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Overexpression of annexin A5 might guide the gemtuzumab ozogamicin treatment choice in patients with pediatric acute myeloid leukemia

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Abstract

Background: Acute myeloid leukemia (AML) is a common hematological malignancy. Gemtuzumab ozogamicin (GO), a humanized anti-CD33 antibody conjugated with the potent anti-tumor antibiotic calicheamicin, represents a promising targeted therapy for AML. Annexin A5 (ANXA5) is a proposed marker for the clinical prognosis of AML to guide treatment choice.

Methods: In total, 253 patients with pediatric AML were enrolled and divided into two treatment groups: conventional chemotherapy alone and conventional chemotherapy in combination with GO. Univariate, multivariate, and Kaplan–Meier survival analyses were conducted to assess risk factors and clinical outcomes, and to estimate hazard ratios (HRs) and their 95% confidence interval. The level of statistical significance was set at $p < 0.05$.

Results: In the GO treatment group, high *ANXA5* expression was considered a favorable prognostic factor for overall survival (OS) and event-free survival (EFS). Multivariate analysis showed that high *ANXA5* expression was an independent favorable factor for OS (HR = 0.629, $p = 0.084$) and EFS (HR = 0.544, $p = 0.024$) distinct from the curative effect of GO treatment. When all patients were again divided into two groups, this time based on the median expression of *ANXA5*, patients undergoing chemotherapy combined with GO had significantly better OS ($p = 0.0012$) and EFS ($p = 0.0003$) in the *ANXA5* high-expression group. Gene set enrichment analysis identified a relevant series of pathways associated with glutathione metabolism, leukocyte transendothelial migration, and hematopoietic cell lineage.

Conclusion: The expression level of *ANXA5* can help optimize the treatment regimen for individual patients, and patients with overexpression of *ANXA5* may circumvent poor outcomes from chemotherapy combined with GO.

Keywords: acute myeloid leukemia, ANXA5, chemotherapy, gemtuzumab ozogamicin, prognosis

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Introduction

Acute myeloid leukemia (AML) is a common hematologic malignancy characterized by excessive proliferation of hematopoietic stem and progenitor cells, accounting for approximately 20% of childhood leukemia cases.[1] The diagnostic classification of AML includes a combination of morphology, immunology, cytogenetics, and molecular biology.[2] Currently, the standard treatment protocol for AML is combination chemotherapy, and patients may also undergo allogeneic stem cell transplantation, especially in high-risk groups for induction failure or recurrence.[3–5] Among patients over the age of 60 years, a

complete remission (CR) rate of 40% to 60% can be achieved, though only 5% to 15% can be cured.[1,6] For the large number of AML cases in children and adolescents, overall survival (OS) and CR rates are 67% and 93%, respectively.[7] Although the diagnostic technology and treatment protocol of AML have gradually improved, the cure rate for pediatric AML lags behind pediatric acute lymphoblastic leukemia, mostly due to relapse.[8,9] Therefore, there is an urgent need for optimizing therapeutic strategies to design personalized therapy for individual patients.

Gemtuzumab ozogamicin (GO), a humanized anti-CD33 antibody conjugated with the potent anti-tumor drug calicheamicin, represents a promising targeted therapy in AML.[10] In May 2000, GO was granted accelerated approval by the US Food and Drug Administration (FDA) for AML patients in first relapse who were not candidates for conventional chemotherapy.[11] However, GO has not demonstrated sufficient effect in different patient populations as a single agent; the overall response rate has been only 25–35%.[12–14] In 2010, Pfizer voluntarily withdrew the drug after a phase III trial that failed to demonstrate superior efficacy of GO.[15] Despite these setbacks, results from subsequent clinical trials administering GO at 3–6 mg/m^2 per dose demonstrated clinical benefit compared with standard chemotherapy in children and adults with AML, and the US FDA announced re-approval of GO in September 2017.[13,16]

Annexin A5 (ANXA5) is a member of the annexin family, which binds phospholipids in a calcium-dependent manner, playing major roles in regulating cellular growth, differentiation, inflammation, and signaling.[17,18] Under stress, *ANXA5* is produced and released into the extracellular medium or bloodstream, functioning as a physiological anticoagulant, anti-inflammatory, and anti-apoptotic agent by protecting stressed or dying cells from contact with inflammatory cells.[17,19] A previous study showed that abnormal expression patterns of *ANXA5* are associated with proliferation, invasion, drug resistance, and tumor treatment.[20–23] Furthermore, annexin family members have distinct prognostic roles in adult and pediatric AML. High expression levels of *ANXA2, ANXA6*, and *ANXA7* have been associated with worse prognoses of patients with AML, whereas *ANXA5* has been correlated with more favorable clinical outcomes.[24,25] Nevertheless, the relevance of annexin family members as predictive molecular markers to guide treatment choice remains largely unexplored.

In this study, we compared GO-containing treatment regimens with non-GO regimens in pediatric patients with AML. From the Therapeutically Applicable Research to Generate Effective Treatment (TARGET) database, a gene expression pattern associated with *ANXA5* expression was derived to investigate pediatric AML. *ANXA5* was identified as a gene that may circumvent poor outcomes in pediatric patients with AML treated with GO. Finally, our study revealed that expression of *ANXA5* can help optimize the treatment regimen for individual patients.

Materials and methods

Patients

A total of 253 patients aged 0–24 years and diagnosed with pediatric/adolescent AML were included in this study. The clinical data and treatment of the patients were analyzed retrospectively for receiving conventional chemotherapy (no-GO group, $n=95$) or the same chemotherapy regimen combined with a dose of 3 mg/m^2 GO (GO group, $n=158$). The datasets used in this investigation were acquired from the publicly available TARGET database (https://ocg.cancer.gov/), and clinical outcome data were included in Children's Oncology Group trials AAML0531 and AAML03P1.[26,27] Data were downloaded and analyzed until 20 June 2019 in this study. Exclusion criteria were as follows: (1) samples without clinical data, (2) samples without complete gene expression data and survival period, and (3) patients with acute promyelocytic leukemia, Down syndrome, or secondary/treatment-related leukemia. As the data were obtained from a publicly available database, further approval from the local ethics committee was not required.

Gene expression profiling

We used Perl language (https://www.perl.org/; version 5.30.0) to extract the gene matrix file, including *ANXA5* expression data and match gene expression array with clinical long-term follow-up data. Then, the Ensembl IDs were transformed into gene names based on the Ensembl database (http://www.ensembl.org). Patients with FLT3-ITD mutations were classified as either positive or negative.[28,29] The Search Tool for the Retrieval of Interacting Genes (STRING 11.0; https://string-db.org/) was used

to predict proteins interacting with a query protein.[30] In the present study, the following parameters were selected: hiding disconnected nodes in the network; medium confidence score > 0.400; no more than 20 interactions. The "pheatmap" package in R 3.5.2 software (https://www.r-project.org/) was used to construct a heat map of coexpression patterns of *ANXA5*. The Cancer Cell Line Encyclopedia (CCLE; https://portals.broadinstitute.org/ccle/about) dataset was used to analyze *ANXA5* expression in cancer cell lines.[31]

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is a knowledge-based method that determines whether a particular set of functionally related genes shows statistically significant, concordant differences between two biological states.[32] In this study, we used GSEA version 4.0.1 software (http://software.broadinstitute.org/gsea/). The 253 AML samples in this investigation were divided into a low- or high-expression group using *ANXA5* median expression level as a cut-off point. To identify potential mechanisms underlying the effects of gene expression, the expression level of *ANXA5* was used as a phenotype label, and gene set permutations were performed 1000 times for each analysis. Finally, the pathways enriched in each phenotype were sorted by normalized enrichment score (NES) and nominal *p*-value.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 8.02; GraphPad Software, Inc., La Jolla, CA, USA). Clinical and molecular characteristics of patients were described by median and/or range. Comparisons between continuous variables were analyzed using the Mann–Whitney *U* test, and the chi-square test or Fisher's exact test were used to compare differences in proportions of variables among groups. OS was defined as the time period from diagnosis to death or the date of last follow up. EFS was defined as the time from diagnosis to relapse, induction failure, death in remission, or the date of last follow up. OS and EFS were estimated by Kaplan–Meier analysis and log-rank test. Univariate and multivariate Cox proportional hazard models were constructed to analyze the impact of clinical prognostic factors in pediatric AML, and to estimate the hazard ratios (HRs) and their 95% confidence interval (CI). The level of statistical significance was set at $p < 0.05$.

Results

Patient characteristics

To establish correlations between *ANXA5* expression and various clinical characteristics in pediatric AML, we assigned patients who underwent chemotherapy combined with ±GO to one of two groups, according to median *ANXA5* expression levels, respectively. Details on the clinical and molecular characteristics of patients in both groups are summarized in Table 1. The median age was 10.4 (range 0.1–23.5) years. In the no-GO treatment group, participants who exhibited downregulated *ANXA5* had a higher percentage of *WT1* mutation compared with upregulated *ANXA5* expression ($p = 0.016$). In addition, high *ANXA5* expression often had more *MLL* ($p = 0.008$) or *CBF*β*-MYH11* ($p = 0.002$) mutations and fewer *RUNX1-RUNX1T1* ($p = 0.004$) fusions. Moreover, patients with lower *ANXA5* expression had higher peripheral blood myeloblast counts ($p = 0.007$), though the median was not different between the two groups. Between the high and low *ANXA5* expression groups, no significant differences were observed in age, sex, ethnicity, white blood cell count, bone marrow blast, complex karyotype, or *FLT3-ITD, NPM1, CEBPA*, and c-KIT mutations in exons 8 and 17. In the GO treatment group, study participants with downregulated *ANXA5* exhibited a higher frequency of *CEBPA* ($p = 0.005$) and *FLT3-ITD* ($p = 0.022$) mutations, whereas c-Kit mutations in exons 17 ($p = 0.007$) and white blood cell counts ($p = 0.001$) were lower. Overall, the clinical and molecular characteristics of the two treatment groups at diagnosis were similar (Supplemental Table S1), excluding the influence of *ANXA5* expression. Interestingly, both treatment groups showed that patients with low *ANXA5* expression were more often diagnosed with M1 or M2 compared with patients with high *ANXA5* expression.

Prognostic value of ANXA5 *expression*

A log-rank test of Kaplan–Meier curves was used to describe the differences in survival to estimate clinical outcomes of *ANXA5* in patients with different chemotherapy regimens. In the no-GO treatment group, survival distribution curves demonstrated that high *ANXA5* expressers had shorter OS (HR = 2.086, 95% CI 1.158–3.758, $p = 0.0141$) and EFS (HR = 2.211, 95% CI 1.225–3.991, $p = 0.0077$) than low expressers [Figure 1(a and b)]. However, high expression of *ANXA5* was considered a favorable prognostic

Table 1. Comparison of clinical and molecular characteristics with *ANXA5* expression in patients.

Characteristic	No gemtuzumab ozogamicin treatment			Gemtuzumab ozogamicin treatment		
	***ANXA5*^{high} (*n* = 47)**	***ANXA5*^{low} (*n* = 48)**	***p*-value**	***ANXA5*^{high} (*n* = 79)**	***ANXA5*^{low} (*n* = 79)**	***p*-value**
Age/years, median (range)	10.6 (0.1–20.3)	10.3 (1.7–23.5)	0.101*	10.4 (0.4–22.5)	10.3 (1.2–18.2)	0.448*
Age group/*n* (%)			0.612§			0.633§
<10 years	23 (48.9)	21 (43.8)		40 (50.6)	37 (46.8)	
⩾10 years	24 (51.1)	27 (56.2)		39 (49.4)	42 (53.2)	
Sex/*n* (%)			0.051§			0.076§
Male	19 (40.4)	30 (62.5)		52 (65.8)	40 (50.6)	
Female	28 (59.6)	18 (37.5)		27 (34.2)	39 (49.4)	
Ethnicity/*n*(%)						
Hispanic or Latino	11 (23.4)	10 (20.8)	0.763§	14 (17.7)	16 (20.3)	0.685§
Not Hispanic or Latino	32 (68.1)	36 (75.0)	0.455§	63 (79.7)	61 (77.2)	0.699§
Unknown	4 (8.5)	2 (4.2)	0.384§	2 (2.5)	2 (2.5)	1.000§
WBC/$\times 10^9$/l, median (range)	33.5 (1.3–519)	34.6 (0.9–439.2)	0.183*	53.5 (4.2–446)	52.6 (1.5–263.1)	0.001*
BM blast/%, median (range)	71 (23–99)	71 (20–99)	0.961*	77 (21–100)	77 (21–99)	0.885*
PB blast/%, median (range)	62 (0–97)	62 (2–97)	0.007*	61 (0–95)	61 (0–97)	0.792*
FAB subtypes/*n* (%)						
M0	0 (0)	2 (4.2)	0.157§	2 (2.5)	4 (5.1)	0.405§
M1	3 (6.4)	10 (20.8)	0.040§	3 (3.8)	16 (20.3)	0.001§
M2	4 (8.5)	19 (39.6)	0.001§	8 (10.1)	26 (32.9)	0.001§
M4	10 (21.3)	4 (8.3)	0.075§	35 (44.3)	8 (10.1)	0.001§
M5	14 (29.8)	2 (4.2)	0.001§	23 (29.1)	9 (11.4)	0.006§
M6	0 (0)	1 (2.1)	0.319§	0 (0)	2 (2.5)	0.155§
M7	5 (10.6)	1 (2.1)	0.087§	1 (1.3)	1 (1.3)	1.000§
Others	11 (23.4)	9 (18.6)	0.578§	7 (8.9)	13 (16.5)	0.151§
Cytogenetics/*n* (%)						
Normal	9 (19.1)	11 (22.9)	0.652§	17 (21.5)	28 (35.4)	0.056§
Complex karyotype	7 (14.9)	7 (14.6)	0.966§	12 (15.2)	11 (13.9)	0.822§
inv(16)/*CBFβ-MYH11*	11 (23.4)	1 (2.1)	0.002§	23 (29.1)	1 (1.3)	0.001§
11q23/*MLL*	14 (29.8)	4 (8.3)	0.008§	14 (17.7)	9 (11.4)	0.259§
t(8;21)/*RUNX1-RUNX1T1*	3 (6.4)	14 (29.2)	0.004§	5 (6.3)	16 (20.3)	0.010§
Others	3 (6.4)	11 (22.9)	0.023§	8 (10.1)	14 (17.7)	0.168§

Characteristic	No gemtuzumab ozogamicin treatment			Gemtuzumab ozogamicin treatment		
	ANXA5^{high} (*n* = 47)	*ANXA5*^{low} (*n* = 48)	*p*-value	*ANXA5*^{high} (*n* = 79)	*ANXA5*^{low} (*n* = 79)	*p*-value
Risk/*n* (%)						
Good	18 (38.2)	21 (43.7)	0.589§	33 (41.8)	31 (39.2)	0.746§
Intermediate	27 (57.4)	19 (39.5)	0.082§	35 (44.3)	34 (43.0)	0.873§
Poor	2 (4.2)	8 (16.6)	0.049§	7 (8.9)	1 0 (12.7)	0.441§
Others	0 (0)	0 (0)	1.000§	4 (5.1)	4 (5.1)	1.000§
***FLT3-ITD*/*n* (%)**			0.473§			0.022§
Positive	8 (17.0)	11 (22.9)		6 (7.6)	16 (20.3)	
Negative	39 (83.0)	37 (77.1)		73 (92.4)	63 (79.7)	
***NPM1*/*n* (%)**			0.751§			0.548§
Mutation	4 (8.5)	5 (10.4)		7 (8.9)	5 (6.3)	
Wild type	43 (91.5)	43 (89.6)		72 (91.1)	74 (93.7)	
***CEBPA*/*n* (%)**			0.176§			0.005§
Mutation	1 (2.1)	4 (8.3)		1 (1.3)	10 (12.7)	
Wild type	46 (97.9)	44 (91.7)		78 (98.7)	69 (87.3)	
***WT1*/*n* (%)**			0.016§			0.148§
Mutation	1 (2.1)	8 (16.7)		4 (5.1)	9 (11.4)	
Wild type	46 (97.9)	40 (83.3)		75 (94.9)	70 (88.6)	
c-Kit mutation exon 8/*n* (%)						
Yes	4 (8.5)	2 (4.2)	0.384§	3 (3.8)	5 (6.3)	0.468§
No	10 (21.3)	13 (27.1)	0.509§	20 (25.3)	10 (12.7)	0.043§
Not done	33 (70.2)	33 (68.8)	0.878§	56 (70.9)	64 (81.0)	0.136§
c-Kit mutation exon 17/*n* (%)						
Yes	2 (4.3)	5 (10.4)	0.250§	7 (8.9)	0 (0)	0.007§
No	12 (25.5)	10 (20.8)	0.587§	16 (20.3)	15 (19.0)	0.841§
Not done	33 (70.2)	33 (68.8)	0.878§	56 (70.9)	64 (81.0)	0.136§
CNS disease/*n* (%)			0.570§			0.230§
Yes	1 (2.1)	2 (4.2)		4 (5.1)	8 (10.1)	
No	46 (97.9)	46 (95.8)		75 (94.9)	71 (89.9)	
CR1 achieved/*n* (%)						
Yes	31 (66.0)	31 (64.6)	0.888§	70 (88.6)	60 (75.9)	0.037§

(Continued)

Table 1. (Continued)

Characteristic	No gemtuzumab ozogamicin treatment			Gemtuzumab ozogamicin treatment		
	ANXA5^{high} (*n* = 47)	*ANXA5*^{low} (*n* = 48)	*p*-value	*ANXA5*^{high} (*n* = 79)	*ANXA5*^{low} (*n* = 79)	*p*-value
No	15 (31.9)	17 (35.4)	0.718§	9 (11.4)	19 (24.1)	0.037§
Unevaluable	1 (2.1)	0 (0)	0.310§	0 (0)	0 (0)	1.000§

BM, bone marrow; CNS, central nervous system; CR1, first complete remission; FAB, French American British; PB, peripheral blood; WBC, white blood cell.
*denotes Mann–Whitney *U* test.
§denotes chi-square test. Complex karyotype is defined as more than or equal to three chromosomal abnormalities.

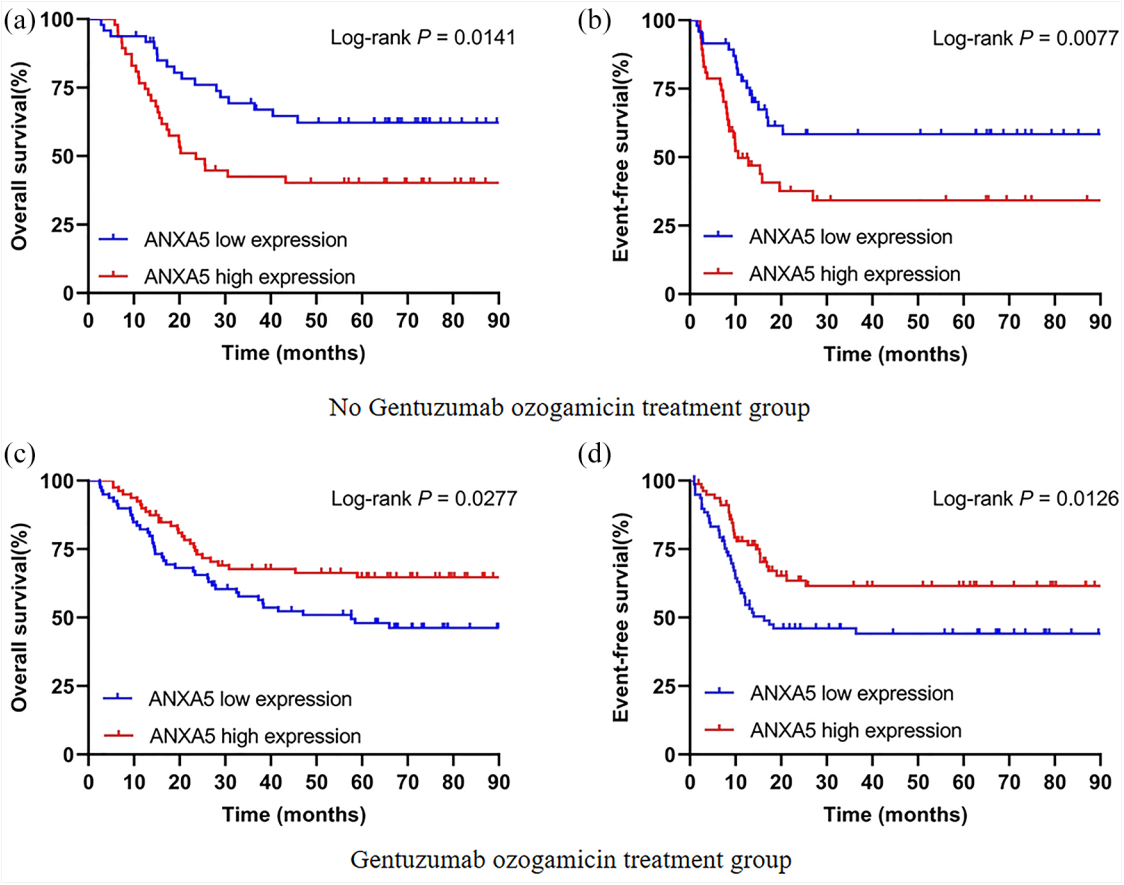

Figure 1. Kaplan–Meier curves of survival in pediatric AML patients with respect to *ANXA5* expression. (a, b) Effect of *ANXA5* expression on OS and EFS in the no-GO treatment group (*n* = 95). (c, d) Patients with high *ANXA5* expression had significantly prolonged OS and EFS in the GO treatment group (*n* = 158).
AML, acute myeloid leukemia; EFS, event-free survival; GO, gemtuzumab ozogamicin; OS, overall survival.

factor for OS (HR = 0.583, 95% CI 0.352–0.939, $p = 0.0277$) and EFS (HR = 0.545, 95% CI 0.338–0.878, $p = 0.0126$) in patients with AML who received GO treatment [Figure 1(c and d)]. These results suggest that the curative effect of GO treatment may be closely related to the level of *ANXA5* expression regulation in pediatric AML.

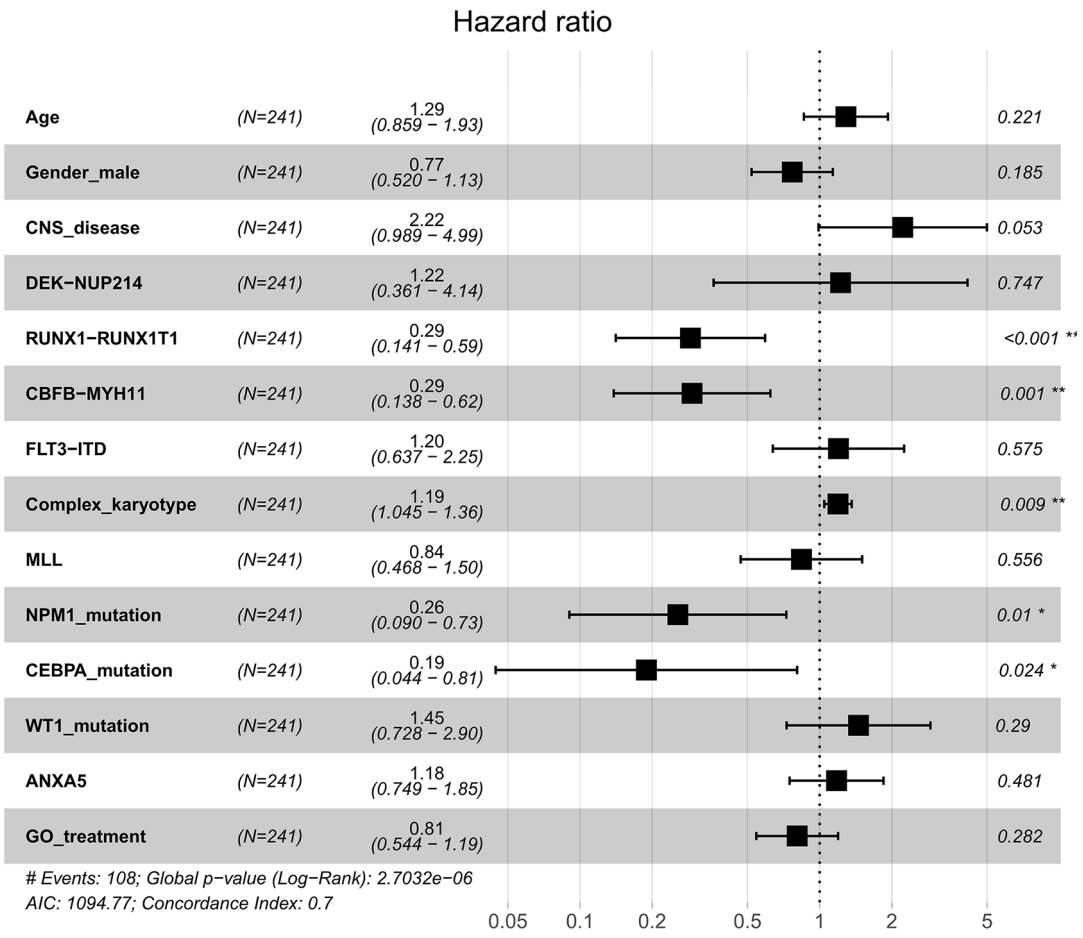

Figure 2. Forest plot of HR for OS according to prognostic factors with all patients with AML. Multivariate analyses of age, sex, CNS disease, *DEK-NUP214, RUNX1-RUNX1T1, CBFB-MYH11, FLT3-ITD*, complex karyotype, *MLL, NPM1* mutation, *CEBPA* mutation, *WT1* mutation, GO treatment and *ANXA5* expression group for OS in all patients. The black squares on the transverse lines represent the HR, and the gray transverse lines represent 95% CI.

AML, acute myeloid leukemia; CI, confidence interval; CNS, central nervous system; GO, gemtuzumab ozogamicin; HR, hazard ratio; OS, overall survival.

*$p < 0.05$ and **$p < 0.01$.

Univariate and multivariate analyses for prognostic factors

To evaluate the impact of clinical and molecular factors associated with *ANXA5* expression level in pediatric AML, Cox proportional hazard models were constructed. The variables included expression levels of *ANXA5* (high *versus* low), age (⩾10 *versus* <10 years), sex (male *versus* female), white blood cells (⩾50 *versus* $<50 \times 10^9$/l), bone marrow blasts (⩾70% *versus* <70%), peripheral blood blasts (⩾50% *versus* <50%), *NPM1* (mutated *versus* wild type), *MLL* (mutated *versus* wild type), *FLT3-ITD* (positive *versus* negative), *CBF*β*-MYH11* (positive *versus* negative), *RUNX1-RUNX1T1* (positive *versus* negative), *WT1* (mutated *versus* wild type), and risk (poor *versus* non-poor). A forest plot was constructed for OS for all patients according to prognostic factors (Figure 2), and the results showed that favorable factors in pediatric AML were found in terms of *RUNX1-RUNX1T1, CBF*β*-MYH11, NPM1* mutation, and *CEBPA* mutation, consistent with the results of the current risk stratification criteria.[33] Meanwhile, we developed a nomogram to predict the probability of the 1- and 5-year OS including *ANXA5* and clinical–biological prognostic index (see Figure 3).

In the no-GO treatment group, univariate analysis showed that high *ANXA5* expression was associated with shorter OS (HR = 2.097, 95% CI 1.146–3.838, $p = 0.016$) and EFS (HR = 2.232, 95% CI 1.218–4.093, $p = 0.009$). Furthermore,

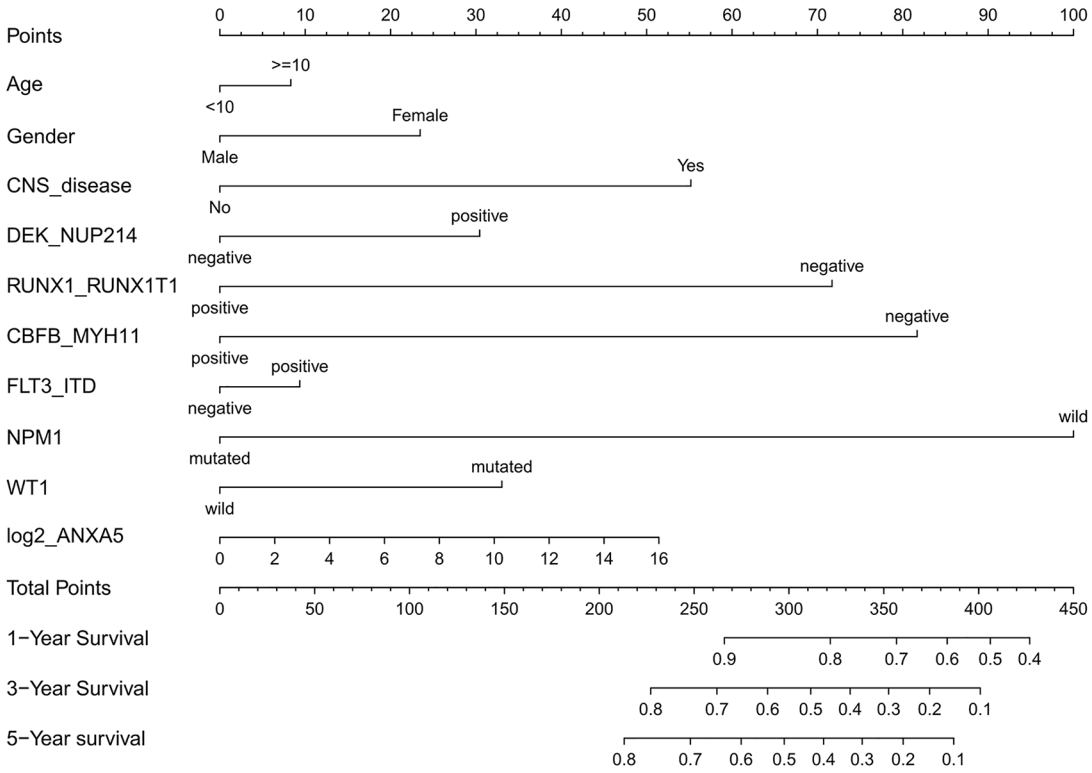

Figure 3. Nomogram for the prediction of OS at 1-, 3- and 5-years according to the clinical–biological prognostic index in patients with pediatric AML. By adding up the points assigned to each predictive variable, the total score on the bottom scale shows the probability of survival.
AML, acute myeloid leukemia; OS, overall survival.

multivariate analysis indicated that *ANXA5* upregulation was an independent risk factor for OS (HR = 2.687, 95% CI 1.355–5.326, $p = 0.005$) and EFS (HR = 2.762, 95% CI 1.365–5.591, $p = 0.005$). Results are shown in Table 2.

In the GO treatment group, univariate analysis showed that high *ANXA5* expression was associated with longer OS (HR = 0.583, 95% CI 0.359–0.948, $p = 0.030$) and EFS (HR = 0.544, 95% CI 0.334–0.884, $p = 0.014$), as well as *CBFβ/MYH11*-positive for OS (HR = 0.364, 95% CI 0.146–0.906, $p = 0.030$) and EFS (HR = 0.356, 95% CI 0.143–0.885, $p = 0.026$). In addition, multivariate analysis showed that high *ANXA5* expression was an independent favorable factor for OS (HR = 0.629, 95% CI 0.372–1.064, $p = 0.084$) and EFS (HR = 0.544, 95% CI 0.321–0.922, $p = 0.024$) in combination with the curative effect of GO treatment. Results are shown in Table 3.

Patients overexpressing ANXA5 *benefited from GO treatment*

The 253 patients were divided into two groups based on median expression levels of *ANXA5* in order to investigate whether GO treatment could overcome the unfavorable outcomes of high *ANXA5* expression in pediatric AML. In the high *ANXA5* expression group, the patients undergoing chemotherapy combined with GO had significantly better OS ($p = 0.0012$) and EFS ($p = 0.0003$) compared with patients treated with conventional chemotherapy alone [Figure 4(a and b)]. For the low *ANXA5* expression group, there were no obvious differences in OS ($p = 0.0679$) regardless of whether or not the chemotherapy regimen was combined with GO [Figure 4(c)]. However, low expression of *ANXA5* might be considered an unfavorable prognostic factor in EFS ($p = 0.0441$) under GO treatment [Figure 4(d)]. Pediatric AML patients with overexpression of *ANXA5* may circumvent poor outcomes from chemotherapy combined with GO.

Table 2. Univariate and multivariate analysis for EFS and OS in patients without gemtuzumab ozogamicin treatment.

Variables	OS		EFS	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Univariate analyses				
ANXA5 (high *versus* low)	2.097 (1.146–3.838)	0.016	2.232 (1.218–4.093)	0.009
Age (⩾10 *versus* <10 years)	1.017 (0.565–1.833)	0.954	0.948 (0.526–1.709)	0.859
Sex (male *versus* female)	1.288 (0.715–2.32)	0.399	1.271 (0.706–2.289)	0.425
Ethnicity (Hispanic or Latino *versus* others)	1.657 (0.854–3.213)	0.135	1.507 (0.777–2.924)	0.225
WBC (⩾50 *versus* $<50\times10^9$/l)	1.003 (0.552–1.821)	0.992	1.315 (0.723–2.393)	0.37
BM blasts (⩾70 *versus* <70%)	0.957 (0.532–1.72)	0.883	0.979 (0.545–1.76)	0.943
PM blasts (⩾50 *versus* <50%)	0.718 (0.395–1.305)	0.277	0.865 (0.476–1.571)	0.634
NPM1 (mutated *versus* wild type)	0.578 (0.179–1.867)	0.36	0.547 (0.169–1.767)	0.313
MLL (mutated *versus* wild type)	1.807 (0.869–3.756)	0.113	1.683 (0.809–3.5)	0.163
FLT3-ITD (positive *versus* negative)	1.163 (0.576–2.351)	0.673	1.288 (0.637–2.603)	0.481
CBFβ-MYH11 (positive *versus* negative)	0.598 (0.214–1.669)	0.326	0.687 (0.245–1.925)	0.476
RUNX1-RUNX1T1 (positive *versus* negative)	0.507 (0.2–1.284)	0.152	0.404 (0.159–1.026)	0.057
WT1 (mutated *versus* wild type)	1.211 (0.433–3.385)	0.715	1.4 (0.498–3.933)	0.523
Risk (poor *versus* non-poor)	2.795 (1.415–5.521)	0.003	3.259 (1.646–6.449)	0.001
Multivariate analyses				
ANXA5 (high *versus* low)	2.687 (1.355–5.326)	0.005	2.762 (1.365–5.591)	0.005
Ethnicity (Hispanic or Latino *versus* others)	1.652 (0.845–3.231)	0.142	1.408 (0.722–2.746)	0.315
MLL (mutated *versus* wild type)	0.783 (0.335–1.828)	0.572	0.709 (0.309–1.626)	0.417
FLT3-ITD (positive *versus* negative)	1.343 (0.62–2.912)	0.455	1.535 (0.696–3.383)	0.288
RUNX1-RUNX1T1 (positive *versus* negative)	2.009 (0.572–7.06)	0.277	1.884 (0.513–6.923)	0.34
Risk (poor *versus* non-poor)	3.806 (1.518–9.542)	0.004	4.492 (1.781–11.33)	0.001

BM, bone marrow; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival; PB, peripheral blood; PM, peripheral blood; non-poor, intermediate and good; WBC, white blood cell.

Biological insights

To generate insight into the biological functions of *ANXA5* among pediatric patients with AML, we analyzed the features of gene expression connected with *ANXA5* expression. An association between the expression of the most frequently altered neighbor genes and *ANXA5* was observed; the network is shown in Figure 5(a). In addition, we extracted the gene matrix file including immune-related genes in pediatric AML, and a

Table 3. Univariate and multivariate analysis for EFS and OS in patients with gemtuzumab ozogamicin treatment.

Variables	OS		EFS	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Univariate analyses				
ANXA5 (high *versus* low)	0.583 (0.359–0.948)	0.03	0.544 (0.334–0.884)	0.014
Age (⩾10 *versus* <10 years)	1.105 (0.687–1.778)	0.68	0.956 (0.594–1.539)	0.853
Sex (male *versus* female)	0.524 (0.325–0.845)	0.008	0.538 (0.334–0.867)	0.011
Ethnicity (Hispanic or Latino *versus* others)	1.19 (0.661–2.142)	0.563	1.179 (0.655–2.124)	0.582
WBC (⩾50 *versus* <50 × 109/l)	0.725 (0.449–1.17)	0.188	0.711 (0.441–1.148)	0.163
BM blasts (⩾70 *versus* <70%)	0.947 (0.584–1.536)	0.827	0.906 (0.558–1.47)	0.688
PM blasts (⩾50 *versus* <50%)	0.981 (0.601–1.6)	0.938	0.905 (0.554–1.476)	0.688
NPM1 (mutated *versus* wild type)	0.328 (0.08–1.34)	0.121	0.303 (0.074–1.237)	0.096
MLL (mutated *versus* wild type)	1.274 (0.632–2.57)	0.499	1.291 (0.64–2.603)	0.476
FLT3-ITD (positive *versus* negative)	1.546 (0.81–2.954)	0.187	1.483 (0.777–2.829)	0.232
*CBF*β-*MYH11* (positive *versus* negative)	0.364 (0.146–0.906)	0.03	0.356 (0.143–0.885)	0.026
RUNX1-RUNX1T1 (positive *versus* negative)	0.545 (0.236–1.261)	0.156	0.528 (0.228–1.222)	0.136
WT1 (mutated *versus* wild type)	1.935 (0.923–4.056)	0.08	2.208 (1.052–4.632)	0.036
Risk (poor *versus* non-poor)	3.115 (1.815–5.346)	0.001	3.451 (2.009–5.928)	0.001
Multivariate analyses				
ANXA5 (high *versus* low)	0.629 (0.372–1.064)	0.084	0.544 (0.321–0.922)	0.024
NPM1 (mutated *versus* wild type)	0.432 (0.103–1.811)	0.251	0.409 (0.098–1.701)	0.219
*CBF*β-*MYH11* (positive *versus* negative)	0.9 (0.297–2.729)	0.852	1.034 (0.34–3.143)	0.953
WT1 (mutated *versus* wild type)	1.569 (0.735–3.35)	0.244	1.753 (0.822–3.738)	0.146
Risk (poor *versus* non-poor)	2.778 (1.476–5.228)	0.002	3.323 (1.764–6.26)	0.001

BM, bone marrow; CI, confidence interval; EFS, event-free survival; HR, hazard ratio; OS, overall survival; PB, peripheral blood; PM, peripheral blood; non-poor, intermediate and good; WBC, white blood cell.

heat map of genes coexpressed with *ANXA5* expression level was constructed. Among these genes, 7 were negatively correlated and 26 were positively correlated with the expression of *ANXA5* [Figure 5(b)]. The results showed that *ANXA5* expression was positively correlated with the expression of *ANXA2, HMOX1, CD4, CD14, C3*, and *MAP2K1*, as well as *HLA-B* and *HLA-C*. Notably, these molecular markers are crucial for the leukemogenesis and immune functions of regulation in AML.[34,35] Figure 5(c) shows the expression of *ANXA5* in different tumor cell lines according to the CCLE database.

To understand the functional biological implications of aberrantly expressed *ANXA5* in pediatric

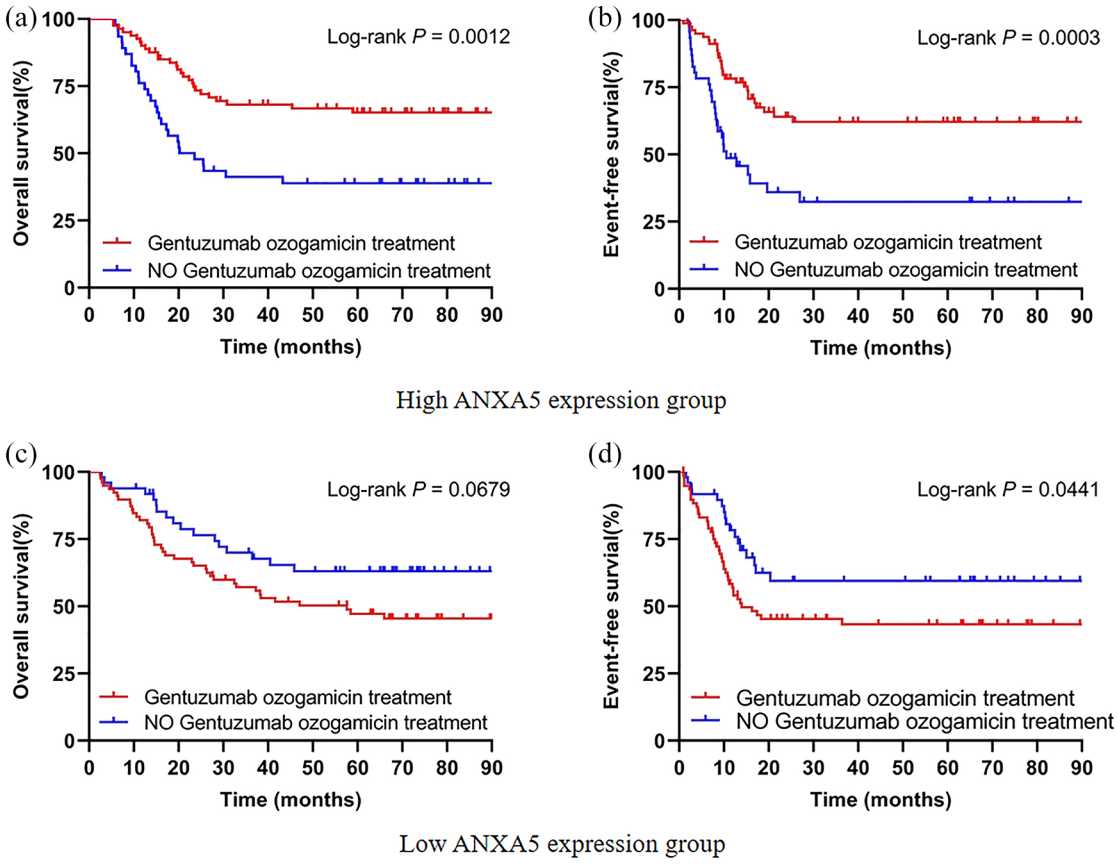

Figure 4. GO treatment circumvents the unfavorable outcomes of high *ANXA5* expression in pediatric AML patients. A total of 253 patients were divided into two groups based on the median expression levels of *ANXA5*. (a, b) Kaplan–Meier curves of OS and EFS in patients with GO treatment ($n = 80$) and without GO treatment ($n = 46$) in the high-*ANXA5*-expression group. (c, d) Kaplan–Meier curves of OS and EFS in patients with GO treatment ($n = 78$) and without GO treatment ($n = 49$) in the low-*ANXA5*-expression group.
AML, acute myeloid leukemia; EFS, event-free survival; GO, gemtuzumab ozogamicin; OS, overall survival.

AML, a top 10 gene ontology enrichment analysis was conducted. Relevant biological processes included were neutrophil activation, neutrophil-mediated immunity, phagocytosis, and innate immune-response-activating signal transduction. Furthermore, the most enriched gene ontology terms in cellular component included lysosomal lumen and secretory granule membrane; in molecular function, the most enriched terms were immunoglobulin binding, cytokine receptor activity, and signaling pattern recognition receptor activity. The results of the bubble diagram are shown in Figure 6.

To explore signaling pathways in which *ANXA5* is differentially activated in pediatric AML, we performed GSEA between high and low *ANXA5* expression data sets. GSEA revealed significant differences (nominal < 0.05, false discovery rate $p < 0.05$) in the gene expression profile from the MSigDB collection (c2.cp.kegg.version 7.0.symbols). The significant pathways for gene sets are listed in order of significance in Table 4. Multiple pathways, including glutathione metabolism, leukocyte transendothelial migration, chemokine signaling pathway, NOTCH signaling pathway, Toll-like receptor signaling pathway, hematopoietic cell lineage, vascular endothelial growth factor (VEGF) signaling pathway, and B-cell receptor signaling pathway, were significant in *ANXA5* high-expression phenotype. Enrichment plots were shown in Figure 7. Our results indicate that *ANXA5* high-expression may be associated with AML progression and sensitivity to chemotherapeutic agents such as GO.

Discussion

The prognosis of pediatric AML has improved tremendously over the last few decades, with the

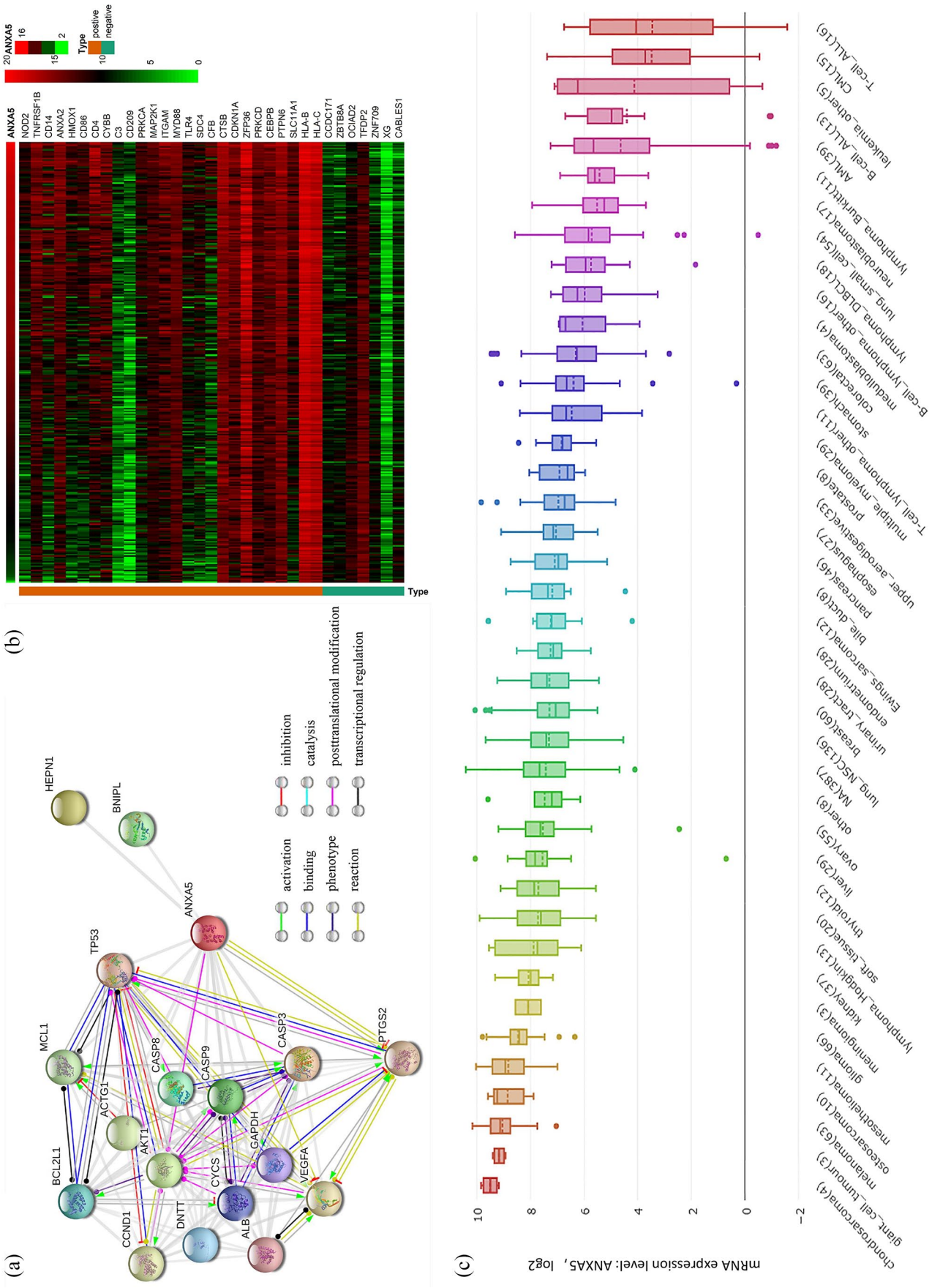

Figure 5. Analysis of *ANXA5* gene expression. (a) The network for *ANXA5* and the most frequently altered neighbor genes, analyzed by STRING. (b) Immune-related genes coexpressed of heat map with *ANXA5* in pediatric AML. (c) The expression of *ANXA5* in tumor cell lines, analyzed by CCLE.
AML, acute myeloid leukemia; CCLE, the Cancer Cell Line Encyclopedia; STRING, Search Tool for the Retrieval of Interacting Genes.

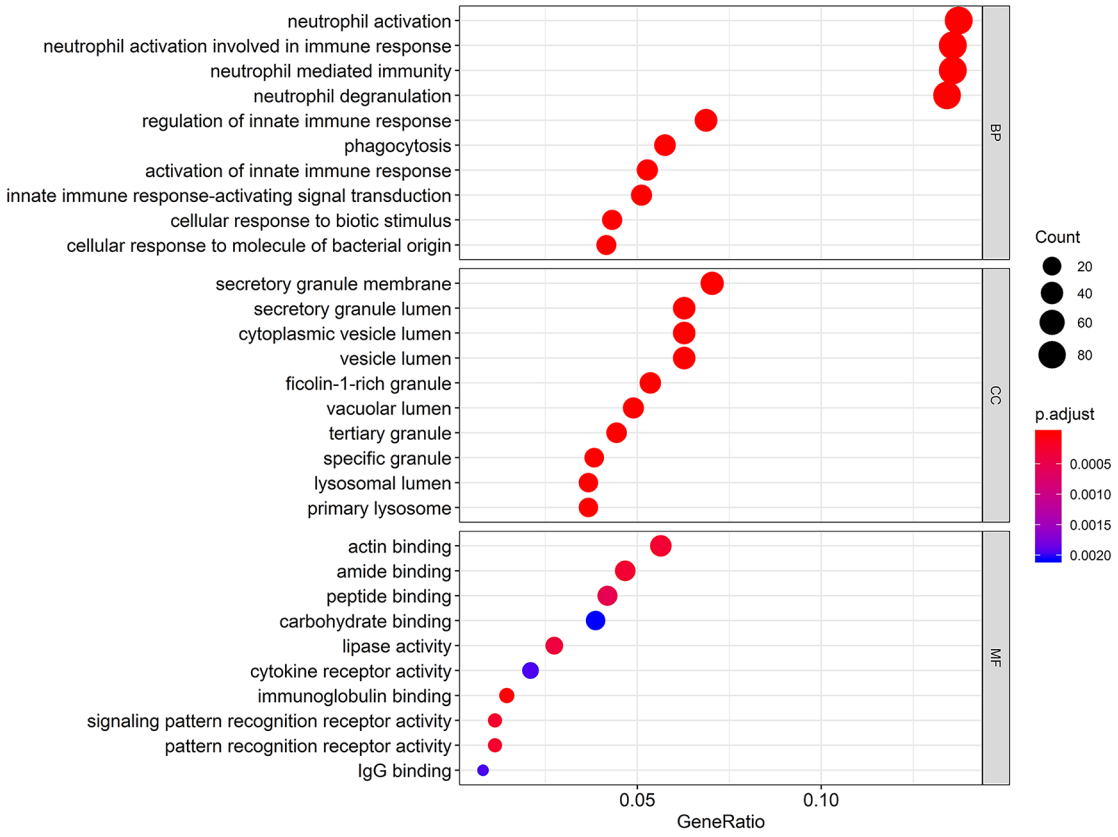

Figure 6. Gene ontology terms of biological processes, cellular component, and molecular function in the *ANXA5* associated expression profile with pediatric AML. The size of each dot represents the count of genes, the color represents the adjusted *p*-value.
AML, acute myeloid leukemia.

survival rate today reaching approximately 75%.[36] Unfortunately, one third of cases eventually relapse.[37] Cytarabine- and anthracycline-based induction chemotherapy for AML has been used for 30–40 years and needs to be optimized to prolong survival and increase CR rate without adopting dose escalation.[38] Recent progress in the genetic and molecular etiology of leukemia has led to molecular-targeted therapies.[39] The first targeted therapy was a combination of all-trans retinoic acid and arsenic trioxide for patients with AML with rearrangement of the retinoic acid receptor.[40] Subsequently, fms-related tyrosine kinase 3 (FLT3) inhibitors targeting *FLT3* mutations emerged, but the effective treatment period of any given drug as a single agent was only 2–6 months.[41] Therefore, another more broad-spectrum *FLT3* inhibitor, midostaurin, combined with chemotherapy, was approved by the US FDA for use in newly diagnosed patients with AML.[42,43] However, patients treated with this regimen still had a high recurrence rate. More recently, inhibitors ivosidenib and enasidenib, targeting *IDH1* and *IDH2* mutations, as well as inhibitors targeting epigenetically related genes such as *EZH2*, *KDM1A* and *DOT1L* mutations were successively approved by the US FDA.[44] These hypomethylated drugs showed good results in specific types of AML, such as patients with mutations in the *TET2* or *p53* genes.[45,46] Venetoclax, an inhibitor of *BCL2* gene mutation, has been used in elderly patients with AML.[47] The effective rate of single-drug therapy in patients with recurrence was about 20%, and combined with hypomethylated drug therapy, 60%.[48] Furthermore, GO can be used in AML induction chemotherapy to improve OS and relapse free survival (RFS) and reduce recurrence rates.[26,38] Several clinical trials have evaluated the efficacy of GO in combination with conventional cytotoxic chemotherapy, but some may increase the toxic load. The cytotoxic effect of GO depends heavily on its intracellular trafficking and processing. Previous research has shown that activation

Table 4. Gene set enrichment analysis demonstrated the correlation between *ANXA5* expression phenotype.

Name	ES	NES	NOM *p*-value	FDR *q*-value	Leading edge
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	0.6027	2.0824	0	0.0069	tags=39%, list=13%, signal=44%
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	0.706	2.0465	0.0021	0.0073	tags=20%, list=2%, signal=20%
KEGG_GLYCOLYSIS_GLUCONEOGENESIS	0.5859	1.984	0	0.0094	tags=44%, list=19%, signal=54%
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	0.5	1.9654	0	0.0096	tags=32%, list=17%, signal=38%
KEGG_GLUTATHIONE_METABOLISM	0.5781	1.9597	0.0021	0.0094	tags=56%, list=24%, signal=74%
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.5061	1.9264	0	0.0113	tags=30%, list=13%, signal=34%
KEGG_CHEMOKINE_SIGNALING_PATHWAY	0.4926	1.9236	0	0.0104	tags=27%, list=13%, signal=31%
KEGG_PENTOSE_PHOSPHATE_PATHWAY	0.6734	1.9087	0.0021	0.0128	tags=65%, list=22%, signal=84%
KEGG_NOTCH_SIGNALING_PATHWAY	0.5746	1.9063	0	0.0125	tags=40%, list=19%, signal=50%
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	0.5263	1.8692	0.002	0.017	tags=47%, list=28%, signal=65%
KEGG_HEMATOPOIETIC_CELL_LINEAGE	0.5143	1.8585	0.004	0.0174	tags=31%, list=11%, signal=34%
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.5053	1.8251	0.0084	0.0223	tags=43%, list=27%, signal=58%
KEGG_VEGF_SIGNALING_PATHWAY	0.5002	1.8199	0	0.0226	tags=36%, list=16%, signal=42%
KEGG_ENDOCYTOSIS	0.4643	1.8076	0	0.024	tags=38%, list=20%, signal=47%
KEGG_CELL_ADHESION_MOLECULES_CAMS	0.4348	1.7818	0.0021	0.0287	tags=31%, list=22%, signal=40%
KEGG_BETA_ALANINE_METABOLISM	0.5792	1.7665	0.0066	0.0304	tags=64%, list=35%, signal=97%
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	0.5403	1.7504	0.0104	0.0336	tags=26%, list=8%, signal=28%
KEGG_PYRUVATE_METABOLISM	0.5485	1.7417	0.0085	0.0359	tags=41%, list=20%, signal=51%
KEGG_TRYPTOPHAN_METABOLISM	0.5075	1.7353	0.0086	0.0365	tags=43%, list=26%, signal=57%
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.5245	1.7279	0.0085	0.0379	tags=31%, list=13%, signal=35%

ES, enrichment score; FDR, false discovery rate; NES, normalized enrichment score; NOM, nominal.

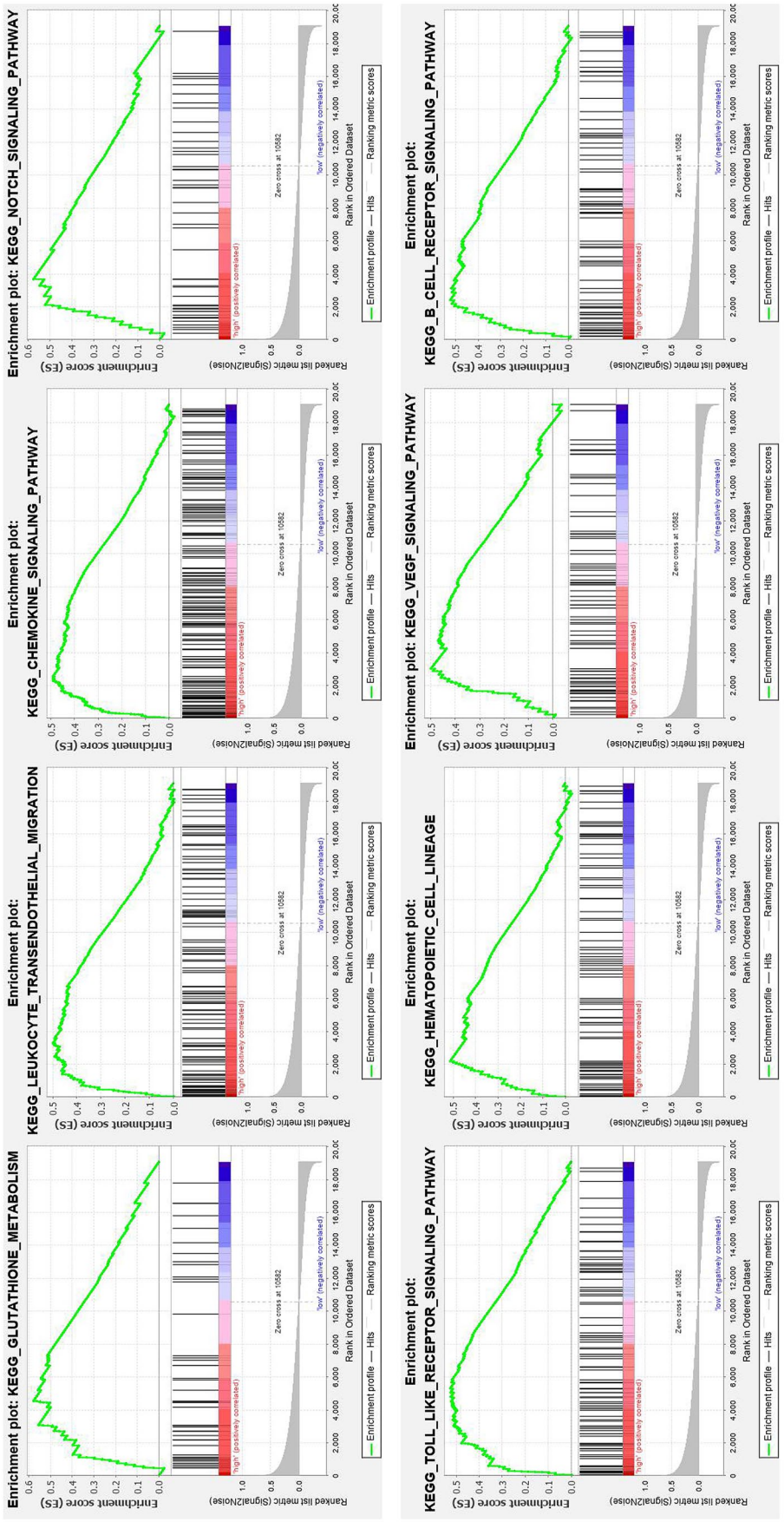

Figure 7. GSEA results showing differential enrichment of genes related to glutathione metabolism, leukocyte transendothelial migration, chemokine signaling pathway, NOTCH signaling pathway, Toll-like receptor signaling pathway, hematopoietic cell lineage, VEGF signaling pathway, and B-cell receptor signaling pathway in pediatric AML with high *ANXA5* expression.
AML, acute myeloid leukemia; GSEA, gene set enrichment analysis; VEGF, vascular endothelial growth factor.

of lysosomal functions in primary leukemia cells could enhance the cytotoxicity of GO.[49] Therefore, it is necessary to integrate available data for evaluation of this drug.

Further research is required to guide clinical therapy for pediatric patients with AML, and to identify molecular predictive markers of efficacy. The present study analyzed the RNA-sequencing data from TARGET databases with a focus on pediatric AML. We evaluated the correlation among *ANXA5* expression with clinical characteristics between GO and no-GO groups, and further described the differences in survival to estimate the clinical outcome of *ANXA5* in patients with different chemotherapy regimens. Furthermore, the results suggest that high *ANXA5* expression correlates with an adverse outcome in patients with pediatric AML treated with conventional chemotherapy. However, patients undergoing conventional chemotherapy combined with GO are able to overcome the adverse effect of high *ANXA5* expression. Our findings suggest that *ANXA5* can be considered a predictive molecule to design personalized therapy for individual patients. Patients with high expression of *ANXA5* will benefit more from GO treatment in pediatric AML. Of note, we made an effort to study the effect of knockdown *ANXA5* on AML cells lines using specific siRNA. *ANXA5* was decreased upon siRNA knockdown; however, it did not significantly alter the expression of CD33. Therefore, *ANXA5* may affect the efficacy of GO *via* an indirect mechanism, and high-throughput sequencing bioinformatics analysis helps to clarify the potential biological implications of this work. We noticed that the expression of *ANXA5* was positively correlated with certain immune-related genes that were crucial for leukemogenesis and immune regulatory functions in AML. Functional enrichment analyses indicated that *ANXA5* was enriched mainly in neutrophil activation, neutrophil-mediated immunity, phagocytosis, lysosomal lumen, immunoglobulin binding, cytokine receptor activity, and signaling pattern recognition receptor activity. GSEA further revealed that 20 pathways were enriched, and primarily involved the following: glutathione metabolism, leukocyte transendothelial migration, chemokine signaling pathway, and hematopoietic cell lineage. In addition, other pathways such as the NOTCH signaling pathway, Toll-like receptor signaling pathway, and VEGF signaling pathway were also related to leukemia.

Pediatric AML lacks an effective prognostic marker to guide the selection of appropriate treatment. In previous research, the expression level of annexin family members predicted clinical outcomes with regard to different variables in combination with clinical characteristics, many of which are frequently dysregulated in human cancers. Tyagi *et al.* demonstrated that patients with AML with high levels of annexin-V expression had significantly inferior OS in univariate analysis. It was also revealed that high apoptosis may be associated with a high-risk phenotype of disease.[50] Several studies have reported that *ANXA5* stimulates reduction of interleukin-6 production in bone-marrow-derived macrophages *in vitro*, reduces the infarcted area after ischemia-reperfusion injury, and improves cardiac function by inhibiting cardiac inflammatory response.[51] *ANXA5* improves diagnostic efficiency of conventional biomarkers in predicting mortality in patients with heart failure.[52] *ANXA5* increases the phosphorylation level of ERK, and ERK inhibitors reverse the activation of *ANXA5* by the ERK/Nrf2 pathway.[53] Linke B *et al.* suggested that manipulating annexin family members mediated immunosuppression may benefit patients with cancer or autoimmune diseases and chronic inflammation.[54] *ANXA5* was associated with monocyte differentiation, which correlates with favorable clinical outcome in AML.[25,55] In addition, *ANXA5* is highly expressed in cells with barrier functions, including vascular endothelium and placental trophoblast cells, and binds to lipopolysaccharide, reducing its endotoxin activity.[56] Since *ANXA5* has an influential biological role based on these findings, we propose *ANXA5* as a predictive molecular marker to guide treatment choice in pediatric AML.

Taken together, the results of this study indicate that *ANXA5* produces different prognostic outcomes in different treatment groups of pediatric AML, and patients with high *ANXA5* expression benefit from GO therapy. Our analysis was based on information obtained from the TARGET database, and strengths of the trial included its strong eligibility criteria and a uniform treatment regimen according to standard guidelines. Despite the fact that our results provide a novel therapeutic option for pediatric AML, there are still certain limitations. Our analysis is a retrospective study design, and so the accuracy rate may drop in small-sample cases. This work is based on the results of an RNA-sequencing dataset, and more

biological insights remain to be explored. Furthermore, whether this scheme is applicable to adult AML needs to be confirmed by research and practice.

In summary, our research results indicate that the curative effect of GO treatment may be closely related to the level of *ANXA5* expression regulation in pediatric AML. This will improve risk stratification and decision-making regarding treatment options. Furthermore, overexpression of *ANXA5* may circumvent poor outcomes from chemotherapy combined with GO. This discovery may further benefit investigations of the therapeutic direction to guide optimal treatment regimens for individual patients.

Conflict of interest

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

References

1. Miller KD, Nogueira L, Mariotto AB, *et al.* Cancer treatment and survivorship statistics, 2019. *CA Cancer J Clin* 2019; 69: 363–385.
2. Weinberg OK, Sohani AR, Bhargava P, *et al.* Diagnostic work-up of acute myeloid leukemia. *Am J Hematol* 2017; 92: 317–321.
3. de Rooij JD, Zwaan CM and van den Heuvel-Eibrink M. Pediatric AML: from biology to clinical management. *J Clin Med* 2015; 4: 127–149.
4. Cornelissen JJ and Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. *Blood* 2016; 127: 62–70.
5. Wiernik PH. Optimal therapy for adult patients with acute myeloid leukemia in first complete remission. *Curr Treat Options Oncol* 2014; 15: 171–186.
6. Laubach J and Rao AV. Current and emerging strategies for the management of acute myeloid leukemia in the elderly. *Oncologist* 2008; 13: 1097–1108.
7. O'Dwyer K, Freyer DR and Horan JT. Treatment strategies for adolescent and young adult patients with acute myeloid leukemia. *Blood* 2018; 132: 362–368.
8. Hofmann S, Schubert ML, Wang L, *et al.* Chimeric Antigen receptor (CAR) T cell therapy in acute myeloid leukemia (AML). *J Clin Med* 2019; 8: E200.
9. Zhang N, Chen Y, Lou S, *et al.* A six-gene-based prognostic model predicts complete remission and overall survival in childhood acute myeloid leukemia. *Onco Targets Ther* 2019; 12: 6591–6604.
10. Godwin CD, Gale RP and Walter RB. Gemtuzumab ozogamicin in acute myeloid leukemia. *Leukemia* 2017; 31: 1855–1868.
11. Bross PF, Beitz J, Chen G, *et al.* Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin Cancer Res* 2001; 7: 1490–1496.
12. Larson RA, Sievers EL, Stadtmauer EA, *et al.* Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. *Cancer* 2005; 104: 1442–1452.
13. Jen EY, Ko CW, Lee JE, *et al.* FDA approval: gemtuzumab ozogamicin for the treatment of adults with newly diagnosed CD33-positive acute myeloid leukemia. *Clin Cancer Res* 2018; 24: 3242–3246.
14. Balaian L and Ball ED. Cytotoxic activity of gemtuzumab ozogamicin (Mylotarg) in acute myeloid leukemia correlates with the expression of protein kinase syk. *Leukemia* 2006; 20: 2093–2101.
15. Fostvedt LK, Hibma JE, Masters JC, *et al.* Pharmacokinetic/pharmacodynamic modeling to support the re-approval of gemtuzumab ozogamicin. *Clin Pharmacol Ther* 2019; 106: 1006–1017.

16. Norsworthy KJ, Ko CW, Lee JE, *et al*. FDA approval summary: mylotarg for treatment of patients with relapsed or refractory CD33-positive acute myeloid leukemia. *Oncologist* 2018; 23: 1103–1108.

17. Bouter A, Carmeille R, Gounou C, *et al*. Review: annexin-A5 and cell membrane repair. *Placenta* 2015; 36(Suppl. 1): S43–S49.

18. Boersma HH, Kietselaer BL, Stolk LM, *et al*. Past, present, and future of annexin A5: from protein discovery to clinical applications. *J Nucl Med* 2005; 46: 2035–2050.

19. van Genderen HO, Kenis H, Hofstra L, *et al*. Extracellular annexin A5: functions of phosphatidylserine-binding and two-dimensional crystallization. *Biochim Biophys Acta* 2008; 1783: 953–963.

20. Xue G, Hao LQ, Ding FX, *et al*. Expression of annexin A5 is associated with higher tumor stage and poor prognosis in colorectal adenocarcinomas. *J Clin Gastroenterol* 2009; 43: 831–837.

21. Tang S, Huang W, Zhong M, *et al*. Identification Keratin 1 as a cDDP-resistant protein in nasopharyngeal carcinoma cell lines. *J Proteomics* 2012; 75: 2352–2360.

22. Lee EK, Cho H and Kim CW. Proteomic analysis of cancer stem cells in human prostate cancer cells. *Biochem Biophys Res Commun* 2011; 412: 279–285.

23. Sofiadis A, Becker S, Hellman U, *et al*. Proteomic profiling of follicular and papillary thyroid tumors. *Eur J Endocrinol* 2012; 166: 657–667.

24. Han SH, Korm S, Han YG, *et al*. GCA links TRAF6-ULK1-dependent autophagy activation in resistant chronic myeloid leukemia. *Autophagy* 2019; 15: 2076–2090.

25. Niu Y, Yang X, Chen Y, *et al*. Distinct prognostic values of Annexin family members expression in acute myeloid leukemia. *Clin Transl Oncol* 2019; 21: 1186–1196.

26. Gamis AS, Alonzo TA, Meshinchi S, *et al*. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III children's oncology group trial AAML0531. *J Clin Oncol* 2014; 32: 3021–3032.

27. Cooper TM, Franklin J, Gerbing RB, *et al*. AAML03P1, a pilot study of the safety of gemtuzumab ozogamicin in combination with chemotherapy for newly diagnosed childhood acute myeloid leukemia: a report from the Children's Oncology Group. *Cancer* 2012; 118: 761–769.

28. Oran B, Cortes J, Beitinjaneh A, *et al*. Allogeneic transplantation in first remission improves outcomes irrespective of FLT3-ITD allelic ratio in FLT3-ITD-positive acute myelogenous leukemia. *Biol Blood Marrow Transplant* 2016; 22: 1218–1226.

29. Döhner H, Estey E, Grimwade D, *et al*. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; 129: 424–447.

30. Szklarczyk D, Morris JH, Cook H, *et al*. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res* 2017; 45: D362–D368.

31. Barretina J, Caponigro G, Stransky N, *et al*. Addendum: the cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 2019; 565: E5–E6.

32. Subramanian A, Tamayo P, Mootha VK, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 2005; 102: 15545–15550.

33. Swerdlow SH, Campo E, Pileri SA, *et al*. The 2016 revision of the world health organization classification of lymphoid neoplasms. *Blood* 2016; 127: 2375–2390.

34. Hobo W, Hutten T, Schaap N, *et al*. Immune checkpoint molecules in acute myeloid leukaemia: managing the double-edged sword. *Br J Haematol* 2018; 181: 38–53.

35. Holmström MO and Hasselbalch HC. Cancer immune therapy for myeloid malignancies: present and future. *Semin Immunopathol* 2019; 41: 97–109.

36. Kuhlen M, Klusmann JH and Hoell JI. Molecular approaches to treating pediatric leukemias. *Front Pediatr* 2019; 7: 368.

37. Zhang N, Chen Y, Shen Y, *et al*. Comprehensive analysis the potential biomarkers for the high-risk of childhood acute myeloid leukemia based on a competing endogenous RNA network. *Blood Cells Mol Dis* 2019; 79: 102352.

38. Li X, Xu SN, Qin DB, *et al*. Effect of adding gemtuzumab ozogamicin to induction chemotherapy for newly diagnosed acute myeloid leukemia: a meta-analysis of prospective randomized phase III trials. *Ann Oncol* 2014; 25: 455–461.

39. Shimada A. Hematological malignancies and molecular targeting therapy. *Eur J Pharmacol* 2019; 862: 172641.

40. Raffoux E, Rousselot P, Poupon J, *et al.* Combined treatment with arsenic trioxide and all-trans-retinoic acid in patients with relapsed acute promyelocytic leukemia. *J Clin Oncol* 2003; 21: 2326–2334.

41. Smith BD, Levis M, Beran M, *et al.* Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. *Blood* 2004; 103: 3669–3676.

42. Stone RM, Mandrekar SJ, Sanford BL, *et al.* Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. *N Engl J Med* 2017; 377: 454–464.

43. Wei AH and Tiong IS. Midostaurin, enasidenib, CPX-351, gemtuzumab ozogamicin, and venetoclax bring new hope to AML. *Blood* 2017; 130: 2469–2474.

44. Bohl SR, Bullinger L and Rücker FG. New targeted agents in acute myeloid leukemia: new hope on the rise. *Int J Mol Sci* 2019; 20: pii: E1983.

45. Itzykson R, Kosmider O, Cluzeau T, *et al.* Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. *Leukemia* 2011; 25: 1147–1152.

46. Welch JS, Petti AA, Miller CA, *et al.* TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. *N Engl J Med* 2016; 375: 2023–2036.

47. Agarwal S, Gopalakrishnan S, Mensing S, *et al.* Optimizing venetoclax dose in combination with low intensive therapies in elderly patients with newly diagnosed acute myeloid leukemia: an exposure-response analysis. *Hematol Oncol* 2019; 37: 464–473.

48. DiNardo CD, Pratz KW, Letai A, *et al.* Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol* 2018; 19: 216–228.

49. Mizutani Y, Inase A, Maimaitili Y, *et al.* An mTORC1/2 dual inhibitor, AZD2014, acts as a lysosomal function activator and enhances gemtuzumab ozogamicin-induced apoptosis in primary human leukemia cells. *Int J Hematol* 2019; 110: 490–499.

50. Tyagi A, Pramanik R, Bakhshi R, *et al.* Apoptosis: a biomarker of high-risk phenotype in pediatric acute myeloid leukemia. *Int J Lab Hematol* 2019; 41: 141–147.

51. de Jong R, Pluijmert NJ, de Vries MR, *et al.* Annexin A5 reduces infarct size and improves cardiac function after myocardial ischemia-reperfusion injury by suppression of the cardiac inflammatory response. *Sci Rep* 2018; 8: 6753.

52. Schurgers LJ, Burgmaier M, Ueland T, *et al.* Circulating annexin A5 predicts mortality in patients with heart failure. *J Intern Med* 2016; 279: 89–97.

53. Zhang L, Qin Z, Li R, *et al.* The role of ANXA5 in DBP-induced oxidative stress through ERK/Nrf2 pathway. *Environ Toxicol Pharmacol* 2019; 72: 103236.

54. Linke B, Abeler-Dörner L, Jahndel V, *et al.* The tolerogenic function of annexins on apoptotic cells is mediated by the annexin core domain. *J Immunol* 2015; 194: 5233–5242.

55. Forthun RB, Aasebø E, Rasinger JD, *et al.* Phosphoprotein DIGE profiles reflect blast differentiation, cytogenetic risk stratification, FLT3/NPM1 mutations and therapy response in acute myeloid leukaemia. *J Proteomics* 2018; 173: 32–41.

56. Rand JH, Wu XX, Lin EY, *et al.* Annexin A5 binds to lipopolysaccharide and reduces its endotoxin activity. *mBio* 2012; 3: e00292-11.