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Original Article

A microRNA signature for clinical outcomes of pediatric ALL patients treated with TPOG protocols

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Abstract: MicroRNA (miRNA) expression is reportedly associated with clinical outcomes in childhood acute lymphoblastic leukemia (ALL). Here, we aimed at investigating whether miRNA expression is associated with clinical outcomes in pediatric ALL patients treated with the Taiwan Pediatric Oncology Group (TPOG) protocols. The expression of 397 miRNAs was measured using stem-loop quantitative real-time polymerase chain reaction miRNA arrays in 60 pediatric ALL patients treated with TPOG-ALL-93 or TPOG-ALL-97 VHR (very high-risk) protocols. In order to identify prognosis-related miRNAs, original cohort was randomly split into the training and testing cohort in a 2:1 ratio, and univariate Cox proportional hazards regression was applied to identify associations between event-free survival (EFS) and expressions of miRNAs. Four prognosis-related miRNAs were selected and validated in another independent cohort composed of 103 patients treated with the TPOG-ALL-2002 protocol. Risk score, including the impact of four prognosis-related miRNAs, was calculated for each patients, followed by grouping patients into the high or low risk-score groups. Irrespective of the training, testing, or validation cohort, risk-score group was significantly associated with EFS and overall survival (OS). Risk-score group combining with clinical characteristics including the age onset (≥10 years), white blood cell counts (≥100 × 10^9/L), cell type (T- or B-cell), sex, and risk groups of the treatment protocols were used as predictors of EFS using the multivariate Cox proportional hazards regression. Results showed that the risk-score group was the strongest predictor. In the validation cohort, hazard ratios (HRs) of the risk-score group were 7.06 (95% CI=1.93-25.84, *p*-value =0.003) and 14.03 (95% CI=3.34-59.04, *p*-value =0.003) for EFS and OS, respectively. High risk-score group had higher risk of having poor prognosis and risk of death than that in the low risk group. Accuracy of the prediction model for 5-year EFS could reach 0.76. For the prediction of 5-year OS, accuracy was 0.75. In conclusion, a miRNA signature was associated with clinical outcomes in childhood ALL patients treated with TPOG protocols and might be a suitable prognostic biomarker.

Keywords: Childhood ALL, microRNA signature, TPOG

Introduction

MicroRNAs (miRNAs) execute diverse functions by simultaneously targeting the mRNAs of multiple genes. Alterations in miRNAs contribute to many human malignancies [1]. In addition, miRNAs are important regulators of hematopoiesis [2-4]. miRNA-mediated control of gene dosage is critical for lineage fate determination of hematopoietic cells, and disruption of this regulation may lead to malignant transformation. Moreover, dysregulation of miRNA expression is frequently associated with cytogenetic abnormalities, and certain abnormalities directly affect the aberrant expression of miRNAs in hematologic malignancies [5-7]. Calin et al. first

demonstrated that a loss of *miR-15a* and *miR-16-1* was the target of 13q14 deletion, a region frequently lost in patients with chronic lymphoblastic leukemia (CLL) [8]. In patients expressing the aberrant fusion protein, AML1/ETO, the most common acute myeloid leukemia-associated fusion resulting from t(8;21), The fusion oncoprotein was first-ever reported to directly repress the expression of miR-223 by triggering chromatin remodeling and epigenetic silencing, which in turn blocks myeloid precursor cell differentiation [9]. miR-155 is essential for B-cell development and is aberrantly upregulated in B-cell malignancies, including diffuse large B-cell lymphoma, follicular lymphoma, and CLL [10-14]. miRNA expression profiles are also used to discriminate different subtypes of acute lymphoblastic leukemia (ALL) [3, 15-18]. The expression of some miRNAs is associated with the clinical outcomes of acute myeloid leukemia [19-21].

Lu et al. used miRNA expression to classify cancers, including childhood ALL. Based on miRNA expression, ALL can be differentiated into major cytogenetic subtypes, including B-ALL with *ETV6-RUNX1*, *BCR-ABL1*, hyperdiploidy, and T-ALL [3]. Bueno et al. showed that the genetic and epigenetic silencing of miRNA-203 enhanced, the expression of *ABL1* and *BCR-ABL1* oncogenes and it might function as a tumor suppressor [22]. The expression of miR-196b was enriched in patients with *KMT2A* fusion leukemia, and its function is necessary for KMT2A fusion-mediated immortalization [16]. miR196b was also linked with the activation of HOXA in pediatric ALL-not restricted to KMT2A-rearranged leukemia-and directly targeted the HOXA/MEIS1 and FAS tumor suppressor genes in KMT2A-rearranged leukemia [23, 24]. In a previous work, we showed that miRNA 181-a was regulated by *ETV6-RUNX1* through a loop feedback [18]. Recently, Malouf et al. showed that miR-130b and miR-128a are downstream targets of MLL-AF4 and can individually drive the transition from a preleukemic stage to an acute leukemia in an murine MLL-AF4 model [25]. miRNA-497/195 is tumor-suppressive and cooperates with *CDKN2A/B* in pediatric ALL [26]. miRNAs are also involved in the pathogenesis of T-ALL via the NOTCH1 and MYB pathways in T-ALL [27, 28].

As, miRNAs are involved in ALL pathways, it is possible that miRNA expression be a prognostic marker for childhood ALL. Several studies have shown that miRNAs are prognostic markers in childhood ALL. Zhang et al. described a miRNA signature that could be used to predict prednisone response in childhood ALL patients, which was validated using a smaller cohort [29]. Schotte et al. demonstrated a correlation between the probability of disease-free survival and the expression levels of 31 distinct miRNAs. Upregulation of miRNA-21 is a poor prognostic marker in patients with childhood B-ALL [30]. Upregulated miR-155 is associated with poor prognosis in childhood ALL and promotes cell proliferation by targeting ZNF238 [31]. The expression of miR-143/miR-182 is associated with the prognosis and risk stratification specificity of BFM-treated childhood ALL [32]. In this study, we used miRNA arrays to identify a signature in pediatric ALL patients treated with Taiwan Pediatric Oncology Group (TPOG) protocols and validated its prognostic impact.

Materials and methods

Patients and protocols

Diagnostic and/or relapsed bone marrow (BM) or peripheral blood samples were obtained from 60 children with newly diagnosed ALL from July 1996 to December 2001 at the National Taiwan University Hospital as the primary cohort. There were 50 patients with B-cell ALL and 10 patients with T-cell ALL. Forty-five patients were treated with the TPOG-ALL-93 protocol, and fifteen patients were treated with the TPOG-97-VHR protocol [33]. These 60 patients were assigned to the training (40 patients) and testing (20 patients) cohorts (**Table 1**). To validate the results, another 103 patients were included as the validation cohort; these included 78 B-cell and 25 T-cell ALL patients who were treated with TPOG-ALL-2002 protocol. The diagnosis of ALL was based on morphologic findings of BM aspirates and immuno-phenotype analyses of leukemic cells by flow cytometry. Conventional cytogenetic analysis were done as part of the routine work-flow [34].

The treatment protocols and risk classifications were described previously [33-35]. The patients were prospectively assigned to one of the three risk groups (standard [SR], high [HR], and very high [VHR]) based on clinical and biological features of Intrathecal chemotherapy replaced cranial irradiation for CNS prophylaxis in HR and VHR patients in the TPOG-ALL-2002

Table 1. Clinical characteristics of the 60 ALL patients in the original cohort. Patients were randomly assigned to the training (n=40) or testing cohort (n=20)

	Total n (%)	Training n (%)	Testing n (%)	*p*-value
Onset age (years)				0.326
<10	47 (78.3)	33 (82.5)	14 (70.0)	
≥10	13 (21.7)	7 (17.5)	6 (30.0)	
Sex				1.000
Female	32 (53.3)	21 (52.5)	11 (55.0)	
Male	28 (46.7)	19 (47.5)	9 (45.0)	
Initial WBC (10^9/L)				0.189
<100	48 (80.0)	34 (85.0)	14 (70.0)	
≥100	12 (20.0)	6 (15.0)	6 (30.0)	
Risk group				0.579
SR+HR	39 (65.0)	27 (67.5)	12 (60.0)	
VHR	21 (35.0)	13 (32.5)	8 (40.0)	
Genetic subtypes*				0.245
BCR-ABL1	1 (3.5)	0 (0.0)	1 (11.1)	
ETV6-RUNX1	8 (27.6)	6 (30.0)	2 (22.2)	
Hyperdiploidy	8 (27.6)	7 (35.0)	1 (11.1)	
DUX4-rearranged	1 (3.5)	0 (0.0)	1 (11.1)	
ZNF384-rearranged	1 (3.5)	1 (5.0)	0 (0.0)	
T-ALL	10 (34.5)	6 (30.0)	4 (44.4)	

*A total of 31 samples had unknown subtype. WBC, white blood cells; SR, standard risk group; HR, high-risk group; VHR, very high-risk group.

protocol. The Institutional Review Board of the National Taiwan University Hospital approved the study, and all participants provided written informed consent in accordance with the Declaration of Helsinki. Details of the protocols and risk group assignments have been published elsewhere [33, 35].

RNA extraction and miRNA detection

Total RNA was extracted from BM or peripheral blood using TRIzol (Invitrogen) at the time of diagnosis. miRNA expression profiling was performed using the ABI PRISM 7900 and stem-loop RT-qPCR miRNA arrays containing 397 mature human miRNAs (Applied Biosystems), as described previously [18]. Individual miRNAs were quantified using TaqMan miRNA assays (Applied Biosystems). All miRNA arrays were run concurrently with a calibration control (U6 snRNA).

Statistical methods

The follow-up care for these patients included physical examination and complete blood analysis after completing the chemotherapy. In case of any abnormality during physical examination or blood tests, the disease relapse possibility will be excluded after BM examination. To identify prognosis-related miRNAs and evaluate the effect of these miRNAs, 60 patients were randomly assigned to the training and testing sets at a ratio of 2:1. Univariate Cox proportional hazards regression was applied to evaluate the association between the event-free survival (EFS) and expression of each miRNA in the training set. Events were defined based on any relapse, death, or secondary malignancy. If the *p*-value of coefficient in the regression model was less than 0.05, then the miRNA was considered as the prognosis-related miRNA. For concluding the effect of prognosis-related miRNAs, the risk score of each patient was calculated using a linear combination of expressions of prognosis-related miRNAs, weighted by the coefficients of the regression model (Supplementary Table 1). In our study, four miRNAs were identified as prognosis-related miRNA. Therefore, the formula of risk score was defined as:

$$\text{Risk score} = \sum_i^4 \beta_i * miRNA_i$$

Where β is the regression coefficient. The cut-off of the risk score is the median of risk score distribution. Patients were grouped into high risk-score group if their risk scores were higher than the cut-off, whereas patients with a risk score lower than the cut-off were grouped into low risk-score group. Finally, the linear equation of risk score calculation and cut-off were also applied in the testing cohort same as the training cohort (**Figure 1**).

To validate the identified miRNAs and prediction model, prognosis-related miRNAs were quantified by RT-qPCR experiments for an independent cohort of 103 patients. Additionally, as the experiment platform was different from the training and testing cohorts, weighting values of risk scores were recalculated using the univariate Cox proportional hazards regression.

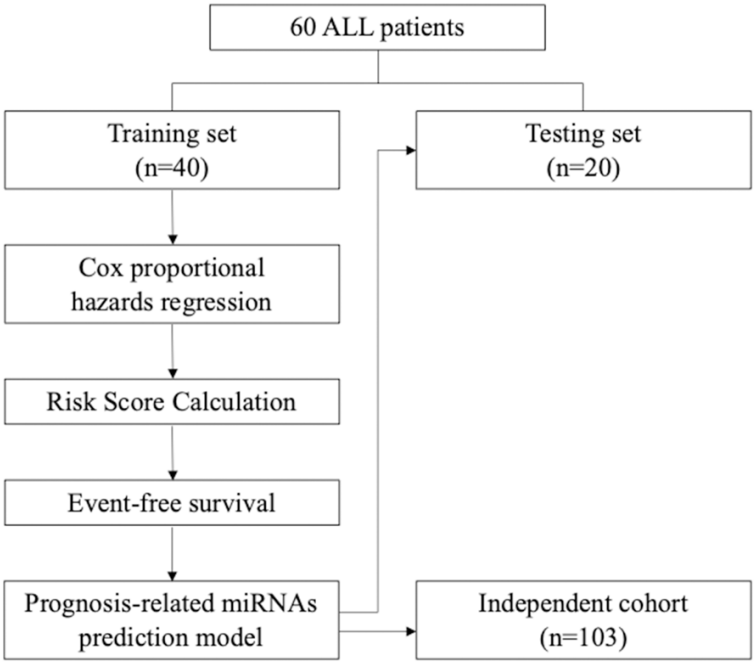

Figure 1. Flowchart for identifying the miRNA signature of childhood ALL for clinical outcome prediction.

Risk score was calculated by summation of the expression values of the four prognosis-related miRNAs, weighted by the coefficients of the regression model. Patients were classified into the two groups based on the threshold value, estimated using the maxstat method.

The association between categorical variables was evaluated using the Fisher's exact test. Survival curves were estimated using the Kaplan-Meier method, and *p*-values were determined using the log-rank test. In order to elucidate the impact of risk-score group and develop the prognostic prediction model, a multivariate Cox proportional hazards regression model was used and risk-score group with clinical variables, including onset age (≥10 years), white blood cell (WBC) counts (≥100 × 10^9/L), cell type (T- or B-cell), and sex, and risk groups of the treatment protocols as predictors. In this model development, complete case analysis was applied. Based on the risk model of the multivariate Cox proportional hazards regression model, Harrell's concordance statistics, and time-dependent ROC (receiver operating characteristics) curves at 5-year EFS and 5-year OS were applied to assess the prediction efficiency. Harrell's C-index near 1 indicates that the prediction model performs well in deciding which patient would have the event first. By contrast, index near 0 indicates that prediction model is worse than the coin flip. All statistical analyses were two-tailed and performed using the SAS software (version 9.4; SAS Institute, Inc., Cary, NC, USA). Statistical significance was set at $P<0.05$.

Results

Clinical features of the original cohort

Sixty patients were included in the original cohort. Among these, 28 were male and 32 were female (a male-to-female ratio of 0.88:1). The median age of the patients at diagnosis was 12.1 years (range, 1.4-17.4 years). The median WBC count was 69.8 × 10^9/L (range, 0.6-1096 × 10^9/L). The clinical features of the original cohort are presented in **Table 1**.

miRNA signature associated with clinical outcomes in patients treated with TPOG-ALL-93 or -97 VHR protocols in the original cohort

Of the 60 samples from the original cohort, 40 were used as the training set and 20 as the validation set. There were no differences in clinical parameters, such as age, risk group, and immunophenotypes, in the original cohort (**Table 1**). A flow chart for identifying the miRNA signature is illustrated in **Figure 1**. We identified that miRNAs 133a, 193a, 151, and 129 were associated with EFS as the prognosis-related miRNAs (Supplementary Table 1). After risk score calculation for each patient, summarizing the impact of the four prognosis-related miRNAs, patients were grouped into the high risk-score and low risk-score group based on the cut-off value. Patients with low risk scores had a better EFS and overall OS than those with high risk scores (95.00%, 69.47-99.28 versus 65.00%, 40.30-81.53 for 5-year EFS and 100%, 100-100, versus 70.00%, 45.05-85.25 for 5-year OS) in the training set (**Figure 2A**). These results were confirmed in the testing set (**Figure 2B**).

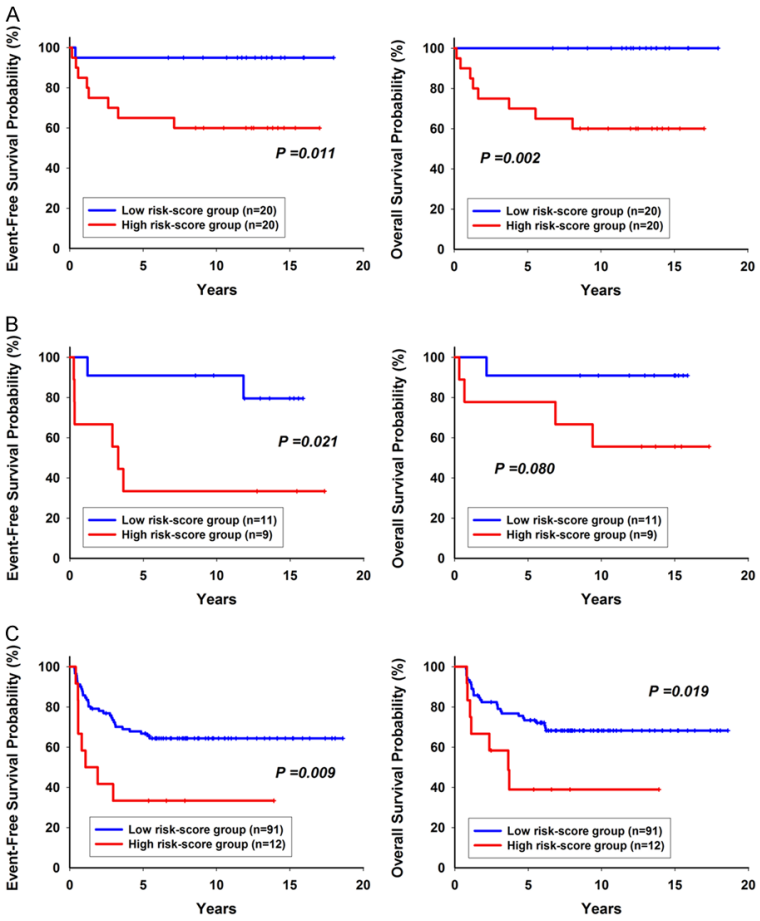

Figure 2. Risk-score group was significantly associated with event-free survival and overall survival (A) in the training cohort (n=40) (B) in the testing cohort (n=20) (C) in the validation cohort (n=103).

Confirmation of the prognostic significance of the miRNA score using another cohort treated with the TPOG-ALL-2002 protocol

Among the 103 additional patients in the validation cohort, 59 were male and 44 were female (male-to-female ratio: 1.34:1). The median age of the patients at diagnosis was 12.1 years (range, 1.4-17.4 years). The median WBC count was 69.8 × 10^9/L (range 0.6-1096 × 10^9/L). The clinical characteristics of the original and validation cohort were not significantly different except the genetic subtypes (Supplementary Table 2). However, half of the patients had unknown genetic subtypes in the original cohort (Supplementary Table 2). Based on the expression profiles of the four selected miRNAs, 91 patients were classified into the low risk-score group and 12 were classified into the high risk-score group. The low risk-score group had better EFS and OS than the high risk-score group (**Figure 2C**). The 5-year EFS and OS rates were 66.72% (55.96-75.42) and 73.35% (62.91-81.27) for patients with a low risk score and 38.89 (12.63-64.98) and 33.33 (10.27-58.87) for patients with a high risk score, respectively.

Development of miRNA signature prediction model

In order to develop the prognostic prediction model, risk-score group in association with clinical variables, onset age (≥10 years), WBC counts (≥100 × 10^9/L), cell type (T- or B-cell), sex, and risk groups of the treatment protocols were used as predictors. It was used to predict EFS using the multivariate Cox proportional hazards regression model (**Table 3**). Irrespective of the training, testing, or validation cohort, the high risk-score group was the strongest predictive factor for unfavorable EFS (**Table 2** and Supplementary Table 3). In the validation cohort, the high risk group had 7.06-fold risk to have the prognosis events than the low risk-score group (95% CI=1.93-25.84, *p*-value =0.003). Furthermore, for the OS prediction, results also show that the high risk-score group had 14.03-fold risk of death than the low risk-score group (95% CI=3.34-59.04, *p*-value =0.003, **Table 2**). In order to assess whether the prediction model has prediction efficacy for clinical outcome, Harrell's concordance statistics and ROC curves at 5-year EFS and 5-year OS were applied. Harrell's C-index for EFS were 0.80, 0.89, and 0.70 in the training, testing, and validation cohort, respectively; the prediction power of overall survival, were 0.89, 0.89, and 0.72 in the training, testing, and validation cohort, respectively (Supplementary Table 4). In terms of EFS or OS, although a lower Harrell's C-index was determined in the validation cohort than in the other cohorts it could reach at least 0.7. Results of

Table 2. Adjusted hazard ratios of predictors in the prognostic model estimated using the multivariate Cox proportional hazards regression model for the validation cohort

Variables	Event-Free Survival				Overall Survival			
	HR	95% CI		*p*-value	HR	95% CI		*p*-value
High risk-score group	7.06	1.93	25.84	0.003	14.03	3.34	59.04	0.003
Initial WBC ≥100	1.74	0.71	4.25	0.224	1.88	0.75	4.67	0.177
T cell	0.31	0.09	1.08	0.066	0.12	0.03	0.55	0.006
Male	1.81	0.91	3.63	0.093	1.80	0.83	3.89	0.134
Age ≥10	1.91	0.95	3.80	0.068	2.00	0.96	4.17	0.064
VHR	1.67	0.60	4.63	0.323	1.88	0.65	5.38	0.242

WBC, white blood cells.

Table 3. Multivariable analysis of 5-year event-free and overall survival

Variable	Hazard ratio (HR)	95% Confidence interval (CI)		*p*-value
High risk-score group	2.73	1.25	5.95	0.012
Variable	HR	95% CI		*p*-value
High risk-score group	7.06	1.93	25.84	0.003
Initial WBC ≥100	1.74	0.71	4.25	0.224
T cell	0.31	0.09	1.08	0.066
Male	1.81	0.91	3.63	0.093
Age ≥10	1.91	0.95	3.80	0.068
Very high risk-score group	1.67	0.60	4.63	0.323
Variable	HR	95% CI		*p*-value
High risk-score group	2.61	1.13	6.01	0.024
Variable	HR	95% CI		*p*-value
High risk-score group	14.03	3.34	59.04	0.003
Initial WBC ≥100	1.88	0.75	4.67	0.177
T cell	0.12	0.03	0.55	0.006
Male	1.80	0.83	3.89	0.134
Age ≥10	2.00	0.96	4.17	0.064
Very high risk-score group	1.88	0.65	5.38	0.242

WBC, white blood cells.

ROC curve at 5-year EFS also showed that accuracy could reach above 0.8 in the training and testing cohort, and accuracy in the validation cohort was 0.76 (**Figure 3**). Accuracy of 5-year OS prediction could reach even higher. The risk model efficiently predicted the clinical outcome, especially for 5-year OS prediction (**Figure 3**).

Discussion

In this study, we analyzed the expression levels of 397 miRNAs in pediatric ALL using specific, stem-loop RT-qPCR miRNA assays. We correlated miRNA expression profiles of 60 patients samples in the original cohort and proposed a miRNA signature associated with clinical outcomes. This signature was validated in samples from another 103 patients. The miRNA signature score included the expression of miR133a, 193a, 151, and 129. The prognostic value was determined to be an independent prognostic marker after the multivariate analysis.

There are several reports on the prognostic value of such signatures in childhood ALL. Schotte et al. used a similar assay to correlate miRNA expression and drug resistance [23]. They identified resistance to vincristine, which was characterized by an approximately 20-fold upregulation of miR-125b, miR-99a, and miR-100 (PFDR≤0.002). A combined expression profile based on 14 miRNAs that were individually associated with prognosis was highly pre-

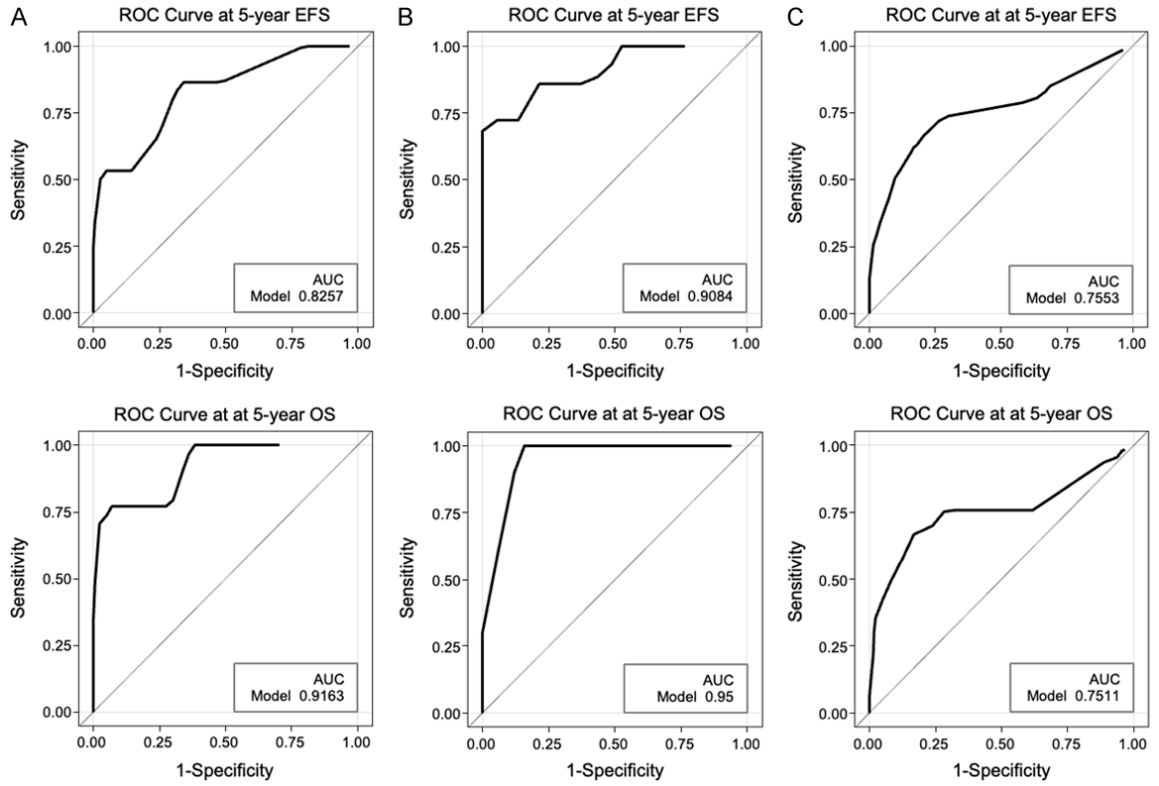

Figure 3. ROC curve at 5-year EFS and 5-year OS. (A) in the training cohort (n=40) (B) in the testing cohort (n=20) (C) in the validation cohort (n=103).

dictive of clinical outcomes in pediatric ALL [23]. One of these 14 miRNAs, miR-193a, was also identified to be associated with clinical outcomes in this study. However, this miRNA did not retain its prognostic significance after the application of correction factors. Avigad et al. identified five miRNAs in a cohort of 48 samples [36]. The authors then used real-time quantitative PCR on a cohort of precursor B-cell ALL patients (n=138). Low expression of miR-151-5p, miR-451, and high expression of miR-1290 or a combination of all three predicted an inferior relapse-free survival. The prognostic relevance of the three miRNAs was evaluated in another B-cell ALL cohort (n=33) treated with other protocols. A significant correlation between aberrant expression of at least one of the three miRNAs and poor outcome was maintained (P<0.0001). Piatopoulou et al. examined the expression profile of miR-143 and miR-182 in 125 childhood ALL patients who received the Berlin-Frankfurt-Münster (BFM) protocol. BM levels of miR-143/miR-182 were significantly decreased in childhood ALL patients at diagnosis, and overexpression of miR-143/miR-182 at the end of induction presented a significantly higher risk for short-term relapse and death. Zamani et al. reported that the expression levels of miR-324-3p and miR-508-5p were different between samples with positive and negative MRD and could serve as potential diagnostic and multidrug-resistant biomarkers in childhood ALL [37].

Our approach was similar to that of Schotte et al. and Avigad et al. and was better than that of Piatopoulou et al. and Zamani et al., as the latter just used two miRNA expressions [23, 36-38]. We used an original cohort to obtain the most significant miRNA signature and have validated this finding with a different cohort, who were treated with another protocol. Moreover, we followed the guidelines of the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis initiative [39]. Thus, the miRNA signature identified herein has a better chance to be successfully validated in a future prospective trial in Taiwan. As the predictive markers might be related to the treatment protocols and genetic

background, the miRNA markers identified by different groups may be distinct [40].

Sengupta et al. identified conserved miR-193a target sites within the 3'-untranslated region of the *MLL1* gene transcript [17]. MiR-193a directly targeted the 3'-untranslated region of the *MLL1* mRNA. Ectopic expression of miR-193a modulated the global H3K4 mono-, di-, and trimethylation levels. Prolonged ectopic expression of miR-193a inhibits growth and cell migration and induces apoptosis. Another similar miR-193 family, miR-193b-3p, was downregulated in several cytogenetically defined subgroups of pediatric and adult AML, and low expression served as an independent indicator of poor prognosis in pediatric AML [20]. This trend is similar to that observed in our study. In knockout mice, loss of miR-193b cooperated with Hoxa9/Meis1 during leukemogenesis, whereas restoring miR-193b expression impaired leukemic engraftment. Similarly, expression of miR-193b in AML blasts from patients diminished leukemic growth *in vitro* and in mouse xenografts. Mechanistically, miR-193b induces apoptosis and G1/S-phase arrest in various human AML subgroups by targeting multiple factors in the KIT-RAS-RAF-MEK-ERK (MAPK) signaling cascade and the downstream cell cycle regulator, CCND1. MiR-133a was downregulated in AMLs bearing the AML1/ETO rearrangements [41]. Fulci et al. identified miR-148, miR-151, and miR-424 as discriminative of T-lineage versus B-lineage ALL [42]. However, how these miRNAs affect drug resistance and the 5-year EFS and OS might require further investigation in the future.

There are several limitations to this study. One of the limitations of this study is its relatively small sample size. Although we performed the validation with another cohort treated with the selected ALL protocol, we did not have other samples with different genetic backgrounds treated with other regimens to validate its significance. There are several limitations of correlation of miRNA expression with clinical outcomes in childhood ALL. In childhood ALL, the RNA-based signature or genetic predictive markers are difficult to validate across protocols or study populations [43-47]. There is also a problem with miRNA expression in ALL. There are several reports discussing its prognosis and drug resistance [23, 32, 36, 37]. However, these miRNAs were not the same, and the miRNA-based signature could be a predictive marker, but this approach might not be easily validated in larger clinical trials. Another limitation is the lack of an MRD parameter in the analysis. Lastly, the subtypes defined by RNA-seq had several novel subtypes, and in this study, there were many patients lacking detailed subtyping. In the current treatment protocol used in Taiwan, these two parameters were used, and a new prospective clinical trial to investigate the clinical significance of this score with MRD levels and novel subtypes might be needed in the future.

In conclusion, the miRNA 133a, 193a, 151, and 129 signature score was associated with clinical outcomes in the selected cohort treated with TPOG-ALL protocols and was validated. In addition to the major cytogenetic alterations, we identified a miRNA signature associated with clinical outcomes. Future larger clinical trials including complete genotyping, MRD, and these miRNAs might be worthy of further investigations.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Four miRNAs associated with EFS were identified using the univariate Cox proportional hazards regression analysis

miRNA	Coefficient	Hazard ratio	95% Confidence interval	*p*-value
miR-129	0.325	1.38	1.09-1.75	0.007
miR-133a	0.409	1.51	1.16-1.95	0.002
miR-151	-0.266	0.77	0.63-0.93	0.007
miR-193a	-0.297	0.74	0.61-0.90	0.002

Supplementary Table 2. Comparison of the clinical characteristics between the original cohort (n=60, randomly split into the training and testing cohort) and validation cohort (n=103)

	Original cohort n (%)	Validation cohort n (%)	*p*-value
Onset age (years)			0.207
<10	47 (78.3)	70 (68.0)	
≥10	13 (21.7)	33 (32.0)	
Sex			0.198
Female	32 (53.3)	44 (42.7)	
Male	28 (46.7)	59 (57.3)	
Initial WBC (10^9/L)			0.565
<100	48 (80.0)	77 (74.8)	
≥100	12 (20.0)	26 (25.2)	
Cell type			0.424
B cell	50 (83.3)	79 (76.7)	
T cell	10 (16.7)	24 (23.3)	
Risk group			0.104
SR+HR	39 (65.0)	53 (51.5)	
VHR	21 (35.0)	50 (48.5)	
Genetic subtypes*			0.038
BCR-ABL1	1 (3.5)	3 (4.0)	
ETV6-RUNX1	8 (27.5)	13 (15.6)	
Hyperdiploidy	8 (27.5)	11 (14.9)	
DUX4	1 (3.5)	0 (0)	
ZNF384	1 (3.5)	2 (2.7)	
T-ALL	10 (34.5)	25 (33.8)	
Other	0 (0)	20 (27.0)	

*A total of 31 and 29 samples had unknown subtype in the original cohort and validation cohort, respectively. WBC, white blood cells; SR, standard risk group; HR, high-risk group; VHR, very high-risk group.

Supplementary Table 3. Adjusted hazard ratios of predictors in the prognostic model estimated using the multivariate Cox proportional hazards regression model for the training and testing cohort

Event-Free Survival Variables	Training group (n=40)				Testing group (n=20)			
	HR	95% CI		*p*-value	HR	95% CI		*p*-value
High risk-score group	10.61	1.24	90.97	0.031	40.42	1.51	1.08E+03	0.028
Initial WBC ≥100	1.36	0.14	12.95	0.788	13.82	1.44	1.32E+02	0.023
T cell	0.72	0.10	5.02	0.736	0.04	0.00	2.51	0.129
Male	0.80	0.18	3.52	0.772	0.11	0.01	1.99	0.136
Age ≥10	6.21	1.26	30.54	0.025	82.43	1.24	5.47E+03	0.039
VHR	0.64	0.13	3.13	0.580	5.13	0.62	42.19	0.128
Overall Survival	Training group (n=40)				Testing group (n=20)			
Variables	HR	95% CI		*p*-value	HR	95% CI		*p*-value
High risk-score group	1.13E+08	0.00		0.995	7.39	0.37	1.49E+02	0.192
Initial WBC ≥100	1.84	0.18	18.69	0.606	8.32	0.43	1.61E+02	0.161
T cell	0.48	0.05	4.27	0.507	2.16E+05	0.00		0.997
Male	1.01	0.18	5.59	0.994	0.52	0.03	8.22	0.644
Age ≥10	11.84	1.77	79.34	0.011	0.00	0.00		0.997
VHR	0.66	0.11	3.90	0.650	2.39	0.18	32.09	0.512

WBC, white blood cells.

Supplementary Table 4. Prediction power of the risk score model

Cohort	EFS		OS	
	Harrell's C-index	95% CI	Harrell's C-index	95% CI
Training (n=40)	0.80	0.65-0.94	0.89	0.82-0.97
Testing (n=20)	0.89	0.79-0.99	0.89	0.75-1.00
Validation (n=103)	0.70	0.62-0.78	0.72	0.63-0.81