clear if the prognostic factors identified in the era of older drugs are still of value in the current era of novel therapies. One new approach for predicting clinical outcome is circulating serum miRNA expression. Manier et al. found that let-7b and miR-18a are significantly associated with both progression-free survival (PFS) and overall survival (OS) in univariate analysis, and remain statistically significant after adjusting for the ISS and adverse cytogenetics in multivariate analysis.[36] In a study by Hao et al. the downregulation of miR-19a was linked to significantly decreased PFS and OS.[37] miR-125b-5p may be another potential clinical biomarker for MM associated with significantly shorter event-free survival (EFS) in myeloma patients.[38] Xu et al. conducted a systematic review and meta-analysis in order to identify the prognostic value of miRNAs in patients with MM. They revealed that the upregulation of miR-92a and downregulation of miR-16, miR-25, miR-744, miR-15a, let-7e, and miR-19b expression were associated with poor prognosis.[39]

Following on from our previous study examining the value of serum miRNA expression signature in predicting refractoriness to bortezomib-based therapy in multiple myeloma patients [31], the present work attempts to determine whether selected miRNAs can have predictive value regarding early mortality. Both hsa-miR-409-3p and hsa-miR-328-3p have already been investigated in various cancers. miR-409 dysregulation has been detected in many neoplasms, including gastric cancer, prostate cancer, bladder cancer and lung adenocarcinoma.[40–44] miR-409-3p reportedly regulates cell proliferation and invasion by targeting zinc-finger E-box-binding homeobox 1 (ZEB1). Overexpression of miR-409-3p inhibits cellular proliferation and was found to suppress cellular migration and invasion *in vitro* and *in vivo* in breast cancer and osteosarcoma.[45,46] It may also potentially have a tumor suppressor function in cervical malignancies by regulating the HPV16/18-E6 mRNA levels.[47] Downregulation of miRNA-409-3p promotes aggressiveness and metastasis in colorectal cancer, while its overexpression sensitizes cells to oxaliplatin by inhibiting Beclin-1-mediated autophagy.[48,49] It was reported that miRNA-328 may decrease chemoresistance in glioblastoma cancer cells and breast cancer cells by down-regulating the ABCG2 gene.[50,51] Downregulation of miR-328-3p was observed in colorectal cancer, while its overexpression reversed the process of rug resistance and inhibited cell invasion.[52] In contrast, studies investigating the role of miR-328-3p in head and neck squamous cell carcinoma and in ovarian cancer found its overexpression to be associated with a more invasive disease.[53,54]

Our findings demonstrated that serum expression of hsa-miR-409-3p and hsa-miR-328-3p is downregulated in patients experiencing early mortality. These results

were used to generate a multiple regression model that may have the potential to predict EM. By extending known prognostic systems with more comprehensive molecular data, such as miRNA expression, it may be possible to increase the chance of identifying high-risk patients.

Our main study limitations are that the group of participants was relatively small, considering the whole MM population, and the fact that only selected miRNAs were tested. Hence, further external validation of our model is necessary to confirm its clinical value.

5. Conclusions

Two miRNAs, hsa-miR-409-3p and hsa-miR-328-3p, are independent factors related to early mortality in MM patients. Our results were used to generate a multiple regression model that may have the potential to predict early mortality. Further study is needed to confirm our findings.

Acknowledgment: We thank Edward Lowczowski from the Medical University of Lodz for editorial assistance.

Author Contributions: Conceptualization: A.P., P.R, D.J., D.M., T.R.; methodology: A.P. P.R., D.J., D.M.; formal analysis: A.P., D.M.; investigation: A.P., P.R.,D.J. ; resources: A.P., P.R., D.J., D.M.; data collection: A.P., P.R., D.J., M.M.; writing—original draft preparation: A.P., D.J.; writing—review and editing: T.R., W.F.; visualization: A.P., D.M.; supervision: T.R., W.F., I.D., J.S.; project administration: P.R., T.R., I.D., J.S.; funding acquisition: A.P., P.R., T.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the NCN (2016/23/B/NZ5/02529) and from the Polish Ministry of Science and Higher Education (MNiSW 0085/DIA/2018/47).

Declaration of interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Institutional Review Board Statement: This study was approved by the Ethical Committee of the Medical University of Lodz (No RNN/103/16/KE).

Informed Consent Statement: Informed consent was obtained from the patients included in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author on request.

Conflict of Interest: All authors declare no conflict of interest.

References

1. Palumbo, A.; Anderson, K. Multiple Myeloma. N. Engl. J. Med. 2011, 364, 1046–1060.
2. Becker, N. Epidemiology of Multiple Myeloma. Recent Results Cancer Res. 2011, 183, 25–35.
3. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2021. CA. Cancer J. Clin. 2021, 71, 7–33, doi:10.3322/CAAC.21654.
4. Fonseca, R.; Abouzaid, S.; Bonafede, M.; Cai, Q.; Parikh, K.; Cosler, L.; Richardson, P. Trends in Overall Survival and Costs of Multiple Myeloma, 2000-2014. Leukemia 2017, 31, 1915–1921, doi:10.1038/LEU.2016.380.
5. Robak, P.; Robak, T. Bortezomib for the Treatment of Hematologic Malignancies: 15 Years Later. Drugs R. D. 2019, 19, 73–92, doi:10.1007/S40268-019-0269-9.
6. Palumbo, A.; Avet-Loiseau, H.; Oliva, S.; Lokhorst, H.M.; Goldschmidt, H.; Rosinol, L.; Richardson, P.; Caltagirone, S.; Lahuerta, J.J.; Facon, T.; et al. Revised International Staging System for Multiple Myeloma: A Report from International Myeloma Working Group. J. Clin. Oncol. 2015, 33, 2863–2869, doi:10.1200/JCO.2015.61.2267.

7. Costa, L.J.; Usmani, S.Z. Defining and Managing High-Risk Multiple Myeloma: Current Concepts. J. Natl. Compr. Canc. Netw. 2020, 18, 1730–1737, doi:10.6004/JNCCN.2020.7673.
8. Augustson, B.M.; Begum, G.; Dunn, J.A.; Barth, N.J.; Davies, F.; Morgan, G.; Behrens, J.; Smith, A.; Child, J.A.; Drayson, M.T. Early Mortality after Diagnosis of Multiple Myeloma: Analysis of Patients Entered onto the United Kingdom Medical Research Council Trials between 1980 and 2002 - Medical Research Council Adult Leukaemia Working Party. J. Clin. Oncol. 2005, 23, 9219–9226, doi:10.1200/JCO.2005.03.2086.
9. Kumar, V.; Ailawadhi, M.; Dutta, N.; Abdulazeez, M.; Aggarwal, C.S.; Quintero, G.; Baksh, M.; Roy, V.; Sher, T.; Alegria, V.; et al. Trends in Early Mortality From Multiple Myeloma: A Population-Based Analysis. Clin. Lymphoma, Myeloma Leuk. 2021, 21, e449–e455, doi:10.1016/J.CLML.2020.12.023.
10. Murakami, H.; Hayashi, K.; Hatsumi, N.; Saitoh, T.; Yokohama, A.; Matsushima, T.; Tsukamoto, N.; Morita, K.; Karasawa, M.; Ogawara, H.; et al. Risk Factors for Early Death in Patients Undergoing Treatment for Multiple Myeloma. Ann. Hematol. 2001 808 2001, 80, 452–455, doi:10.1007/S002770100330.
11. Kastritis, E.; Terpos, E.; Roussou, M.; Eleutherakis-Papaiakovou, E.; Gavriatopoulou, M.; Kalapanida, D.; Migkou, M.; Kanellias, N.; Christoulas, D.; Gika, D.; et al. Very Early Death (<2 Months) In Myeloma Is Associated With Advanced Age, Poor Performance Status and Reduced Use Of Novel Agents, While Early Death Within 12 Months Is Associated With High Risk Features Of Both The Disease and The Patient. Blood 2013, 122, 3195, doi:10.1182/BLOOD.V122.21.3195.3195.
12. Costa, L.J.; Gonsalves, W.I.; Kumar, S.K. Early Mortality in Multiple Myeloma. Leukemia 2015, 29, 1616–1618, doi:10.1038/LEU.2015.33.
13. Kumar, S.K.; Dispenzieri, A.; Lacy, M.Q.; Gertz, M.A.; Buadi, F.K.; Pandey, S.; Kapoor, P.; Dingli, D.; Hayman, S.R.; Leung, N.; et al. Continued Improvement in Survival in Multiple Myeloma: Changes in Early Mortality and Outcomes in Older Patients. Leuk. 2014 285 2013, 28, 1122–1128, doi:10.1038/leu.2013.313.
14. Dimopoulos, M.A.; Delimpasi, S.; Katodritou, E.; Vassou, A.; Kyrtsonis, M.C.; Repousis, P.; Kartasis, Z.; Parcharidou, A.; Michael, M.; Michalis, E.; et al. Significant Improvement in the Survival of Patients with Multiple Myeloma Presenting with Severe Renal Impairment after the Introduction of Novel Agents. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 2014, 25, 195–200, doi:10.1093/ANNONC/MDT483.
15. Hsu, P.; Lin, T.W.; Gau, J.P.; Yu, Y. Bin; Hsiao, L.T.; Tzeng, C.H.; Chen, P.M.; Chiou, T.J.; Liu, J.H.; Liu, Y.C.; et al. Risk of Early Mortality in Patients With Newly Diagnosed Multiple Myeloma. Medicine (Baltimore). 2015, 94, doi:10.1097/MD.0000000000002305.
16. Terebelo, H.R.; Shah, J.J.; Durie, B.G.; Abonour, R.; Gasparetto, C.; Mehta, J.; Narang, M.; Thomas, S.P.; Toomey, K.; Pashos, C.L.; et al. Early Mortality (EM) for Newly Diagnosed Multiple Myeloma (NDMM) in the Connect MM U.S. Registry. https://doi.org/10.1200/jco.2013.31.15_suppl.8596 2013, 31, 8596–8596, doi:10.1200/JCO.2013.31.15_SUPPL.8596.
17. Holmström, M.O.; Gimsing, P.; Abildgaard, N.; Andersen, N.F.; Helleberg, C.; Clausen, N.A.T.; Klausen, T.W.; Frederiksen, M.; Kristensen, D.L.; Larsen, H.; et al. Causes of Early Death in Multiple Myeloma Patients Who Are Ineligible for High-Dose Therapy with Hematopoietic Stem Cell Support: A Study Based on the Nationwide Danish Myeloma Database. Am. J. Hematol. 2015, 90, E73–E74, doi:10.1002/AJH.23932.
18. Chen, Y.K.; Han, S.M.; Yang, Y.; Lin, T.H.; Tzeng, H.E.; Chang, K.H.; Hwang, W.L.; Teng, C.L.J. Early Mortality in Multiple Myeloma: Experiences from a Single Institution. https://doi.org/10.1080/10245332.2015.1101969 2016, 21, 392–398, doi:10.1080/10245332.2015.1101969.
19. Zonder, J.A.; Crowley, J.; Hussein, M.A.; Bolejack, V.; Moore, D.F.; Whittenberger, B.F.; Abidi, M.H.; Durie, B.G.M.; Barlogie, B. Lenalidomide and High-Dose Dexamethasone Compared with Dexamethasone as Initial Therapy for Multiple Myeloma: A Randomized Southwest Oncology Group Trial (S0232). Blood 2010, 116, 5838–5841, doi:10.1182/BLOOD-2010-08-303487.
20. San Miguel, J.F.; Schlag, R.; Khuageva, N.K.; Dimopoulos, M.A.; Shpilberg, O.; Kropff, M.; Spicka, I.; Petrucci, M.T.; Palumbo, A.; Samoilova, O.S.; et al. Bortezomib plus Melphalan and Prednisone for Initial Treatment of Multiple Myeloma. N. Engl. J. Med. 2008, 359, 906–917, doi:10.1056/NEJMOA0801479.
21. Rajkumar, S.V.; Jacobus, S.; Callander, N.S.; Fonseca, R.; Vesole, D.H.; Williams, M.E.; Abonour, R.; Siegel, D.S.; Katz, M.; Greipp, P.R. Lenalidomide plus High-Dose Dexamethasone versus Lenalidomide plus Low-Dose Dexamethasone as Initial Therapy for Newly Diagnosed Multiple Myeloma: An Open-Label Randomised Controlled Trial. Lancet. Oncol. 2010, 11, 29–37, doi:10.1016/S1470-2045(09)70284-0.
22. Mateos, M.V.; Oriol, A.; Martínez-López, J.; Gutiérrez, N.; Teruel, A.I.; de Paz, R.; García-Laraña, J.; Bengoechea, E.; Martín, A.; Mediavilla, J.D.; et al. Bortezomib, Melphalan, and Prednisone versus Bortezomib, Thalidomide, and Prednisone as Induction Therapy Followed by Maintenance Treatment with Bortezomib and Thalidomide versus Bortezomib and Prednisone in Elderly Patients with Untreated Multiple Myeloma: A Randomised Trial. Lancet. Oncol. 2010, 11, 934–941, doi:10.1016/S1470-2045(10)70187-X.
23. Palumbo, A.; Bringhen, S.; Caravita, T.; Merla, E.; Capparella, V.; Callea, V.; Cangialosi, C.; Grasso, M.; Rossini, F.; Galli, M.; et al. Oral Melphalan and Prednisone Chemotherapy plus Thalidomide Compared with Melphalan and Prednisone Alone in Elderly Patients with Multiple Myeloma: Randomised Controlled Trial. Lancet (London, England) 2006, 367, 825–831, doi:10.1016/S0140-6736(06)68338-4.

24. Facon, T.; Mary, J.Y.; Hulin, C.; Benboubker, L.; Attal, M.; Pegourie, B.; Renaud, M.; Harousseau, J.L.; Guillerm, G.; Chaleteix, C.; et al. Melphalan and Prednisone plus Thalidomide versus Melphalan and Prednisone Alone or Reduced-Intensity Autologous Stem Cell Transplantation in Elderly Patients with Multiple Myeloma (IFM 99-06): A Randomised Trial. Lancet 2007, 370, 1209–1218, doi:10.1016/S0140-6736(07)61537-2.
25. Rajkumar, S.V.; Rosiñol, L.; Hussein, M.; Catalano, J.; Jedrzejczak, W.; Lucy, L.; Olesnyckyj, M.; Yu, Z.; Knight, R.; Zeldis, J.B.; et al. Multicenter, Randomized, Double-Blind, Placebo-Controlled Study of Thalidomide Plus Dexamethasone Compared With Dexamethasone As Initial Therapy for Newly Diagnosed Multiple Myeloma. J. Clin. Oncol. 2008, 26, 2171, doi:10.1200/JCO.2007.14.1853.
26. Handa, H.; Murakami, Y.; Ishihara, R.; Kimura-Masuda, K.; Masuda, Y. The Role and Function of MicroRNA in the Pathogenesis of Multiple Myeloma. Cancers (Basel). 2019, 11, doi:10.3390/CANCERS11111738.
27. Puła, A.; Robak, P.; Robak, T. MicroRNA in Multiple Myeloma - A Role in Pathogenesis and Prognostic Significance. Curr. Med. Chem. 2021, 28, 6753–6772, doi:10.2174/0929867328666210504104419.
28. Papanota, A.M.; Tsiakanikas, P.; Kontos, C.K.; Malandrakis, P.; Liacos, C.I.; Ntanasis-stathopoulos, I.; Kanellias, N.; Gavriatopoulou, M.; Kastritis, E.; Avgeris, M.; et al. A Molecular Signature of Circulating MicroRNA Can Predict Osteolytic Bone Disease in Multiple Myeloma. Cancers (Basel). 2021, 13, 3877, doi:10.3390/CANCERS13153877/S1.
29. Durie, B.G.M.; Harousseau, J.L.; Miguel, J.S.; Bladé, J.; Barlogie, B.; Anderson, K.; Gertz, M.; Dimopoulos, M.; Westin, J.; Sonneveld, P.; et al. International Uniform Response Criteria for Multiple Myeloma. Leukemia 2006, 20, 1467–1473, doi:10.1038/SJ.LEU.2404284.
30. Rajkumar, S.V.; Dimopoulos, M.A.; Palumbo, A.; Blade, J.; Merlini, G.; Mateos, M.V.; Kumar, S.; Hillengass, J.; Kastritis, E.; Richardson, P.; et al. International Myeloma Working Group Updated Criteria for the Diagnosis of Multiple Myeloma. Lancet Oncol. 2014, 15, e538–e548.
31. Robak, P.; Dróżdż, I.; Jarych, D.; Mikulski, D.; Węgłowska, E.; Siemieniuk-Ryś, M.; Misiewicz, M.; Stawiski, K.; Fendler, W.; Szemraj, J.; et al. The Value of Serum MicroRNA Expression Signature in Predicting Refractoriness to Bortezomib-Based Therapy in Multiple Myeloma Patients. Cancers (Basel). 2020, 12, 1–18, doi:10.3390/CANCERS12092569.
32. Charliński, G.; Tyczyńska, A.; Małecki, B.; Fornagiel, S.; Barchnicka, A.; Kołkowska, A.; Kopińska, A.; Usnarska-Zubkiewicz, L.; Robak, P.; Waszczuk-Gajda, A.; et al. Risk Factors and Causes of Early Mortality in Patients with Newly Diagnosed Multiple Myeloma in a "Real-World" Study: Experiences of the Polish Myeloma Group. Polish Arch. Intern. Med. 2021, 131, 527–534, doi:10.20452/PAMW.15980.
33. Ríos-Tamayo, R.; Sáinz, J.; Martínez-López, J.; Puerta, J.M.; Chang, D.Y.L.; Rodríguez, T.; Garrido, P.; de Veas, J.L.G.; Romero, A.; Moratalla, L.; et al. Early Mortality in Multiple Myeloma: The Time-Dependent Impact of Comorbidity: A Population-Based Study in 621 Real-Life Patients. Am. J. Hematol. 2016, 91, 700–704, doi:10.1002/AJH.24389.
34. O'Donnell, E.K.; Kabrt, J.; Ezenwajiaku, N.; Yee, A.J.; Horick, N.; Raje, N.S. Early Mortality in Newly Diagnosed Multiple Myeloma in the Context of Novel Drugs. Blood 2015, 126, 3315, doi:10.1182/BLOOD.V126.23.3315.3315.
35. Ross, F.M.; Avet-Loiseau, H.; Ameye, G.; Gutiérrez, N.C.; Liebisch, P.; O'Connor, S.; Dalva, K.; Fabris, S.; Testi, A.M.; Jarosova, M.; et al. Report from the European Myeloma Network on Interphase FISH in Multiple Myeloma and Related Disorders. Haematologica 2012, 97, 1272–1277, doi:10.3324/haematol.2011.056176.
36. Manier, S.; Liu, C.J.; Avet-Loiseau, H.; Park, J.; Shi, J.; Campigotto, F.; Salem, K.Z.; Huynh, D.; Glavey, S. V.; Rivotto, B.; et al. Prognostic Role of Circulating Exosomal MiRNAs in Multiple Myeloma. Blood 2017, 129, 2429–2436, doi:10.1182/blood-2016-09-742296.
37. Hao, M.; Zang, M.; Wendlandt, E.; Xu, Y.; An, G.; Gong, D.; Li, F.; Qi, F.; Zhang, Y.; Yang, Y.; et al. Low Serum MiR-19a Expression as a Novel Poor Prognostic Indicator in Multiple Myeloma. Int. J. Cancer 2015, 136, 1835–1844, doi:10.1002/ijc.29199.
38. Jiang, Y.; Luan, Y.; Chang, H.; Chen, G. The Diagnostic and Prognostic Value of Plasma MicroRNA-125b-5p in Patients with Multiple Myeloma. Oncol. Lett. 2018, 16, 4001–4007, doi:10.3892/ol.2018.9128.
39. Xu, P.; Xia, T.; Ling, Y.; Chen, B. MiRNAs with Prognostic Significance in Multiple Myeloma: A Systemic Review and Meta-Analysis. Med. (United States) 2019, 98, doi:10.1097/MD.0000000000016711.
40. Zheng, B.; Liang, L.; Huang, S.; Zha, R.; Liu, L.; Jia, D.; Tian, Q.; Wang, Q.; Wang, C.; Long, Z.; et al. MicroRNA-409 Suppresses Tumour Cell Invasion and Metastasis by Directly Targeting Radixin in Gastric Cancers. Oncogene 2012, 31, 4509–4516, doi:10.1038/ONC.2011.581.
41. Li, C.; Nie, H.; Wang, M.; Su, L.; Li, J.; Yu, B.; Wei, M.; Ju, J.; Yu, Y.; Yan, M.; et al. MicroRNA-409-3p Regulates Cell Proliferation and Apoptosis by Targeting PHF10 in Gastric Cancer. Cancer Lett. 2012, 320, 189–197, doi:10.1016/J.CANLET.2012.02.030.
42. Josson, S.; Gururajan, M.; Sung, S.Y.; Hu, P.; Shao, C.; Zhau, H.E.; Liu, C.; Lichterman, J.; Duan, P.; Li, Q.; et al. Stromal Fibroblast-Derived MiR-409 Promotes Epithelial-to-Mesenchymal Transition and Prostate Tumorigenesis. Oncogene 2015, 34, 2690–2699, doi:10.1038/ONC.2014.212.
43. Xu, X.; Chen, H.; Lin, Y.; Hu, Z.; Mao, Y.; Wu, J.; Xu, X.; Zhu, Y.; Li, S.; Zheng, X.; et al. MicroRNA-409-3p Inhibits Migration and Invasion of Bladder Cancer Cells via Targeting c-Met. Mol. Cells 2013, 36, 62, doi:10.1007/S10059-013-0044-7.
44. Wan, L.; Zhu, L.; Xu, J.; Lu, B.; Yang, Y.; Liu, F.; Wang, Z. MicroRNA-409-3p Functions as a Tumor Suppressor in Human Lung Adenocarcinoma by Targeting c-Met. Cell. Physiol. Biochem. 2014, 34, 1273–1290, doi:10.1159/000366337.

45. Ma, Z.; Li, Y.; Xu, J.; Ren, Q.; Yao, J.; Tian, X. MicroRNA-409-3p Regulates Cell Invasion and Metastasis by Targeting ZEB1 in Breast Cancer. IUBMB Life 2016, 68, 394–402, doi:10.1002/IUB.1494.
46. Wu, L.; Zhang, Y.; Huang, Z.; Gu, H.; Zhou, K.; Yin, X.; Xu, J. MiR-409-3p Inhibits Cell Proliferation and Invasion of Osteosarcoma by Targeting Zinc-Finger E-Box-Binding Homeobox-1. Front. Pharmacol. 2019, 10, 137, doi:10.3389/FPHAR.2019.00137/BIBTEX.
47. Sommerova, L.; Anton, M.; Bouchalova, P.; Jasickova, H.; Rak, V.; Jandakova, E.; Selingerova, I.; Bartosik, M.; Vojtesek, B.; Hrstka, R. The Role of MiR-409-3p in Regulation of HPV16/18-E6 MRNA in Human Cervical High-Grade Squamous Intraepithelial Lesions. Antiviral Res. 2019, 163, 185–192, doi:10.1016/J.ANTIVIRAL.2019.01.019.
48. Liu, M.; Xu, A.; Yuan, X.; Zhang, Q.; Fang, T.; Wang, W.; Li, C. Downregulation of MicroRNA-409-3p Promotes Aggressiveness and Metastasis in Colorectal Cancer: An Indication for Personalized Medicine. J. Transl. Med. 2015, 13, 1–9, doi:10.1186/S12967-015-0533-X/FIGURES/7.
49. Tan, S.; Shi, H.; Ba, M.; Lin, S.; Tang, H.; Zeng, X.; Zhang, X. MiR-409-3p Sensitizes Colon Cancer Cells to Oxaliplatin by Inhibiting Beclin-1-Mediated Autophagy. Int. J. Mol. Med. 2016, 37, 1030–1038, doi:10.3892/IJMM.2016.2492/HTML.
50. Li, W.-Q.; Li, Y.-M.; Tao, B.-B.; Lu, Y.-C.; Hu, G.-H.; Liu, H.-M.; He, J.; Xu, Y.; Yu, H.-Y. Downregulation of ABCG2 Expression in Glioblastoma Cancer Stem Cells with MiRNA-328 May Decrease Their Chemoresistance. Med. Sci. Monit. Int. Med. J. Exp. Clin. Res. 2010, 16, HY27-30.
51. Pan, Y.Z.; Morris, M.E.; Yu, A.M. MicroRNA-328 Negatively Regulates the Expression of Breast Cancer Resistance Protein (BCRP/ABCG2) in Human Cancer Cells. Mol. Pharmacol. 2009, 75, 1374–1379, doi:10.1124/MOL.108.054163.
52. Xu, X.T.; Xu, Q.; Tong, J.L.; Zhu, M.M.; Nie, F.; Chen, X.; Xiao, S.D.; Ran, Z.H. MicroRNA Expression Profiling Identifies MiR-328 Regulates Cancer Stem Cell-like SP Cells in Colorectal Cancer. Br. J. Cancer 2012, 106, 1320–1330, doi:10.1038/BJC.2012.88.
53. Ma, H.; Liu, C.; Zhang, S.; Yuan, W.; Hu, J.; Huang, D.; Zhang, X.; Liu, Y.; Qiu, Y. MiR-328-3p Promotes Migration and Invasion by Targeting H2AFX in Head and Neck Squamous Cell Carcinoma. J. Cancer 2021, 12, 6519–6530, doi:10.7150/JCA.60743.
54. Srivastava, A.K.; Banerjee, A.; Cui, T.; Han, C.; Cai, S.; Liu, L.; Wu, D.; Cui, R.; Li, Z.; Zhang, X.; et al. Inhibition of MiR-328-3p Impairs Cancer Stem Cell Function and Prevents Metastasis in Ovarian Cancer. Cancer Res. 2019, 79, 2314–2326, doi:10.1158/0008-5472.CAN-18-3668/653645/AM/INHIBITION-OF-MIR-328-3P-IMPAIRS-CANCER-STEM-CELL.
55. Stawiski K, Kaszkowiak M (2022). OmicSelector: OmicSelector - a package for biomarker selection based on high-throughput experiments.. R package version 1.0.0, https://biostat.umed.pl/OmicSelector/.

Captions to complementary tables:

Table 1. The list of 31 selected miRNAs.
Table 2. The results of differential expression analysis for all miRNAs.
Table 3. The results of univariate logistic regression analyses for all miRNAs.